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12th International Conference on Thrombosis and Hemostasis Issues in Cancer

Bergamo, Italy | May 17-19, 2024

Guest Editors: Anna Falanga, Benjamin Brenner, Alok A. Khorana

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Bleeding, Thrombosis and Vascular Biology

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Papers and Abstracts of the 12th International Conference on Thrombosis and Hemostasis Issues in Cancer

May 17-19, 2024 | Bergamo, Italy

Guest Editors:

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Preface to the Proceedings of the 12th International Conference on Thrombosis and Hemostasis Issues in Cancer, 2024

Dear Colleagues,

We are pleased to present this volume of the Proceedings of the 12th International Conference on Thrombosis and Hemostasis Issues in Cancer (ICTHIC) being held in Bergamo, Italy, May 17-19, 2024.

Cancer-associated thrombosis – which includes both venous and arterial events in its clinical manifestations and involves cancer biology, hemostatic proteins, platelets and many other players in its subclinical terrain – is an old problem. Yet it makes its way into the newest of new themes in cancer medicine, including new paradigms of treatment which continue to be complicated by this very old effect. The ICTHIC conference since its inception has focused on integrating varied perspectives from different disciplines involved in both the investigation of cancer biology, and the treatment of cancer patients, into the ultimate goal of reducing the burden and consequences of cancer-associated thrombosis. A secondary aim has been to provide emerging investigators and research themes a platform to carry the field forward.

Our 2024 edition's opening set of articles focuses on **emerging trends** in cancer-associated thrombosis. Jamie O'Sullivan from Ireland describes endothelial cell dysfunction in cancer, and its role as a modulator of both thrombosis and metastasis. Deborah Siegal from Canada reviews the impact of arterial thromboembolism in this setting, with a focus on stroke in patients with cancer. Ang Li from the United States provides updates on trends in the epidemiology of cancer-associated thrombosis. Maria Barca-Hernando from Spain provides information on the association between the location of metastasis and complications of anticoagulant treatment. A major highlight of the ICTHIC meeting is the **Simon Karpatkin Memorial Lecture**, which honors the memory of the late Professor Karpatkin of New York University. The 2024 awardee is Agnes Y. Y. Lee from Canada, who may be said to have pioneered the field of treatment of cancer-associated venous thromboembolism (VTE) by leading the first randomized trial of treatment in the cancer setting. This groundbreaking study resulted in the first regulatory approval of an anticoagulant specifically for the treatment of cancer-associated VTE and created a model for future agents and classes of drugs to follow. She provides her perspective in a major overview of the topic of *VTE Treatment In Patients with Cancer: Reflections On An Evolving Landscape*.

A second set of articles focuses on **anticoagulation management in hematologic malignancies**. Kristen Sanfilippo from the United States provides an overview of the association of VTE with mortality specifically in people with hematologic cancers. Simon Stanworth from the United Kingdom provides a description of the uncertainties in managing thrombosis and anticoagulation in patients with thrombocytopenia which is a major concern in the hematologic malignancy population. A third theme of the meeting explores new insights into the relationship between **hemostasis and cancer**. Janusz Rak from Canada describes the intricate and complex interplay between the coaguloma and the tumor microenvironment. In a special article, Simon Noble from the United Kingdom speaks to the importance of patient and public involvement in research into cancer-associated thrombosis.

The **past, present and future of cancer-associated thrombosis** is another theme explored by various investigators. One of us (A.A.K. from the United States) evaluates the emerging association of VTE in cancer patients treated with immune checkpoint inhibitors, drugs that are changing the paradigm of cancer treatment yet seem afflicted with the same "old" problem. Marcello Di Nisio from Italy looks ahead to an emerging class of agents – factor XI inhibitors – and how they could potentially be new options for the prevention and treatment of cancer-associated thrombosis. The future may be altered not just by treatment options but also by **novel biomarkers**. Jeffrey Zwicker from the United States evaluates emerging new technology of proteomics and how it can be used to both predict cancer-associated VTE, and to provide mechanistic insights into its pathophysiology. Simon Mantha, also from the United States, provides an overview of the use of machine learning approaches for the prediction of cancer-associated thrombosis. The final set of articles evaluates knowledge gaps in **anticoagulation in cancer patients**. Gary Raskob from the United States evaluates the risk of recurrent VTE in cancer patients after discontinuation of anticoagulant therapy.

We are grateful to all the authors, researchers and meeting participants for their contributions to this meeting. As cancer medicine evolves, the roles of the hemostatic system and of anticoagulants in cancer biology and cancer outcomes, respectively, are becoming increasingly clear. We are delighted that ICTHIC remains at the cutting-edge of this paradigm shift, and that it continues to serve as a major platform for the various disciplines involved in this important area of cancer research.

The Conference Chairmen
Anna Falanga, Benjamin Brenner, Alok A. Khorana

Venous thromboembolism treatment in patients with cancer: reflections on an evolving landscape

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ABSTRACT

Cancer is a leading cause of morbidity and mortality worldwide. It is also one of the strongest risk factors for venous thromboembolism (VTE), reported in approximately 20% of all cases of VTE diagnosed. The thrombotic effect of cancer and its treatments, however, is highly variable among patients and changes over the course of their cancer. Anticoagulant therapy remains the cornerstone of VTE treatment, but it is associated with a substantial rate of VTE recurrence and the potential for serious bleeding. The risk of bleeding in patients with cancer is also dependent on the cancer type and its treatments, often revealing underlying tumor invasion of mucosal or parenchymal tissues, and treatment complications such as thrombocytopenia or coagulopathy. Over the past few decades, efforts to improve the efficacy and safety of anticoagulant therapy for the treatment and prevention of cancer-associated thromboembolism have resulted in changes in the standard of practice. This evolution has been made possible largely through the development of new anticoagulants. This review will reflect on the major advances in the treatment of cancer-associated thrombosis and offer insights on how to address unmet needs in this field.

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Introduction

Cancer is a leading cause of morbidity and death worldwide. In 2020, over 19 million new cases of cancer were diagnosed globally, and the annual incidence is continuing to climb.[1] Among these patients, just over 5 million were diagnosed with cancers of the pancreas, liver/biliary, lung, ovary, or stomach. These 5 tumor types are associated with the highest risks of venous thromboembolism (VTE) with incidence rates ranging from 66.4 to 156.0 per 1000-person years within the first 6 months after the cancer diagnosis.[2-4] However, because breast, lung, colorectal, and prostate cancers are the most commonly diagnosed cancers worldwide, they are the most prevalent cancers in patients diagnosed with cancer-associated thrombosis despite the relatively lower risk of thrombosis for breast and prostate (Figure 1).[2] The incidence of cancer-associated thrombosis is rising over time and it is associated with an increased mortality for all tumor types.[3] In addition to the tumor type, the extent of the cancer (*e.g.*, metastatic versus localized) and the prescribed systemic therapies (*e.g.*, surgery, chemotherapy, immunotherapy) contribute to each individual's risk of thrombosis.[5] Patient-specific factors, such as age, sex, race/ethnicity, and the presence of other prothrombotic conditions also influence the overall risk of thrombosis. The risk of bleeding in patients with cancer is also dependent on some of the same factors as thrombosis, along with a history of bleeding, chronic kidney disease, and use of antiplatelet agents.[6] Consequently, providing optimal patient-centered care for the treatment of cancer-associated thrombosis requires balancing a multitude of factors, including patient values and preferences, all of which can also change over time. Furthermore, managing patients with cancer-associated thrombosis has become more complex over the past two decades because of the rapidly changing landscape of cancer therapeutics, prolonged survival of patients with advanced disease, and the availability of more anticoagulant options with variable costs, convenience and accessibility.

Before the 1990s: heparin and vitamin K antagonists

Heparin and vitamin K antagonists (VKAs) were the only available anticoagulants for over half a century.[7] Heparin was discovered in 1916 and came into clinical use in the 1930s. The first randomized trial demonstrating the efficacy of anticoagulant therapy with unfractionated heparin in patients with pulmonary embolism was published in 1960.[8] Dicoumarol, a VKA no longer in use, was introduced for clinical use in the 1950s, and other VKAs, such as warfarin, remained the only long-term oral anticoagulant option for the next 5 decades. Although heparin and VKAs are cumbersome to use because of the requirement for laboratory monitoring and dose adjustments to achieve blood levels within a narrow therapeutic range, they remain in common use today for managing venous and arterial thrombosis. In fact, unfractionated heparin is still the drug of choice for coronary bypass surgery and critically ill or unstable patients with acute thrombotic events, while VKA is the drug of choice for thrombosis prevention in mechanical heart valves and antiphospholipid syndrome. Their 'reign' is expected to continue given their established efficacy in these settings, their low cost, and their rapid reversibility.

Between the 1970s and early 2000s, VTE treatment with heparin followed by warfarin was the standard of care for all patients, regardless of their cancer status.[9,10] It was recognized that outcomes were worse in patients with cancer, with higher rates of recurrent thrombosis, major bleeding and mortality. But without other anticoagulant options, cancer patients were sometimes treated with warfarin at a higher intensity which often resulted in more bleeding and worse outcomes.[9-11]

1990s: low molecular weight heparin is the new standard of care

To overcome the unpredictable pharmacodynamics of unfractionated heparin, low-molecular-weight heparin (LMWH) was developed in the 1990s.[7] Numerous randomized trials directly compared these agents for the initial treatment of acute VTE and meta-analyses of these studies demonstrated subcutaneous, weight-based dosing of various LMWHs is superior to intravenous heparin in reducing recurrent thrombosis and major bleeding.[12] LMWH also allowed outpatient treatment and thus revolutionized acute care delivery in VTE. But the need to use warfarin for long-term treatment and secondary prevention (because no other oral agents were available) remained unsatisfactory. This was particularly challenging in patients with cancer, in whom the time-in-therapeutic range for the INR was suboptimal because of drug-drug interactions, poor nutrition, and gastrointestinal toxicity.[9,10] The requirement for venipunctures is especially traumatic to patients with difficult venous access after multiple rounds of chemotherapy. This prompted the investigation of using LMWH for initial and long-term treatment, instead of transitioning to warfarin. Following the publication of the CLOT trial and several other randomized trials, all major clinical practice guidelines endorsed using LMWH over VKA as first-line treatment for cancer-associated thrombosis.[13]

Meta-analyses showed LMWH offered a risk reduction of 53% in symptomatic recurrent thrombosis without increasing the risk of major bleeding compared with VKA.[14] However, the lack of survival benefits, the unpleasantness of daily injections and the high cost of LMWH are major barriers in implementing the change in practice and maintaining adherence.[15] Worldwide, VKA

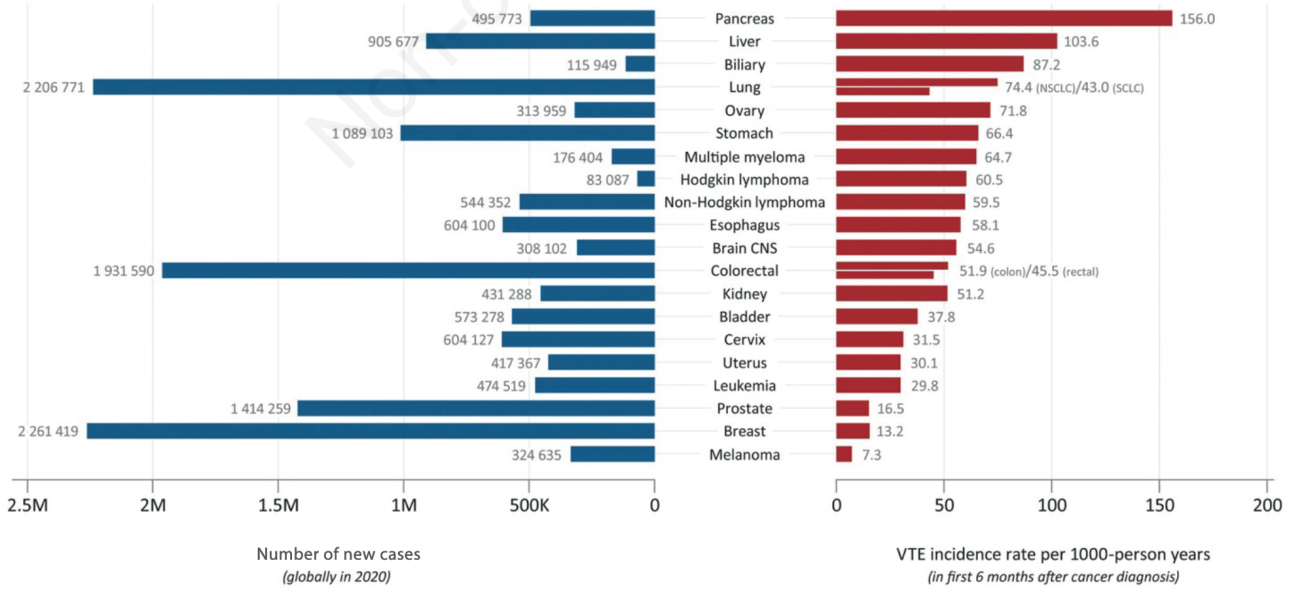

Figure 1. The burden of cancer and thrombosis in patients with cancer. This figures summaries the estimated number of new cases of cancer for major types of cancer reported globally in 2020 and the incidence rate of thrombosis per 1000-person years in the first 6 months after cancer diagnosis.[1,4]

therapy remains a commonly used anticoagulant, especially in those with limited government reimbursement or insurance coverage, living in low-income areas, and in patients who are unable to inject.[16-18]

Early 2000s: specific factor inhibition with fondaparinux and direct thrombin inhibitor

Development of fondaparinux in 1997 delivered the proof of concept that selective inhibition of activated factor X (FXa) alone was effective and safe in treating acute VTE.[7] Fondaparinux was the first synthetic, small molecule parenteral anticoagulant that can be given at a fixed dose as a once-daily subcutaneous injection. Clinical trials demonstrated that fondaparinux was comparable to LMWH in efficacy and safety but a subgroup, post-hoc analysis of patients with cancer in the Matisse-DVT trial suggested that fondaparinux was less efficacious than LMWH in this population.[19] Further studies were not done to verify this finding and the lack of any practical advantage (in terms of cost and route of administration) over LMWH likely made fondaparinux a less attractive alternative to LMWH. Further development of small molecules that were selective and potent inhibitors of FXa or thrombin followed.[7] Dabigatran became the first direct oral anticoagulant (DOAC) that showed efficacy and safety compared with VKA for the acute and long-term treatment for VTE. Although a lead-in period of 5 days of LMWH prior to dabigatran use is required, the convenience of this direct thrombin inhibitor with a fixed, twice-daily dosing regimen and far fewer drug and food interactions compared with warfarin was obvious. A subgroup, post-hoc analysis also suggested that dabigatran could be potentially useful in patients with cancer and thrombosis.[20] However, dabigatran has not been compared directly with LMWH and it is not recommended for use in this setting by most clinical practice guidelines.[21,22]

2010s: direct oral anticoagulant in cancer-associated thrombosis

On the heels of dabigatran, randomized trials of oral FXa inhibitors in VTE treatment were published between 2010 and 2013.[23] All were of similar design and showed that each DOAC was non-inferior to standard treatment with heparin/LMWH followed by VKA in reducing recurrent thrombosis. The risks of major and clinically relevant non-major bleeding were also similar between DOAC and VKA. About 6% of patients in these studies had active cancer or a history of cancer and the subgroup analyses of these highly selected patients with cancer suggested DOAC is comparable with warfarin.[24] However, it was evident that the cancer patient populations enrolled in DOAC *vs*. VKA trials were *healthier* than those in LMWH *vs*. VKA trials, as the rates of recurrent VTE, bleeding and mortality were lower in DOAC trials.[24] Network meta-analyses of these early trials suggested that DOAC would be comparable to warfarin and also LMWH for treatment of cancer-associated thrombosis.[25]

Cancer-associated thrombosis treatment studies comparing DOAC directly with LMWH soon followed.[26] The first randomized trial (Hokusai VTE Cancer) studying edoxaban was published in 2018 and the largest trial (Caravaggio) evaluating apixaban was reported in 2020. Smaller studies (SELECT-D and CASTA-DIVA) described the outcomes for rivaroxaban. Some studies excluded certain types of cancer, such as primary brain cancers, and all studies excluded patients with a high risk of bleeding, hepatic impairment or severe renal dysfunction, or poor performance status (Eastern Cooperative Oncology Group 3-4).[26] They demonstrated that DOAC is non-inferior to LMWH in efficacy but varied in the relative risk of clinically relevant bleeding. A meta-analysis combining the results of all randomized controlled trials demonstrated that DOAC, compared with LMWH, is associated with a significantly lower risk of recurrent VTE [relative risk (RR), 0.67 (95% CI, 0.52-0.84)], a non-significant increased risk of major bleeding [RR, 1.17 (95% CI, 0.82-1.67)], and a significant increase in clinically relevant non-major bleeding [RR, 1.66 (95% CI, 1.31-2.09)].[27] The higher rates of bleeding were largely driven by gastrointestinal bleeding, occurring mostly in patients with gastrointestinal cancers and particularly in those with unresected luminal tumors.[28,29] Other sites of clinically relevant bleeding included hematuria, abnormal uterine bleeding or epistaxis. Although real-world data have also emerged to suggest that DOACs may carry different bleeding risks, head-to-head comparisons are needed to verify these observations given the significant heterogeneity of the patient populations. Other clinically important differences among DOACs include mechanisms of drug-drug interactions, oral bioavailability and sites of gastrointestinal absorption.[21,22,26]

The totality of evidence and major clinical practice guidelines to date indicate that direct oral FXa inhibitors (apixaban, edoxaban and rivaroxaban), LMWH and warfarin all have important roles and limitations in the treatment of cancer-associated thrombosis.[21,22,26] The complexity of this patient population demands individualized therapy that cannot be met with any single class of these anticoagulants. For example, many clinical scenarios associated with a higher risk of bleeding lack high-quality evidence to guide management.[26,30] Up to 13% of cancer patients on anticoagulant therapy experience major bleeding, with a case-fatality rate of 8.9% in patients with cancer.[6,31]

2020s: factor XI inhibition is the new frontier

To reduce the risk of anticoagulant-related bleeding, new targets in the coagulation cascade are being examined. The most promising of these is factor XI (FXI) in the contact pathway.[32] Based on epidemiology data, observational studies and animal models, selective inhibition FXI could be effective in reducing thrombosis without interfering with hemostasis.[32,33] Inhibition of the contact pathway might also offer improved efficacy in management of thrombosis associated with foreign materials in medical devices such as central venous catheters and mechanical heart valves. Currently, this upstream blockade approach is being investigated in clinical trials for stroke prevention in atrial fibrillation, treatment of acute coronary syndrome, thromboprophylaxis in total joint arthroplasty, and cancer-associated thrombosis.

Abelacimab, a human IgG1 monoclonal antibody that binds to FXI and blocks its activation by activated FXII or thrombin, is the first FXI inhibitor being evaluated for treatment of cancer-associated thrombosis. Two complementary phase 3 randomized trials are currently enrolling patients with active cancer and VTE. The ASTER trial (NCT05171049) is comparing abelacimab with

apixaban in patients who are eligible for DOAC therapy, while the MAGNOLIA trial (NCT051171075) is comparing abelacimab with LMWH in patients with gastrointestinal or genitourinary cancers. Potential drawbacks of abelacimab are that it has a half-life of about 20 days, and it is a parenteral agent. But some argue that once-monthly subcutaneous injection could improve persistence and adherence with therapy over the long term if administration is timed with regular oncology visits. Abelacimab also does not rely on gastrointestinal absorption or renal or hepatic clearance, which are barriers for DOAC use in some patients. But unlike DOACs which are short-acting and have rapid reversal agents available, abelacimab has a prolonged anticoagulant effect and there are no proven methods for controlling serious bleeding.[34] Shorter-acting, small molecules that block the active site of FXIa, such as asundexian and milvexian, are also under investigation in a number of cardiovascular settings, but studies of these oral agents in cancer-associated thrombosis are not yet available.[33]

Unmet clinical needs: more work ahead

Without a doubt, the effectiveness, convenience and lower cost of DOACs have made treatment of cancer-associated thrombosis more accessible and acceptable for many patients. Still, there are many areas where DOAC and other anticoagulants fall short in the treatment of thrombosis in these complex and heterogeneous patients.[26,30]

Anticoagulant-related bleeding poses one of the biggest challenges and fears in treating patients with cancer-associated thrombosis. FXI inhibition might improve the risk-benefit profile but there may be other ways to reduce bleeding. For example, avoiding unnecessary invasive procedures and paying closer attention to renal and hepatic function will help to reduce iatrogenic instances of bleeding, and primary prophylaxis with proton pump inhibition might reduce upper gastrointestinal bleeding.[35] Using non-anticoagulant agents that target pathways (*e.g.*, complement system) that can activate coagulation or the vascular endothelium is also worthy of investigation.[36-38] Also, as we learn more about cancer-specific mechanisms of thrombosis, targeting the molecular pathways involved might offer even more precise therapy.[39]

Patients with unusual site thrombosis (*e.g.*, splanchnic vein thrombosis), primary brain cancer, untreated intracranial metastasis, severe thrombocytopenia, and shorter life expectancy are routinely excluded from clinical trials participation. Yet, these patients might experience the most harm when it comes to anticoagulant therapy.[40,41] Reluctantly, clinicians extrapolate findings from clinical trials and often rely on retrospective analyses from administrative data sets. Results from such *real world* studies are often outdated and contain inherent biases (*e.g.*, confounding by indication). More organized and collaborative research efforts are needed to provide higher-quality evidence to manage these vulnerable patients.

Another area where data are lacking is in the management of refractory or 'breakthrough' thrombosis, when patients develop recurrent thrombosis despite being on therapeutic anticoagulation. This is a common outcome and yet little therapeutic advancement has been made over the past decades. The best available evidence remains small, retrospective studies and registries that reported dose escalation of LMWH can be effective and appears safe.[42] Applying the same principle by using higher doses of DOAC has not been studied and carries a heightened concern for bleeding. Importantly, drug-drug interaction, reduced gastrointestinal absorption and poor treatment adherence should be excluded as potential causes of refractory thrombosis before concluding there is true treatment failure.[26,42]

Optimal duration and dosing for extended anticoagulation, an issue that is encountered in all patients, remains inadequately addressed in cancer-associated thrombosis.[42] One randomized trial has shown that treatment of cancer patients with isolated distal DVT with edoxaban for 12 months reduced symptomatic recurrent VTE or VTE-related death compared with 3 months.[43] An ongoing randomized trial (APICAT NCT03692065) is comparing standard- with low-dose apixaban for secondary prevention after 6 months or more of full-dose anticoagulation.[44] Guideline recommendations to continue anticoagulant therapy beyond 6 months in patients with active cancer, metastatic disease or who are receiving anticancer therapy are largely based on expert experience. This seems reasonable when the risk of recurrent thrombosis remains at 5-15% even after the first 6 months of anticoagulant therapy, but information on thrombosis and bleeding beyond the first year of the thrombotic event is scarce.[2,45,46] Nevertheless, we do recognize now that the risk-benefit balance might be tipped towards avoiding anticoagulant therapy in patients in the palliative phase of their cancer.[47] It is also important to note that advancements in cancer care have further complicated this decision process as an increasing number of cancer patients enjoy extended survival. For example, as maintenance therapy becomes the standard of care for a multitude of malignancies (*e.g.*, immunomodulatory therapy for myeloma, check-point inhibitors in non-small cell lung cancer), identifying when it is safe to stop anticoagulant therapy for patients with metastatic cancer will have a substantial impact. Biomarkers and risk assessment models might play an important role in risk prediction in this setting.[45,48]

Targeting tumor growth might be an essential strategy to manage cancer-associated thrombosis.[49] Experimental data in the 1950s first offered plausible mechanisms of anticancer effects for anticoagulants and this hypothesis was then tested in an observational study in 1964, which reported a beneficial effect of VKA on mortality in patients with cancer.[50] Almost 20 years later, this field of research was ignited when a randomized trial in 1981 showed that warfarin was associated with significant improvement in overall survival in patients with small-cell lung cancer.[51] However, enthusiasm dimmed when subsequent studies in other types of cancer provided negative results.[52] Over the past 20 years, a similar cycle of research studying heparins and LMWH followed, with preclinical studies continuing to provide evidence that anticoagulants, particularly LMWH, may have antitumor effects (*e.g.*, antiangiogenesis) while clinical studies in different settings (*e.g.*, tumor type and stage) produced provocative but inconsistent results on cancer patient survival.[53,54] Now, 60 years later, we are circling back to the hypothesis that warfarin, compared with LMWH or DOAC, is associated with a survival benefit in cancer patients.[55] Since none of the randomized controlled trials comparing warfarin with LMWH or DOAC demonstrated any difference in 6-month survival, these recent observations from administrative databases might reflect confounding by indication from the selection of patients with better prognosis to receive warfarin. It remains uncertain if anticoagulant therapy has any meaningful antitumor effects, and if present, in what specific clinical scenarios.

Finally, patient quality of life of and racial, ethnic and social disparities have not been well studied in cancer-associated thrombosis.[56,57] Evidence available indicates that thrombosis is a dramatically adverse event that is under estimated by the medical community and vulnerable populations might be affected more negatively.[57-59] While the incidence of thrombosis varies among Blacks, Whites and Asians, the bulk of published literature on epidemiology, prevention and treatment are largely derived from White populations and from higher-income nations.[58,60] Across many parts of the world, the anticoagulant choices made may be less dependent on science and more dictated by practical issues such as accesibility.[16-18,60]

Conclusions

Major achievements have been made in the management of cancer-associated thrombosis over the past decades. The availability of more anticoagulant options and a better appreciation of patient preferences and values have changed clinical practice. Further improvements will require novel approaches, such as inhibiting coagulation without disturbing hemostasis, adopting innovative research methodologies, embracing risk in challenging clinical settings, and broadening research collaboration around the globe. We must also pay more attention to equity, inclusion and diversity; so that as we march forward to break new ground, we must also look back, look outside the box, and look beyond *the usual suspects*.

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Patient and public involvement in cancer-associated thrombosis research: necessary or glorified tokenism?

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ABSTRACT

The advantages of patient and public involvement (PPI) in research are becoming more widely known, however different research organizations have different rates of adoption. Comparably, some groups provide tokenistic participation in the research process, which is inconsistent with the extent to which PPI partners are truly involved. Recent developments in the field of cancer-associated thrombosis (CAT) research have shown how crucial PPI is to the foundation of the entire research process, from formulating the research question to disseminating the findings. This manuscript aims to present an overview of PPI within the framework of CAT research and demonstrate how, when used appropriately, PPI can improve a project's overall success, rigor, and relevance.

Introduction

There is an Apocryphal story about the great Louis Armstrong who, when asked the question "What is jazz?" replied with the often misquoted answer:[1] "Man! If you gotta ask, you'll never know!"

Such a response comes to mind when I am asked by academic colleagues to explain the point of public involvement and public engagement in biomedical research. The question usually arises following a discussion in which I have challenged their assertion that having a layperson cast their eyes over a patient information sheet for a research study constitutes sufficient public involvement and public engagement. Recent years have seen an increased interest in patient and public involvement in research such that many research groups will have representation in their trial management groups. However, the contribution of public involvement representatives is variable, particularly when researchers have considered their activities to be a "box-ticking exercise" rendering their involvement tokenistic.[2,3] This paper shall discuss the merits of embracing public involvement within our research and shall include suggestions on how to optimize such activities. Whilst it is readily acknowledged that many examples of excellent public involvement exist around the globe, for the purposes of this paper, the focus shall be on undertakings within the United Kingdom (UK). It shall also give examples of where public involvement has been used successfully in cancer-associated thrombosis (CAT) research.

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Definitions

One of the biggest challenges in embedding meaningful public involvement in research is to inform people what it is in a way that the research community can understand, what the benefits are and how to embed it in such a way that it makes a real difference rather than just look good. In order to implement a new development, one must first understand what new concept or activity you are trying to introduce. For the sake of this paper definitions of public involvement and public engagement shall be as follows:

Public involvement (PI) in research is defined by the UK Health Research Authority as "research carried out 'with' or 'by' members of the public, rather than 'to', 'about' or 'for' them. It means that patients or other people with relevant experience contribute to how research is designed, conducted and disseminated. It does not refer to research participants taking part in a study".[4]

Public engagement (PE) as defined by the UK National Coordinating Centre for Public Engagement describes "the myriad of ways in which the activity and benefits of higher education and research can be shared with the public. Engagement is by definition a two-way process, involving interaction and listening, with the goal of generating mutual benefit".[5]

Confusion often arises when the term PE is erroneously used

as an umbrella term to describe both PE and PI. Many descriptions for public involvement and public engagement exist with some being used interchangeably, inevitably leading to confusion and sub-optimal practice.

Drivers for public involvement

The main overarching driver for PI in research is the fact that it supports the development, conduct and overall success of good research. The renowned theoretical physicist Richard Feynman was considered one of the most influential and inspiring scientists of the 20th century. An alumnus of Robert Oppenheimer's Manhattan Project, Nobel Laureate for his work on quantum physics and member of the Rogers Commission into the Challenger space shuttle disaster, he was also an amazing raconteur with a reputation for making science exciting and interesting. He suggested we should undertake research for "the pleasure of finding things out" a sentiment used as the title of a documentary made about him and a separate book of papers and correspondence collated by his children after his death.[6]

Whilst this is an admirable sentiment and no doubt a sufficient and achievable driver in some specialties, such philosophy lends itself less well to research in clinical medicine. For one, clinical studies are required to satisfy the scrutiny of research ethics committees to ensure there is a cogent case for the research and involvement of patient participants is justified. Funding bodies are unlikely to fund a study just because it sounds interesting, they will want to be convinced that there is not only a need for the research but also there is a strong likelihood of recruiting participants. Furthermore, they will want to know if there is a strong likelihood that the results will impact on clinical practice. These answers are unlikely to be accurately answered without the input and advice from laypersons who will see the rational importance of the study and acceptability of the intervention through a different viewpoint.

Many funders in the UK have patient and public partners on their funding panel and will expect applications to outline the amount of PI in the development of the application and how they plan to involve PI partners in the ongoing project. From the author's experience, some funding requests have been rejected on the grounds of insufficient PI or for not costing it into the bid.

Whilst PI representation is not mandatory when presenting the project at the research ethics committee meeting, it can be very useful if any questions arise regarding the patient perspective or concerns raised by lay members of the committee. Finally, the role of PI is becoming more important when looking to publish the results in peer-reviewed journals. The British Medical Journal amongst others, requires authors to outline how patients were involved in the delivery of the research, with more of their manuscripts including patient perspectives within the narratives.

Benefits of public involvement

Rather than undertaking PI in research because funding bodies, ethics committees and journals say we should, it is worth considering that rather than being the "new fashion", it is also a means by which we can undertake better research. The UK Health Research Authority suggests that academic teams engaged in PI conduct better research and undertake better studies because: i) they design studies that are of greater relevance to participants; ii) studies are more likely to be acceptable to participants; iii) participants find the study information to be more understandable; iv) the patient experience of research is better and provide a better experience of research; v) study results are communicated better to participants at the end of the study.[4]

Conducting research with public involvement

Undertaking meaningful involvement of patients and the public in health and social care research should follow four agreed principles which are: involve the right people, involve enough people, involve those people enough, and describe how it helps. These are discussed in the following paragraphs.

Principle 1: involve the right people

This means you should be involving people with lived experience of the condition being studied. Sometimes this may not be possible for various reasons; when studying patients with incurable illnesses or who are too unwell to contribute, it may be better to involve carers or significant others who have some experience and understanding. Sometimes representation from patient groups may be possible especially if they are able to act as a conduit between the researchers and a wider public population. In addition to having lived experience of the condition, it is also important to ensure the PI contributor is representative of the population associated with the condition. For example, some conditions may be associated with lifestyle choices or socioeconomic deprivation. Others might target patients of a particular gender, age, ethnicity or geographical region. It does not make sense, therefore, to only have a pool of PI contributors consisting solely of white middle-class retired gentlemen who divide their time between golf and meetings at the Rotary Club.

Principle 2: involve enough people

In order to understand the breadth of views on issues of importance to the target recruitment population, there is a need for sufficient PI representation to gain an accurate perspective of the different people whom the research is intended to benefit. Numbers will vary according to the scope of the study but a single contributor will rarely be able to covey the views and needs of the whole study population. Most of the CAT studies we have undertaken will have a minimum of two PI contributors, from different backgrounds and experiences.

It is also worth considering different roles for different contributors; not everyone needs to be a member of the project team. Some may wish to focus on reviewing the recruitment pathway or patent information literature. Others may review the acceptability of planned assessment tools or even the form of how the assessment will be conducted.

Principle 3: involve those people enough

PI contributors should be given the opportunity to be involved in as many aspects of the research project as possible. Ideally, they should be involved at the planning stage, before

funding has been awarded. It will enhance the planning of the study and ensure its relevance to the patient population. It may also identify potential pitfalls to recruitment. Examples of activities that contributors might undertake are listed in Figure 1.

Principle 4: describe how it helps

There is an expectation that researchers inform funders and regulatory authorities including the REC to describe: i) those involved in the study as PI contributors including the relevant experience they brought to the project; ii) what activities PI contributors undertook; iii) how their involvement benefit the study, *i.e.*, in what way they helped the study become more relevant, acceptable to study participation; iv) how study results are to be shared with study participants (if they wish to know) and other stakeholders.

Evaluating public involvement contribution

The UK Public Involvement Standards Development Partnership developed a set of standards against which researchers could benchmark their activity.[7] These are outlined in Figure 2. Within CAT research, the UK standards were used to evaluate PI during the Hospice Inpatient Deep vein thrombosis Detection study (HIDDen), a multicentre, prospective, longitudinal, observational study to explore the prevalence, symptom burden and natural history of venous thromboembolism in people with advanced cancer.[8,9] This was led by the study PI contributor lead who had also been involved in the development of UK standards. They concluded that all six standards were met with the greatest opportunities in 'working together' and 'support and learning'. Meeting the 'governance' standard was less complete; with evidence of participation in decision-making but little involvement in management, regulation, and leadership. The experience of benchmarking PI activity against the UK standards revealed that such appraisal was largely subjective and ideally PI involvement should be evaluated in real time so that involvement can be proactively managed. Recently, the Marie Curie Research Group and Wales Cancer Research Centre at Cardiff University have developed the Public Involvement in Research Impact Toolkit (PIRIT).[10] This is free to use and available online (https://www.cardiff.ac.uk/marie-curie-research-centre/patient-and-public-involvement/public-involvement-in-research-impact-toolkit-pirit). It was designed as a set of pragmatic tools to support researchers working with public contributors who aim to: i) proactively integrate PI into the research project through planning; ii) allow teams to track the activity of PI public contributors and evaluate the difference they have made to the research; iii) allow teams to report this in a format that benchmarks activities against the UK standards for PI.

These consist of the PIRIT planning tool and the PIRIT tracking tool. The PIRIT planning tool is a checklist of possible PI activities that may be available and follows the research pathway allowing teams to objectively measure if and how they meet the relevant standards. The PIRIT tracking tool comprises a spreadsheet to record when and how the public contributed to the research. More specifically, it will record what the activities hoped to achieve, whether their involvement made any difference, why this mattered and how this relates to the standards.

Public involvement in cancer-associated thrombosis research

The role of PI in the HIDDen study has already been discussed.[9] However, following its publication, PI in the dissemination and reflection stage of the research further influenced the next research project. With the support of the lead PI contributor, a round table discussion was organized with representation from all relevant UK professional and patient organizations. The data were presented and discussed, with particular emphasis on how the research would influence practice and whether there were ongoing unanswered questions to answer. Through this forum, the patient organization representatives gave a very strong steer on what questions were important to them and this formed the basis of the follow-up study HIDDEN2.[11]

Currently, the SERENITY study is underway; this is an ambitious 7-phased multicenter European mixed methods research program that aims to develop and subsequently evaluate a shared decision-making tool for the continuation or deprescribing of

Before funding awarded
- Developing and prioritizing research questions;
- Identifying outcome measures of relevance to the population being studied;
- Contributing to research methods with respect to practicalities such as how to optimize recruitment, minimize participant burden, identify potential pitfalls and obstacles;
- Contributing to grant application.

Once funding awarded
- Commenting on full protocol;
- Contribute to Research Ethics Committee (REC) applications;
- Attending REC meeting to discuss acceptability of study to participants;
- Co-designing and commenting on participant information sheets and other patient facing documents.

During the research
- Active participation on research advisory groups and steering groups;
- Supporting aspects of the research e.g. co-facilitating focus groups, questionnaires or distributing questionnaires.

After the research completed
- Contributing to dissemination.

Figure 1. Examples of activities undertaken by public involvement contributors.

- Inclusive opportunities:
  - Offer public involvement opportunities that are accessible and that reach people and groups according to research needs;
- Working together:
  - Work together in a way that values all contributions, and that builds and sustains mutually respectful and productive relationships;
- Support and learning:
  - Offer and promote support and learning opportunities that build confidence and skills for public involvement in research;
- Communications:
  - Use plain language for well-timed and relevant communications, as part of involvement plans and activities;
- Impact:
  - Seek improvement by identifying and sharing the difference that public involvement makes to research;
- Governance:
  - Involve the public in research management, regulation, leadership and decision making.

Figure 2. United Kingdom public involvement standards for research.[7]

antithrombotic medicines in patients with advanced cancer near the end of life.[12] Public involvement has been embedded in the project with each phase having PI activity planned. The study is being conducted across fourteen different academic institutions in eight European countries, which have differing levels of knowledge, experience and confidence in PI activity. It has thus been a dynamic, iterative educating experience for many researchers. One very apparent thing, however, is the consensus that the PI partners and the PI coordinator are an integral and essential component of the study group. Any thoughts of tokenism have long dissipated.

Conclusions

For many, public involvement is one more of a long line of tokenistic activities that do little other than symbolize academic institutions acceding to public pressure. They see it as a necessary hurdle to jump over in order to get a study funded, ethically approved and, ultimately, published in a high-profile journal. Such attitudes do result in tokenism being practiced within their own particular research groups. However, organizations that embrace the public as true partners and advisers, derive the benefit of their involvement very early on and reap the rewards of better-designed, successfully recruited-to studies of true relevance to the population being studied.

Thinking back to the original quote in this paper regarding Louis Armstrong's response is, in fact, an urban myth: as accurate as quotes such as "Play it again Sam", or "Luke, I am your father."

The true response by Armstrong, and on reflection, far more apt when considering it in the context of defining public involvement, is: "If you still have to ask, shame on you".[1]

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Endothelial cell dysfunction in cancer: a not-so-innocent bystander

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ABSTRACT

The body's homeostasis depends on the vascular endothelium, which controls angiogenesis, vascular tone, inflammation, cell trafficking, hemostasis, and the movement of nutrients and waste out of the body. Endothelial cells (ECs) are the primary gatekeepers of many of these vessel wall functions, despite only having a single cell's thickness. Normally quiescent ECs in the context of cancer are activated by anti-cancer therapies, the tumor microenvironment, and factors secreted by the tumor. Crucially, this dysfunctional endothelium actively participates in tumor metastasis and progression rather than just acting as a passive bystander. Compared to the healthy vasculature, ECs in the tumor vasculature are heterogeneous and have a different gene expression profile. Tumor-associated ECs, in particular, exhibit increased pro-angiogenic characteristics and upregulated expression of adhesion molecules and proinflammatory cytokines, facilitating the intra- and extravasation of spreading tumor cells. Furthermore, the downregulation of important anticoagulant molecules and increased endothelial secretion of prothrombotic molecules can directly encourage cancer-associated thrombosis. Many anti-cancer therapies are also less effective in their delivery and function when there is dysfunction in the tumor endothelium. The review highlights some of the most recent research showing how tumor-associated ECs influence angiogenesis, inflammation, coagulation, and metastasis to contribute to the progression of tumors. Undoubtedly, a better understanding of how the tumor microenvironment subverts quiescent ECs and how phenotypic alterations in the vessel wall support the survival and spread of tumor cells will aid in the identification of new therapeutic targets to slow the advancement of cancer.

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Introduction

The vascular endothelium is the largest organ in the body. The total surface area of the vessel wall has been estimated to be 20 times the size of a tennis court and is lined with over one trillion endothelial cells (ECs).[1] These highly specialized cells are key in maintaining vessel homeostasis, regulating vessel integrity and barrier function, contributing to immune-cell trafficking, and facilitating nutrient delivery, and waste removal.[2] ECs display a high degree of plasticity and are responsive to environmental stimuli including infection, drugs, and oxygenation status all of which can feature in patients' cancer journey.[3] Consequently, the endothelium can become significantly dysregulated by tumor-specific factors as well as anti-cancer therapies, and in turn promote tumor proliferation, angiogenesis, chemoresistance, and metastasis.[2,4]

Additionally, endothelium plays an important role in maintaining the balance between hemostasis and thrombosis.[5] Malignancy is a key driver of both arterial and venous thrombosis which not only contributes to the overall disease burden and impacts anti-cancer treatments but is the second leading cause of death for these patients.[6] While the etiology of cancer-associated thrombosis is certainly multifactorial, EC activation and damage play a significant role. Despite all this, little consideration is given to the vasculature or tumor-associated ECs (TECs) in current treatment strategies. Crucially, cancer may not only alter the function of the endothelium within the primary tumor but also systemically throughout the body, thus priming the formation of the pre-metastatic niche, facilitating extravasation, and survival of disseminated tumor cells.[7]

Rather than being a binary event, EC activation is more of a graded response. For example, activation can be specific to the stimulus and may occur locally, as seen in atherosclerosis, or systemically as seen in sepsis.[8] In the setting of cancer

specifically, EC activation has been reported to result in loss of tight- and adherens junctions, permitting EC migration and formation of new blood vessels. The mediators of endothelial activation include a diverse array of factors such as changes in shear stress induced by compression forces of a large solid tumor or a variety of proinflammatory molecules such as interleukins and cytokines, including transforming growth factor beta (TGFβ), all of which can be secreted by the tumor cells directly or secreted by tumor-associated macrophages within the tumor microenvironment (TME). Other means of EC activation that have been reported include elevated tumor secretion of angiogenic mediator, vascular endothelial growth factor (VEGF), or tumor-induced hypercoagulability resulting in thrombin-mediated protease-activated receptor (PAR) signaling on the endothelium. Finally, the direct cytotoxic effects of chemotherapy agents also have a significant impact on endothelial function.[9] This prolonged chronic EC activation also significantly impacts blood vessel morphology and contributes to poor drug delivery to the tumor. Deformed branches with poor coverage of pericytes and remodeled basement membranes result in leaky endothelial vessels, with increased interstitial pressure, decreased blood flow, and perfusion into the tissue.[10,11] This culminates in a hypoxic TME which further propagates EC dysfunction and contributes to poor drug delivery and efficacy.[11]

In this review, we discuss how malignancy can significantly disrupt endothelial homeostasis to promote angiogenesis, thrombosis, inflammation, and metastasis, which ultimately contribute to cancer progression. This also shines some light on novel therapeutic avenues aimed at vasculature normalization in cancer that are under investigation.

Angiogenesis

Tumors that excess 1-2mm^3 switch to an angiogenic phenotype to meet growing metabolic demands.[12] To this end, EC angiogenesis within the TME is orchestrated by a variety of mediators including VEGF, interleukin-8 (IL-8), angiopoietins (Ang), platelet-derived growth factors (PDGF) and TGFβ-1.[13] Elevated plasma levels of these factors are associated with a poorer prognosis and more aggressive tumor phenotype.[14,15] Consequently, anti-angiogenic therapies, including anti-VEGF antibody bevacizumab, were an attractive and promising addition to the oncologist's armamentarium. Despite the

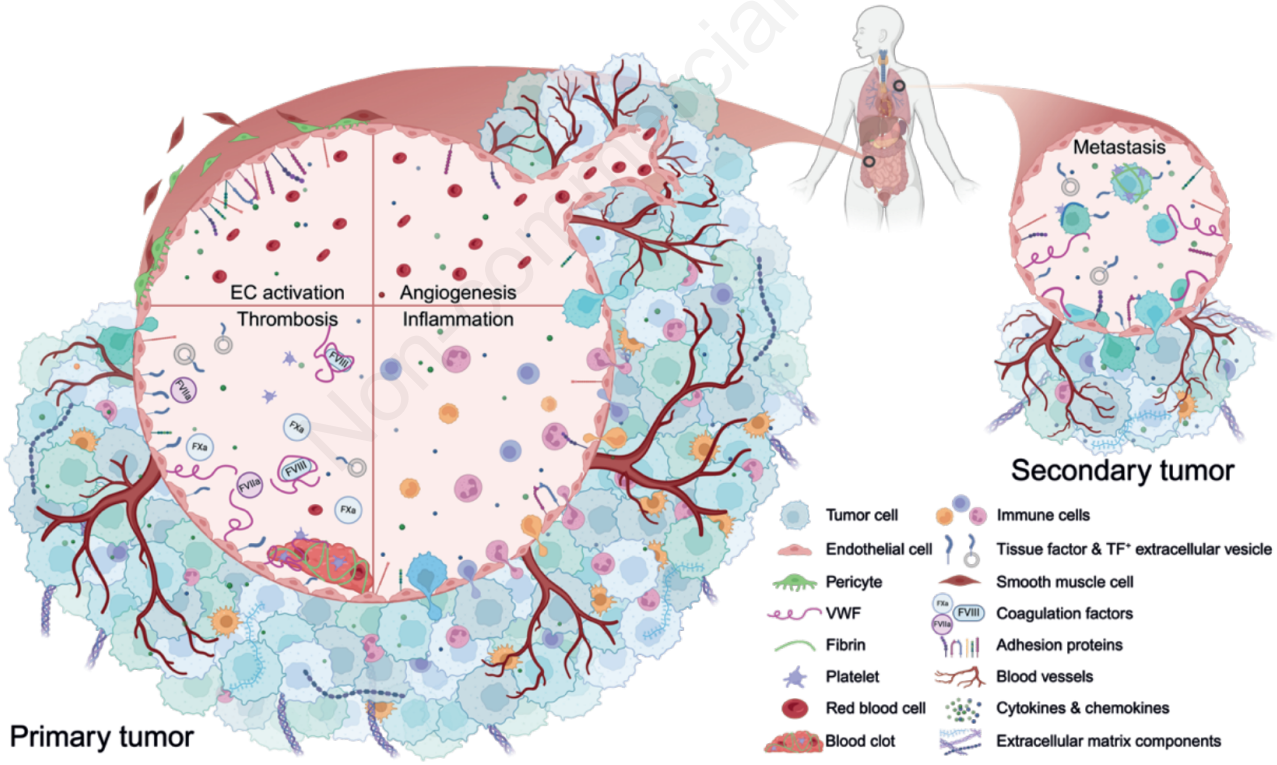

Figure 1. Schematic overview of endothelial cell dysfunction in the contribution to tumor progression. In the primary tumor, endothelial cell (EC) activation results in increased expression of adhesion molecules, secretion of several cytokines and chemokines as well as enhanced vascular permeability mediated by loss of interactions between ECs, smooth muscle cells and pericytes. Tumor-associated ECs display enhanced proliferation and migration properties, contributing to neovascularization within the tumor to support tumor growth. A pro-inflammatory phenotype is also a hallmark of ECs within the tumor vasculature, with increased recruitment and activation of immune cells to the tumor microenvironment. Tumor-associated ECs promote a prothrombotic milieu, with elevated tissue factor expression on the endothelium and enhanced von Willebrand factor/factor VIII secretion. Furthermore, the endothelium prepares the premetastatic niche and facilitates homing and transendothelial invasion of circulating tumor cells.

mechanistic rationale for these anti-angiogenic agents, they had limited clinical efficacy.[16] The precise biological mechanisms underpinning tumor neovascularization remain unclear, however recently, TECs have been postulated to contribute to resistance to anti-angiogenic therapies. For instance, targeting ECs with an anti-VEGF antibody in mice with glioblastoma did not significantly reduce tumor vascularization owing to the fact that expression of its specific receptor (VEGFR) was decreased via PDGF-mediated differentiation of ECs towards a mesenchymal phenotype.[17] TECs consist of a distinct and heterogeneous population of cells that are unique to the TME, recognized by the irregular thickness of the vessel, loose pericyte binding, hyperpermeability and a stiffened extracellular matrix.[18] Consequently, blood flow within tumor vasculature is disorganized creating a hypoxic TME, which affects the efficient delivery of chemotherapies, and the infiltration of immune cells. In fact, up to 40% of invasive breast cancer tumors exhibit hypoxic regions resulting in the upregulation of hypoxia-inducible transcription factor (HIF), thus further stimulating angiogenesis. This reciprocal cycle has been shown to contribute to drug resistance in breast tumors.[19]

Compared to normal ECs, TECs display elevated expression of cancer stem-like markers endoglin, endothelial protein-disulfide isomerase, and aldehyde dehydrogenase (ALDH), associated with high proliferative capacity.[20,21] For instance, $ALDH^{high}$ TECs were able to form tubular networks on Matrigel and were more resistant towards the 5-Fluorouracil.[20,22] Furthermore, some TECs display enhanced angiogenic potential with elevated expression of VEGFR, TIE2, Ang1, E-selectin and intercellular adhesion molecule-1.[23] In addition, TECs help recruit hematopoietic cells and endothelial progenitor cells to the TME to maintain tumor angiogenesis.[24] Interestingly, TECs isolated from highly metastatic tumors displayed increased pro-angiogenic properties and increased chemoresistance than TECs isolated from low metastatic tumor *in vivo*, highlighting their role in supporting the TME.[25] As a result, TECs mirror a chronically activated endothelium with high angiogenic potential (Figure 1).

To improve the understanding of TEC heterogeneity, recent studies have focused on single-cell transcriptomics.[26,27] Single-cell RNA sequencing of malignant and non-malignant ECs from lung and breast cancer patients as well as tumor-bearing mice has helped identify subpopulations of ECs with different phenotypes and proportions. These data may provide a basis for new therapies to specifically target TECs. For instance, TECs display a high glycolytic metabolic profile. The inhibition of the upstream glycolytic activator PFKFB3 upregulated VE-cadherin expression on ECs. Consequently, a more mature vascular endothelium was present with a restored barrier function, resulting in fewer metastases and improved chemotherapy delivery.[18] Checkpoint inhibitors are an ever-growing family of anti-cancer drugs. However, tumors can become resistant when infiltration of immune cells is blocked. Interestingly, the high expression of Ang2 in the TME can cause T-cell exclusion in melanoma tumors. Improved T-cell infiltration was observed when the Ang/TIE2 signaling axis on ECs was inhibited, consequently increasing the sensitivity of melanoma cells towards the checkpoint inhibitor anti-PD1.[28] Collectively, this highlights the therapeutic potential in targeting TECs in combination with standard cancer treatments to improve efficacy.

More recently, studies show plasticity of tumor cells and how they can circumvent anti-angiogenic therapy by acquiring a pseudo-endothelial-like phenotype, coining the term 'vasculogenic mimicry'.[29,30] These endothelial-like tumor cells form a stable tubular structure, resulting in 'mature' vessels with sustained blood flow.[29] To date, knowledge on the underlying mechanisms of vasculogenic mimicry is limited, however, in melanoma, there are indications that they might have derived from cancer stem cells, as they are CD271 positive; or via Oct4a in lung cancer stem cells, respectively.[30,31] Furthermore, hypoxia-mediated HIF-1α can transdifferentiate cancer stem cells into an endothelial-like phenotype.[32] Clinically, vasculogenic mimicry is associated with a poorer prognosis in cancer patients, *e.g.*, lung adenocarcinoma.[33] These studies highlight that an improved understanding of the tumor vasculature and its heterogenic population of ECs will be key to help identify novel therapeutic targets and attenuate the pro-angiogenic TME.

Thrombosis

Plasma of cancer patients displays a hypercoagulable state, and approximately 20% of cancer patients develop thrombosis.[6] Specific anti-cancer treatment regimens also exacerbate this risk, for example, chemotherapies can activate the endothelium, inducing a pro-inflammatory and pro-thrombotic state.[6] The etiology of cancer-associated thrombosis remains poorly understood but is certainly likely to be multifactorial. Intriguingly, however, this interplay between cancer and coagulation appears to be bidirectional, with coagulation activation providing positive feedback for cancer progression.[34] Within the circulation, coagulation factors including fibrin(ogen), as well as platelets can protect tumor cells from attack by immune cells, thereby facilitating successful metastasis (Figure 1).[35] Furthermore, coagulation factor Xa (FXa) expressed on tumor-associated macrophages contributes to tumor-evasive immune signaling, thus limiting the efficacy of immune checkpoint inhibitors.[36]

In cancer, chronically activated ECs display elevated secretion of von Willebrand factor (VWF) and factor VIII (FVIII).[37,38] Both VWF and FVIII have been associated with significant risk factors for cancer-associated thrombosis.[39] Furthermore, high VWF plasma levels are associated with poor overall survival in cancer patients. VWF-multimers secreted from ECs can tether circulating platelets and promote aggregation.[5] Bauer *et al*. demonstrated that VWF-mediated platelet aggregation occurs via melanoma-derived VEGF-A, which promotes the release of VWF from ECs in mice. The inhibition of VWF secretion by treatment with low molecular weight heparin (LMWH), which sequesters melanoma VEGF-A, significantly attenuated tumor metastasis as well as angiogenesis *in vivo*.[40] In addition, we have shown that these adhesive VWF multimers also contributed directly to circulating breast tumor cell binding under shear flow, a key prerequisite step for metastasis.[13] In support of this, several studies have reported reduced metastasis *in vivo* following treatment with anti-VWF antibodies.[38,41]

A well-studied driver of hypercoagulability in malignancy is tissue factor (TF), the initiator of the extrinsic coagulation

pathway.[34] In cancer, elevated TF expression strongly associates with cancer proliferation and progression.[42] In tumors, hypoxia-mediated signaling increases TF expression in tumors and stromal cells, like monocytes and ECs. This leads to the production of proangiogenic factors like VEGF, CXCL1, and IL-8, consequently leading to neovascularization.[34] Furthermore, cancer cells can secrete TF-positive extracellular vesicles (TF^+ EVs). *In vitro*, these TF^+ EVs were endocytosed by ECs and recycled to the cell surface, resulting in procoagulant phenotype while also increasing E-selectin expression and IL-8 secretion culminating in enhanced angiogenesis, endothelial permeability, and metastasis.[43]

TF-mediated coagulation can be inhibited by EC-expressed tissue factor pathway inhibitor-1 (TFPI-1) as it directly suppresses FXa activity and the TF/FVIIa complex.[44] Downregulation of TFPI1 increases the invasive and migratory capacity of tumor cells via integrin-mediated adhesion.[44] In breast cancer patients reduced expression of TFPI1 in tumors is associated with poor prognosis.[45] Furthermore, soluble TFPI in the plasma of non-small cell lung carcinoma patients revealed to be a biomarker for both thrombosis and metastasis.[46] Treatment with a chemically modified LMWH, termed S-NACH, increased TFPI1 expression on ECs and consequently reduced hypercoagulation and metastasis of pancreatic tumor cells *in vivo*.[47,48] Although TFPI1 is predominantly expressed on ECs, and exerts its anticoagulant effects there, it is unclear if these associations come from tumor- or EC-expressed TFPI1.

Anticoagulants, as part of anti-cancer therapies, increased the potential bleeding risks, and their use thus far has been limited to thromboprophylaxis in high-risk cancer-associated thrombosis patients.[36] Notwithstanding this, preclinical studies have provided evidence of anti-tumor properties for LMWH, with improved vessel barrier function, inhibition of angiogenesis and transendothelial migration.[13] Furthermore, LMWHs have been shown to inhibit the expression of selectins on ECs, and block the interaction between Integrin $\alpha_4\beta_1$ and vascular cell adhesion protein 1 (VCAM-1) on tumor and ECs, respectively. Correspondingly, reduced tumor-endothelium adhesion-mediated migration has been observed following LMWH treatment.[49] Treatment of breast cancer with LMWH in combination with chemotherapeutic agents paclitaxel or doxorubicin reduced tumor growth and liver metastasis in a mouse xenograft model, as LMWH improved intratumoral drug delivery.[50] Similarly, the combination of LMWH with checkpoint inhibitor anti-PD1 reduced tumor growth and metastasis of murine colon adenocarcinoma. These tumors displayed enhanced lymphocyte infiltration, with improved tumor vascular normalization by LMWH.[51] These data suggest a novel therapeutic strategy wherein combining anticancer therapies with LMWH might possess great potential to improve drug efficacy and attenuate disease progression and metastasis.

Inflammation

Under normal conditions, the endothelium appears to serve a more tumor-suppressive role. For example, the secretome from quiescent tumor-native ECs reduced breast and lung tumor cell proliferation and invasion *in vitro* and *in vivo* by reducing NF-κB and STAT3 inflammatory signaling.[52] Interestingly, however, silencing of endothelial perlecan, a heparan sulfate proteoglycan, reversed these anti-inflammatory effects, increasing tumor cell growth and invasiveness in a manner that was dependent on the pro-inflammatory cytokine IL-6, thus promoting metastasis in lung carcinoma xenograft models. The biology of EC dysregulation in the tumor vasculature is not fully understood, but pro-inflammatory mediators secreted by the tumor cell and other stromal cells within the TME are postulated to play a role (Figure 1). For example, tumor cell-derived CCL2 directly activates CCR2 on ECs, thereby enabling efficient tumor cell extravasation.[53] In fact, colon carcinoma extravasation and metastasis were highly dependent on endothelial expression of CCR2 which induced downstream JAK2, STAT5, and MAPK signaling pathways to increase vessel permeability and transendothelial migration.

In addition, direct tumor-endothelial interactions can also augment vascular homeostasis. For example, cell-cell interactions mediate increased Notch1 activation in the tumor microvasculature of lung, breast, colorectal, ovarian cancer and malignant melanoma.[54] This significantly impacts vessel permeability and induces the expression of several endothelial cytokines and chemokines, including IL-1β, CCL1, CXCR4 as well as upregulating adhesion molecule VCAM-1. Collectively, this pro-inflammatory endothelial phenotype has been shown to promote neutrophil infiltration, tumor-endothelial adhesion and migration, lung colonization, and metastasis in murine models of lung carcinoma and melanoma.[54]

Recent in-depth single-cell transcriptomic analysis of TECs has revealed enhanced gene signatures for leukocyte recruitment, cytokine production and scavenger activity.[27] There is evidence to suggest that the upregulation of these inflammatory processes can directly promote cancer aggressiveness.[55] In particular, genes related to TNF-α receptor signaling pathways including NF-κB and interferon family members were all enriched in TECs isolated from tumor-bearing mice.[56] Crucially, TEC secretome accelerated the growth of human colon tumors in immunosuppressed mice. Moreover, Pitroda *et al*. reported a panel of six inflammatory endothelial-derived genes which displayed a significant prognostic value in predicting overall survival in breast, lung, and glioma cancer patient cohorts. Collectively, these findings suggest that the pro-inflammatory tumor milieu, propagated at least in part by dysfunctional tumor endothelium, contributes to cancer progression and poorer patient outcomes.

ECs can also negatively influence immune cell recruitment and activation within the TME. For instance, TECs can display immunosuppressive properties with repressed leukocyte infiltration. Altered T-cell responses have been described due to elevated expression of inhibitory immune checkpoints from ECs, like programmed death-ligand-1/-2, IL-10 or prostaglandin-E2.[57] Moreover, Notch1 signaling in ovarian cancer cells can induce endothelial expression of CXCL2. This triggers the infiltration of monocyte-derived macrophages into the TME and promotes an immunosuppressive environment.[58] Similarly, in a murine model of glioblastoma, EC-specific knockdown of IL-6 inhibits macrophage polarization to an immunosuppressive phenotype, thus improving survival *in vivo*.[59] Additionally, EC hyperglycolysis inhibition with Osimertinib in colorectal tumor-bearing mice restored

vasculature and immune infiltration. Combinational therapy of Osimertinib with PD-1 blockade even showed synergistic effects with significantly smaller tumor volumes *in vivo*.[60] The ability of ECs to contribute to an immunosuppressive environment and altered T-cell responses is likely to impact the efficacy of immunotherapies,[61] and a combinational approach of targeting both the vasculature and tumor cells could be beneficial.

Dysregulated ECs can facilitate resistance to chemotherapy through altered pro-inflammatory signaling. For instance, studies have shown that chemotherapeutic drug exposure triggers TECs to secrete TNF-α and promotes CXCL1/2 expression in both breast and lung tumor cells, contributing to chemoresistance and relapsed disease.[62] Similarly, targeting focal adhesion kinase (FAK) in TECs promoted tumor cell sensitization to doxorubicin and radiation-based therapies, thus inhibiting tumor growth in murine models of melanoma and lung carcinoma.[63] The inhibition of endothelial FAK was shown to reduce NF-κB signaling, therefore resulting in a significant decrease in pro-inflammatory cytokines, including IL-1α, and IL-6, again highlighting the interplay between tumor biology and its inflammatory microenvironment. The improved understanding of the mechanisms through which the tumor vascular niche drives resistance may help identify novel drug targets to mitigate refractory and relapsed disease for cancer patients.

Metastasis

Classically, metastatic dissemination is focused on the tumor cell, how it remodels the extracellular matrix, invades and transmigrates through the endothelial layer into the bloodstream. At a distant organ, the circulating tumor cell will cross the endothelial barrier once more to establish micro-metastatic foci.[64] Even in this simplistic view, it is clear that tumor-endothelial interactions play a key role in metastatic spread. The immature structure and organization of tumor-associated vasculature is postulated to enable enhanced migration of tumor cells through its leaky barrier (Figure 1). Moreover, the secretome of TECs can directly modulate the invasiveness of tumor cells.[65] For example, Akt signaling in TECs isolated from highly metastatic tumors was significantly upregulated compared to TECs from low metastatic tumors. Consequently, the secretion of cytokines such as IL-6, and matrix metalloproteases (MMPs), including MMP-9, are known to drive invasion in tumors.[24,25] Epigenetically upregulated expression of biglycan from TECs activated NF-κB and ERK signaling pathway in tumor cells increasing the number of circulating tumor cells and lung metastases *in vivo*. Accordingly, biglycan plasma levels in patients correlated with the presence of metastatic disease.[66] Furthermore, vascular expression of CXCR7 was associated with improved tumor cell survival and metastasis. Stacer *et al*. reported that endothelial-specific knock-out of CXCR7 in mice was associated with significantly greater recurrence of breast cancer following resection, increased numbers of circulating tumor cells, and enhanced spontaneous metastases.[67]

As part of metastasis, tumor cells undergo epithelial-to-mesenchymal transition (EMT),[42] during which they lose cell-cell contacts, and enhance migratory and invasive properties. TECs can directly potentiate EMT via secretion of epidermal growth factor, plasminogen activator inhibitor-1, and CCL5.[65] In addition, TECs are capable of remodeling the extracellular matrix through the secretion of ADAM17 and MMPs,[25] which leads to the release of key growth factors including TGFβ, fibroblast growth factor-2, and insulin-like growth factor-1, consequently creating a positive feedback loop to further augment tumor cell migration.[68] *Vice versa*, tumors can induce endothelial-to-mesenchymal transition (EndMT) via TGFβ1/2, Wnt/β-catenin, and Notch signaling. This disrupts tight junctions, causing leaky vessels and increased tumor transendothelial migration.[65] Additionally, knockout of EC-specific c-MET in glioblastoma, restored temozolomide sensitivity *in vivo*, which resulted in smaller tumors and improved vasculature.[69] Likewise, the restoration of the brain microvasculature via inhibition of endothelial rho kinase ROCK suppressed migration of small cell lung cancer cells.[70] EndMT can drive ECs to transition to cancer-associated fibroblasts (CAFs). These cells correlate with increased tumor development and growth.[71] Approximately 40% of all CAFs are derived from ECs.[68,71] The elevated secretion of MMPs allows CAFs to remodulate the extracellular matrix.[71] Erdoğan *et al*. have shown that patient-derived CAFs produce a stiff, fibronectin-rich matrix in the tumor milieu, where fibronectin orientation laid down by the CAFs promotes directional migration for invasive prostate tumor cells.[72]

ECs can also actively contribute to the formation of the pre-metastatic niche with the primary tumor-secreting factors that precondition distal tissues for the arrival of disseminating tumor cells.[73] Specifically, tumors secrete extracellular vesicles and inflammatory cytokines to enhance vascular permeability and thus promote tumor cell extravasation. For example, TGFβ-mediated upregulation of angiopoietin-like 4 in breast tumor cells decreased cell-cell junctions in ECs *in vitro*. This directly facilitated tumor colonization in the lungs of murine breast cancer models.[74] Similarly, endomucin, which is involved in the tube formation of ECs, is downregulated in several cancers. Deficiency of endomucin on ECs results in increased vascular permeability and recruited tumor-supporting N2-type neutrophils at the premetastatic niche in a murine lung carcinoma model.[75] Direct interaction between disseminating tumor cells and normal, tumor-naïve ECs at the premetastatic niche can also promote drug resistance. Specifically, breast tumor integrin $\alpha_v\beta_3$ facilitates interaction with endothelial-expressed VWF within the perivascular niche of the bone marrow.[76] Targeting this axis via knockdown of VWF expression or $\alpha_v\beta_3$ blocking antibody sensitized breast tumor cells to doxorubicin and reduced bone marrow metastasis *in vivo*. Further studies are warranted to better understand tumor-specific factors that induce vascular dysregulation at distal tumor-free sites, as these may be key to attenuating metastasis at early disease stages.

Overall, considering the tumor endothelium in the design and development of novel anti-cancer strategies may not only improve the targeting and delivery of standard-of-care treatments but also serve to attenuate pro-tumor properties of the vasculature. However, as previously described, TECs are a heterogeneous population with altered genetic, transcriptomic, and metabolomic profiles.[57,77] On top of that,

it is entirely likely that TECs are tumor-subtype specific.[26] Recent reviews have extensively described TEC heterogeneity and how these cells could be reprogrammed to restore EC function.[18,57,77] Further studies defining TECs in specific cancers will help elucidate their complex contribution to disease and potentially identify specific means by which to target them and contribute to novel therapeutic approaches.

Conclusions

The endothelium plays an important role in modulating tumor progression. There is a bidirectional crosstalk between the vasculature and tumor cells with dysfunctional ECs promoting angiogenesis to sustain tumor growth, fostering a pro-inflammatory TME and a systemic pro-thrombotic state to aid dissemination and drug resistance. Moreover, enhanced expression of adhesion molecules facilitates direct tumor-endothelial binding and contributes to transendothelial migration and metastasis. The dysregulated endothelium can no longer be considered an epiphenomenon of malignancy but rather an active player in tumor progression. Further studies will be required to help identify novel therapeutic targets aimed at vasculature normalization in cancer. Importantly this approach may also help improve the delivery and efficacy of current standard of care treatments for patients with cancer.

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Machine learning in cancer-associated thrombosis: hype or hope in untangling the clot

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ABSTRACT

The goal of machine learning (ML) is to create informative signals and useful tasks by leveraging large datasets to derive computational algorithms. ML has the potential to revolutionize the healthcare industry by boosting productivity, enhancing safe and effective patient care, and lightening the load on clinicians. In addition to gaining mechanistic insights into cancer-associated thrombosis (CAT), ML can be used to improve patient outcomes, streamline healthcare delivery, and spur innovation. Our review paper delves into the present and potential applications of this cutting-edge technology, encompassing three areas: i) computer vision-assisted diagnosis of thromboembolism from radiology data; ii) case detection from electronic health records using natural language processing; iii) algorithms for CAT prediction and risk stratification. The availability of large, well-annotated, high-quality datasets, overfitting, limited generalizability, the risk of propagating inherent bias, and a lack of transparency among patients and clinicians are among the challenges that must be overcome in order to effectively develop ML in the health sector. To guarantee that this powerful instrument can be utilized to maximize innovation in CAT, clinicians can collaborate with stakeholders such as computer scientists, regulatory bodies, and patient groups.

Introduction

Cancer-associated thrombosis (CAT) is now a well-established disease entity and is recognized to substantially impact the overall survival, morbidity, quality of life and healthcare costs of the cancer subpopulation.[1-3] Management of cancer itself has evolved rapidly since CAT was first described in the 1800s, with major breakthroughs in surgical, radiation and medical interventions. However, data suggest that the rates of CAT continue to rise perhaps reflecting improving diagnostics and/or increased survival in patients with cancer, including patients treated with novel therapeutic agents such as targeted agents and immunotherapies.[4,5] Moreover, despite the advances in cancer treatment, venous thromboembolism in patients with cancer continues to be associated with increased mortality in contemporary cohorts.[6,7]

Machine learning (ML) refers to a specialized field of computer science that leverages algorithms to automatically identify patterns in data and ultimately perform a task. This approach has led to numerous transformative applications in diverse fields from voice recognition to virtual assistants, traffic prediction, financial market analysis and forecasting, fraud/criminal recog-

nition and even self-driving vehicles.[8] Considerable interest exists in developing applications of ML in healthcare to enhance diagnostic accuracy, improve efficiency, safety and quality, and substantially offload physicians. However, the high stakes inherent to healthcare as well as limitations intrinsic to ML science bring about somewhat unique challenges to its implementation in medicine, tempering enthusiasm and progress.[9,10] It is essential that clinicians work in close partnership with computer scientists to ensure that ML models developed are practical, unbiased and meet standards required to be integrated into patient care.

Applications for ML in the arena of hemostasis and thrombosis are growing. In this review, we catalog the potential areas where ML can enhance clinical care for patients with thrombotic disorders, with a focus on CAT. We also briefly review future directions and pitfalls that researchers and clinicians will need to be cognizant of as these technologies grow from research projects to more practical applications in the clinic.

Opportunities for application of machine learning to prevention and treatment of cancer-associated thrombosis

Certain features of thrombotic disorders may make these diseases particularly suitable to apply ML.[11] A training dataset is a large pool of data used to adjust a ML model's parameters and learn the underlying patterns in data; subsequently, the model is tested on an independent dataset to test its performance, known as validation dataset. Thrombotic conditions are relatively common and thus curating real-world datasets for training and validation of ML models is potentially feasible. Moreover, thrombosis is a frequent complication in cancer patients and feature-rich datasets already exist that could be targeted to develop and use ML models.[12,13]

Secondly, although the precise etiology of thrombosis in individual patients can be hard to pinpoint, there are several potential factors that are often available in electronic health records contributing to the risk of thrombosis and thus can be used as 'features' in ML models. Risk factors for CAT are extremely diverse and range from patient factors, (such as age and habitus), tumor features (such as site and stage), laboratory values, interventions (including surgery and procedures) as well as systemic medications (including cytotoxic chemotherapy, hormonal therapy and targeted agents).[14]

The interventions used to prevent and treat thrombotic disorders usually involve anticoagulants, and thus bleeding risk needs to be balanced in patients with or at risk for thrombosis. Models based on ML can be envisioned to be developed not only to calculate risks associated with thrombosis but also bleeding and thus facilitate informed and tailored decisions for clinicians and patients. Patients with cancer are not only at increased risk of thrombosis but also have high rates of major and fatal bleeding, which makes anticoagulation a challenge for clinicians.[15,16] Finally, patients with malignancy are relatively complex and can have significant temporal changes in thrombotic and hemorrhagic risk factors due to changes in cancer status (disease progression/recurrent or metastatic disease in critical sites), alterations in therapeutic interventions and general health status leading to institutionalization or immobilization. Thus, CAT risk is dynamic and continuous risk assessment would be beneficial to account for variations in risk with time.[17]

We identified three specific applications of ML to the research and clinical management of thromboembolism: i) natural language processing to optimize automated identification of thrombotic complications in patients; ii) computer vision to classify radiology images; iii) predictive ML modeling for thrombosis (Figure 1, Table 1).

Natural language processing and venous thromboembolism

Natural Language Processing (NLP) refers to the application of ML technology and linguistics to enable computers to automatically interpret, manipulate, and comprehend human language.[18] Within healthcare, this allows automated interpretation of textual data within the electronic health record such as those in medical notes or laboratory and radiological reports for accurate case detection that can, in turn, aid surveillance efforts, augment hospital triage systems, and allow for automated measurement of quality metrics.[19] For computers to analyze human language, one can rely on keyword extraction, predetermined rule-based technology or more advanced techniques that apply ML algorithms to make inferences, all approaches which have been studied in text into case-detection algorithms in the electronic health records.[20] Furthermore, with the advent of generative artificial intelligence technology, such as generative pre-trained transformer (also known as GPT) models, there is interest in developing NLP applications to reduce burdens and time for providers by assisting in tasks such as automation of documentation with human review, prepare orders or compute and synthesize information from electronic health records and medical literature.[21,22]

The application of NLP for the detection of thrombotic disorders including deep vein thrombosis (DVT) and pulmonary embolism has been developed for over a decade.[23-26] Although manual extraction is considered the gold standard, this labor-intensive process is not feasible for long-term and continuous case extraction. The use of billing or administrative diagnostic codes lacks accuracy for VTE detection and compares unfavorably to NLP algorithms.[27] In a multicenter study that compared NLP to manual chart extraction in two orthogonal datasets, the NLP-based VTE identification system was found to score >90% on all performance measures calculated including accuracy, sensitivity, specificity, and positive and negative predictive in both datasets.[28] This supports that NLP could be a promising tool for automated surveillance systems. This technology has also been studied for VTE surveillance in specific settings such as post-surgery, pediatric populations and patients hospitalized with COVID-19.[29-31]

Various researchers have worked on developing NLP models that can aid acute CAT case detection within cohorts of patients with malignancy. Ostensibly CAT may differ from thrombosis in the general population given higher patient complexity, cancer-directed medications, more frequent interventions such as central access catheters as well as the high prevalence of preexisting thrombosis which could make detection of recurrent acute events challenging. A transformer NLP model utilizing a combination of clinical notes and radiology re-

ports to detect CAT longitudinally was developed that achieved a precision (positive predictive value, PPV) and recall (sensitivity) of about 93%.[32] A separate group demonstrated the successful use of a customized NLP pipeline for clinical notes, used in combination with a keyword search of radiology reports and extraction of anticoagulation data from pharmacy records to detect VTE events in 14,223 adult patients with solid tumor malignancy.[33] Li *et al*. used a longitudinal single-center retrospective dataset of patients with cancer to demonstrate that a combined algorithm based on billing codes and anticoagulation with a ruled-based NLP classifier had a weighted PPV of 98% and a weighted sensitivity of 96%, with a C statistic of 0.98 (95% CI, 0.97-0.98) that out-performed either approaches individually.[34] This suggests that combining information related to VTE from both structured data (billing and procedural codes and laboratory results) and unstructured data (such as radiology reports, clinical notes) could lead to optimal event detection. The use of NLP to detect thrombotic events in more specific oncologic populations such as patients undergoing allogenic stem cell transplants has also been described.[35]

Machine learning applications for image recognition in venous thromboembolism

Diagnosis of VTE is routinely established by radiological investigations including computed tomography angiograms, pulmonary ventilation perfusion scans and duplex ultrasound for extremity DVT.[36] This is performed historically with trained physicians reviewing imaging visually to identify pathologies and make diagnoses. The field of computer vision leverages ML algorithms to recognize patterns in imaging data fields that exceed the limits of the human eye. Those models can be integrated into workflow to improve efficiency.[37] Moreover, within oncology, ML offers the ability to optimize image acquisition sequences to maximize efficiency and reduce radiation exposure and costs, develop personalized screening programs for patients, create precise and reliable volumetric-based tumor responses to guide cancer-directed therapies and potentially elucidate otherwise imperceptible radiographic patterns to investigate cancer biology, as well as predict treatment response (also known as 'radiomics').[38]

Given that pulmonary embolism can be clinically misdiagnosed or missed in up to one-fourth of patients,[39] several groups have worked on ML-based automatic detection models for this clinical event.[38,40,41] A deep learning model (PENet) for automatic detection of pulmonary embolism from volumetric computed tomography (CT) pulmonary angiograms was developed that achieved an AUROC of 0.85 [95% CI 0.81-0.87] on an external dataset.[42] Such tools can be envisioned to serve as secondary reading tools and also prioritize scans in radiologist review queues to prevent delays in diagnosis. Beyond the detection of PE, deep learning-based models to quantify clot burden are also being developed that have been shown to correlate with risk stratification markers in acute pulmonary embolism, including right ventricular metrics.[43,44] Similarly, ML-based tools have been developed for computer-aided diagnosis of DVT, although the majority utilize MR/CE-MRI or CT-venography, while the most widely employed diagnostic technique is compression ultrasound.[45-48] Aiming to equip non-specialists to detect DVT, a deep learning approach to compression ultrasound images was developed and externally validated with a negative predictive

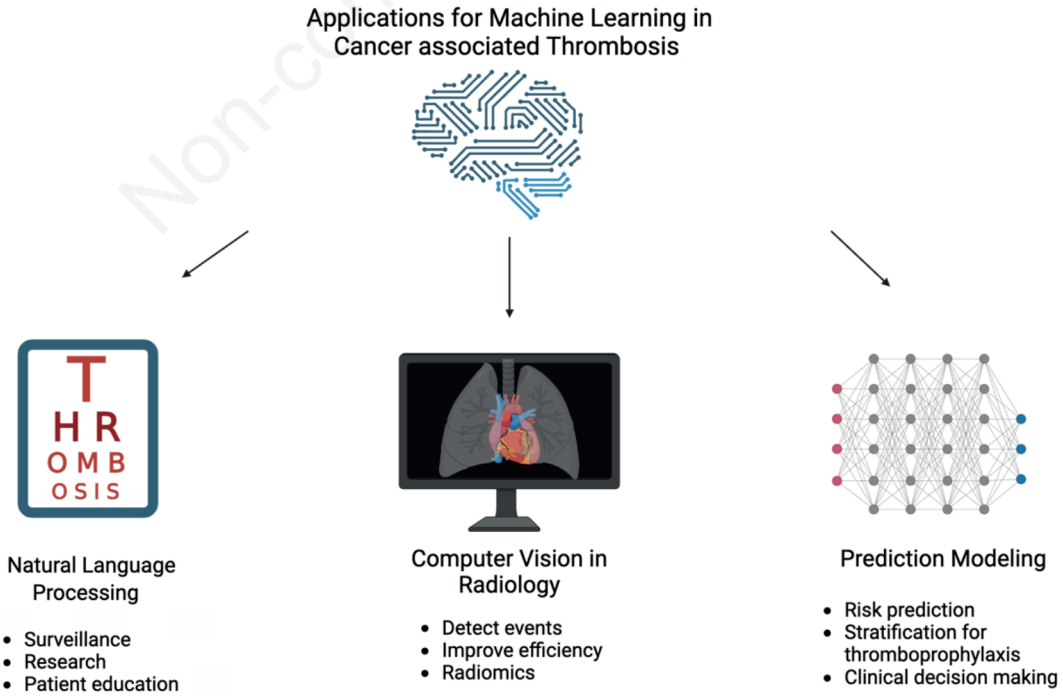

Figure 1. Applications for machine learning in cancer-associated thrombosis.

value of 98-99%. The authors also performed a cost analysis of integrating this ML tool into their current diagnostic pathway and estimated the net monetary benefits.[49]

Studies exploring the role of ML-assisted radiologic diagnosis of pulmonary embolism, extremity-associated vein thrombosis and thrombosis in unusual sites such as splanchnic and cerebral vasculature specifically in patients with underlying cancer are pending. However, several potential uses of ML-assisted radiological imaging at several stages in the cancer journey including screening, disease detection, treatment assessment and surveillance have already been identified.[50] Surveillance imaging is frequent among patients with malignancy, and ML could assist in automated detection of thrombosis in patients where a diagnosis is not otherwise suspected. Estimating the composition

Table 1. Selected examples of applications of machine learning in cancer-associated thrombosis.

Study	Population/ study design	Dataset size	Corpus datasets	ML model	Metrics				
					Precision/ PPV	Recall/ sensitivity	C-statistic	NPV	Specificity
Natural language processing and venous thromboembolism									
Maghsoudi *et al.*[32]	Single center; retrospective		Clinical notes; radiology notes	ClinicalBERT (Bidirectional Encoder Representations from Transformers) large language model	0.93	0.93	-	-	-
Dunbar *et al.*[33]*	Single center; retrospective		Clinical notes	Custom NLP	-	-	-	-	-
Li *et al.*[34]‡	Single center; retrospective		Radiology notes	Rule based NLP pipeline	0.98	0.96	0.98		-
Computer vision to identify thrombotic events from radiologic data†									
Huang *et al.*[42†]	Retrospective study included internal and external datasets		CT pulmonary angiography scans	3D CNN (PENet)	-	-	.84 (Internal) .85 (external)	-	-
Li *et al.*[84†]	Retrospective multicenter study		CT pulmonary angiography scans	CNN + U-NET	-	-	0.93	-	-
Kainz *et al.*[49†]	Prospective study included internal and external cohorts		Ultrasound videos	Dual-task CNN (AutoDVT)	-	0.82-0.94 (95% CI)	-	0.99-1.00 (95% CI)	0.70-0.082 (95% CI)
Machine learning based prediction modeling for venous thromboembolism									
Ferroni *et al.*[59†]	Retrospective	608	Tabular	Kernel method	-	-	0.72	-	-
Li *et al.*[64†]	Retrospective	Derivation: 9,769 validation: 79,517	Tabular	Logistic regression	-	-	0.68 (0.67-0.69)	-	-
Muñoz *et al.*[66]	Retrospective; goal was to predict recurrent VTE	16,407	Tabular	Logistic regression and decision trees (individual trees and random forests)	-	-	0.68 (0.63-0.72) for random forests	-	-
Verstovsek *et al.*[68††]	Retrospective	Derivation: 1,012 validation: 100	Tabular	Decision trees (individual trees and random survival forests)	-	-	0.84	-	-

*Model was used to supplement an approach using pharmacological data for therapeutic anticoagulation to identify thrombotic episodes. Performance measures not reported; ‡algorithm combined billing codes and NLP on radiology reports. Combined approach was found to be better than and NLP or coding algorithm alone; †did not describe separately patients with cancer; ††neutrophil percentage, lymphocyte percentage and red blood cell distribution width are important predictors in decision trees. ML, machine learning; PPV, positive predictive value; NPV, negative predictive value; NLP, natural language processing; CT, computed tomography; CNN, convolutional neural network; DVT, deep vein thrombosis; CI, confidence interval.

of thrombus using artificial intelligence is also an emerging method that has shown to be potentially impactful for prognostic and therapeutic decision-making in ischemic stroke.[51] Such an approach can be envisioned in CAT for determinations that have therapeutic significance such as to differentiate chronicity of a thrombus as well as distinguish between bland thrombus and intravascular involvement by tumor.[52,53]

Machine learning for prediction of cancer-associated thrombosis

Modeling the risk of CAT is a potentially impactful application of ML given the importance of risk stratification for prophylaxis. The yearly risk of CAT is relatively low overall, with a cumulative incidence of less than 10% in most reports.[54] Anticoagulant prophylaxis in this patient population has not been shown to be associated with a significant increase in the risk of major bleeding overall, however specific subgroups might have a higher risk.[55] Monetary costs and inconvenience for patients constitute additional downsides of pharmacological prophylaxis. In order to maximize net benefit, it is desirable to carefully select candidates for thromboprophylaxis, focusing on individuals with the highest risk of thrombosis and the lowest risk of bleeding. ML predictive models could conceivably be applied to both sides of this equation in order to optimize preventive efforts.

The first broadly used risk stratification algorithm for CAT is the Khorana score.[56] Still very prevalent in the clinical arena, this clinical prediction rule is derived from a simple logistic regression model. It is easy to use and has been extensively validated.[57] It has been applied in randomized studies of pharmacological prophylaxis for CAT, in which a clinical benefit was demonstrated in the intervention group.[58] However, in general, the Khorana score has exhibited disappointing performance. It does not have an appreciable capacity to discriminate thrombosis risk within cancer strata, as the most important predictor in this model is tumor type. Using a score threshold of 2, typically half of patients in a diverse solid cancer cohort will be retained for prophylaxis, however, left untreated less than 10% of those individuals would go on to develop a CAT episode by the 6-month mark.[57]

Based on those considerations it becomes evident that improved CAT prediction models are needed. Beyond additive models like logistic regression, more advanced algorithms could conceivably improve model discrimination and accuracy by leveraging complex relationships between predictors. Also, doing away with the clinical prediction rule format and switching to a model deployed directly from the electronic health record would allow the inclusion of a far greater number of predictors than otherwise possible, along with more granularity in model inputs.

In the last few years, several authors have explored varied ML algorithms to improve risk prediction for CAT. The approaches used include additive models (*e.g.*, logistic regression and Fine-Gray regression), tree-based models (*e.g.*, random forests), kernel methods (*e.g.*, support vector machines), gradient boosting, ensembles and deep learning.[59-69] The predictors featured in those models included cancer type and stage, routine laboratory test results (*e.g.*, hemoglobin, total white blood cell count, *etc*.), basic demographic characteristics, chemotherapy type, circulating procoagulant vesicles, circulating tumor DNA levels, germline molecular markers and tumor somatic genetic alterations. As a general rule, model discrimination as measured with the C-index did not surpass 0.72 in the test set. External validation is lacking for most of those studies, with few instances of a satisfactory assessment.

While the findings above are stimulating, much remains to be done to change the paradigm of CAT prediction and prevention. At this juncture, it appears unlikely that more complex modeling algorithms using the usual static risk markers will improve model metrics. Incorporating large amounts of omics data, unstructured data, novel orthogonal biomarkers or time series data of predictors commonly available in the electronic health record are all approaches with the potential to move the needle further and meaningfully increase the net benefit of pharmacological prophylaxis for CAT. Survival methods could generate CAT incidence predictions which factor in the competing risk of death, allowing the clinician to estimate risk at different arbitrary time points. Deep learning models can be customized extensively and offer the added benefit of transfer learning but are more technically difficult to implement and require larger datasets than other ML algorithms to reach their full potential. Model generalizability between locales will remain a challenge and federated learning is a promising modality to alleviate privacy concerns surrounding the sharing of multiple large patient datasets.

Future challenges for the application of machine learning to clinical management of cancer-associated thrombosis

Despite the exciting avenues for ML in clinical medicine, researchers and clinicians involved in the development of this novel technology need to be mindful of challenges and potential pitfalls (Table 2).[9,53] Although electronic health records do contain enormous amounts of data that could be relevant to CAT, these are often unstructured and siloed in medical imaging archival systems, pathology systems, documentation fields, electronic prescribing tools and insurance databases which would need to be processed and unified so they are accessible to an algorithm. Moreover, datasets for most current ML studies in VTE are retrospective and fixed; however, in reality, a ML model for thrombosis would need to handle non-stationary input data due to changes in clinical, operational practices as well as dynamic patient populations and changing individual health status. Thus, methods to address dataset shift and update models prospectively would need to be built in beforehand to ensure optimal performance.[70] Prospective testing of these computer systems and periodic or continuous performance checks are also critical to ensure the models remain accurate despite changes in the environment, to detect issues and deploy updates to address them.

Generalizability, so that tools can be utilized outside their training environments, is an important goal in developing ML applications.[71] Moreover, ML algorithms that operate without human oversight can be prone to over-fitting or utilization of unknown confounders that would not be reliable in a different setting or dataset.[72] Given that, different institutions can vary

widely in clinical practices, record keeping, and technical equipment; this can be a particular challenge in building tools for widespread clinical use. Transfer learning is a ML technique that allows computer systems to apply knowledge learned from a task to be reused to improve performance on related tasks. This can save computing and time resources, and thus can be leveraged to enhance generalizability.[73] Another attractive approach that has emerged to improve generalizability is federated learning. Federated learning can be used to derive a global model from several distinct datasets belonging to different organizations without sharing sensitive clinical data between the participants, thus preserving patient privacy.[74]

A serious concern is that ML algorithms can contain discriminatory biases, that can inadvertently affect already disadvantaged groups in healthcare and enhance health inequities.[75,76] In order to avoid unintentional bias in ML algorithms that could further worsen existing racial and ethnic disparities in CAT, developers need to be sensitive of potential issues in the databases where the models are trained.[77,78] Clinicians should also be mindful of testing and evaluating models by population subgroups (such as race, age, socioeconomic strata, or location) before they are deployed. Moreover, rigorous regulatory frameworks need to be developed and updated in pace with technological innovation to ensure guardrails are in place for the supervised and controlled development of clinical ML models.[79,80] Towards this goal, the World Health Organization recently outlined six key areas for regulation of AI in health including transparency, risk management, data validation, data quality, privacy and collaboration between various stakeholders including regulatory agencies, healthcare providers and industry partners.[81]

There is also concern about reluctance and mistrust among clinicians and patients that can be a hurdle to the uptake of ML at the bedside. The explainability of a model can be viewed as its inner mechanics and behavior being interpretable and understandable by human observers. Deep learning models in particular often feature a large number of parameters which in isolation do not have any well-defined meaning, which can lead to a perception by users that the algorithm is a "black box", which can decrease confidence in its accuracy and reliability. A nationally representative online panel of patients was surveyed and found that over half believed that artificial intelligence would improve healthcare delivery.[82] In a study of paired surveys of clinicians and informaticians that focused specifically on diagnosis and prevention of VTE, a majority of clinicians (70%) and informaticians (58%) indicated that they believed that AI can ensure appropriate VTE in hospital prophylaxis. However, lack of transparency was the most frequently cited barrier by both clinicians and informaticians to the use of AI in clinical care of thrombosis.[83] Finally, ensuring that ML-based tools built for CAT are adequately and rigorously studied prospectively with clinically meaningful endpoints (such as recurrent thrombosis, major bleeding and mortality) prior to deployment in clinical practice will be essential to ensure that these tools are relevant and safe in healthcare and improve patient and physician trust in their use.

Conclusions

ML has the potential to create impactful changes in clinical medicine including cancer-associated thrombosis. NLP can facilitate VTE case detection from unstructured fields including clinical notes and radiological reports to enhance research and surveillance activities. Computer vision can optimize detection of thrombotic events from radiological data which can decrease missed diagnosis and assist radiologists in triaging studies to avoid treatment delays. Finally, ML algorithms are being developed to accurately predict patients' risk of developing CAT, which could in turn be utilized to assign thromboprophylaxis to patients who would benefit from this intervention and avoid exposing individuals with a higher bleeding risk to unnecessary anticoagulant administration. Experts and clinicians need to familiarize themselves with this novel technology to ensure that tools being developed are relevant, safe and minimize the risks of inherent bias during development. ML needs to be tested for safety and clinically relevant outcomes under the emerging regulatory landscape that can ultimately promote safe and effective innovation. Lastly, the ML models need to be continuously monitored and periodically retrained.

Table 2. Key barriers to building machine learning applications in healthcare.

Barriers	Comments
Dataset quality	Feature rich, well annotated high-quality datasets need to be developed and made publicly available to train models. Testing datasets would ideally be prospective and external to establish validity.
Evolution of medical care and patient populations over time	Predictive and diagnostic models in clinical use should be audited periodically to ensure persistence of satisfactory performance metrics. Transfer learning and other model updating techniques can be used to fine tune an older model.
Changes in individual patient medical condition over time	Predictive models should be used to make clinical decisions only for the time period used in the original validation studies. Dynamic modeling should be explored to mitigate loss of predictor information over time.
Generalizability	Models need to be developed and validated on diverse datasets to ensure performance is unform across institutions and networks. Transfer learning and Federated learning can be incorporated to ensure generalizability.
Bias	Preexisting biases within datasets and clinical practice need to be identified to ensure algorithms are not flawed. Machine learning applications need to be evaluated in population subgroups to compare performance.
Regulatory framework	Regulatory agencies should work with stakeholders to establish guardrails that can keep up with technology updates t to ensure innovation is safety, efficacy and health equity
Clinicians mistrust/reluctance	Increased transparency, robust external and prospective validation to establish efficacy and safety and patient and physician education as well as effective and open regulation

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Cancer-associated ischemic stroke: current knowledge and future directions

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ABSTRACT

Because cancer is a strong prothrombotic, there is an increased risk of thromboembolism, which includes ischemic stroke, especially in the first six to twelve months following a cancer diagnosis. The risk of ischemic stroke differs according to the location and stage of cancer. Given that the risk increases prior to a cancer diagnosis, stroke may be the initial sign of occult cancer. Although data on the risk, treatment, and outcomes of cancer-associated stroke are more limited than those on cancer-associated venous thromboembolism, the condition is still recognized as a thrombotic complication of cancer. Up to 10% of ischemic stroke patients also have a concurrent cancer diagnosis, and these patients seem to have higher short-term mortality and morbidity rates. With more people expected to survive longer after cancer treatment and an increasing number of cancer survivors, the burden of stroke among cancer patients is predicted to rise. This narrative review aims to provide an overview of the pathophysiologic mechanisms, treatment options, and epidemiology of ischemic stroke, including cancer screening for those who have cryptogenic (unexplained) stroke.

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Ischemic stroke in patients with cancer: burden of the problem

Cancer survivorship, "the process of living with, through and beyond cancer",[1] is increasing. Advances in the early detection and treatment of cancer, and population growth and aging have resulted in higher numbers of cancer survivors. In the United States, there are currently an estimated 15 million people living with a history of cancer, a number which is expected to reach 21 million by 2026.[2,3] Up to one-third of Europeans and one-half of Canadians are expected to develop cancer in their lifetime.[4,5] Over 60% of those diagnosed with cancer are expected to survive for 5 years or longer after a cancer diagnosis.

Cancer is highly thrombogenic and increases the risk of venous and arterial thromboembolism.[6,7] Unlike cancer-associated venous thromboembolism (VTE), data regarding the risk of cancer-associated stroke, its management, and outcomes are more limited. Approximately 4% to 10% of patients with ischemic stroke have a concurrent diagnosis of cancer.[8] Ischemic stroke appears to have higher morbidity and short-term mortality in patients with cancer and may interfere with the provision of optimal cancer therapies thereby impacting disease prognosis.[9,10] The burden of stroke among individuals with cancer is expected to grow due to the increasing number of cancer survivors and longer survival after cancer treatment.

In this narrative review, we describe the epidemiology of ischemic stroke in patients with cancer, summarize the existing evidence for treatment, and propose future directions for prevention and treatment. Table 1 reports a brief summary of previously released cohort studies that looked at cancer patients' risk of stroke.

Epidemiology of ischemic stroke in patients with cancer

Early evidence for an association between cancer and ischemic stroke was demonstrated in an autopsy study in which pathological evidence of cerebrovascular disease was found in 14.6% of patients with non-central nervous system cancer.[11] Subsequent analyses of data from the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute revealed a higher 6-month risk of ischemic stroke in individuals newly diagnosed with cancer when compared to those without cancer [3.0% *vs*. 1.6%, hazard ratio (HR) 1.9, 95% confidence interval (CI) 1.8-2.0] which decreases over time after diagno-

Table 1. Summary of previously published cohort studies that examined the risk of stroke in cancer patients.

First author	Title	Journal/year	Cancer population	Comparator	Outcome	Measure of association
Jang *et al.*	The long-term effect of cancer on incident stroke: a cohort study in Korea	Front Neurol 2019	New diagnosis of digestive organs, lip/oral/pharynx, respiratory, thyroid, others (breast and reproductive organs)	Individuals without cancer (propensity score matched)	Stroke within 7 years	Subdistribution hazard ratio: 1.13; 95% CI 1.02-1.26
Lun *et al.*	Previous ischemic stroke significantly alters stroke risk in newly diagnosed cancer patients	Stroke 2023	New diagnosis of cancer (non-melanoma skin cancer and primary central nervous system cancer excluded)	N/A	Ischemic stroke at 1 year	Incidence rate: 107.8 per 10,000 person-years
Navi *et al.*	New diagnosis of cancer and the risk of subsequent cerebrovascular events	Neurology 2018	New diagnosis of cancer (cutaneous basal cell or squamous cell carcinoma excluded)	N/A	Cerebrovascular events at 30 days	Hazard ratio: 6.1; 95% CI 2.7-13.7
Navi *et al.*	Risk of arterial thromboembolism in patients with cancer	J Am Coll Cardiol 2017	New diagnosis of breast, lung, prostate, colorectal, bladder, pancreatic, or gastric cancer or non-Hodgkin lymphoma	Individuals without cancer (matched)	Arterial thromboembolism at 6 months	Hazard ratio: 2.2; 95% CI 2.1 to 2.3
Navi *et al.*	Recurrent thromboembolic events after ischemic stroke in patients with cancer	Neurology 2014	Active systemic cancer (diagnosis of, or treatment for, systemic within the prior 6 months, or known recurrent or metastatic disease (local basal cell or squamous cell carcinoma of the skin and primary brain tumors excluded)	N/A	Recurrent thromboembolic events after ischemic stroke	Incidence 34%; 95% CI 28-40%
Navi *et al.*	Association between incident cancer and subsequent stroke	Ann Neurol 2015	New diagnosis of breast, colorectal, lung, pancreatic or prostate cancer	Individuals without cancer (matched)	Stroke at 3 months	Analyzed individually
Mulder *et al.*	Arterial thromboembolism in cancer patients	JACC: CardioOncology 2021	First-time diagnosis of all cancers (skin cancers excluded)	Individuals without cancer (matched)	Arterial thromboembolism at 12 months	Cumulative incidence of 1.50%; 95% CI 1.47-1.54% in cancer patients
Wei *et al.*	Stroke rate increases around the time of cancer diagnosis	Front Neurol 2019	New diagnosis of lung, colorectal, hepatocellular, urogenital, gastric, prostate, brain malignancy, esophageal, nasopharyngeal, breast, ovarian, thyroid, lymphoma, leukemia	Individuals without cancer (matched)	Stroke within 1 year after cancer diagnosis	Subdistribution hazard ratio 1.72; 95% CI 1.48-2.01
Zoller *et al.*	Risk of haemorrhagic and ischaemic stroke in patients with cancer: a nationwide follow-up study from Sweden	Eur J Cancer 2012	New diagnosis of cancer (all types)	N/A	Ischemic stroke	Standardized incidence ratio: 1.2; 95% CI 1.2-1.2

CI, confidence interval; N/A, not applicable.

sis.[6,12] The risk of arterial thromboembolism precedes cancer diagnosis with a 69% increase beginning about 5 months before the date of cancer diagnosis.[12] Canadian population cohort data similarly show that individuals with a new diagnosis of cancer have a 1.5-fold higher risk of ischemic stroke compared to matched cancer-free controls within 1.5 years (HR 1.40, 95% CI 1.34-1.47).[13] In a recent meta-analysis, the 1-year incidence of ischemic stroke after a new diagnosis of cancer was about 1.3% (95% CI 1.0-1.8%).[4]

Specific characteristics intrinsic to cancer including site, histology, and stage appear to play a role in reflecting unique pathophysiological mechanisms associated with stroke in this context.[5] Stroke risk varies across cancer sites; in a systematic review, survivors of pancreatic, hematologic, lung, head and neck, and stomach cancers had a higher risk for stroke compared to cancer-free controls, but not other cancer sites.[6] Other studies have shown excess risk after colorectal cancer as well.[13,14] Adenocarcinoma is a high-risk histology associated with circulating cancer-cell-derived extracellular vesicles and elevated biomarkers of hypercoagulability such as D-dimer.[7] Stroke risk increases with cancer stage and is highest among patients with stage 4 disease.[6]

Cardiovascular risk factors such as obesity, glucose intolerance, and smoking contribute to the increased stroke risk among patients with cancer.[15] Atrial fibrillation is more prevalent in the cancer population and confers a high 1-year risk of stroke of 3.3% (95% CI 2.4-4.6%).[4,8] A history of previous ischemic stroke increases the risk for stroke after a new diagnosis of cancer [aHR, 2.68 (95% CI, 2.41-2.98)], with events occurring within 1 year of diagnosis associated with the highest risk [aHR, 3.68 (95% CI, 3.22-4.22)].[9]

Individuals with cancer have unfavorable outcomes following ischemic stroke, characterized by high rates of recurrent stroke (11% to 16%), thromboembolic events (up to 37% within 6 months), increased mortality, and functional impairment.[16,17] Arterial thromboembolic events (including stroke) carry a 3-fold higher among patients with cancer compared to those without cancer.[18] In a small retrospective study, patients with cancer experienced high mortality rates (47%) and half of the survivors had a poor neurological outcome at 3 months post-stroke as measured by the modified Rankin scale.[16] Cryptogenic stroke (*i.e.*, no known stroke mechanism) is more common in cancer patients and portends poor survival [median 55 days (IQR 21-240 days) and an increased risk of death (HR 1.64, 95% CI 1.2-2.1].[19] However, these studies were limited by small sample size and clinically important outcomes such as bleeding were not captured.

Mechanism of ischemic stroke in patients with cancer

Multiple factors contribute to the risk of stroke in patients with cancer including but not limited to shared risk factors (*e.g.*, older age, smoking, obesity, alcohol), cancer-associated hypercoagulability, and the effects of cancer therapies (*e.g.*, systemic therapies, surgery, radiation-induced complications including vasculopathy, *etc*) (Figure 1).[20] A number of mechanisms that promote hypercoagulability have been implicated including activation of coagulation (*e.g.*, increased D-dimer, thrombin-antithrombin, tissue factor release), platelet function (P-selectin), and endothelial integrity (thrombomodulin, soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule-1) and formation of neutrophil extracellular traps.[21-24] Patients with cancer who experience stroke have a distinct blood mRNA expression profile and higher levels of cancer cell-derived extracellular vesicles compared to those without stroke and without cancer.[25,26] Antiphospholipid antibodies appear to be highly prevalent among patients with active cancer and are associated with the development of thrombotic events.[27-30] Diffusion-weighted-imaging patterns on magnetic resonance imaging showing the involvement of multiple vascular territories in individuals with cancer suggest a central embolic source, and may point to cancer-associated coagulopathy as the underlying mechanism.[31,32]

Cancer-associated hypercoagulability can manifest as stroke through unconventional mechanisms including non-bacterial thrombotic endocarditis (NBTE) and or paradoxical embolism through right-to-left intra-cardiac shunt (*i.e.*, patent foramen ovale). NBTE is a rare condition in the general population characterized by non-infectious organized thrombi on native cardiac valves and is associated with hypercoagulable states such as antiphospholipid antibody syndrome.[33] NBTE appears to be an underappreciated etiology of cryptogenic stroke in patients with cancer.[34] In an autopsy series, NBTE was present in 1.6% of individuals of whom 80% had concurrent cancer.[35] Venous thromboembolism is a frequent complication of cancer and can cause ischemic stroke via paradoxical embolism through right-to-left intra-cardiac shunt which is present in about 25-35% of individuals.[36] A prospective cohort study found a right-to-left intra-cardiac shunt in 18% of patients with ischemic stroke, 5% of whom had cancer.[37] The prevalence of right-to-left intra-cardiac shunt was higher among patients with cancer compared to those without (55% *vs*. 15%, P=0.001). All patients with cancer and right-

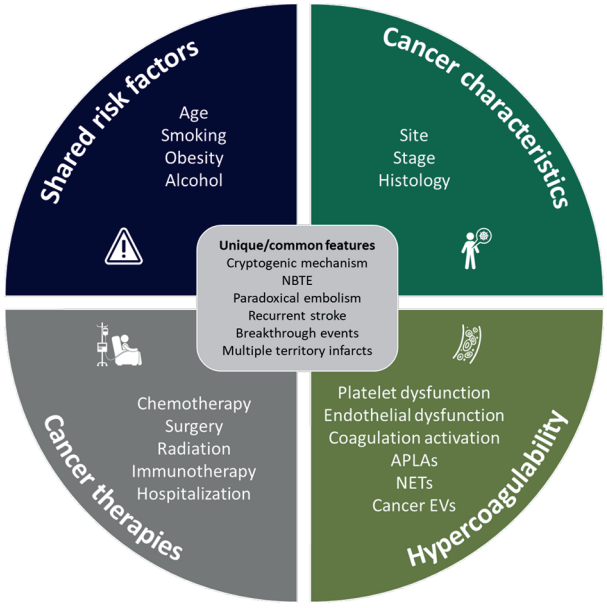

Figure 1. Factors contributing to ischemic stroke in patients with cancer. APLAs, antiphospholipid antibodies; NETs, neutrophil extracellular traps; EV, extracellular vesicles; NBTE, non-bacterial thrombotic endocarditis.

to-left shunt also had venous thromboembolism (*i.e.*, lower extremity deep vein thrombosis or pulmonary embolism).

Cryptogenic ischemic stroke and undiagnosed cancer

Cryptogenic stroke refers to ischemic stroke with no known pathogenic mechanism after standard diagnostic evaluation. The term "embolic stroke of undetermined source" (ESUS) was coined in 2014 to describe a non-lacunar (*i.e.*, embolic) ischemic stroke that remains cryptogenic after evaluation.[38] Cryptogenic stroke accounts for 10% to 40% of all ischemic strokes.[39-42]

Given that the risk of arterial thromboembolism increases before cancer diagnosis, ischemic stroke may be the first clinical manifestation of underlying cancer possibly reflecting prothrombotic effects of occult cancer. Patients without known cancer who present with cryptogenic ischemic stroke are at increased risk of cancer diagnosis within the subsequent year. In a recent meta-analysis, the 1-year incidence of cancer diagnosis after cryptogenic stroke was 6.2% (95% CI 1.4 to 13.9).[43] Therefore, detection of occult cancer after cryptogenic stroke may lead to earlier cancer diagnosis and treatment, and possibly improved survival.

Although cancer may underlie unexplained thrombosis, for patients with cryptogenic stroke there are no high-quality data regarding the potential benefits and harms of cancer screening and the optimal screening strategy is unknown. Although expert guidance endorses consideration of underlying cancer as an etiology of cryptogenic stroke, specific recommendations beyond cancer screening according to sex, age and risk for the general population are lacking.[44,45] This approach may be inadequate for the cryptogenic stroke population at higher risk of occult cancer and younger age. Professional guidelines (*e.g.*, American Heart Association/American Stroke Association Guidelines 2021, Canadian Stroke Best Practice Guidelines 2022) do not make specific recommendations as to how and when to screen for occult cancer after cryptogenic stroke which likely reflects the paucity of data regarding the utility of screening approaches.[46,47]

A systematic review evaluating the frequency and predictors of cancer after ischemic stroke found that the cumulative incidence of a new cancer diagnosis in a general ischemic stroke population was low: 13.6 per thousand (95% CI 5.6-24.8).[43] However, studies restricted to the cryptogenic stroke population had a higher cancer incidence as compared to those including all stroke subtypes (62.0 per thousand; 95% CI 13.6-139.3 *vs*. 9.6 per thousand; 95% CI: 4.0-17.3; P=0.02). The most predictive clinical factors for occult cancer in ischemic stroke patients were older age, a history of smoking, cryptogenic etiology, and involvement of multiple vascular territories on brain imaging. Laboratory indices associated with cancer were lower hemoglobin levels, higher C-reactive protein, higher D-dimer, and higher fibrinogen. Given the burden of financial, time-related, and healthcare resource costs associated with cancer screening, an evidence-based approach to screening is needed.

A recent registry and population-based study of 390,398 patients in the Netherlands that was published after the above-mentioned meta-analysis found that the cumulative incidence of new cancer at 10 years after a first-ever stroke was 3.7% (95% CI 3.4-4.0%) among patients aged 15-49, and 8.5% (95% CI 8.4-8.6%) among those 50 years or older.[48] However, when compared with age-matched peers from the general population, patients aged 14-49 were more likely to receive a diagnosis of new cancer after ischemic stroke (standardized incidence ratio 2.6, 95% CI 2.2-3.1). These results suggest that patients younger than 50 were about 3 times more likely to receive a new diagnosis of cancer compared to peers from the general population, and this risk remained elevated for 8 years after ischemic stroke. Among younger adults aged 15-49 years, the three most common cancers diagnosed were breast cancer (22.2%), gastrointestinal cancer (20.0%), and lung cancer (19.8%). Conversely, among older adults, the most common cancers were gastrointestinal (28.5%), urogenital (24.3%), and lung cancer (18.8%).

A risk prediction model was developed to identify patients at the highest risk for occult cancer diagnosis after ischemic stroke.[49] The incidence of a new occult cancer diagnosis was 3% at 1 year (34/1157) and 5% at 3 years (55/1158). The independent predictors of cancer included levels of white blood cells >9,600/µl [subdistribution (SHR) 3.68, P=0.014, platelets >400,000/µl (SHR 7.71, P=0.001), and D-dimer ≥3 mg/l (SHR 3.67, P=0.007); ischemic strokes in ≥2 vascular territories not attributed to a cardioembolic etiology was associated with cancer diagnosed within 1 year after stroke only in univariate analysis (SHR 3.69, P=0.001). A score of 2 or higher had a sensitivity of 43% and a specificity of 92% for prediction of new cancer diagnosis within 1 year after stroke. However, given its retrospective nature and low number of outcomes (*i.e.*, only 34 patients were diagnosed with cancer within 1 year after stroke), this study requires external validation.

In a survey of 138 physicians who manage stroke in patients with cancer, approximately half of respondents indicated they defer cancer screening investigations to primary care providers. (Poirier *et al*. manuscript embargo). Less than a third of physicians ordered tests that are commonly used for screening such as body imaging, mammograms or fecal occult blood tests even guideline-directed age-, sex- and risk-appropriate screening tests.

Given the clinical equipoise about screening and a lack of evidence-based guidelines to inform clinical practice, the Intensive Cancer Screening After Cryptogenic Stroke (INCOGNITO) Randomized Pilot Trial is evaluating to evaluate whether fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT) in addition to usual care screening increases the number of occult cancers detected in patients with cryptogenic ischemic stroke compared to usual care alone (NCT05733416).[18] FDG PET/CT, which is an established imaging technique used for the diagnosis, staging and restaging of cancers, is a promising candidate for occult cancer screening in this setting. It is a non-invasive, whole-body test with acceptable diagnostic accuracy. In patients with unexplained venous thrombosis, there was a lower rate of missed cancer among patients who had screening that included FDG-PET/CT compared to those who did not (0.5% *vs*. 4.6%).[50]

Antithrombotic treatment considerations

Guidelines regarding antithrombotic therapies for acute reperfusion and secondary prevention of ischemic stroke may not be generalizable to patients with cancer who are at a

uniquely high risk of both bleeding and thrombosis, especially given the predominance of alternative stroke mechanisms.

Intravenous (systemic) thrombolysis is the mainstay of treatment for acute ischemic stroke, the benefit of which is time-dependent. Patients with cancer and acute ischemic stroke appear less likely to be offered and to receive systemic thrombolysis which may reflect the presence of absolute or relative contraindications (*e.g.*, thrombocytopenia, renal/hepatic dysfunction, surgery, brain metastases).[51,52] For example, thrombocytopenia with platelets of $<100\acute{}10^9$/L is a contraindication to systemic thrombolysis.[53] Although timely administration of thrombolysis is crucial, if there is a high suspicion for thrombocytopenia (*e.g.*, patients with cancer receiving myelosuppressive chemotherapy) it may be reasonable to wait for initial laboratory tests prior to administration. Given concerns regarding bleeding complications, mechanical endovascular thrombectomy (EVT) appears to be feasible and effective for the management of acute ischemic stroke in this setting based on limited data. In a substudy of the MR CLEAN EVT registry, patients with active cancer who underwent EVT had similar rates of successful reperfusion and symptomatic intracerebral hemorrhage, but higher rates of recurrent stroke and worse functional outcomes compared to those without cancer.[9] Although there are limited data regarding optimal acute management, a diagnosis of cancer *per se* should not exclude patients from receiving thrombolysis or EVT given the substantial mortality and life-altering functional impairments of ischemic stroke. Decisions regarding acute reperfusion therapy should be individualized with multidisciplinary input if possible and shared decision-making with patients/caregivers.

The optimal antithrombotic regimen for secondary stroke prevention is not known. Antithrombotic choice in this setting is complicated by a paucity of data including limited studies comparing antithrombotic regimens. Anticoagulation is often favored for cancer-associated stroke based on the role of hypercoagulability in its pathogenesis and indirect extrapolation from cancer-associated VTE literature. In the general (non-cancer) population, large, randomized trials failed to demonstrate benefit of direct oral anticoagulants (DOACs) compared to aspirin for patients with ESUS.[54,55] However, anticoagulants may be preferred for cancer-associated stroke given that the pathophysiologic mechanisms appear to be distinct with a greater role of hypercoagulability contributing to cryptogenic etiology. In a NAVIGATE ESUS sub-study limited to participants with a history of cancer, the rates of recurrent stroke were similar among participants receiving rivaroxaban (7.7%) and those receiving aspirin (5.4%), while the rate of major bleeding was higher in the rivaroxaban group (2.9%) compared to the aspirin group (1.1%).[56] Given that only 9% of participants were diagnosed with cancer in the previous year, these results may not be generalizable to individuals with a recent diagnosis of cancer which is the highest risk time for cancer-associated stroke.

In a non-randomized study of patients with active cancer and acute ischemic stroke, anticoagulation with low molecular weight heparin or warfarin was associated with lower D-dimer levels and 1-year mortality, although methodological limitations preclude firm conclusions.[57] Another non-randomized study showed that patients treated with antiplatelet therapy had similar odds of recurrent stroke compared to those receiving anticoagulation.[17] The pilot trial of Enoxaparin *vs*. Aspirin in patients with cancer and stroke (TEACH) was designed to assess feasibility and showed that 40% of participants crossed over from enoxaparin to aspirin suggesting that anticoagulation with DOACs may be a more feasible approach.[58] The Edoxaban for the Treatment of Coagulopathy in Patients with Active Cancer and Acute Ischemic Stroke (ENCHASE) pilot trial is evaluating edoxaban *vs*. enoxaparin for cancer-associated ESUS (NCT03570281).

Given the role of coagulation and platelets in the pathogenesis of cancer-associated thrombosis and stroke, dual pathway inhibition with anticoagulants and antiplatelet therapy is a potential candidate for evaluation in this setting. The combination of very low dose rivaroxaban (2.5 mg twice daily) in addition to aspirin has been studied in patients with stable peripheral artery and coronary artery disease, and acute limb ischemia resulting in cardiovascular benefit at a cost of more major bleeding events.[59,60]

Conclusion and future directions

While acute ischemic stroke is a known complication of cancer, particularly within the first year after diagnosis, significant uncertainty remains with respect to prevention and treatment. First, there are no clinically available risk prediction models to identify patients at high risk who may benefit from prevention strategies. For example, the Khorana score is used to identify ambulatory cancer patients starting chemotherapy at high risk for VTE who are candidates for thromboprophylaxis based on the results of randomized trials.[61-63] Similarly, ischemic stroke risk assessment at cancer diagnosis may be used to evaluate prevention strategies in randomized trials for patients at high risk. Second, outcomes after cancer-associated stroke are not well characterized including bleeding which limits the use of antithrombotics and is key for establishing the net clinical benefit of therapies. Third, because of the uniquely high thrombotic and bleeding risk associated with cancer, antithrombotic data from non-cancer populations may not be generalizable and dedicated randomized trials are needed. Finally, cryptogenic ischemic stroke is associated with undiagnosed cancer, but there are no evidence-based strategies for cancer screening. Age-, sex-, and risk-directed screening may not be adequate in younger, high-risk populations. Like unprovoked VTE, randomized trials are needed to evaluate the benefits and harms of occult cancer screening strategies among patients with cryptogenic stroke.

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Trends and updates on the epidemiology of cancer-associated thrombosis: a systematic review

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ABSTRACT

For cancer patients, cancer-associated thrombosis (CAT) is a serious complication. An updated epidemiology of CAT over the last ten years is summarized in this review. A comprehensive analysis of pertinent population cohort research released between 2011 and 2024 was carried out. In patients with unselected cancers, the 12-month incidence of CAT is roughly 3-5% (9-fold increase vs to the matched non-cancer population); however, in patients with advanced cancers requiring systemic therapy, the risk rises to 6-8% (20-fold increase *vs*. to the matched non-cancer population). Anticoagulation use and adherence have improved, but the risk of recurrence is still high, at 5-8% at 6 months and 7-15% at 12 months. The type, stage, and treatment of cancer, a history of venous thromboembolism (VTE), prolonged hospitalization or immobilization, and obesity are significant clinical predictors of the development of CAT. The modified Vienna-CATS and EHR-CAT have the best performance (area under the curve 0.68-0.71) among the clinical risk prediction scores for CAT using the original Khorana score backbone that has been externally validated. However, additional research is required to guarantee appropriate implementation and utilization of these models. Even with contemporary antineoplastic treatments, CAT is still a major complication for cancer patients. We encourage interdisciplinary partnerships among hematologists, data scientists, epidemiologists, and oncologists to guarantee the integration of customized VTE risk evaluation into standard oncologic treatment.

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Introduction

Cancer-associated thrombosis (CAT) is defined as venous thromboembolism (VTE) involving pulmonary arteries (PE), deep veins of the legs (LE-DVT) or arms (UE-DVT), and sometimes abdominal or splanchnic veins (SpVT) during initial cancer diagnosis or active cancer treatment. In one estimate, CAT accounts for as many as 20% of all VTEs that affect 900,000 people from the United States each year.[1] Since VTE mostly occurs within the first year of cancer diagnosis, its development may lead to prolonged hospitalization, delayed treatment initiation, and consequently a detrimental effect on the quality of life and survival of cancer patients.[2,3] Given significant changes in anti-neoplastic treatment over the past decade, an increased understanding of the updated epidemiology of CAT in the modern era through this systematic review is crucial for both hematologists and oncologists.

The current review will describe the updated incidence, trends, risk factors, recurrence, and mortality of CAT in patients with active cancers. Treatment of CAT is beyond the scope of this review. We will primarily focus on population observational cohort studies with large sample size and valid methodological study designs. We will present both absolute and relative risks when available.

Methodology

A systematic review was performed using Title and MeSH term search with ("neoplasm" OR "cancer") AND ("thrombosis" OR "thromboembolism") AND ("epidemiology" OR "cohort studies" OR "incidence" OR "recurrence" OR "mortality" OR

"risk factors") in MEDLINE/PubMed for published studies in the English language from 2011 to 2024. Retrospective cohort studies >10,000 patients or prospective cohort studies >1,000 were preferentially included. The inclusion criteria for study size were set lower for prospective studies because they require greater effort and expenses and thus often have smaller study populations than retrospective studies. Abstracts only, review articles, clinical trials, case-control studies, systematic reviews, and studies isolated to singular cancer types were excluded. Cited references were further examined for additional inclusion. When multiple studies referenced the same cohort or population database, the most updated one was chosen. Two reviewers (AL and EZ) screened the title and abstract of the 380 resulting articles (date of search 1/15/24) and chose the relevant studies in this review.

Definition of cancer-associated thrombosis in studies

A total of 11 studies (7 retrospective and 4 prospective) were included to assess the incidence of CAT (Table 1). These studies represented diverse geographic locations across the world, including the United States (USA), the United Kingdom (UK), Denmark, Austria, Italy, France, Israel, Japan, and Taiwan. The definition of

Table 1. Incidence and trend of cancer-associated thrombosis in selective population studies.

Author	Location	Design	N	Year	Study population	VTE definition[1]	VTE trend	Incidence at 12 mo[2]
All cancers at time of diagnosis								
Martens JAMA Open 2023	USA	Retrospective cohort	434,203	2006-2021	US veterans with newly diagnosed solid + heme cancers Median age 67; 97% male; 26% metastatic	Inpatient or outpatient PE, LE-DVT, and UE-DVT (excluding abdominal thrombosis) (ICD code + NLP algorithm)	Minor increase 4.2% to 4.7%	4.5% VTE3.6% PE/LE-DVT
Mahajan Blood Adv 2022	California, US	Retrospective cohort	942,109	2005-2017	California residents with newly diagnosed solid + heme cancers Median age 65; 47% male; 20% metastatic	Inpatient + emergency department PE, LE-DVT (ICD code)	Major increase for most cancers	Cancer-specific PE/LE-DVT See Table 2
da Costa AJH 2021	Harris County, TX	Retrospective cohort	15,342	2011-2020	Harris county residents with newly diagnosed solid + heme cancers Mean age 54; 45% male; 29% metastatic; 74% uninsured; 84% disadvantaged neighborhood	Inpatient + outpatient PE, LE-DVT, UE-DVT, or non-tumor abdominal vein thrombosis (ICD code + chart review)	No change	11.2% VTE 8.1% PE/LE-DVT
Mulder Blood 2021	Denmark	Retrospective cohort	499,092	1997-2017	Danish residents with newly diagnosed solid + heme cancers Median age 68; 49% male; 21% metastatic Comparison cohort matched on age, sex, year	Inpatient or outpatient PE, DVT, abdominal thrombosis, and other VTE (ICD code)	Major increase 1.0% to 3.4%	2.3% VTE 2.8/100-py HR 8.5 *vs.* matched non-cancer
Moser RPTH 2021	Israel	Retrospective cohort	15,388	2010-2018	Israel residents with newly diagnosed solid + heme cancers Median age 60; 35% male; 49% early stage on adjuvant hormone therapy	Inpatient + outpatient PE, LE-DVT, UE-DVT (ICD code)	N/A	2.2% VTE
Yu T&H 2012	Taiwan	Retrospective cohort	497,180	1997-2005	Taiwan residents with newly diagnosed solid + heme cancers (catastrophic illness database) Mean age 61; 56% male; unknown stage	Inpatient PE, DVT, abdominal thrombosis (ICD code)	Minor increase 0.3% to 0.6%	0.5%

To be continued on next page

CAT varied across the studies due to limitations in outcome ascertainment strategies. Most studies included inpatient and outpatient diagnosis of PE and LE-DVT, though some only reported hospital discharge diagnosis (missing outpatient diagnosis).[4-6] There was significant heterogeneity in the inclusion of UE-DVT and SpVT across the studies. Since 10-20% of patients with hepatobiliary and pancreatic cancers develop SpVT,[7] indiscriminate inclusion of SpVT in CAT outcome definition could lead to overinflated incidence estimation. Most of the retrospective cohort studies relied on country-specific International Classification of Diseases (ICD) codes to determine VTE outcomes, though a few also incorporated chart review or natural language processing verifications.[5,8,9] Most

Table 1. Continued from previous page.

Author	Location	Design	N	Year	Study population	VTE definition[1]	VTE trend	Incidence at 12 mo[2]
Ohashi Thromb Res 2022	Japan	Prospective cohort	9,630	2017-2019	Japanese residents with newly diagnosed solid cancers Mean age 67; 51% male; 23% metastatic	Inpatient or outpatient symptomatic PE, LE-DVT (adjudicated)	N/A	0.5%
Advanced cancers receiving systemic therapy								
Martens JAMA Open 2023 Subset	USA	Retrospective cohort	118,731	2006-2021	US veterans with newly diagnosed solid + heme cancers receiving systemic therapy within 3 months	See above	See above	7.7% VTE
Mulder Blood 2021 Subset	Denmark	Retrospective cohort	64,397	2011-2017	Danish residents with newly diagnosed solid + heme cancers receiving systemic therapy within 4 months	See above	See above	5.3% VTE 6.3/100-py HR 19.7
Cohen T&H 2017	UK	Retrospective cohort	112,738 person years	2001-2011	UK residents with "active" solid + heme cancer episodes (primary Dx of cancer as hospital discharge diagnosis; OR receipt of chemotherapy, radiation, or transplantation) Mean age 69; 49% male; unknown stage	Inpatient PE, LE-DVT, UE-DVT (excluding cerebral and abdominal vein thrombosis) (ICD code + chart review)	N/A	5.8/100-py
Englisch Blood Adv 2022	Austria CATS	Prospective cohort	1,708	2003-2019	Vienna resident with newly diagnosed or recently progressed solid + heme cancers with 68% receiving chemotherapy during observation Mean age 61; 46% female; 32% metastatic	Inpatient or outpatient PE, LE-DVT, UE-DVT (adjudicated)	N/A	7.8%
Verzeroli JTH 2023	Italy HYPERCAN	Prospective cohort	1,286	2012-2019	Italian residents with lung, colon, gastric, breast solid cancers receiving chemotherapy Median age 65; 55% male; 100% metastatic	Inpatient or outpatient PE, LE-DVT, symptomatic UE-DVT (adjudicated)	N/A	9.7% (6 months)
Van Es Haematologica 2017	Multinational	Prospective cohort	876	2008-2016	Netherlands, Italy, France, Mexico residents with solid cancers receiving chemotherapy last 3 months Mean age 64; 59% male; 66% metastatic	Inpatient or outpatient PE, LE-DVT, symptomatic UE-DVT (chart review or telephone contact)	N/A	6.5% (6 months)

[1]Most large epidemiology studies relied on the use of country-specific ICD codes from either inpatient or inpatient/outpatient data sources. Significant heterogeneity existed on the inclusion or exclusion of upper extremity DVT and abdominal venous thrombosis; [2]either cumulative incidence (%) or incidence rate (per 100-person-year) was provided depending on the individual study design. VTE, venous thromboembolism; mo, months PE, pulmonary embolism; DVT, deep vein thrombosis; LE-DVT, lower extremity deep vein thrombosis; UE-DVT, upper extremity deep vein thrombosis; ICD, international classification of diseases; NLP, natural language processing; US, United States; py, patient-year; HR, hazard ratio; OR, odds ratio.

of the prospective cohort studies required independent outcome adjudications. To ensure uniformity in outcome reporting, a 12-month follow-up window was chosen for this review to report the cumulative incidence (%) or incidence rate per 100-patient-year (py). Across all 11 studies, the cumulative incidence of CAT ranged from 0.5% to 11.2%. Given the differences in study populations across each study in the following sections, we will examine the data in further granularity by examining incidence rates in different cancer types, stages, and treatments.

Trend of cancer-associated thrombosis in active cancer

A few studies examined the trends of CAT over time. Mulder *et al*. reported a significant increase in 12-month CAT from 1.0% in 1997 to 1.9% in 2004 to 3.4% in 2017.[10] Mahajan *et al*. also reported an increase in CAT incidence from 2005-2017, although the rate of increase was cancer-dependent.[11] In contrast, Martens *et al*. reported a minor increase in CAT incidence from 4.2% in 2005 to 4.5% in 2017.[9] Advances in VTE awareness, imaging modalities, and anti-neoplastic treatment likely all contributed to the increase in CAT incidence over the past 2 decades. Nonetheless, changes in ICD codes used in studies could also have influenced the outcome reporting. For example, ICD-CM (USA) for VTE diagnosis had a significant expansion in years 2004, 2009, and 2015; therefore, it is important for studies to use epoch-specific ICD code for outcome ascertainment.

Incidence of cancer-associated thrombosis by cancer stage/treatment

Due to significant heterogeneity in the study populations, we divided the CAT incidence assessment into those with cohort entry at the time of diagnosis (all cancers) *vs*. at the time of systemic therapy (advanced cancers). Accounting for differences in study design, CAT occurrence appeared to have geographic variations. Among representative studies at the time of initial cancer diagnosis, the 12-month cumulative incidence of CAT was 4.5% in 1 study (USA),[9] 2.3% in 2 studies (Denmark, Israel),[10,12] and 0.5% in 2 studies (Taiwan, Japan).[6,13] One additional cohort study from Harris County, USA reported a higher incidence of 8.1% PE/LE-DVT at 12 months,[8] though the patient population was diverse (50% Hispanic, 28% Black), young (mean age 54), uninsured (74%) and living in disadvantaged neighborhoods (84%), and with more aggressive cancer and advanced stage (48%). In contrast to the studies reporting CAT incidence in all patients from time of cancer diagnosis, among representative studies at the time of systemic therapy treatment, the 12-month cumulative incidence of CAT was significantly higher ranging 5.8-7.8% in 4 studies (USA, Denmark, UK, Austria).[5,9,10,14] In two additional cohorts that included selective solid tumor patients with high proportion of metastatic disease, the 6-month cumulative incidence of CAT was even higher at 6.5% (MICA, 66% metastatic) and 9.7% (HYPERCAN, 100% metastatic).[15,16]

Taken together, relative to matched non-cancer populations, the hazard ratio (HR) for VTE was 8.5 for patients with cancers and 19.7 for patients with advanced cancers receiving systemic therapy.[10] Therefore, cancer patients with advanced disease requiring systemic therapy have a significantly higher risk of CAT than those with limited resectable disease. For example, gynecologic and non-prostate genitourinary cancers are considered "high-risk" in risk prediction models but "low-risk" in population studies (most of these cancers normally present at early stages).[9,17]

Incidence of cancer-associated thrombosis by cancer type

Cancer type is likely the most important determinant of CAT risk. Table 2 summarizes the 12-month cumulative incidence of CAT across different cancer types in 5 large epidemiology studies. Yu *et al*. did not report significant variations among cancer types in Taiwan.[6] Among solid tumors in the 4 other studies, pancreatic, stomach, biliary, and brain cancers consistently had the highest 12-month incidence of CAT (8-10%). Lung, colorectal, ovarian, sarcoma, lymphoma, and myeloma had the next highest incidence (5-7%). Non-prostate genitourinary cancers had variable risks depending on staging and treatment as discussed previously. Among leukemias, acute lymphocytic leukemia had the highest incidence of 12-month CAT at 18.6% (11.8% PE/LE-DVT) followed by acute myeloid leukemia at 7.3% (3.6% PE/LE-DVT).[9] It is important to note that UE-DVT (mostly catheter-associated thrombosis) was significantly more common in acute leukemias than most other cancer types due to prolonged insertion of indwelling peripherally inserted central catheter for chemotherapy administration. Overall, the previously identified very high risk and high-risk cancer types associated with CAT continue to be associated with greater incidences of CAT in Western countries.

Risk factors of cancer-associated thrombosis occurrence

As discussed above, different cancer types, stages, and treatments are all important drivers for CAT development and contribute to the varying incidences of CAT. The relative impact of each can only be discerned in studies adequately powered to adjust for each factor. Table 3 highlights studies that examined the cancer- and patient-specific risk factors for CAT. In cohort studies, the specific cancer type had the highest association. For example, pancreatic cancer and stomach cancer had a 6-9-fold and 4-5-fold increase in CAT risk *vs*. prostate cancer, respectively. Cancer stage was the second most significant cancer-specific predictor with a 2-fold increase for stage III and a 4-fold increase for stage IV (*vs*. stage I). A case-control study by Ashrani *et al*. (not included in Table 3) reached a similar finding as these cohort studies.[18] In contrast, treatment type (received within the first 3-4 months of diagnosis) had a more attenuated effect. Cytotoxic chemotherapy, immune checkpoint inhibitor, and targeted/endocrine therapy had 1.5-2-fold, 1.5-fold, and 1.2-fold increased risks, respectively, compared to no treatment. Two recently published comparative cohort studies further demonstrated that patients receiving immunotherapy had a similar or slightly lower risk of VTE as those receiving cytotoxic chemotherapy in the first-line setting.[19,20] Based on these studies, we can conclude that while treatment choice impacts CAT risk, the underlying disease (cancer histology) and aggressiveness (cancer stage) likely had significantly higher association.

In addition to cancer-specific risk factors, there are also more

traditional patient-specific risk factors for VTE. Unsurprisingly, the strongest risk factor was the history of VTE at 2-8-fold increased risk.[8-10] Other risk factors included older age, male sex, higher body mass index, and recent hospitalization (each ~1.2-fold higher risk). Interestingly, comorbidity score (marker of underlying comorbid illness) and area of deprivation index (social determinant of health) had no association with CAT.[8,9]

Racial disparity and cancer-associated thrombosis incidence

The impact of race and ethnicity on the development of CAT (or VTE in general) remains a debated topic. Previous papers have posited that biological and sociological mechanisms contribute to racial disparities in incidence rates of CAT events. It is important to remember that race is inherently a sociopolitical construct and that racial and ethnic categories do not always correlate with genetic differences. While the reports of lower CAT risk among east Asians were consistent in studies across USA, Europe, and Asia (Table 1 and Table 4), the comparison between non-Hispanic Black (NHB) and Hispanic *vs*. non-Hispanic White (NHW) has mostly been reported in epidemiology studies in the USA. Specifically, the adjusted HR for CAT for Asians *vs*. NHW was 0.6-0.8 across multiple studies.[4,8,9,21] In contrast, the adjusted HR for NHB *vs*. NHW was consistently elevated at 1.2-1.4. Finally, the comparison between Hispanic *vs*. NHW was less pronounced with adjusted HR of 0.9-1.0. In absolute terms, Raskob *et al*. also reported highest incidence of CAT in NHB (40.9/100,000-py), followed by NHW (32.5/100,000-py), Asian Pacific islanders (7.7/100,000-py), and Hispanics (5.6/100,000-py) among active cancers in a population surveillance study in Oklahoma.[22]

Table 2. Incidence of cancer-associated thrombosis in individual cancers at 12 months in selective studies.

	Martens	Mahajan	Mulder	Cohen	Yu
	PE, LE-DVT, UE-DVT	PE, LE-DVT	PE, LE-DVT, UE-DVT, SpVT	PE, LE-DVT, UE-DVT	PE, LE-DVT, UE-DVT, SpVT
Location	US	US	Denmark	UK	Taiwan
Number	434,203	942,109	942,109	112,738 py	497,180
Breast	3.4	1.0	1.0	3.2	0.7
Lung	6.9	6.8	2.2-3.3	10.1	1.5
Prostate	1.5	1.0	1.2	4.4	1.4
Testicular	5.3		1.3		1.0
Bladder	5.8	5.1	2.7	2.7	0.9
Kidney	3.9	3.6	2.7		1.2
Colorectal	6.7	3.9	2.8	6.7	1.0
Esophageal	10.0		3.0		0.6
Stomach		6.7	3.2	10.8	1.1
Pancreas	12.1	10.7	5.5	14.6	1.2
Bile/gallbladder	9.1		3.8		
Liver	2.7		3.4		1.0
Neuroendocrine	4.3				
Ovarian		8.2	3.9	11.9	1.8
Uterine	4.9	3.7	2.0	7.0	
Cervical			2.0		1.8
Head & Neck	4.1				0.4
Sarcoma	6.2				1.4
Melanoma	1.7		0.6		0.8
Brain	11.1	9.7	3.3	12.1	1.3
Endocrine	1.6				0.3
Myeloma	7.7	5.3	3.8		1.6
NHL	11.0	4.3	3.2		1.0
HL	9.5		3.8		
ALL	18.6			4.5	
CLL	2.0				
AML	7.3				
MDS	2.7		1.7		0.9
CML	2.1				

PE, pulmonary embolism; LE-DVT, lower extremity deep vein thrombosis; UE-DVT, upper extremity deep vein thrombosis; SpVT, splanchnic veins; US, United tates; UK, United Kingdom; py, patient-year; NHL, non-Hodgkin lymphomas; HL, Hodgkin's lymphoma; ALL, acute lymphoblastic leukaemia; CLL, chronic lymphocytic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndromes; CML, chronic myelogenous leukemia.

Validated clinical risk prediction scores for cancer-associated thrombosis

The American Society of Hematology 2021 Guidelines suggest thromboprophylaxis (conditional recommendation, moderate certainty of evidence) with a low-dose direct oral anticoagulant (DOAC) if the 6-month VTE risk is high (defined as 9.5% or higher by the consensus panel) while the bleeding risk is low-to-moderate.[23] Since the original Khorana score in 2008,[17] there have been various adaptations and improvements, including the Vienna CATS in 2010,[24] PROTECHT in 2012,[25] COMPASS-CAT in 2017,[26] modified Vienna CAT in 2018,[27] ONCOTHROMB in 2023,[28] and electronic health record (EHR) CAT in 2023.[29] Each of these risk scores relied primarily on the cancer type (with variation based on the inclusion criteria of each study) as the backbone of the models. Three risk scores included additional biomarkers such as P-selection,[24] D-dimer,[27] and a 9-single nucleotide poly-

Table 3. Risk factors for cancer-associated thrombosis in selective studies (multivariable regression).

Location	Martens US	da Costa US	Mulder Denmark
Number	434,203	15,342	942,109
Cancer type/site			
Breast	1.85 (1.62-2.10)	Baseline	1.56 (1.27-1.92)
Non-small cell lung cancer	3.23 (3.08-3.39)	1.59 (1.26-2.00)	4.94 (4.07-6.00)
Small cell lung cancer			2.51 (1.97-3.20)
Prostate	Baseline	0.79 (0.57-1.10)	1.71 (1.39-2.10)
Testicular	2.49 (1.91-3.25)		2.23 (1.58-3.14)
Bladder	2.76 (2.57-2.96)	2.37 (1.86-3.0)	3.68 (2.94-4.59)
Kidney	2.17 (2.02-2.33)		4.41 (3.53-5.50)
Colon	2.45 (2.31-2.59)	1.34 (1.08-1.66)	4.28 (3.52-5.21)
Rectal			4.10 (3.34-5.03)
Stomach	4.03 (3.78-4.30)	1.67 (1.28-2.18)	4.94 (3.95-6.19)
Esophageal	4.03 (3.78-4.30)		3.86 (3.03-4.91)
Pancreatic	6.42 (5.98-6.90)	2.89 (2.21-3.77)	9.23 (7.54-11.30)
Biliary	4.38 (3.84-4.98)	1.92 (1.39-2.66)	6.43 (4.79-8.62)
Liver	1.84 (1.70-2.00)	2.13 (1.61-2.82)	6.68 (5.25-8.49)
Neuroendocrine	1.97 (1.78-2.18)	1.06 (0.69-1.62)	
Ovarian	2.93 (2.35-3.61)	1.50 (1.21-1.85)	5.52 (4.44-6.86)
Uterine			3.68 (2.91-4.65)
Cervical			3.74 (2.88-4.86)
Head & neck	1.32 (1.24-1.41)	0.65 (0.47-0.89)	
Sarcoma	2.82 (2.46-3.23)	1.41 (0.99-1.99)	
Melanoma	1.38 (1.26-1.52)	1.40 (0.72-2.74)	Baseline
Brain	5.65 (4.96-6.44)		9.11 (7.19-11.54)
Endocrine	1.05 (0.90-1.22)	0.34 (0.16-0.72)	
Myeloma	1.72 (1.57-1.87)	0.93 (0.57-1.51)	5.66 (4.46-7.19)
Aggressive NHL	2.65 (2.43-2.89)		4.61 (3.75-5.67)
Indolent NHL	1.38 (1.26-1.51)	1.39 (1.09-1.76)	
Hodgkin	2.00 (1.68-2.38)		5.73 (4.24-7.74)
ALL	4.98 (3.71-6.68)		
AML	2.10 (1.82-2.41)		
CLL	0.77 (0.68-0.87)	0.86 (0.56-1.32)	2.76 (2.17-3.50)
CML	0.57 (0.49-0.66)		
MDS	0.76 (0.66-0.87)		
Cancer stage			
Stage I	Baseline	Baseline	Baseline
Stage II	1.47 (1.41-1.54)	1.87 (1.49-2.35)	
Stage III	1.88 (1.80-1.97)	2.68 (2.17-3.30)	2.34 (2.19-2.50)
Stage IV	2.78 (2.68-2.90)	3.89 (3.17-4.78)	4.00 (3.74-4.27)

To be continued on next page

morphism genetic risk score.[28] Three risk scores expanded on additional clinical risk factors.[25,26,29] In external validation studies, the original Khorana score and most of the earlier adaptations had an area under the curve (AUC) of 0.57-0.64.[15,16,29-32] The two recently published risk prediction scores had improved AUC of 0.66-0.68 in modified Vienna CAT and 0.68-0.71 in EHR-CAT.[16,27,29,30] The modified Vienna CAT from Pabinger *et al*. relied on cancer type and D-dimer nomogram with relatively high degree of accuracy.[27] The EHR-CAT from Li *et al*. was a modern adaptation of the Khorana score. It was derived and validated in elec-

Table 3. Continued from previous page.

	Martens	da Costa	Mulder
Location	**US**	**US**	**Denmark**
Cancer treatment			
No treatment	Baseline	Baseline	Baseline
Chemotherapy	1.44 (1.40-1.49)	1.92 (1.68-2.18)	2.16 (1.98-2.36)
Immune checkpoint inhibitor	1.49 (1.22-1.82)	1.29 (0.67-2.50)	1.78 (1.02-3.10)
Targeted therapy	1.21 (1.13-1.30)	1.07 (0.78-1.47)	
Hormone therapy	1.20 (1.12-1.28)	1.89 (0.94-3.79)	0.95 (0.81-1.12)
Older age	1.02 (1.01-1.04) per year	1.16 (1.01-1.34) For 65+ vs. <65	Increasing per decade
Male sex	1.15 (1.06-1.24)	1.04 (0.94-1.16)	1.02 (0.97-1.06)
Race			
Non-hispanic white	Baseline	Baseline	
Non-hispanic black	1.23 (1.19-1.27)	1.15 (1.00-1.33)	
Non-hispanic asian pacific islander	0.84 (0.76-0.93)	0.58 (0.44-0.77)	
Hispanic	1.04 (0.98-1.10)	0.86 (0.75-0.99)	
Area of deprivation index			
1st quartile	Baseline	Baseline	
2nd quartile	0.96 (0.92-1.00)	1.01 (0.88-1.15)	
3rd quartile	0.95 (0.91-0.99)	1.07 (0.94-1.22)	
4th quartile	0.94 (0.90-0.98)	0.94 (0.82-1.08)	
BMI 35+	1.27 (1.23-1.31)	1.29 (1.12-1.49)	
VTE history	2.75 (2.65-2.86)	1.59 (1.13-2.25)	8.24 (7.81-8.69)
Recent hospitalization	1.17 (1.13-1.21)	1.54 (1.39-1.70)	
Immobilization or paralysis history	1.20 (1.08-1.35)		
Comorbidity score	0.97 (0.95-1.00)	0.98 (0.87-1.10)	0.79 (0.67-0.94)

All numbers in this table represent hazard ratios from cause-specific Cox regression models. Only risk factors present in multiple studies are shown here. US, United States; NHL, non-Hodgkin lymphomas; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous

Table 4. Racial Disparities for cancer-associated thrombosis observed in the United States.

Author	Population	N	Years	Non-hispanic white	Non-hispanic black	Hispanic	Asian/Pacific islander
Raskob JTH 2022	Oklahoma county, Oklahoma	Incidence rate	2012-2014	32.5/100,000-py	40.9/100,000-py	5.6/100,000-py	7.7/100,000-py
Martens JAMA Open 2023	Veterans, USA	434,203	2006-2021	Baseline	1.23 (1.19-1.27)	1.04 (0.98-1.10)	0.84 (0.76-0.93)
da Costa AJH 2021	Harris county, Texas	15,342	2011-2020	Baseline	1.15 (1.00-1.33)	0.86 (0.75-0.99)	0.58 (0.44-0.77)
Mahajan Semin Thromb Hemost 2019	California cancer registry, California	942,109	2005-2017	Baseline	1.43 (1.37-1.49)	0.93 (0.89-0.96)	0.62 (0.59-0.65)

Py, patient-year.

tronic health record data and composed of cancer type (4 categories), advanced stage, cancer treatment (chemo/immune *vs*. targeted/endocrine), pre-treatment leukocyte, hemoglobin, and platelet count, body mass index, VTE history, paralysis/immobilization history, recent hospitalization, and Asian race (https://dynamicapp.shinyapps.io/EHR-CAT/).[29] Inherently, clinical risk prediction score is a trade-off between complexity and accuracy.[33] Given the rapid development in artificial intelligence and health informatics, it is conceivable that these models will be soon incorporated into clinical decision-support tools to aid decision-making.

Recurrence risk after cancer-associated thrombosis diagnosis

After the first CAT event, the risk of VTE recurrence remains elevated despite anticoagulation treatment. In 4 randomized controlled trials comparing DOAC *vs*. low-molecular-weight heparin (LMWH), the pooled 6-month recurrence was 5.6% in the DOAC arm and 8.3% in the LMWH arm.[34] The exact recurrence rate in epidemiology studies is difficult to assess due to the lack of a validated algorithm for the recurrent VTE outcome. Many studies have utilized a combination of ICD codes at the principal inpatient discharge diagnosis position, anticoagulant medication interruption/resumption, and/or imaging procedure codes. However, no study to date has presented validation of such algorithms in cancer patients. Despite potential ascertainment bias, the 12-month VTE recurrence rate was reported to be 6.7%-15.3% in 6 large cohort studies (Table 5).[5,22,35-38] All except one of the studies were performed before the DOAC era. Reliable longer-term VTE recurrence data beyond 1-year are lacking.

Mortality risk after cancer-associated thrombosis diagnosis

The impact of CAT on the mortality risk in cancer patients remains another challenging topic. Since VTE occurrence has strong associations with aggressive cancer and advanced staging, it is inevitably correlated with worse survival. To account for confounding and selection bias, Sorensen *et al*. performed a retrospective cohort study using the Danish cancer registry to compare cancer patients with concurrent cancer and VTE diagnosis *vs*. those with cancer but no VTE after matching on age, sex, cancer type, and stage. The authors found that 1- and 5-year cumulative incidence for mortality was 68% and 84% in the CAT cohort *vs*. 38% and 67% in the non-CAT cohort (HR 4.34, 95% CI 3.95-4.78). Among

Table 5. Incidence of cancer-associated thrombosis recurrence in selective population studies.

Author	Location	Design	N	Year	Study population	VTE definition	Recurrence risk/rate
Raskob JTH 2022	Oklahoma county, Oklahoma	Surveillance study	3,422	2012-2014	Oklahoma county residents with cancer and VTE	Inpatient and outpatient PE and DVT (ICD code + imaging)	12 months: 12.5%
Cohen Thromb Haemost 2017	UK clinical practice research datalink	Retrospective cohort	6,592	2001-2011	UK residents with "active" solid + heme cancer and VTE	Inpatient PE, LE-DVT, UE-DVT (excluding cerebral and abdominal vein thrombosis) (ICD code + chart review)	6 months: 7.4% 12 months: 9.2%
Ording Int J Cardiol 2023	Danish cancer registry	Retrospective cohort	34,702	2003-2018	Danish residents with active cancer and first-time diagnosis of VTE	Inpatient and outpatient PE and DVT (ICD code + imaging)	6 months: 5.1% 12 months: 6.7%
Lecumberri Thromb Haemost 2022	Computerized registry of patients with venous thromboembolism (RIETE) registry	Retrospective cohort	16,694	2001-2020	Spanish residents with cancer and VTE	Inpatient and outpatient symptomatic PE, LE-DVT, UE-DVT (imaging)	10.5/100-py at median 150d in solid cancers 7.7/100-py at median 127d in heme cancers
Hwang Clin Exp Thromb Hemost 2021	Korean health insurance review and assessment	Retrospective cohort	19,725	2004-2013	Korean residents with cancer and VTE	Inpatient and outpatient PE, LE-DVT (ICD code + medication)	7.1% at median 1.6 years
Khorana AJH 2019	Truven health MarketScan database	Retrospective claims database	13,804	2013-2016	Commercially-insured patients with cancer and VTE	Inpatient primary discharge diagnosis (ICD code only)	12 months: Rivaroxaban: 11.3-13.3% LMWH: 14.7%-15.3% Warfarin: 11.6-13.3%

VTE, venous thromboembolism; PE, pulmonary embolism; DVT, deep vein thrombosis; ICD, International Classification of Diseases; LE-DVT, lower extremity deep vein thrombosis; UE-DVT, upper extremity deep vein thrombosis; py, patient-year.

patients with CAT onset after cancer diagnosis, the 1- and 5-year cumulative incidence for mortality was 45% (HR 3.48, 95% CI 3.37-3.60) and 69% in the CAT cohort (HR 2.57, 95% CI 2.50-2.63).[39] Similarly, Mahajan *et al*. reported that the adjusted hazard ratios ranged from 1.89 to 4.79 across various cancer types when the onset of CAT was treated as a time-varying covariate to account for immortal time bias.[11]

Future direction

Significant advances have been made in the past decade to better elucidate the epidemiology, prevention, and management of CAT. Nonetheless, despite improved risk prediction scores, randomized trials, and guideline recommendations, most oncologists are not aware of the available tools.[40] Future areas of research include some of the following: i) hybrid implementation projects aimed at physician and patient education; ii) integration of risk prediction scores into electronic health records with longitudinal real-time updates; iii) development and validation of artificial intelligence-assisted but transparent VTE risk models; iv) incorporation of comprehensive plasma biomarkers using commercially available assays to measure proteomics or cell-free DNA; v) development of validated natural language processing algorithms for recurrent VTE to ascertain short- and long-term epidemiology of VTE recurrence.

Conclusions

In summary, the 12-month incidence of CAT among patients with unselected cancers in the modern era is approximately 3-5% in Western countries (9-fold increase *vs*. matched non-cancer population) and 1% in eastern Asian countries; although the risk increases to 6-8% in patients with selectively advanced cancers requiring systemic therapy (20-fold increase *vs*. matched non-cancer population). Despite improvement in anticoagulation usage and adherence, recurrence risk remains high at 5-8% at 6 months and 7-15% at 12 months. The most important clinical predictors of CAT development are cancer type, cancer stage, cancer treatment, prior VTE history, prolonged hospitalization and immobilization, and obesity. Several clinical risk prediction scores for CAT utilizing the initial Khorana score backbone have been developed and externally validated, though more studies are needed to ensure adequate implementation and usage of these models. We encourage multidisciplinary collaborations between hematologists, oncologists, epidemiologists, and data scientists to ensure the adoption of personalized VTE risk assessment in routine oncologic care.

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Location of metastasis and complications in patients with venous thromboembolism and cancer: systematic review

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ABSTRACT

According to current guidelines, patients with venous thromboembolism (VTE) and active cancer should receive prolonged anticoagulant treatment (>6 months). In patients with cancer-associated thrombosis (CAT), metastasis is a factor in recurrent VTE and bleeding; however, the function of metastasis location remains unclear. In order to assess the risk of complications (such as bleeding or recurrent VTE) based on the location of metastases in patients with CAT, we conducted a systematic review. The PubMed database search was used to perform a systematic review. MESH terms pertaining to metastasis, VTE, and neoplasms were employed. Patients with CAT who were at least eighteen years old and receiving therapeutic doses of anticoagulants were included, as were details regarding the locations of metastases and the availability of patients who had complications (bleeding or recurrent VTE). Among the 1,447 articles found by the search, 7 retrospective studies met all eligibility requirements and were added to the analysis. The majority of these studies addressed brain metastases. Studies found that intracranial hemorrhage occurred between 4% and 19% of the time. In the context of brain metastases and VTE, other studies examined the safety and effectiveness of direct oral anticoagulants in comparison to low-molecular-weight heparin. This systematic review draws attention to the paucity of data regarding the impact of metastasis location on complications in CAT patients. Further research is required to assess the effect of metastasis location on the risk of VTE complications in patients with CAT.

Introduction

Venous thromboembolism (VTE) that includes pulmonary embolism (PE) and deep vein thrombosis, is the second leading cause of death in cancer patients.[1] These patients have a 6 to 14-fold higher risk of developing VTE compared to patients without cancer,[2] and higher rates of recurrent VTE and bleeding complications during VTE treatment.[3]

Current clinical practice guidelines recommend at least 6 months of anticoagulant therapy in patients with cancer-associated thrombosis (CAT).[4-7] Beyond 6 months, the decision to discontinue or continue anticoagulation should be based on individual evaluation of the benefit-risk ratio, paying special attention to cancer activity (metastatic disease or oncological treatment).[4-7] Therefore, understanding the variables associated with complications in patients with CAT could be highly useful for decision-making in clinical practice.

The presence of metastasis has been described as a variable

associated with recurrent VTE and bleeding in patients with CAT. A systematic review of 10 studies (6 observational and 4 retrospectives), that included 4,791 patients with CAT, found a significantly higher risk of VTE recurrence in patients with metastasis [relative risk (RR): 1.4, 95% confidence interval (CI), 1.1-1.7; P=0.01].[8] In addition, the *post-hoc* analysis of the CLOT trial (comparison of low-molecular-weight-heparin *vs*. oral anticoagulant therapy for the prevention of recurrent venous thromboembolism in patients with cancer) observed that the risk of recurrent VTE was higher in patients with metastatic disease (P=0.018).[9,10] On the other hand, investigators of the *Registro Informatizado de la Enfermedad TromboEmbólica* (RIETE) registry performed a study that included 2,945 patients with CAT whom 1.0% had fatal bleeding during the first 3 months of anticoagulation therapy. Moreover, they observed that metastatic cancer was an independent risk factor for fatal bleeding [odds ratio (OR): 3.1, 95% CI, 1.4-7.1; P=0.006].[11] Furthermore, an analysis of the Caravaggio trial (Apixaban for the treatment of venous thromboembolism associated with cancer) that included 1,034 patients, showed that patients with metastatic cancer had numerically increased major bleedings compared to those with localized cancer [5.2%, hazard ratio (HR): 1.65, 95% CI, 0.7-3.8].[12]

Recent studies have shown that the rate of complications in patients with CAT varies based on the location of the primary tumor. However, the rates of recurrent VTE and bleeding according to the location of metastasis are unclear. Therefore, we performed a systematic review to evaluate the risk of complications (recurrent VTE or bleeding) according to the location of metastasis in patients with CAT.

Materials and Methods

To find an answer to the raised issue, we formulated the following PICO question: population (patients with CAT), intervention (anticoagulant treatment), comparison (patients with CAT without metastasis or with metastasis in other locations), and outcomes (bleeding and recurrent VTE). We performed a search in the PubMed database on 24th September 2023. The following MESH terms were used to search original articles, reviews, and guidelines: Neoplasms[mesh] OR neoplas*[tiab] OR cancer[tiab] OR malign*[tiab] OR tumor [tiab] OR tumour [tiab]) AND Venous Thromboembolism[Mesh] OR venous thromboem*[tiab] OR Venous Thrombosis[Mesh] OR venous thrombosis[tiab] OR deep vein thrombosis[tiab] OR deep venous thrombosis OR Pulmonary Embolism[Mesh] OR pulmonary embolism[tiab])) AND (Metastasis[mesh] OR metasas*[tiab] OR mestastatic*[tiab] (Table 1 shows the search strategy). There were no restrictions for year of publication or language. The references of the different articles included were examined to identify other articles of interest.

Two reviewers (MBH and VGG) independently assessed the eligibility of studies using a data extraction form. Study selection was initially performed by review of titles, subsequently, selected abstracts and finally full texts were reviewed. Discrepancies between reviewers were resolved by a third reviewer (LJP).

Study selection

Inclusion criteria were: i) patients aged ≥18 years with CAT that received anticoagulant treatment; ii) information about location of metastasis; iii) availability of the number of patients who experienced complications (recurrent VTE or bleeding). Case reports and case series with <3 patients and studies not involving humans were excluded.

Study objective

The objective of this systematic review was to evaluate the risk of complications (recurrent VTE or bleeding) according to the location of metastasis in patients with CAT. Active cancer was defined as any cancer diagnosed within the previous six months, recurrent, regionally advanced or metastatic cancer, cancer for which treatment had been administered within six months, or hematological cancer that is not in complete remission.[13] VTE was accepted as any objectively confirmed symptomatic or incidental thrombosis event, except superficial vein thrombosis. Complications were defined such as recurrent VTE or bleeding. Recurrent VTE was defined as objectively confirmed symptomatic or incidental VTE by imaging with evidence of thrombus progression or involvement of the thrombus in another region. Bleeding was evaluated according to the criteria of the International Society on Thrombosis and Hemostasis.[14]

Results

The PubMed database and manual search of references in included articles provided a total of 1,447 articles. After a review of titles, 1,396 articles were excluded because they did not meet the inclusion criteria. After reviewing 51 abstracts, 24 possible valid articles were identified. Finally, after a full-text assessment,

Table 1. Search strategy.

1	Neoplasms[mesh] OR neoplas*[tiab] OR cancer[tiab] OR malign*[tiab] OR tumor[tiab] OR tumour[tiab] (n=5,036,885)
2	Venous thromboembolism[Mesh] OR venous thromboem*[tiab] OR venous thrombosis[Mesh] OR venous thrombosis[tiab] OR deep vein thrombosis[tiab] OR deep venous thrombosis OR pulmonary embolism[Mesh] OR pulmonary embolism[tiab] (n=159,865)
3	Metastasis[mesh] OR metasas*[tiab] OR mestastatic*[tiab] (n=222,545)
4	"Infant"[Mesh] OR "infant"[MeSH Terms:noexp] OR "child"[MeSH Terms] OR "child"[MeSH Terms:noexp] OR infant, newborn[Mesh] OR child, preschool[Mesh] (n=2,806,435)
5	Animals[mh] NOT humans[mh] (n=5,174, 295)
6	4 OR 5
7	1 AND 2 AND 3 NOT 6 (n=1,440)

7 articles were included in the systematic review. All of them were retrospective studies. Figure 1 shows the study flow diagram. The characteristics of the studies included are specified in Table 2.

Schiff *et al*. observed that 7% of 51 patients with brain metastases and VTE developed symptomatic intracranial hemorrhage (ICH) and 7% an asymptomatic ICH.[15] Alvarado *et al*. found that the incidence of ICH in a cohort of 74 patients with melanoma with brain metastases and VTE who received anticoagulant treatment was 4%.[16] Moreover, they observed that the number of brain metastasis correlated with survival from VTE among the patients receiving systemic anticoagulation (2.6 months for 1-4 brain metastasis *vs*. 5.9 months for >4 brain metastases, P<0.0001).[16] In 2015, Donato *et al.*, matched 293 patients with VTE and brain metastasis (104 with enoxaparin at therapeutic doses and 189 controls) in a retrospective study.[17] The cumulative incidence of ICH at 1 year was 19% in the enoxaparin cohort and 21% in the control cohort (HR 1.02, 90% CI 0.66-1.59).[17] They concluded that patients with brain metastasis had a high incidence of spontaneous intracranial bleeding, and that this risk was not increased by anticoagulation. The risk for ICH was four-fold higher in patients with melanoma or renal cell carcinoma compared with those with lung cancer (adjusted HR 3.98, 90% CI 2.41-6.57; P<0.001).[17] In 2017, Chai-Adisaksopha *et al*. performed a retrospective study matching patients with primary brain tumor versus brain metastasis on low-molecular-weight heparin (LMWH) treatment for more than four weeks for VTE. The incidence rate of recurrent VTE was similar in both groups (11.0% in patients with brain tumors and 13.5% in brain metastasis). However, the incidence of major bleeding was 8.6% (95% CI, 4.8-14.7) in patients with primary brain tumor and 5.0% (95% CI 2.8-9.2) in patients with brain metastasis. In addition, rate of ICH was higher in brain tumor patients (4.4% *vs.* 0%, P=0.004).[18] In 2019, a retrospective study analyzed, in patients with brain tumors and venous thromboembolism (n=105), the cumulative incidence of ICH with direct oral anticoagulants (DOACs) compared with LMWH. Compared with LMWH, DOACs did not increase the risk of any ICH.[19] A retrospective study of 96 patients evaluated the incidence of ICH in patients with brain metastasis receiving DOACs (n=41) or LMWH (n=55) for VTE or atrial fibrillation, showed that the 12-month cumulative incidence of ICH in the DOAC group was 10.1% compared with 12.9% in LMWH group (HR: 0.77, 95% CI, 0.23-2.59).[20] Likewise, Lee *et al*. evaluated, in a retrospective study, the safety and efficacy of DOACs and LMWH for CAT in patients with primary brain tumor or brain metastasis.[21] In the brain metastasis cohort (n=85), the incidence of recurrent VTE events was 4.9% in DOAC group and 4.5% in LMWH group. However, the incidence of ICH was 4.9% and 2.3% with DOACs and LMWH, respectively.[21]

Discussion and future research

This systematic review shows that patients with brain metastasis and VTE present a high incidence of ICH during anticoagulant treatment. However, these studies did not evaluate the risk of ICH in patients with brain metastasis. Hunter *et al*. in a meta-analysis that included 4 of the 7 previously discussed studies, showed that there was no higher risk of ICH under anticoagula-

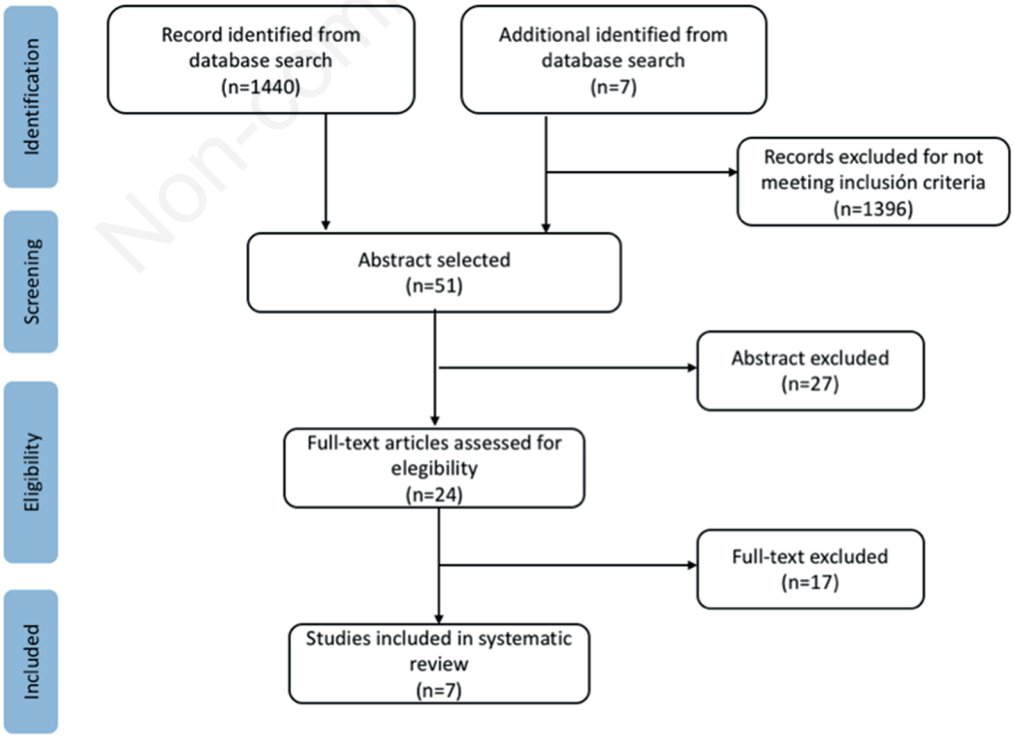

Figure 1. Flow diagram.

tion in patients with brain metastasis and VTE (OR 1.37, 95% CI, 0.86-2.1; P=0.18).[22] The meta-analysis by Zwicker *et al*. that evaluated whether therapeutic anticoagulation is associated with an increased risk of ICH in patients with brain tumors, observed that there was no statistically increased risk of ICH in patients with brain metastasis treated with anticoagulation compared with no anticoagulation (OR 1.07, 95% CI 0.61-1.88; P=0.81; I^2=0%).[23] A recent meta-analysis that evaluated ICH in primary or metastatic brain cancer patients with or without anticoagulant treatment, demonstrated that the risk of ICH was significantly higher in patients with metastatic brain cancer than in patients with primary brain cancer (RR 3.26, 95% CI 2.69-3.94; I^2=92.8%), although we need to be cautious with these findings due to quality of the studies and high heterogeneity.[24] On the other hand, in patients with metastatic brain cancer, anticoagulant therapy was not associated with an increased rate of ICH (RR 0.86, 95% CI 0.45-1.65; P=0.287).[24]

The presence of metastasis has been described as a variable associated with bleeding and recurrent VTE in patients with CAT. Cohen *et al*. provided a model to predict the risk of bleeding in patients with CAT and observed that the presence of metastasis was an independent predictor of significant bleeding.[25] Furthermore, an analysis of RIETE registry that included 2,945 patients with CAT in whom 1.0% had fatal bleeding during the first 3 months of anticoagulation therapy, showed that the presence of metastasis was an independent risk factor for fatal bleeding (OR 3.1, 95% CI 1.4-7.1; P=0.006).[11] Moreover, metastatic cancer was independently associated with an increased risk for fatal PE (OR 2.9; 95% CI 1.8-4.8; P<0.001).[11] On the other hand, a retrospective study that evaluated the risk factors of recurrent VTE after discontinuation of anticoagulation in 311 patients with CAT, found that the presence of metastasis was associated with a higher risk of recurrent VTE (sub-distribution hazard ratio: 3.8, 95% CI 1.54-

Table 2. Main clinical features of the studies included.

Author (year)	Study design	Sample size BM	Outcome	Complications	RR to complications (95% CI)
Schiff (1994)[17]	R	51	The efficacy and complications of IVC filters and anticoagulation in patients with brain metastases and VTE	IVC filter, ICH: 40% Anticoagulation: - Symptomatic ICH: 7% - Asymptomatic ICH: 7% - Global bleeding: 19%	NR
Alvarado (2012)[18]	R	74	The relative risk and benefits of systemic anticoagulation in patients with brain metastasis from melanoma and VTE	ICH: 4%	NR
Donato (2015)[19]	R	293	The risk for ICH associated with the administration of therapeutic doses of LMWH in patients with brain metastases	Cumulative incidence of ICH at 1 year: 19% LMWH	NR
Chai-Adisaksopha (2017)[20]	R	115	The effectiveness and safety of extended duration LMWH in adult patients with primary brain tumor *vs* metastatic intracranial tumors	- ICH=4.5% - Major bleeding: 8.6% (4.8-14.7%) - Clinically relevant bleeding: 12.4% (7.8-19.7%) - Recurrent VTE: 11% (6.7-17.9%)	NR
Carney (2019)[21]	R	105	The cumulative incidence of ICH in DOACs compared with LMWH in patients with brain metastases and VTE	12-month cumulative incidence ICH: - DOAC group: 27.8% - LMWH: 52.9%	NR
Leader (2020)[22]	R	96	The incidence of ICH in patients with brain metastases receiving DOACs (n=41) or LMWH (n=55) for VTE or AF	12-month cumulative incidence ICH: - DOAC group: 10.1% - LMWH: 12.9%	NR
Lee (2021)[23]	R	85	The safety and efficacy of DOACs in comparison with LMWH for cancer-associated VTE in patients with primary brain tumor or brain metastases	ICH: - DOAC group: 4.9% - LMWH: 2.3% Systemic bleeding: - DOAC group: 17.1% - LMWH: 20.4% Recurrent VTE: - DOAC group: 4.9% - LMWH: 4.5%	NR

RR, relative risk; CI, confidence interval; IVC, inferior vena cava; VTE, venous thromboembolism; ICH, intracranial hemorrhage; LMWH, low-molecular-weight heparin; DOACs, direct oral anticoagulants; AF, atrial fibrillation.

9.52; P=0.0039).[26] Likewise, a systematic review of 10 studies (6 observational and 4 retrospectives) that included 4,791 patients with CAT found a significantly higher risk of VTE recurrence in patients with metastasis (RR 1.4, 95% CI 1.1-1.7; P=0.01).[8]

This work has several limitations. First, the search was performed in a single database which increases the risk of excluding relevant studies that may be indexed in other databases. This may introduce a selection bias in the review process. However, previous studies conclude that searching only one database can be sufficient as searching other databases has no effect on the outcome.[27,28] Second, publication bias was not analyzed but we only found publications related to brain metastases and not in other locations, so we were unable to conduct such an analysis in our study. Third, all available studies were observational and retrospective. In addition, the sample sizes of these studies were limited, and may not be representative of the population. Finally, all included studies that collected information about metastasis location were focused on brain metastasis, without considering other locations.

Interestingly, there are previous studies that have evaluated the risk of VTE in cancer patients according to the location of the metastasis. In a cross-sectional analysis of data from the Nationwide Inpatients Sample database that included 850,570 patients with metastatic cancer, 6.6% developed a VTE.[29] Patients with metastasis to adrenal glands, liver, brain, lung and bone had an increased risk of developing VTE, while those with metastasis to genital organs and lymph nodes had a lower risk.[29] In addition, patients with multiple metastasis (≥2 locations) had a higher risk of VTE compared with patients with single metastasis (OR 1.09, 95% CI 1.05-1.13; P=0.001).[29] Nevertheless, these findings contrast with those observed in other works. Conteduca *et al*. performed a prospective biomarker analysis to evaluate the association between plasma tumor DNA fraction and risk of VTE in 180 patients with metastatic castration-resistant prostate cancer (mCRPC) and observed a cumulative incidence at 12 months of VTE of 17.1% (95% CI 10.3-23.9).[30] In the multivariable analysis, the presence of metastases in the liver (HR 2.22, 95% CI 0.25-19.28; P=0.470) and in the lung (HR 2.57, 95% CI 0.70-9.42; P=0.153) and number of metastasis (>7) (HR 0.73, 95% CI 0.24-2.22; P=0.584) were not associated with an increased risk of VTE.[30] However, the sample size of the study was small, and it included a group of highly selected patients that may not be representative of real clinical practice. Therefore, future studies are needed to evaluate the impact of metastasis location on the risk of VTE.

Conclusions

This systematic review highlights the lack of evidence on the role of metastasis location in complications in patients with CAT. Recent studies postulate that patients with brain metastases appear to have an elevated risk of ICH. Our work has identified a gap for future studies to analyze the risk of complications of VTE according to the location of the metastasis in patients with CAT. Future studies are needed to evaluate the impact of the location of metastasis on the risk of complications of VTE in patients with CAT. It may be relevant for the management of anticoagulant treatment in these patients.

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Venous thromboembolism and mortality in patients with hematological malignancies

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ABSTRACT

Mortality and venous thromboembolism (VTE) are major risks for patients with hematological malignancies. However, they are commonly underrepresented in major clinical trials of VTE. Treatment decisions are further complicated by the unique characteristics they frequently exhibit, such as thrombocytopenia. In addition to discussing treatment challenges, knowledge gaps, and future directions, our goal in this narrative review is to provide an overview of the epidemiology and risk factors of mortality in patients with hematological malignancies and VTE.

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Introduction

Malignancy is a known strong risk factor for venous thromboembolism (VTE). Hematological malignancies (HM) include a variety of distinct types, such as acute or chronic leukemia, lymphoma, multiple myeloma (MM), and myeloproliferative neoplasms (MPNs). HM frequently carry special characteristics such as thrombocytopenia that can complicate treatment decisions of VTE and increase rates of treatment-related adverse events (*e.g.*, anticoagulant-related major bleeding).

With advances in anticancer and supportive care therapies, survival in patients with HM is rising.[1,2] In some HM (*e.g.*, lymphoma), non-cancer causes of death are surpassing those of cancer-related deaths.[3] Of secondary causes of death in cancer patients in one study, thromboembolism was noted to be the leading cause accounting for 9.2% of all deaths.[4] Thus, the interplay between VTE and mortality in this high-risk population is worth exploring. In this narrative review as an accompanying paper of the 12th International Conference on Thrombosis and Hemostasis Issues in Cancer Congress 2024, we intend to summarize some of the available literature on VTE and mortality in patients with hematological malignancy, highlight the knowledge gaps and future research directions. As MPNs have distinct characteristics including thromboembolism as a hallmark of the disease, directly related to the associated pathological mutation (*i.e.*, JAK2 V617F mutation), which affects disease management strategies and likely mortality, we elected to exclude MPNs in the current review as it deserves its own discussion.

Mortality and venous thromboembolism in hematological malignancies

Numerous studies have demonstrated an increased risk of mortality in patients with cancer and VTE *vs*. those with cancer without VTE. The association with mortality is higher when VTE is diagnosed concurrently with cancer diagnosis (as compared to VTE diagnosed after cancer). In a population cohort study using the Danish healthcare registry, the 1-year mortality rate was high

at 68% in patients with VTE diagnosed at the time of cancer diagnosis, 4-fold higher compared to a matched cohort of newly diagnosed cancer without VTE [38%, mortality rate ratio (MRR) 4.34, 95% confidence interval (CI): 3.95-4.78].[5] VTE diagnosed after cancer was also associated with a 3-fold higher risk of mortality (45% *vs*. 17%, MRR 3.48, 95% CI: 3.37-3.60).

Whilst much data shows the risk of death is higher in patients with cancer and VTE, the direct causation of VTE with mortality in these patients remains debated. Historically, progression of the underlying malignancy has been demonstrated to be the leading cause of death in patients with cancer. In a prospective, observational study by Khorana *et al.*, progression of the underlying cancer was the leading cause of death (70.9%) in ambulatory cancer patients receiving chemotherapy, followed by thromboembolism (9.2% total; arterial 5.6%, venous 3.5%) and infection (9.2%).[4] A more recent systematic review and meta-analysis including data from 29 studies (N=8000 patients) showed a combined all-cause mortality of 28.8%, with cancer progression accounting for 82.9% of all deaths.[6] With death from cancer as the leading cause of death for most cancers, VTE occurrence may be a surrogate for more aggressive cancer and the mortality may be related to such.

A higher association between aggressive cancer histology and VTE has been demonstrated and is significant in patients with HM. In a recent cohort study of more than 400,000 United States (US) veterans diagnosed with cancer between 2006 and 2021, 10% of the cohort (n=40,010) had a diagnosis of HM.[7] The cancer type with the highest 12-month cumulative incidence of VTE in the cohort was acute lymphoblastic leukemia (ALL) (18.6%). Overall, the aggressive-type HM had higher 12-month cumulative incidences of VTE [*i.e.*, ALL 18.6%, aggressive non-Hodgkin's lymphoma (NHL) 11%, Hodgkin's lymphoma (HL) 9.5%, MM 7.7%, and acute myelogenous leukemia (AML) 7.3%] when compared to more indolent HM types [*i.e.*, indolent NHL 4.5%, myelodysplastic syndrome (MDS) 2.7%, chronic myelogenous leukemia (CML) 2.1%, and chronic lymphocytic leukemia (CLL) 2.0%]. In multivariable analysis adjusting for baseline demographics, VTE risk factors, cancer type (reference = prostate cancer), anticoagulant or antiplatelet therapy, cancer stage, and cancer therapy, the hazard of VTE remained increased for the aggressive HM: ALL [hazard ratio (HR) 4.98, 95% CI: 3.71-6.68], aggressive NHL (HR 2.65, 95% CI: 2.43-2.89), AML (HR 2.10, 95% CI: 1.82-2.41), HL (HR 2.00, 95% CI: 1.68-2.38), MM (HR 1.72, 95% CI: 1.57-1.87) and was lower for more indolent HM: indolent NHL (HR 1.38, 95% CI: 1.26-1.51). Patients with CLL, MDS, and CML had a reduced hazard of VTE (compared to prostate cancer), with HR of 0.77, 0.76, and 0.57, respectively. These findings provide support for the relation between aggressive HM histology and VTE with mortality and call for continued attention to thromboembolism in HM and understanding of the role of VTE in death in this patient population.

In the aforementioned Danish cohort study, less than 10% of patients included had a diagnosis of HM; however, when focusing on patients with HM, the 1-year MRR increased beyond that of the full cohort (MRR 11.37-38.02 in patients with lymphoma and leukemia who presented with VTE concurrently with cancer diagnosis compared to 4.34 in the cohort at large).[5] Given the higher rate of mortality in patients with HM and VTE and the association of aggressive HM histology with VTE, histology-specific studies are needed to determine the exact role of VTE in mortality in these patients. We highlighted the pertinent data specific to cancer types below focusing on multiple myeloma, lymphoma, and acute leukemia given the higher incidence of VTE in these HMs (Table 1). Given the lower risk of VTE in some HM (*e.g.*, CLL, MDS, and CML), there is a paucity of data on the associated morbidity and mortality and future studies are needed. Finally, as monoclonal gammopathy of undetermined significance is a precursor state, it was not included in this narrative review.

Table 1. Incidence of venous thromboembolism and mortality in patients with hematologic malignancy and venous thromboembolism.

Type of malignancy/reference	12-month Cumulative incidence of VTE after cancer diagnosis, N, % (95% CI) 12-month		Hazard/risk of death in patients with VTE *vs.* no VTE, risk, (95% CI)
Leukemia*[45]	N=14,841	1.7 (1.5-2.0)	aHR 1.41 (1.01, 1.98)*
ALL[7,17]	N=193	18.6 (NR)	aHR 0.87 (0.83-0.91)*
AML[7,16,17]	N=2,657	7.3 (NR)	aHR 1.42 (1.13, 1.80)*
CLL[7]	N=6,486	2.0 (NR)	
CML[7]	N=3,840	2.1 (NR)	
HL[7,45]	N=1,129	9.5 (NR)	
	N=2,459	3.8 (3.1-4.6)	
NHL[12,45]	N=18,473	3.2 (3.0-3.5)	
Indolent NHL[7]	N=8,987	4.5 (NR)	HR 1.7 (1.5-1.9)#
Aggressive NHL[7]	N=5,351	11.0 (NR)	
MDS[7]	N=4,897	2.7 (NR)	
MM[7,45]	N=6,470	7.7 (NR)	aHR 1.66 (1.19-2.33)^
	N=6,693	3.8 (3.4-4.3)	aHR 2.9 (2.4-3.5)^
			HR 2.27 (1.26-4.08)^

*Not specified; #6-month risk; ^12-month risk. VTE, venous thromboembolism; CI, confidence interval; aHR, adjusted hazard ratio; HR, hazard ratio; NR, not reported; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphoblastic lymphoma; CML, chronic myeloid leukemia; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma.

Multiple myeloma

The available data on patients with MM is controversial. In a large retrospective cohort study of US veterans with newly diagnosed MM between 2006 and 2014, diagnosis of VTE was associated with a 2-fold increased risk of death at 6-months [adjusted hazard ratio (aHR) 2.31, 95% CI: 1.52-3.51] with risk persisting at 12-months (aHR 1.66, 95% CI: 1.19-2.33).[8] Findings were similar in the Swedish Cancer Registry where patients with MM and VTE had a 3-fold increase in risk of death at 12 months compared to those without VTE (aHR 2.9, 95% CI: 2.4-3.5).[9] However, for patients eligible for and treated in the randomized control (RCT) myeloma IX and XI trials, there was no association between the development of VTE and the risk of death.[10] It is unclear if this discrepancy between real-world data and that of the RCT is related to additional patient- or disease-related factors (*e.g.*, medical comorbidities, therapeutic intensity, *etc.*), or follow-up durations. In a systematic review and meta-analysis which included these 3 studies in addition to 6 others for a total of 38,047 patients, VTE was associated with a 2-fold increased risk of early mortality (HR 2.27, 95% CI: 1.26-4.08) in 3 studies and decreased overall survival in 3 studies (HR 0.79, 95% CI: 0.64-0.98) (a fourth study was excluded for heterogeneity).[11]

Lymphoma

In a retrospective study of 16,755 patients with NHL, acute VTE was associated with a 70% increase in the risk of death at 6 months (HR 1.7, 95% CI: 1.5-1.9) after adjusting for stage, comorbidities, age, and histopathology (*i.e.*, intermediate- or aggressive lymphoma).[12] Similarly, in HL, the presence of VTE was associated with increased mortality (32.3% *vs*. 5.9%, P<0.001); however, on examination of the causation of death in patients with VTE, all deaths were attributed to either infectious etiologies due to treatment or progression of the underlying lymphoma.[13] In primary central nervous system lymphoma, the 12-month cumulative incidence of VTE and major bleeding were high, 13.6% and 12.4%, respectively.[14] Pulmonary embolism (PE) [+/- deep vein thrombosis (DVT)] and major bleeding were both associated with significantly increased risks of mortality (HR 1.61 and HR 2.36, respectively).

Acute leukemia

Most studies focused on acute leukemia did not find an increased risk of mortality in patients with VTE and acute leukemia compared to those without VTE. In a cohort of 1088 patients with ALL from SEER-Medicare data, risk factors for VTE included kidney disease, heart failure, use of central venous catheters, and chemotherapy. Diagnosis of VTE was not associated with an increased hazard of death in the overall cohort, or when separating patients by ALL treatment status. Conversely, VTE appeared protective against the hazard of death in patients with ALL on chemotherapy-sparing treatment (*i.e.*, tyrosine kinase inhibitors) in adjusted analysis.[15] Findings for patients with AML within the SEER-Medicare data were similar to those with ALL with no increase in the hazard of death in patients with VTE. In adjusted analysis that accounted for AML treatment, VTE was associated with a 13% decreased risk of all-cause mortality.[16] The high mortality rate from acute leukemia itself could contribute to these findings (*i.e.*, the competing risk of death from acute leukemia prior to the development of VTE). However, another population-based cohort study that focused on VTE in acute leukemia did find an association between VTE and an increased hazard of mortality.[17] In this study, the outcome of interest focused on leukemia-specific mortality while censoring patients at the time of death for non-leukemia-related causes of death. In the adjusted analysis for AML (N=3252), both upper extremity (UE) DVT and PE +/- lower extremity DVT were associated with an increased risk of AML-specific death (aHR 1.42 for both, P 0.001 and P 0.003, respectively). Similar results were noted in the cohort of patients with ALL (N=1820). UE DVT was associated with an 80% increase in risk of ALL-specific death (aHR 1.80, P=0.0003), and PE +/- lower extremity DVT was associated with a 41% increase in risk of ALL-specific death (aHR 1.41, P=0.04).

Mortality associated with non-cancer causes

While available histology-specific studies provide mixed results, contemporary results may be changing. Some data shows that as survival for cancer increases, secondary causes of death (*i.e.*, non-cancer-related deaths) are rising and may replace cancer as the leading cause of death. In a study assessing trends in death from index cancer *vs*. secondary causes for patients diagnosed with cancer between 1973 to 2012, patients with lymphoma (NHL or HL) had significant declines in death from their index cancer.[3] Death due to index cancer decreased from >60% at study start to ≤40% in 2012. Accordingly, deaths from secondary causes outnumbered deaths from index cancer by 2012. Declines in death from MM were also noted in the study, however, death from MM remained the leading cause of death in these patients. In a similar study assessing causes of death in a combined cohort of solid and hematological cancers over a 40-year period, secondary (non-cancer) causes of death increased from 20% for persons diagnosed with cancer between 1975 and 1979 to 26% for those diagnosed between 2010 and 2014.[18] Of the secondary causes of death, "diseases of heart" was most common, with this category including PE. Ongoing research is needed to determine the contemporary mortality burden of VTE and VTE treatment in patients with HM.

Mortality associated with types of venous thromboembolism

Many studies assessing the association between VTE and mortality in HM combine VTE subtypes into a single category including all events within the definition of VTE (*e.g.*, UE DVT, lower extremity DVT, and PE), with some studies also including splanchnic vein thromboses in the definition of VTE. However, the risk of death with location of VTE may differ, especially for UE DVT where the risk of PE is known to be less than that of lower extremity DVT.[19] When focusing on VTE subtypes in the Danish cohort, patients diagnosed with PE had worse survival compared to those diagnosed with extremity DVT, with higher 1-year mortality rates and MRRs, regardless if PE was diagnosed concurrently with cancer or after.[5] For example, patients with PE diagnosed concurrently with cancer had a 1-year mortality rate as high as 73% (matched control 39.3%), as compared to 60.9% in those with DVT (matched control 37.3%). This indicates that at least in some patients, VTE (*i.e.*, PE) directly contributed to the poor outcome, which could be related to the PE itself, the need

for hospitalization for VTE and/or interruption of anticancer therapies, or complications related to treatment (*i.e.*, anticoagulant-related major bleeding). The high risk of death attributable to PE was demonstrated in the RIETE registry where PE-related death was a leading cause of 3-month mortality, second only to death from cancer itself in a cohort of 1,605 patients with cancer.[20]

Up to 50% of VTE events in patients with HM are due to UE thromboses, often due to the frequent use of central venous catheters in this patient population.[7] Despite the common occurrence, management of catheter-associated VTE in patients with cancer remains controversial as this patient population is largely excluded from the pivotal RCTs.[21] Therefore, high-quality evidence to guide the optimal management of catheter-related UE DVT, including the type and duration of anticoagulant therapy variable, is lacking.[22,23] Fortunately, as noted above, risks of PE are less with UE DVT (compared to lower extremity DVT) and mortality is rarely reported or noted to be directly related to catheter-related UE DVT alone. Accordingly, in the aforementioned study of UE DVT and acute leukemia, the presence of UE DVT (all patients were presumed to have central venous catheters) was associated with an increase in leukemia-specific mortality in both ALL (aHR 1.80; 95% CI: 1.31-2.47, P=0.0003) as well as AML (aHR 1.42; 95% CI: 1.16-1.73, P=0.001) patients after adjusting for the presence of confounders including PE.[17] However, the investigators were unable to quantify deaths directly attributable to VTE and/or VTE-directed treatment. In fact, the presence of a UE DVT was associated with an increased risk of subsequent bleeding for both patient populations ALL (aHR 1.62; 95% CI: 1.02-2.57, P=0.04) and AML (aHR 2.07; 95% CI: 1.60-2.68, P=<0.0001). Future well-designed studies are needed for catheter-related VTE therapy in patients with cancer.

Mortality associated with treatment of venous thromboembolism

Mortality during treatment of cancer-associated VTE can be related to recurrent VTE or anticoagulation-related bleeding. Patients with cancer have a 3- to 7-fold increased risk of recurrent VTE despite anticoagulant therapy compared to non-cancer counterparts.[24] In addition, cancer patients have a 2-fold increased risk of major hemorrhage on anticoagulant therapy compared to non-cancer patients.[25] Analysis of the RIETE registry showed that within the first 12 months of diagnosis of cancer-associated VTE, fatal PE was the second leading cause of death while major bleeding was the 4th most common.[26] In the CATCH trial, a RCT comparing low molecular weight heparin (LMWH) *vs*. vitamin K antagonist in patients with active cancer, most deaths were related to the progression of cancer (69%) as previously discussed.[27] However, of the remaining 31% causes of death, almost half were related to treatment of or recurrence of VTE (12.5% and 2.1% were caused by fatal recurrent PE and anticoagulant-related bleeding, respectively).[27] Similarly, a systemic review and meta-analysis of patients with cancer-associated VTE found that the case fatality rate of recurrent VTE in the oncology population was high at 14.8%, while the case fatality rate of anticoagulant-related major bleeding events was 8.9%.[6] Given the high case-fatality rates of recurrent VTE and anticoagulant-related bleeding, optimal management of VTE in cancer remains an active area of need.

While the focus of this review is not on thrombocytopenia in HM, this topic warrants discussion as thrombocytopenia is a major risk factor for anticoagulant-related bleeding (and thus, bleeding-related death) and may significantly contribute to mortality in patients with HM and VTE. Compared to solid tumors, the prevalence of thrombocytopenia in HM is also higher, with longer durations and severity of thrombocytopenia. This likely results from the disease-based marrow involvement and as toxicity from cancer-directed therapy (*e.g.*, high-dose chemotherapy). In a retrospective cohort study of 3,549 patients with active cancer and newly diagnosed VTE, thrombocytopenia (defined as platelet count $<100´10^9$/L) was present in 47% of patients with HM (n=647), as compared to 22% of patients with solid cancers (n=2,902).[28] Furthermore, 30% of patients with HM had a platelet count $<50´10^9$/L compared to only 7% of those with solid cancers. The presence of thrombocytopenia complicates VTE treatment decisions. In a prospective observational study (TROVE study) of 121 patients with active cancer with newly diagnosed VTE and thrombocytopenia (platelet count $<100´10^9$/L), 70% of enrolled patients had an underlying HM.[29] Patients treated with full-dose anticoagulant therapy had a higher risk of major bleed compared to those treated with modified-dose anticoagulant therapy (12.8% *vs*. 6.6%, Fine and Gray HR 2.18, 95% CI: 1.21-3.93), including one fatal hemorrhagic event *vs*. zero fatal events, respectively. Another prospective cohort study (CAVEaT study) included 105 patients with HM, new VTE, and platelet count $<50´10^9$/L).[30] Within 28 days, the mortality rate was high at 15%, while 8% of patients experienced VTE recurrence or progression, and 7% had major bleeding.

These studies provide a rationale for high-quality data from prospective RCTs to provide better guidance for the treatment of cancer-associated VTE in the setting of concurrent thrombocytopenia. A pilot RCT – START (STrategies for Anticoagulation in patients with thRombocytopenia and cancer-associated Thrombosis) (NCT05255003) – is currently underway to evaluate the feasibility of conducting such a trial, as well as the efficacy and safety of different management strategies in this setting. Based on the limited evidence currently available, the International Society on Thrombosis and Haemostasis and the European Hematology Association proposed guidance for the management of VTE in patients with cancer and thrombocytopenia, including those with HM (Figure 1).[31,32] In general, full-dose LMWH is recommended for patients with platelet counts of $\geq$40-$50´10^9$/L while anticoagulation dose modifications are recommended for platelet counts of 25 to 40-$50´10^9$/L, depending on the acuity and severity of VTE. The adoption of these or similar treatment strategies within clinical practice has resulted in variable VTE-related outcomes.[33]

With the case fatality rate of recurrent VTE being higher than that of anticoagulant-related bleeding in patients with cancer,[6] optimal anticoagulant strategies have been studied in large RCTs. To date, six randomized studies have been published assessing the treatment of cancer-associated VTE with direct oral anticoagulation (DOAC) *vs*. LMWH.[34-39] Meta-analysis of these studies found that treatment of cancer-associated VTE with DOAC is associated with a decrease in risk of recurrent VTE [risk ratio (RR) 0.67, 95% CI: 0.52-0.85].[40] However, the use of DOAC is associated with a significant increase in the risk of clinically relevant non-major bleeding (RR 1.66, 95% CI: 1.31-2.09) and a trend towards increased risk of major bleeding (RR 1.17, 95% CI: 0.82-1.67). While findings from these studies guide current practice, insight into the significance for patients with HM is lacking. Of the 3,703

patients enrolled in these trials, less than 10% (n=315, 8.5%) had a HM as their qualifying cancer. In addition, most of the trials did not report HM-specific outcomes (Table 2). Accordingly, treatment of cancer-associated VTE in HM relies on extrapolating findings from these trials that were predominately focused on patients with solid tumors.[41-43] Given the sparse data and unique treatment considerations (*i.e.*, thrombocytopenia) in patients with HM, and the potential implications on mortality, studies are urgently needed to improve outcomes and reduce VTE-associated mortality in this growing patient population.

Table 2. Representation of hematologic malignancies in randomized control trials comparing direct oral anticoagulants to low molecular weight heparin for acute venous thromboembolism in cancer (Table modified from Wang *et al.*, 2022).[44]

Randomized trials	Hokusai VTE cancer*	Select-D	ADAM VTE	Caravaggio	CASTA-DIVA	CANVAS
N, total participants	1046	406	300	1155	158	638^
N, HM (%)	111 (10.6%)	31 (7.6%)	28 (9.3%)	85 (7.4%)	13 (8.2%)	47 (7.4%)
Recurrent VTE (DOAC *vs.* LMWH)	2/56 (3.6%) *vs.* 4/55 (7.2%) Risk difference: 3.7%, 95% CI: -13.9% to 6.5%	N/A	NR	2/33 (6.1%) *vs.* 2/52 (3.8%)	NR	NR/24 *vs.* NR/23 Risk difference: -4.3%, 95% CI: -12.6% to 4.0%
Major bleeding (DOAC *vs.* LMWH)	1/56 (1.8%) *vs.* 2/55 (3.6%) Risk difference: 1.9%,95% CI: -9.7% to 6.0%	0/14 *vs.* 0/17	NR	0/33 *vs.* 0/52	NR	NR
CRNMB (DOAC *vs.* LMWH)	NR	2/14 (14.3%) *vs.* 0/17	NR	NR	NR	NR

*Hokusai reported on 12 months follow-up while the remaining studies report on 6 months follow-up; ^Randomized cohort data presented. VTE, venous thromboembolism; HM, hematological malignancies; DOAC, direct oral anticoagulants; CI, confidence interval; LMWH, low molecular weight heparin; NR: not reported; CRNMB, clinically relevant non-major bleeding.

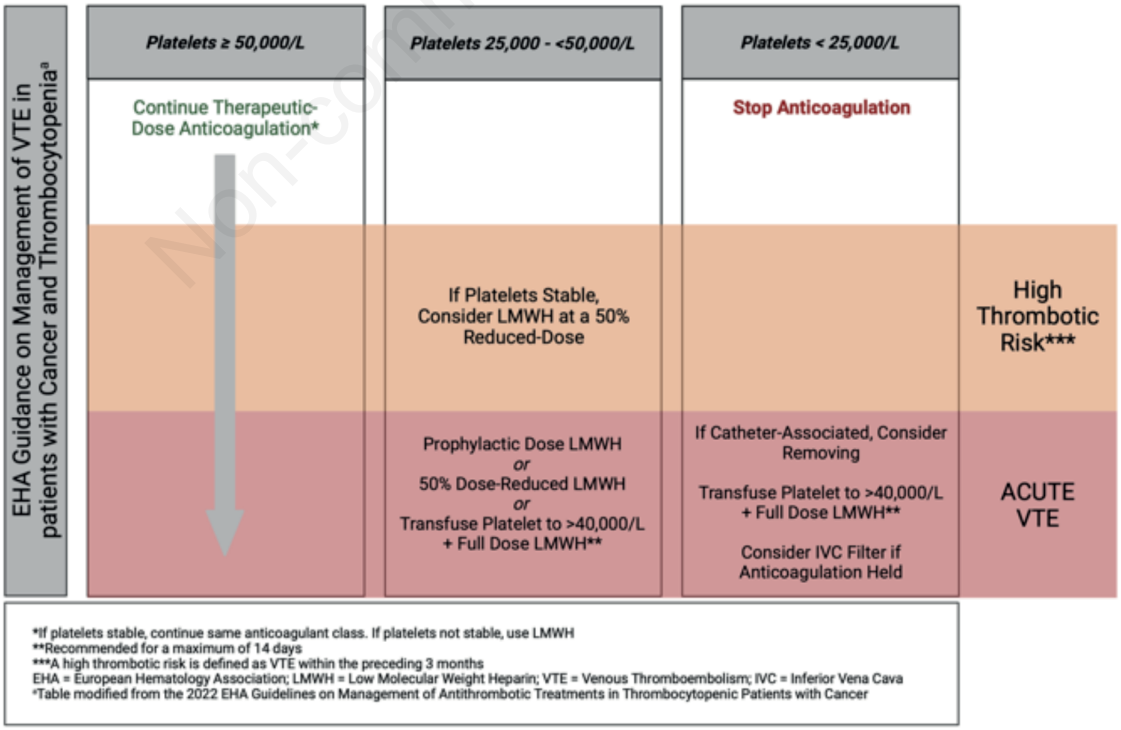

Figure 1. European Hematology Association guidance on management of venous thromboembolism in patients with cancer and thrombocytopenia.[31] Created with BioRender.com.

Conclusions

Patients with HM have unique characteristics and challenges that can contribute to their risks of VTE, VTE-related treatment complications (*i.e.*, recurrent cancer-associated VTE and anticoagulant-related bleeding), and mortality. However, these patients are commonly under-represented in cohort studies and clinical trials assessing outcomes. Future studies focusing on this high-risk population are needed.

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Cancer complicated by thrombosis and thrombocytopenia: still a therapeutic dilemma

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ABSTRACT

Individuals who have thrombocytopenia and cancer-associated thrombosis (CAT) are difficult to manage because they have a high risk of bleeding and recurrent thrombosis. The International Society on Thrombosis and Haemostasis guidelines for the management of thrombocytopenia in patients with CAT suggest two main approaches: either complete anticoagulation with transfusion support if necessary, or dose-modified anticoagulation while the platelet count is $<50\times10^9$/L. Nevertheless, rather than being based on information from randomized controlled trials (RCTs), these recommendations were based on expert consensus. Recent research from two different countries has shown how this cohort's management and results vary widely. While the United Kingdom study, Cancer-Associated Venous Thrombosis and Thrombocytopenia, found no significant differences in bleeding or recurrent thrombosis between full dose and modified dose groups, the North American Thrombocytopenia Related Outcomes with Venous thromboembolism study demonstrated a significantly lower risk of bleeding events in those receiving modified dose anticoagulation compared to full dose, without an increased risk of recurrent VTE. Therefore, an RCT is required to assess the best course of action for patients with CAT and thrombocytopenia. To define the standard of care for the management of patients with CAT and thrombocytopenia, a full-scale trial called the START randomized trial (STrategies for Anticoagulation in patients with thRombocytopenia and cancer-associated Thrombosis) is an international, multi-site pilot study that compares the use of platelet transfusions plus higher dose anticoagulation to modified dose anticoagulation in patients with thrombocytopenia and CAT receiving anticoagulation.

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Introduction

Healthcare professionals are commonly faced with the dilemma of managing patients with cancer complicated by both thrombocytopenia and thrombosis. Treatment decisions need to balance the risks of bleeding and the extension of thrombosis. Thrombocytopenia is common in patients with cancer and may be multifactorial, with contributing systemic chemotherapy, malignant bone marrow infiltration, or infection.[1] Whilst thrombocytopenia may increase the risk of bleeding, it confers no protection against thrombosis recurrence in patients with cancer-associated thrombosis (CAT).[2,3] This adds an extra layer of complexity to an already difficult balance between the competing risks of bleeding and thrombotic complications.

How common is cancer-associated thrombosis in thrombocytopenic patients with solid and hematological malignancies?

The estimated lifetime risk of developing cancer in the United Kingdom (UK) is 50% and cancer is a significant risk factor for venous thromboembolism (VTE).[4] The risk of VTE is 7-11-fold higher in patients with cancer compared to those without cancer,[5] with the risk rising to 23-fold if receiving chemotherapy or immunotherapy.[6] The incidence of CAT is increasing, likely due to thrombotic risks observed with some newer therapeutic agents, patients living longer with cancer due to advancement in therapies,[6] as well as increased vigilance of CAT over the last 20 years.[5]

CAT is the second leading cause of mortality in patients with cancer.[7] Thrombocytopenia (platelet count 100×10^9/L or less) is present in approximately 1 in 2 patients with CAT and hematological malignancies and 1 in 5 patients with CAT and solid tumors.[8]

Amongst patients with hematological malignancies, cohorts that are particularly at risk of thrombosis include patients with: myeloma and on immunomodulatory drugs;[9] acute lymphoblastic leukemia receiving L-asparaginase; acute promyelocytic leukemia, who are prone to thrombotic as well as bleeding complications due to disseminated intravascular coagulopathy;[10,11] and patients who have undergone hematopoietic stem cell transplant.[12]

Risk factors for thrombosis in ambulatory patients with solid organ tumors receiving chemotherapy include cancer type and treatment-related factors. Although risk prediction scores have been validated to predict patients at higher risk of CAT,[13] most patients who developed CAT were not identified as high risk by current risk assessment models.[14] It should also be noted that the majority of patients included in the development of the risk scores had solid tumors rather than hematological malignancies.[11]

What are the consequences of cancer-associated thrombosis in thrombocytopenic patients with cancer?

A recent systematic review and meta-analysis found high risks of both recurrent VTE (2-4%/100 patient months) and bleeding (major bleeding: 2-4%/100 patient months, total bleeding: 3-13%/100 patient months) in patients with CAT and thrombocytopenia (platelet count $<100\times10^9$/L), regardless of the anticoagulation management strategies.[15] This adds to the findings of a previous systematic review in 2018, which found that 27% of patients with CAT experienced recurrent VTE regardless of their management, whilst 13% of anticoagulated patients developed major bleeding.[3] In addition, CAT has a significant impact, including increased morbidity, reduced quality of life, interruptions in cancer treatment, significant healthcare system costs, as well as a 3-fold reduction in one-year survival rate compared to cancer patients without VTE.[6,16,17]

Overview of current management of cancer-associated thrombosis in thrombocytopenic patients

The optimal management options need to consider the competing risks of bleeding and thrombosis extension. Therefore, one common practice has seen a dual approach of raising platelet count by platelet transfusion and treating with anticoagulants,[18] based on the unproven assumption that anticoagulation would be safer above a certain platelet threshold. However, here is where the uncertainties start, with no randomized controlled trials (RCTs) guiding the target platelet count, dose of platelet transfusion, or frequency of monitoring and dose of anticoagulation.

Both platelet transfusions and anticoagulants have inherent risks. Platelet transfusions have risks common to all biological agents and blood components and have been implicated in bacterial and viral transfusion-transmitted infections, transfusion-related acute lung injury, allergic reactions, and febrile non-hemolytic transfusion reactions.[19] These risks of platelet transfusion have been reinforced by findings of recent randomized trials comparing more liberal and restrictive policies for platelet transfusion. For example, several RCTs have reported evidence of additional harm in patient cohorts needing platelet transfusions, including neonates with thrombocytopenia and patients presenting with acute hemorrhagic strokes associated with antiplatelet medications.[20,21] It is likely that these risks reflect the immunological effects of platelets, which have *in vivo* actions beyond hemostasis.

What do guidelines say about management?

There is a lack of consensus on the management of CAT in patients with thrombocytopenia, with current international guidance informed by observational studies and expert opinions rather than evidence from RCTs.[18,22] The 2018 guidance from the International Society on Thrombosis and Haemostasis (ISTH) Scientific and Standardization Committee recommends a risk-stratified management approach according to the acuity of the thrombus, the risk of thrombosis progression, and platelet count (Table 1).

What is the current practice?

Despite the international guidelines, audits and studies demonstrate inconsistent and variable practice, likely reflecting the lack of strong evidence behind the guideline recommendations. Two international studies have recently demonstrated the heterogeneity in the management of this cohort, the key findings of which are summarized in Table 2.[23,24] The Cancer-Associated Venous Thrombosis and Thrombocytopenia (CAVEaT) UK study in patients with hematological malignancies showed that 47% of patients with higher risk thrombosis and 5% with lower risk thrombosis were managed according to the ISTH guidance. There was variation in the use of platelet transfusions. Changes in anticoagulation were observed in 51% of patients by 90 days. Mortality was 15% at 28 days and significant morbidity was

demonstrated.[24] The North American Thrombocytopenia Related Outcomes with Venous thromboembolism (TROVE) study also found that changes in anticoagulation choice were frequent, with less frequent alterations in anticoagulation intensity.[23]

Interestingly, the two studies revealed different findings. The TROVE study showed a significantly reduced risk of bleeding events in those receiving modified dose anticoagulation compared to full dose, without an increased risk of recurrent VTE. In contrast, the CAVEaT study showed no significant differences in bleeding or recurrent thrombosis between full-dose and mod-

Table 1. Summary of the 2018 International Society on Thrombosis and Haemostasis Scientific and Standardization Committee guidance on the management of cancer-associated thrombosis in patients with thrombocytopenia.

Risk category	Baseline platelet count at the time of index VTE	Management
Any	>50×10^9/L	Therapeutic dose anticoagulation without platelet transfusion support
Higher risk* acute# CAT	<50×10^9/L	Platelet transfusion support, target >40-50×10^9/L, and therapeutic anticoagulation (LMWH/UFH)
Lower risk& acute CAT, subacute or chronic^ CAT	25-50×10^9/L	Reduced dose (50% of therapeutic dose) LMWH, or Prophylactic dose LMWH
	<25×10^9/L	Withhold anticoagulation while platelet <25×10^9/L

*Higher risk CAT, including but not limited to: symptomatic segmental or more proximal pulmonary embolism (PE), proximal deep vein thrombosis, history of or recurrent/progressive thrombosis; #Acute CAT, within the first 30 days of index venous thromboembolism; &Lower risk CAT, including but not limited to: distal deep vein thrombosis, incidental subsegmental pulmonary embolism, catheter related thrombosis; ^Subacute or chronic CAT, >30 days since index venous thromboembolism.
VTE, venous thromboembolism; CAT, cancer-associated thrombosis; LMWH, low molecular weight heparin; UFH, unfractionated heparin.

Table 2. A comparison between Thrombocytopenia-Related Outcomes with Venous thromboembolism and Cancer-Associated Venous Thrombosis and Thrombocytopenia studies.23,24

	TROVE	CAVEaT
Region	North America	United Kingdom
Design	Prospective, observational, multicentre cohort study	Prospective, observational, multicentre cohort study
Number of patients	121	105
Type of malignancy		
• Hematological	85/121 (70%)	105/105 (100%)
• Solid tumor	36/121 (30%)	0%
Index VTE event:		
• Upper limb DVT	48/121 (40%)	44/105 (42%)
• Lower limb DVT	49/121 (40.5%)	16/105 (15%)
• PE	45/121 (37%)	35/105 (33%)
• Other	4/121 (3%)	10/105 (9.5%)
Baseline platelet threshold for enrolment	<100×10^9/L	<50×10^9/L
Initial anticoagulation	• Full dose LMWH, UFH or DOAC: 75/121 (62%) • Modified dose LMWH, UFH or DOAC: 33/121 (27%) • No anticoagulation: 13/121 (11%)	• Full dose LMWH or UFH: 56/105 (53%) • Modified dose LMWH: 33/105 (31%) • DOACs: 4/105 (4%) • No anticoagulation: 12/105 (11%)
Thrombosis recurrence according to initial anticoagulation	At 60 days: • Full dose anticoagulation: 5.6% (95% CI 0.2-11) • Modified dose anticoagulation: 0%	At 28 days: • Full dose LMWH or UFH: 4% • Modified dose LMWH: 4% • DOACs: 0% •No anticoagulation: 0%
Major bleeding according to initial anticoagulation	At 60 days: • Full dose anticoagulation: 12.8% (95% CI 4.9-20.8) • Modified dose anticoagulation: 6.6% (95% CI 2.4-15.7) • Fine Gray hazard ratio 2.18 (95% CI, 1.21-3.93)	At 28 days: • Full dose LMWH or UFH: 3% • Modified dose LMWH: 4% • DOACs: 0% • No anticoagulation: 0%
Conclusions as reported by authors	Modified dose anticoagulation may be a safe alternative to treatment dose anticoagulation	No clear relationship between platelet transfusion threshold, anticoagulation dose reduction threshold and risk of thrombosis progression or major bleeding

TROVE, Thrombocytopenia Related Outcomes with Venous thromboembolism; CAVEaT, Cancer-Associated Venous Thrombosis and Thrombocytopenia; VTE, venous thromboembolism; DVT, deep vein thrombosis; PE, pulmonary embolism; LMWH, low molecular weight heparin; UFH, unfractionated heparin; DOAC, direct oral anticoagulants; CI, confidence interval.

ified-dose groups. The reasons for these differences were not clear but might reflect aspects of the study methodology including inclusion criteria and differences in baseline characteristics (Table 2). While these studies advanced the field by providing prospective data for the first time for patients with CAT and thrombocytopenia, the observational nature and non-randomized design of the studies were potential confounders and limited the strength of any conclusions. The results are hypothesis-generating and not yet practice-changing.

What current research is happening?

There is a pressing need for a more robust design of the study to evaluate the optimal management strategies (including anticoagulation and platelet transfusion) in patients with CAT and thrombocytopenia. Patient groups are integral to designing studies and disseminating findings. Patients with hematological cancers frequently emphasize the importance of quality of life and functional recovery in addition to outcomes such as survival, and complications such as thrombosis are viewed as a barrier to rehabilitation.

An example of a currently recruiting study is the START randomized trial (STrategies for Anticoagulation in patients with thRombocytopenia and cancer-associated Thrombosis) (NCT05255003) (Figure 1). This is an international, multi-site pilot trial assessing the use of platelet transfusions plus higher dose anticoagulation compared to modified dose anticoagulation in patients with thrombocytopenia and CAT receiving anticoagulation, with planned participating sites in Canada and the UK. The study has been reviewed and supported by patient representatives at the Canadian Venous Thromboembolism Research Network, and Thrombosis UK.

Potential participants who have developed an acute CAT within 14 days, received <72 hours of anticoagulation for index CAT and have platelet count <50×10^9/L are randomized to one of two study arms and followed up for 30 +/-3 days:

1) Study arm without platelet transfusion:
   I. Platelet count 25-50×10^9/L: 50% dose low-molecular-weight heparins (LMWH).
   II. Platelet count <25×10^9/L: hold anticoagulation.
2) Study arm with platelet transfusion:
   I. Pre-transfusion platelet count 25-50×10^9/L: 100% dose LMWH after one adult unit of platelet transfusion.
   II. Pre-transfusion platelet count <25×10^9/L: 50% dose LMWH after one adult unit of platelet transfusion.

Recruitment has begun for the feasibility phase of the study in Canada, with the aim of recruiting 50 patients internationally. The pilot trial is important to assess the feasibility and potential barriers to patient recruitment in this challenging area of study. It will allow assumptions about key parameters to be tested/validated and hence influence the study design for a future full-scale definitive trial. This is especially important in this patient population with a high risk of complications and where clinicians may have uncertainties in equipoise for recruitment to follow a protocol. Designing a definitive study that is pragmatic and provides important data to guide clinical practice is a major endeavor and will be best accomplished by international collaboration. Definitive studies also need to consider cost-effectiveness, given, for example, that more aggressive platelet transfusions also require more intense resource allocation.

The aim is that the full-scale trial will define the standard of

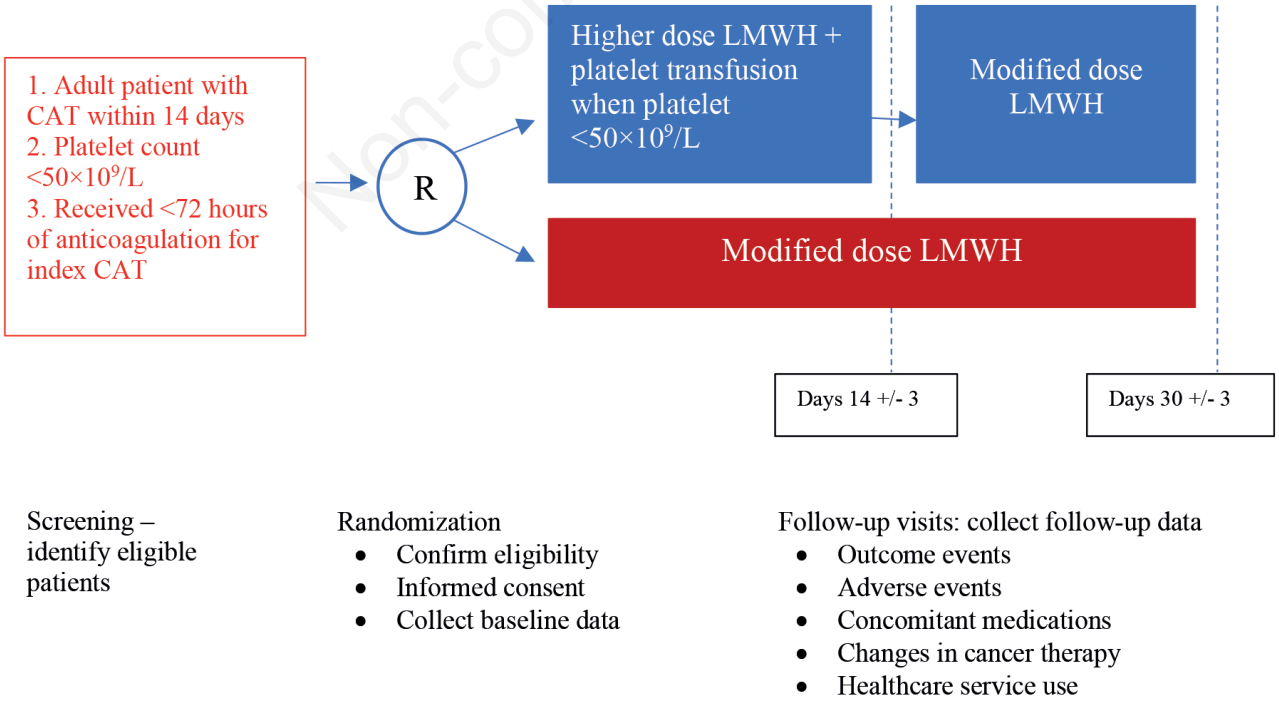

Figure 1. Study design of START trial. CAT, cancer-associated thrombosis; LMWH, low molecular weight heparin.

care for the management of patients with CAT and thrombocytopenia when treated with LMWH. As this is a patient group with high bleeding risk, future studies will then center around comparison of this newly defined standard of care with the use of alternative anticoagulants.

In conclusion, patients with CAT and thrombocytopenia are at high risk of both bleeding and thrombosis. Identification of the optimal management strategy is urgently needed which can best be established by the conduct of RCTs.

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Coagulome and tumor microenvironment: impact of oncogenes, cellular heterogeneity and extracellular vesicles

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ABSTRACT

Cancer-associated thrombosis (CAT) results from the hemostatic system being dysregulated by the progression of cancer. Despite common clinical manifestations, the mechanisms of CAT may vary greatly because cancers develop along distinct biological trajectories that are imposed by the interaction between the tumor cell genome, the epigenome, the surrounding microenvironment, and the tissue of origin. The coagulome, or repertoire of coagulation effectors, expressed by stromal, inflammatory, and cancer cells at the tumor-vascular interface and systemically, reflects this biological variability. Complex landscapes of coagulant and non-coagulant cellular populations are revealed by single-cell RNA sequencing analyses conducted on unperturbed human cancer tissues. Additionally, through mediators of cell-cell interactions, soluble coagulants, and extracellular vesicles containing tissue factor, podoplanin, and other effectors, coagulomes are projected into the pericellular milieu and systemic circulation. As this complexity is currently outside of the clinical paradigm, one could argue that better CAT management could result from a more individualized analysis of coagulomes in cancer patients.

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Key words: cancer; thrombosis; oncogenes; coagulome; extracellular vesicles.

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Introduction: the vasculature as a gateway for systemic manifestations of cancer

Among the multiple complex facets of the tumor microenvironment, the vascular compartment plays a unique and integrative role.[1] The vasculature, including networks of blood vessels, lymphatics, lymph and the circulating blood, all shape the local tumor milieu and link the anatomically circumscribed cancer foci with the systemic circulation. This crucial connection is responsible for the widespread biological responses, comorbidities and, ultimately, for the metastatic progression of the disease.

Thus, tumor microcirculation plays both local and systemic roles in cancer. The local role of the tumor vasculature encompasses a plethora of perfusion-dependent and -independent processes. For example, the vasculature controls the behavior, metabolism and survival of cancer cells through the supply of blood enriched in oxygen, nutrients, regulatory plasma proteins, hormones and cells. Sustained blood flow through the tumor microcirculation regulates the influx of immune effectors, and drugs while mediating the removal of metabolites and shedding of tumor cells and their products into the general circulation.

Alteration within the blood vessel wall (endothelial cells, perivascular cells, extracellular matrix) across the tumor microvasculature enables the flux of fluids, molecules and cells between the circulating blood and the surrounding tissue. In this regard, cancer-related impact on vascular permeability and transmissivity may encompass processes such as regional modification of the blood-brain barrier, formation of the blood-tumor barrier,[2] different degrees of vascular leakiness, microhemorrhage, along with other structural and functional abnormalities triggered at the tumor-vascular interface.[3]

These crucial alterations occur in the course of events leading to formation, expansion and remodeling of the tumor micro-

circulation, including the onset of angiogenesis,[1] vascular cooption,[4] vascular dilatation (vasectasia),[5] lymphangiogenesis,[6] vasculogenic mimicry,[7] emergence of transient lymphoid structures,[8] and changes in immunoregulatory functions of endothelial cells,[9] among other effects.[10] These responses are increasingly well understood, well described, and, at least in some cases, have already served to identify therapeutic targets in cancer, as illustrated by the advent of antiangiogenic agents directed at the vascular endothelial growth factor pathway. Several of these agents have been approved for cancer treatment over the past two decades.[11-13]

Somewhat less explored are the perfusion-independent aspects of the tumor microcirculation, especially the potent secretory activity of endothelial cells (possibly also of pericytes, perivascular fibroblasts and myeloid cells).[14,15] Indeed, endothelial cell secretome has been described as an important regulatory force in mediating changes in the tissue and tumor microenvironments, impacting migratory behavior of cancer cells (possibly also other cells), their growth,[16] stemness and other responses.[17,18] This paracrine effect, initially described decades ago,[16,19] has more recently been brought to light in various biological contexts under the term of the 'angiocrine' regulation.[17,20,21]

Similarly, circulating blood components, such as red blood cells, leukocytes,[15] platelets,[22] coagulation proteases (*e.g.*, thrombin) and plasma proteins often play multiple roles, either related to their canonical homeostatic (and hemostatic) functions, or involving induction of cellular signaling responses across multiple organ sites, with consequences for cancer progression.[23]

As mentioned earlier, access to the vascular system enables the transition of a localized neoplastic growth to a complex, systemic disease. Indeed, even ostensibly non-metastatic cancers often elicit profound and morbid systemic effects on multiple organ systems. Some of the most striking examples of such 'remote' influences include functional alterations in the liver,[24] pancreas,[25] brain,[26] bone marrow and immune system,[27] as well as clinically overt paraneoplastic syndromes, such as cachexia,[28] or cancer-associated thrombosis (CAT).[29] These alterations may be further exacerbated in the course of a more advanced or metastatic disease. Conversely, the systemic effects of cancer progression mediated by the vasculature often precede and enable subsequent metastatic dissemination.[30-32] For example, the conditioning of distant organs by cancer-derived extracellular vesicles (EVs), cytokines and clotting factors leads to the formation of pre-metastatic niches that serve as sites of subsequent colonization by incoming cancer cells.[33-36]

Thus, cancers represent complex and highly interactive, multifactorial and multicellular processes that highjack, alter, and exploit elements of the circulation, including the hemostatic system, which becomes engulfed by, and alters, cancer progression. Amidst this complexity, the nexus between cancer and the coagulation system represents the focus of our remaining comments.

Cancer-associated thrombosis: implications for disease progression and heterogeneity

The formation of tumor-vascular interface represents a common feature of virtually all cancers, with implicit consequences for both blood vessels and blood.[3] Yet, the hemostatic consequences of this interaction are hardly straightforward, or uniform. Thus, in some cancers, the manifestations of CAT are relatively subtle, while in others the impact of the disease on the coagulation system may be more profound, morbid, and biologically, as well as clinically, manifest requiring prophylaxis and intervention.[37] In the latter case, the elevated hypercoagulability is often associated with heightened systemic risk for arterial and especially venous thromboembolism (VTE).[29] Moreover, in certain cancers, such as subsets of high-grade glioma, CAT may be associated with extensive microvascular thrombosis within the tumor mass) coupled with an impact on peripheral circulation in the form of dramatically heightened VTE risk.[38-41]

In its severe forms, CAT poses considerable clinical concerns due to morbidity associated with VTE, which may escalate to life-threatening pulmonary embolism.[29] In addition, the co-existing thrombosis leads to poor overall outcomes in cancer patients.[42] At the same time, the activated coagulation system and platelets often deploy disease-modifying mechanisms that may facilitate cancer progression and dissemination. For example, the formation of fibrin matrix and release of growth factors from activated platelets may facilitate tumor invasion, while activated sticky platelets in blood stream can coat extravasated cancer cells creating a shield for circulating cancer cells against immune effectors.[22,31,32,34] However, while thrombosis in cancer patients in its various forms has been recognized for over 150 years, the exact molecular chains of causation, mechanistic pathways leading to CAT and precise points at which clotting intersects with the biology of specific cancers still remain poorly defined.[37,43] It seems reasonable to suggest that CAT (or CATs) could become less intractable if a system of biologically based stratification could be developed and applied in a context-specific manner to defined populations of cancer patients.

Cancer coagulome: at the crossroads of thrombosis and biological regulation

Operationally, the upstream triggers of CAT implicitly lie within the molecular apparatus of cancer cells that evoke CAT, either directly or indirectly. Indeed, cancer progression may exert multiple indirect influences in the vascular system, leading to hemostatic perturbations. For example, the formation of aberrant and poorly perfused intratumoral vascular networks may lead to stasis and thereby promote microthrombosis. Moreover, the exposure to blood of procoagulant surfaces within perivascular tissues of the tumor bed may occur due to porosity and anatomical abnormalities of tumor blood vessels, resulting in the activation of the coagulation system. Similarly, the recruitment of procoagulant inflammatory cells, endothelial cell activation and other processes may compromise the anticoagulant functions of the vasculature.[37]

Cancer cells may also possess the molecular apparatus enabling them to interact with the hemostatic system directly. Some of the best-described effectors of such interactions include the expression by different tumor cell types of tissue factor (TF) podoplanin (PDPN), coagulation factor VII (FVII), prothrombin, or antifibrinolytic serpins, such as plasminogen activator inhibitor 1 (PAI-1).[44-51] To describe this cancer-associated molecular interface the term 'coagulome' has been coined previously, initially to capture the totality of relevant molecular features affected by disease progression (coagulation, fibrinolytic, and platelet regulating factors).[52] This term was later used to define the complex reper-

toire of putative regulators of clotting processes associated with cancer cells themselves,[53,54] or to characterize a wider procoagulant network of interactions involving multiple components of the disease, such as tumor cells, inflammatory cells, stroma, and blood elements, all of which may contribute to CAT in various ways and in different contexts.[55]

Defining cancer coagulome is important for at least three main reasons. First, the triggers of CAT could be markedly different than those leading to thrombosis in the course of other procoagulant conditions, such as major surgery, cardiovascular disease, or genetic thrombophilia. This is because cancer cells possess unique molecular makeup and functionalities incomparable to normal tissues. Second, different cancers exhibit vastly different VTE risks,[56,57] which suggests that different cancer-specific mechanisms of CAT may be operative between distinct diagnostic entities. It could also be argued that, although different cancers may carry comparable global VTE risks, they may differ in their abilities to activate specific prothrombotic pathways (*e.g.*, coagulation system or platelets) due to stark differences in their molecular profiles. Moreover, cancers originating from similar tissue sites may trigger vastly different CAT activating mechanisms. The cases in point are recent studies on high-grade glioma, where oncogenic mutations of the isocitrate dehydrogenase 1 and 2 (IDH1/2) genes had a protective effect against microthrombosis and VTE risk, while histologically similar IDH1/2 wild-type tumors, currently classified as proper glioblastomas (GBMs),[58] were associated with pronounced incidence of VTE, upward of 20%.[39,41] Interestingly, while the mechanistic basis of these differences remains to be conclusively elucidated, the IDH1/2-related changes in CAT correlate with the differential expression by cancer cells of at least two different prothrombotic effectors, such as TF and PDPN.[41,51]

Third, a better definition of cancer coagulome in specific disease contexts may enable a more targeted and personalized intervention, based on what can be gleaned from molecular causality and its impact on coagulome. For example, the identification of cancer-associated coagulant effectors (*e.g.*, TF), or mediators leading to activation of procoagulant inflammatory responses or platelets may enable directing anticoagulant therapy at upstream triggers of these events.[37,47,49,51] This could complement and improve the current paradigm built around therapies aiming at elements of the common coagulation pathway, such as factor Xa or thrombin, which are burdened with bleeding risks due to global perturbances in hemostatic requirements they induce.[59] Thus, molecular causation and composition of the cancer coagulome may have practical implications that are, perhaps, worthy of some consideration.

Oncogenic drivers of cancer coagulome: lessons from cancer genome and epigenome

While the impact of cancer progression on CAT may stem from multiple, sometimes non-specific, or indirect influences, cancer-specific factors are also clearly a play. For example, marked differences in VTE risk exist between different cancer types,[56] and along the path of cancer progression. In this sense, progression of pancreatic,[60] or colorectal cancer (CRC) has been linked to upregulation of TF by tumor cells,[61] and parallels corresponding increases in the VTE risk.[37] In patients with primary brain tumors, not only VTE but also microvascular thrombosis was found to correlate with the increasing tumor grade.[38] These and other examples illustrate the emerging interrelationship between biological properties of cancer cells and their ability to promote thrombosis.

At the root of progressive changes in the cancer cell phenotype are oncogenic events (mutations) affecting the cellular genome and epigenome, with a profound impact on the expression of multiple downstream genes.[62] It is, therefore, reasonable to suggest that oncogenic changes may influence cancer coagulome and have some bearing on VTE. This notion was originally proposed and later directly examined using experimental models of human and rodent cancer cell lines with precisely defined (or engineered) oncogenic alterations.[63] Some of these studies included a series of human isogenic CRC cell lines expressing either the wild-type KRAS gene, or its oncogenic mutant KRAS G13D allele, either in the presence or in the absence of TP53 tumor suppressor gene. Interestingly, this comparison revealed that more advanced mutational status correlates with increased cellular aggressiveness, higher expression of TF and greater release of TF-carrying procoagulant extracellular vesicles.[47] Similarly, the loss of PTEN tumor suppressor in the experimental glioma model resulted in the upregulation of TF,[64] while oncogenic MET receptor drove the upregulation of PAI-1 in a model of murine hepatoma.[49] In another study involving a series of isogenic human GBM cell lines, the enforced expression of oncogenic EGFRvIII stimulated the aggressive tumor phenotype *in vivo,* along with a dramatic upregulation of TF, FVII and thrombin receptor (PAR-1) by cancer cells.[65,66] Interestingly, in the same series of cell lines, the expression of platelet-activating PDPN ligand was down-regulated in concert with EGFRvIII expression by cancer cells. This may suggest that oncogenic events (such as EGFRvIII status) may control the switch between two qualitatively different pro-thrombotic cellular phenotypes/states (TF/coagulation-dependent and PDPN/platelet-dependent).[51]

In some of these experimental studies, the source of a systemic hypercoagulability readouts could be traced to the tumor microcirculation. For example, in mice harboring EGFRvIII-driven and TF-expressing GBM xenografts, the levels of D-dimer were predictably elevated in peripheral blood, but these readings were orders of magnitude higher within the tumor mass, compared to systemic circulation. These observations may indicate that, in this case, D-dimer could largely originate from the highly procoagulant tumor microenvironment rather than being generated systemically.[51] Whether this is generalizable, or not, the underlying processes were driven by the oncogenic mutation. Moreover, such a link between oncogenic events and procoagulant phenotypes of cancer cells has been repeatedly described in experimental studies employing different tumor models, as reviewed recently.[54]

In keeping with these findings the subsequent analyses of several clinical cohorts suggested that in cancer patients the incidence of VTE,[41,67-69] or upregulation of some of its effectors (*e.g.*, TF) may also be a function of oncogenic mutations.[70] For example, VTE was markedly more frequent in CRC patients with KRAS mutations relative to those whose tumors did not carry this genetic alteration.[67] In a large cohort of patients with different cancer types, mutations in STK11, KRAS, CTNNB1, KEAP1, CDKN2B, and MET were generally linked to the elevated VTE risk. Conversely, in the same cohort, certain onco-

genic mutations had protective effects leading to lower VTE risk, either in general (SETD2) or in specific tumor types (IDH1). In this regard, IDH1/2 status has been extensively validated as an element of the VTE risk prediction algorithm recently developed for high grade glioma.[39]

The impact of genomic mutations on the phenotype of cancer cells is not absolute, and it can be modulated by the cellular epigenome. This is in keeping with the role of chromatin structure, chemical modification, DNA methylation and other processes in the execution of the cellular genetic program. These effects underlie gene expression changes involved in normal cellular differentiation, adaptation, and plasticity, as well as their epigenetic aberrations driving malignant transformation.[71] Thus, while cancer cells may carry common genetic mutations, they may also respond to residual lineage-specific programs, or microenvironmental cues that could profoundly reshape their coagulome. This interplay is at the core of many aspects of cellular heterogeneity pervading cancer progression, including the formation of stem cell populations, progenitor cell pools and diversification of their progenies.[72] Indeed, gene promoter methylation, chromatin modifications and regulatory effects of non-coding RNAs, including microRNA, may mold the molecular repertoire of cancer cells including effectors of thrombosis, often acting in a cancer-specific manner.[54] For example, experimentation with *in vitro* model systems suggests that markers of cancer cell stemness may, in some cases converge with,[73] while in others diverge from,[74] effectors of the coagulation pathway, such as TF. In GBM-derived cell lines, EGFRvIII suppresses the expression of PDPN in a manner potentially involving the epigenetic modifier EZH2, while in patients with high-grade glioma expression of mutant IDH1, downregulates both TF and PDPN due to its global impact on gene methylation.[51,75] Likewise, specific microRNAs may control the levels of TF,[76] or impact other elements of the cancer coagulome.[37-77]

Cancer models and coagulome: advantages and possible pitfalls

It should be noted that while cancer cell lines and transgenic mouse models provide invaluable and well-controlled resources for studies on molecular causality impinging upon the regulation of cancer coagulome, they are often not identical to (or directly predictive of) their 'real life' counterparts in unperturbed human tumor microenvironments.[51] This important limitation is infrequently discussed in the literature and may be attributed to the genetic drift in long-term cultures, epigenetic modifications induced under *in vitro* conditions,[52] selection of cancer subclones, species-specific factors, changes imposed by experimental manipulation, and the absence of natural complexity and cellular diversification processes, which occur during natural cancer progression *in vivo*. It is surprising that more advanced and complex models of cancer, such as spheroids, tumor spheres, organoids, organs on chip or patient-derived orthotopic xenografts have scarcely been studied in terms of their ability to emulate CAT in cancer patients.[78,79] While greater investment in this regard could be valuable, the accurate recapitulation of the cancer-specific complexity of tumor cell 'communities', and dynamic aspects of the tumor-vascular interface may be difficult to achieve under purely experimental settings.

Cancer coagulome: lessons from single-cell RNA sequencing

One way to circumvent these limitations is to extract features of cancer coagulome and its upstream regulators directly from clinical cancer datasets increasingly available in the literature and achievable technologically. Such data often report on multiomic molecular profiles and single-cell sequencing (scRNAseq) results of cancer tissues that have never been subjected to experimental manipulations *in vitro*.[51,80] In particular, the advent of scRNAseq technology has fundamentally changed the outlook at the multicellular cancer 'architecture' and the dynamic of transitory phenotypic states of cancer cells as they interact with their microenvironment.[72,81,82] For example, in high-grade brain tumors, single-cell transcriptomes illuminated the fact that traditional distinctions between molecular subtypes of GBM, such as proneural, classical and mesenchymal disease,[58] are not reflective of the corresponding differences between seemingly phenotypically uniform cellular masses populating these tumors, as bulk RNA sequencing would seem to suggest.[83] Rather, these subtypes emerge as a function of complex equilibria that form between heterogeneous cancer cell subsets, among which the predominant population dictates the global molecular signature of the tumor as a whole.[81] The exact forces that control these cellular 'mosaics' are not entirely clear.[84] However, the phenotypic biases driving these brain cancer cell 'ecosystems' toward one equilibrium or another, appear to be imposed by prevalent oncogenic drivers, such as EGFR for astrocytic-type GBMs, or NF1 loss for mesenchymal tumors, which are also enriched for inflammatory stroma.[85]

These findings may potentially redefine the meaning of cellular coagulome in GBM and likely in other cancers, as well.[54] For example, the analysis of single-cell datasets suggested that transcripts for TF and PDPN may be expressed preferentially (though not exclusively) by specific cellular subpopulations, such as astrocytic or mesenchymal cancer cells, respectively (Figure 1).[51] Interestingly, progenitor GBM cells were relatively devoid of these pro-thrombotic effectors. Moreover, at the single-cell level, the impact of oncogenic drivers was more complex than could be inferred from cell culture studies. For example, a large proportion of EGFR expressing GBM cells did not express PDPN, which instead was enriched among EGFR non-expressing subsets of cancer cells. A fraction of cancer cells, however, expressed both TF and PDPN.[51] Thus, in complex cancers, such as GBM, tumor cells form coagulant mosaics, which contain subpopulations of highly coagulant cells interspersed with their counterparts expressing low (or no) apparent prothrombotic phenotypes.[51] How this coagulant heterogeneity impacts intra-tumoral microthrombosis, or projects its effects systemically, to trigger VTE is presently poorly understood.

Extracellular vesicles: emerging regulators of vascular responses and thrombosis in cancer

How could genetic and epigenetic alterations in cancer cells trigger thrombosis at remote organ sites and in anatomically distant, peripheral blood vessels? In this regard, several mutually non-exclusive scenarios could be considered. For example, systemic hypercoagulability originating from within the tumor

microcirculation may precipitate clotting processes at vulnerable sites, such as venous valves in lower limbs or in areas of vascular stasis.[86] Alternatively, cancer cells could trigger a systemic or peripheral hypercoagulable state through the release of circulating procoagulant mediators. In fact, several such cancer-related candidate mediators have been studied over the years, including enzymatic activities associated with cancer coagulant,[59] neutrophile extracellular traps (NETs),[87] or cancer-derived procoagulant microparticles,[46] more recently referred to as EVs.[88]

EVs and smaller membrane-less extracellular particles (EPs) (collectively referred to here as EVPs) represent an intriguing element in the cellular secretome with a possible role in thrombosis.[89] EVPs are highly heterogeneous due to diversity of biological processes leading to their formation. While small EVs (<100 nm) may originate from the cellular endosome (exosomes) and represent a part of the membrane protein recycling processes, other EVs originate at the cellular surface (ectosomes) following membrane blebbing, budding and protrusion. These EVs vary in size from ~100 nm (small microvesicles, ARMMs) to >2 μm in diameter (large oncosomes, migrasomes, exophers, apoptotic bodies) and in terms of molecular cargo, as well as function.[89,90] The biogenesis of EPs is currently unclear, but it leads to the formation of molecularly distinct particles, such as exomeres and supermeres, ranging in size from <50 nm to <35 nm respectively.[89] Different EVPs contain distinctive repertoires of proteins, lipids and nucleic acids and possess a remarkable ability to interact with biofluids and cells, whereupon they serve as hubs for macromolecular complexes, or as vehicles that transfer their cargo to cellular recipients, respectively. In the latter case EVP-cell interactions may elicit a range of biological responses, including changes in cellular phenotype.[91]

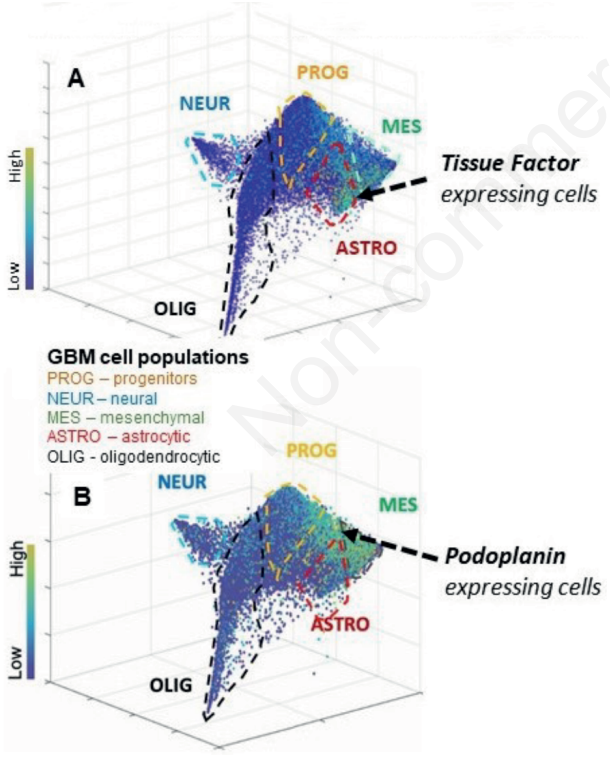

Figure 1. Heterogeneous cellular carriers of glioblastoma coagulome. Single-cell mRNA sequencing. Roadmap analysis of developmental programs expressed in glioblastoma cell subpopulations reveals cell subsets enriched in tissue factor (panel A; mostly astrocytic cells) or podoplanin (panel B; mostly mesenchymal cells). The plots were adapted with permission from N. Tawil Ph.D. Thesis (2021); analysis based on the pipeline described by Couturier *et al.*[82] and applied to coagulome.[51]

EVPs have long been known to carry potent vascular mediators.[92] While some of these molecules may directly interact with the hemostatic system,[46,93] others may exert their vascular effects through interaction with circulating cells, or the vascular wall, and by impacting angiogenesis, vascular permeability, inflammation and other processes.[17,94,95] TF, PDPN, phosphatidyl serine, mucins, inorganic polyphosphate are among the EVP-associated effectors found capable of impacting the hemostatic system under various pathological conditions, including cancer.[46,47,93,95-97]

There is mounting evidence that procoagulant EVPs may serve as an export mechanism for TF, PDPN and other effectors from cancer cells to their surroundings and to peripheral blood.[46,47,51,93,98] Particularly rich, in this regard, is the literature on TF-carrying, cancer-derived EVPs, which appear to possess the capacity to activate the coagulation cascade in several experimental systems, especially in models of pancreatic cancer, a tumor enriched in cellular TF.[98] Similarly, the release of TF-carrying EVPs has been documented in CRC,[47] GBM and other cancers.[93,99] However, the role of TF-EVPs in triggering and predicting VTE remains a subject of some debate, with some studies supporting,[99] and others questioning the role of this mechanism in the clinic.[100]

While the analysis of EVPs poses significant pre-analytical, technical and standardization challenges,[101] it is also possible that the cellular architecture of the respective cancers would need to be taken into consideration as a source of EVP cargo and variability. For example, in experimental models of GBM, the positivity of cancer cells for two or more putative prothrombotic effector molecules, such as TF and PDPN was paralleled by the release of EVs with the corresponding dual positivity (TF+/PDPN+; Figure 2). However, the same cells also exported EVs containing single, or none of these molecules. Since the cargo assembly during EV biogenesis is non-random, it is important to understand how these different, coagulant, or non-coagulant EVs, are formed and regulated.

Nonetheless, the enrichment in EVs carrying specific molecular cargo (TF or PDPN) was found to correlate with their potential to activate coagulation cascade and/or platelets in experimental settings.[51] As mentioned earlier, cancer cells positive for either PDPN, or TF, both, or none, are also readily detectable in scRNAseq datasets of human GBM.[51] It is therefore of considerable interest to determine whether VTE risk prediction that may be difficult to establish while monitoring TF-EVs alone, could be improved by analyzing EVs for multiple effectors, including through the use of technology platforms capable of generating multiplex data at the single EV resolution (Figure 2).[102] It is possible that comprehensive multidimensional molecular landscapes of coagulant EV subpopulations in cancer patients with the help of super-resolution technologies and machine learning may become diagnostically informative in the context of CAT.[102,104]

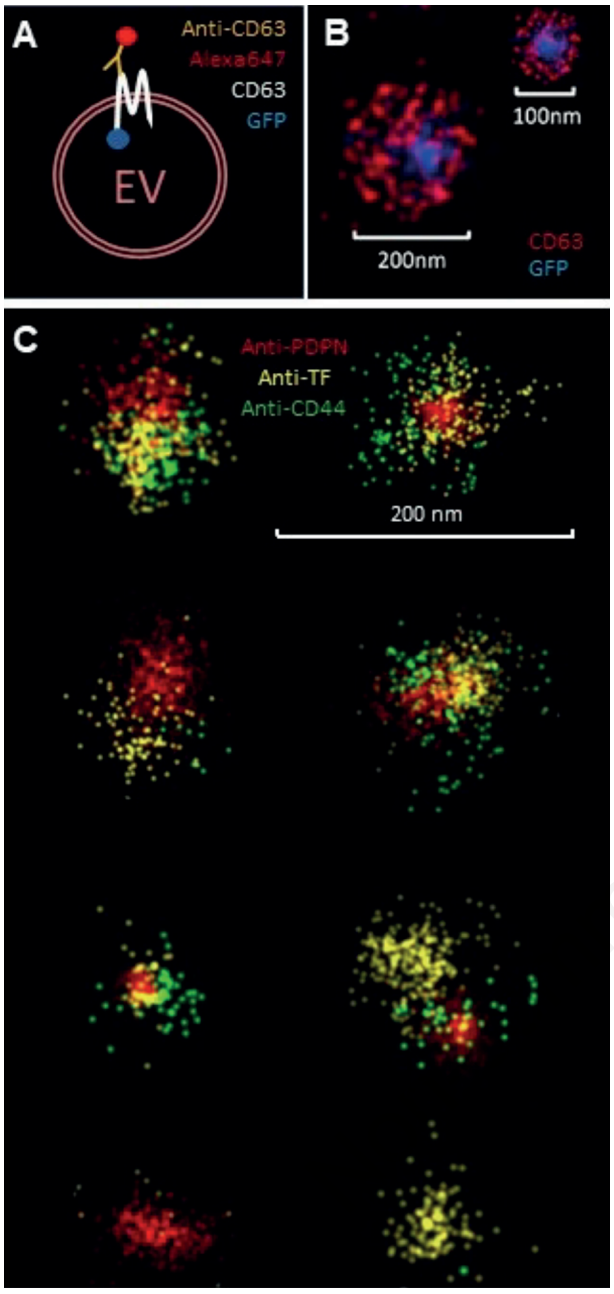

Figure 2. Structural complexity and coagulome of cancer-derived extracellular vesicles. A) Diagrammatic representation of extracellular vesicles from A431 epithelial cancer cells expressing CD63-GFP fusion protein; B) A431 extracellular vesicles imaged by ONI super-resolution microscope (dSTORM mode) for the expression of CD63 ectodomain (red) labeled with fluorescent antibody (Alexa 647) and for the intraluminal CD63-GFP tag (blue); C) heterogenous coagulant repertoires of individual extracellular vesicles derived from glioma stem cells-engineered to express podoplanin, and tissue factor, with endogenous CD44 expression (staining with fluorescent antibodies). All extracellular vesicles were imaged at the Centre for Applied Nanomedicine, RIMUHC (https://rimuhc.ca/research-initiatives/centre-for-applied-nanomedicine; with support from the ONI team and Mahsa Jalali). EVs, extracellular vesicles; GFP, green fluorescent protein; PDPN, podoplanin; TF, tissue factor.

Conclusions

While CAT may encompass all complexities of Virchow's triad, including unspecific and indirect influences, it is causally and molecularly triggered by the unique nature of the underlying neoplastic process. It may, therefore, be useful to consider (as one of the relevant factors) the drivers of cancer progression operating upstream of cancer coagulome, or of immediate clotting mechanisms. Both the biology of the underlying disease and the corresponding anticancer therapy may shape processes leading to VTE. Since these upstream effects are highly heterogeneous so could be the mechanisms triggering VTE, as well as its nature. Moreover, these may not be linear relationships. Rather, the consequences of oncogenic mutations may intersect with epigenetic alterations and interactions between cancer cells and their surroundings collectively impacting coagulome. Single cell profiling of cancers revealed that previously uncovered global properties of the tumor mass conceal more complex equilibria of cancer, stromal and inflammatory cells that underlie the malignant process and its vascular components. It is of interest to ask whether cellular landscapes of coagulant cancer types could help understand and address the VTE risks in individual cancer patients.

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New drugs, old problems: immune checkpoint inhibitors and cancer-associated thrombosis

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ABSTRACT

A frequent side effect of cancer treatment is venous thromboembolism (VTE), which is made more likely by systemic anticancer medication. Immune checkpoint inhibitors (ICIs) have emerged as a paradigm-shifting treatment for many cancers. Early trials investigating the efficacy of ICIs did not identify thrombosis as a significant adverse event of concern. An initial meta-analysis reported a 1.1% [95% confidence interval (CI) 0.5-2.1] risk of arterial thromboembolism (ATE) and a 2.7% (95% CI 1.8-4.0) rate of vein thrombosis. ICIs have, however, been linked to ATE and VTE in an increasing number of post-marketing investigations. The reported incidence rates of cumulative VTE range from 5-8% at 6 months to 10-12% at 12 months, while the rates of ATE vary from 1-2% at 6 months to 17 months. Furthermore, a number of studies show a correlation between reduced survival and ICI-related thromboembolism. In order to provide a compiled and thorough narrative on the mechanisms, incidence, risk factors, and survival related to ICI-associated VTE and ATE, this narrative review summarizes the literature.

Introduction

Cancer patients have a greater risk of both venous thromboembolism (VTE) and arterial thromboembolism (ATE).[1] Chemotherapy and other anti-cancer therapies increase the risk of VTE, including deep vein thrombosis (DVT), and pulmonary embolism (PE).[2-5] Cancer patients have a four-to-twelve-fold higher incidence of VTE.[6,7] This risk increases 23-fold in chemotherapy or targeted treatment patients.[8] ATE includes myocardial infarction, stroke, and peripheral arterial embolism.

The advent of immune checkpoint inhibitors (ICIs) is a paradigm shift in cancer therapeutics. ICIs target programmed cell death protein 1 (PD-1) or its ligand (PD-L1) or cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) to prevent tumor immune evasion.[1,9] The United States Food and Drug Administration and the European Medicines Agency both granted approval to the first ICI, ipilimumab, in 2011.[10] ICIs are now widely used to treat lung cancer, melanoma, renal cell carcinoma, head and neck cancer, and colorectal cancer subsets.[9,11] A meta-analysis of these randomized controlled trials (RCTs) found a low 1.1% [95% confidence interval (CI) 0.5-2.1] risk of ATE and a moderate 2.7% (95% CI 1.8-4.0) risk of VTE.[12] Similar rates (VTE rates: 1.5% in melanoma and 1.9% in lung cancer) were also revealed by another meta-analysis of RCTs and prospective studies of ICI use in patients with melanoma and lung cancer.[13]

Here we provide a comprehensive review of the existing data about the occurrence of VTE or ATE in association with ICI therapy of various malignancies. We examine the candidate mechanisms of thrombosis in this setting, provide an overview of the

documented incidence in different cohort studies, and analyze risk factors for thromboembolism in patients receiving ICIs.

Immune checkpoint inhibitors as therapeutics in cancer

ICIs target 'immune checkpoints' stimulatory and inhibitory processes that directly affect immune cells. Homeostatic immune checkpoints balance pro- and anti-inflammatory signals.[14] Tumor microenvironment immune cells become anergic due to regulatory T cells recruitment, persistent inflammation, and the production of chemicals such CTLA-4 or PD-L1, which drive immune cell proliferation and evasion. The most researched immunological checkpoints are CTLA-4, PD-1, and PD-L1. T-cell receptors activate T cells.[14] T-lymphocytes express CTLA-4 protein receptors, which compete with CD28 to generate cytokines like interleukin (IL)-1. T cells produce less proinflammatory cytokines and have lower overall survival (OS) when CTLA-4 is activated.[15]

PD-1 is another anti-tumor T-lymphocyte receptor. T-lymphocyte survival, proinflammatory cytokine production, and proliferation are inhibited by PD-1 activation. Tumor cells reduce T-lymphocyte anti-tumor activity by producing PD-L1. ICIs assault cancer cells via CTLA-4 and PD-1/PD-L1 activation.[15] A full list of approved ICI agents to date is shown in Table 1.[14]

Incidence of venous thromboembolism and arterial thromboembolism

ICIs can cause immune-related gastrointestinal, cutaneous, thyroid, or hematological disorders like autoimmune hemolytic anemia or thrombocytopenia collectively known as immune-related adverse events; although initial studies did not recognize VTE or ATE as such.[9] The earlier systematic review and meta-analyses of 68 studies (18 retrospective studies and the remaining clinical trials examining the efficacy of ICIs, N=20,273) found 1.1% (95% CI 0.65-1.45%) and 2.7% (95% CI 1.4-5.4%) of ATE and VTE in cancer patients receiving ICIs.[12] A recent meta-analysis found that ICI users had no higher risk of VTE than non-ICI users [odds ratio (OR) 0.99, 95% CI 0.82-1.19].[16] However, the challenge with relying on RCT data is that RCTs may have underreported thrombosis events.[17]

Multiple post-marketing cohort studies have examined rates of thromboembolism in patients with various malignancies receiving ICIs, ICI plus chemotherapy, or chemotherapy alone. Table 2 lists ICI patients' VTE and/or ATE characteristics and published study results. A large single-institution study evaluated the incidence of VTE; of 1,686 patients, 404 (24%) experienced VTE during immunotherapy (using an expanded definition of VTE to include visceral thrombotic events). In a similar retrospective study by the Vienna group, of 672 patients, 47 VTE events occurred during a median follow-up of 8.5 months [cumulative incidence 12.9% (95% CI, 8.2-18.5)].[1] Another large cohort study of 2854 patients found a VTE rate of 7.4% at 6 months and 13.8% at 1 year.[18] The risk of VTE increased over 4-fold after initiating ICI therapy [hazard ratio (HR) 4.98, 95% CI 3.65-8.59, P<0.001]. DVT risk increased by 5.7-fold (HR 5.70, 95% CI 3.79-8.59, P<0.001) and PE risk increased by 4.75-fold (HR 4.75, 95% CI 3.20-7.10, P<0.001).

Thrombosis in ICI patients was evaluated in three Danish population cohort studies.[8,19,20] Two reported both ATE and VTE,[19,20] whereas one reported only VTE.[8] These studies reported a 2-4% VTE rate at 6 months and a 4-7% rate at 12 months, lower than retrospective studies. The difference in incidence rates may be due to the use of ICD10 codes and/or imaging codes in population research, rather than individual record review.[17]

Table 1. Immune checkpoint inhibitors listed by generic names, their year of approval (Food and drug administration), and cancers for which they are used.[15,53]

Generic name	Approval year	Indications
Anti-CTLA-4 antibody		
Ipilimumab	2011	Melanoma, CRC, RCC
Anti-PD-1 antibodies		
Pembrolizumab	2014	Cervical cancer, RCC, urothelial carcinoma, gastroesophageal adenocarcinoma, esophageal cancer, HCC, HNSCC, NSCLC, Hodgkin's lymphoma, large B-cell lymphoma, melanoma, MCC
Nivolumab	2014	CRC, HCC, HNSCC, Hodgkin's lymphoma, melanoma, NSCLC, SCLC, RCC, urothelial carcinoma
Cemiplimab	2018	Cutaneous SCC
Dostarlimab (with chemo)[54]	2023	Primary advanced or recurrent endometrial cancer that is mismatch repair deficient (dMMR), as determined by an FDA-approved test, or microsatellite instability-high[54]
Anti-PD-L1 antibodies		
Avelumab	2015	MCC, RCC, urothelial carcinoma
Atezolizumab	2016	Breast cancer, NSCLC, SCLC, MCC, urothelial carcinoma, RCC
Durvalumab	2016	NSCLC, urothelial carcinoma

CRC, colorectal cancer; RCC, renal cell carcinoma; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer; MCC, Merkel cell carcinoma; SCLC, small cell lung cancer; SCC, squamous cell carcinoma; PD-L1, programmed cell death protein ligand-1.

However, Mulder *et al*. found that ICI patients had a 6-month cumulative incidence of VTE of 4.1% (95% CI 2.3-6.7%), similar to chemotherapy patients' 3.5%.[8]

These studies show a high frequency or incidence of VTE, although cancer patients in general have high rates of VTE throughout therapy. Since control groups receiving chemotherapy alone were rarely included, the retrospective cohort studies alone cannot definitively prove an increased risk compared to chemotherapy. A US claims-based study examined VTE risk variables and incidence in advanced lung cancer patients under-

Table 2. Summary of incidence rates of venous and arterial thrombosis from select studies of cancer patients receiving immune checkpoint inhibitors.[17]

Study	Country	Study design	N (cohort size)	Type of cancer	Follow-up [Median (IQR)]	VTE incidence % (95% CI)	ATE incidence % (95% CI)
Hegde *et al.*, 2017[39]	USA	Retrospective	76	Lung	10.8 mo	18.4	2.6
Ibrahimi *et al.*, 2017[55]	USA	Retrospective	154	Lung 20.8% Melanoma 20.1% Ovarian 12.3%	7 mo (198 days)	10.4	0
Hsu *et al.*, 2018[56]	Taiwan	Retrospective	50	Non-small cell lung cancer (n=24, 48%) Hepatocellular carcinoma (n=4, 8%)	N/A	2	N/A
Bar *et al.*, 2019[28]	Israel	Retrospective	1215	All cancers Melanoma 40.5% Lung 28.7%	12 mo	AVE (including MI, stroke, PE, multisite DVT): 6 mo: 2.6 12 mo: 3.0 AVE plus single site DVT:6 mo: 4.9 12 mo: 5.8	
Nichetti *et al.*, 2019[47]	Italy	Retrospective analysisfrom prospective APOLLO cohort	217	NSCLC	37.8 mo	7.4	6.5
Ando *et al.*, 2020[41]	Japan	Retrospective	122	Lung, kidney, stomach, urothelial, melanoma	N/A Time to thrombosis 90 days (range 6-178)	4.1 Likely 6 mo rate	4.9
Drobni *et al.*, 2020[29]	USA	Retrospective	2842	All cancers NSCLC 28.8% Melanoma 27.9%	2 years	N/A	Composite: 5.35/100 person-years MI: 2.49 Stroke: 2.08
Deschênes-Simard *et al.*, 2021[46]	Canada	Retrospective	593	NSCLC	12.7 (4.9-22.7) mo	9.9 (7.5-12.3) 76.5 (59.9-97.8) per 1000 person-years	1.3
Gong *et al.*, 2021[18]	USA	Retrospective	2854	All cancers NSCLC 28.4% Melanoma 28.2%	194 days (IQR 65-412)	6 mo: 7.4 12 mo: 13.8	N/A
Gutierrez-Sainz *et al.*, 2021[40]	Spain	Retrospective	229	Lung 48% Melanoma 23.6% RCC 11.8%	9.8 mo	7 (4-10)	N/A
Guven *et al.*, 2021[48]	Turkey	Retrospective	133	RCC 26.3% Melanoma 24.1% NSCLC 18.8%	10.1 (5.8-18.5) mo	11.3	N/A
Haist *et al.*, 2021[57]	Germany	Retrospective	280	Melanoma	28 mo (95% CI 23.4-32.6)	12.5	4.3
Hill *et al.*, 2021[26]	USA	Retrospective	435 (a) ICI: 171 (b) ICI+chemo: 157 (c) chemo then durvalumab: 107	NSCLC	N/A	6 mo: (a) 7.6 (4.3-12.2) (b) 9.9 (5.8-15.3) (c) 9.4 (4.8-15.8) 12 mo: (a) 9.0 (5.3-14.0) (b) 12.8 (7.8-19.0) (c) 12.2 (6.8-19.2)	N/A
Icht *et al.*, 2021[51]	Israel	Retrospective	176	NSCLC	6 mo (187 days)	4.5 (2.1-8.3)	N/A

To be continued on next page

going first-line ICI-, chemotherapy-, or ICI + chemotherapy regimens.[21] Among 2299 eligible patients (ICI-based, n=605; chemo-based, n=1092; ICI + chemotherapy, n=602) with a median follow-up of 9.1 months, the VTE incidence rates (95% CI) per 100 person-years were 17.8 (95% CI 16.0 to 19.5) overall, 13.5 (95% CI 10.6 to 16.5) for ICI-based, 18.0 (95% CI 15.5 to 20.5) for chemo-based, and 22.4 (95% CI 20.2 to 24.5) for ICI + chemotherapy.[21] Due to the wide diversity of underlying malignancies, accompanying cancer therapies such as chemotherapy, and variable follow-up periods, thrombosis rates varied quite widely between studies. Overall, VTE incidence was 5-8% at 6 months and 10-15% at 12 months.

In general, retrospective cohort studies showed higher rates than RCTs (1-2% in meta-analysis),[12,13,17] but not substantially higher when considering the 9-10% 6-month VTE risk in ambulatory cancer patients with a Khorana score of ≥2 undergo-

Table 2. Continued from previous page.

Study	Country	Study design	N (cohort size)	Type of cancer	Follow-up [Median (IQR)]	VTE incidence % (95% CI)	ATE incidence % (95% CI)
Kewan *et al.*, 2021[50]	USA	Retrospective	552	All cancers NSCLC 47.3%	12.1 mo	12.1	1.3
Madison *et al.*, 2021[58]*	USA	Retrospective	6127	Lung	6 mo	6.3	2.6
Moik *et al.*, 2021[1]	Austria	Retrospective	672	Melanoma 30.4% NSCLC 24.1% RCC 11%	8.5 mo	6 mo: 5.0 (3.4-6.9) 12mo: 7.0 (5.1-9.3) Overall: 12.9 (8.2-18.5)	6 mo: 1.0 (0.4-2.0) 12 mo: 1.8 (0.7-3.6) Overall 1.8 (0.7-3.6)
Mulder *et al.*, 2021[8]**	Denmark	Population cohort	370	All cancers	6 12	4.1 (2.3-6.7) 7.1 (4.2-11.1)	N/A
Roopkumar *et al.*, 2021[25]	USA	Retrospective	1686	Lung 49.6% Melanoma 13.2%	438 days (range 7-1971)	6 mo: 7.1 12 mo: 10.9 Overall: 24	N/A
Sheng *et al.*, 2021[24]	USA	Retrospective	351	RCC	12.8 mo	11 Total thromboembolism: 6 mo: 4.4 (2.6-6.9) 12 mo: 9.8 (6.8-13.4)	2
Sussman *et al.*, 2021[45]	USA	Retrospective	228	Melanoma	27.3 mo	6 mo: 8.0 (4.9-12.0) 12 mo: 12.9 (8.9-17.7)	6 mo: 2.2 (0.8-4.8) 12 mo: 4.5 (2.3-7.8)
Alma *et al.*, 2022[49]	France	Retrospective	481	Lung	9.8 mo	9.8	N/A
Bjornhart *et al.*, 2023[42]	Denmark	Retrospective	146 prospective (A) * 426 retrospective (B)	NSCLC	16.5 mo	6 mo: 13.0 12 mo: 14.4 Overall: 14 6 mo: 4.9 12 mo: 5.6 Overall: 6	N/A
Canovas *et al.* 2022[43]	Spain	Retrospective	665 291	Lung Melanoma	14 mo 17 mo	6.9 All thrombosis: 8.4 (6.23-10.6) 4.8 All thrombosis: 5.8 (3.34-9.18)	1.5 1
Endo *et al.* 2022[44]	Japan	Retrospective	120	Lung	Within 6 mo	2.5	4.2
Khorana *et al.* 2023[21]*	USA	Retrospective	(a) ICI: 605 (b) ICI+chemo: 602	N SCLC	9.1 mo	6 mo: (a) 8.1 (b) 12.8 12 mo: (a) 13.5 (10.6-16.5) (b) 22.4 (20.2-24.5)	N/A
May *et al.* 2022[59]*	USA	Retrospective	1823	All cancers	6 mo	7.3	N/A
Sanfilippo *et al.* 2022[27]*	USA	Retrospective	1754	All cancers	6 mo	4.1	N/A
Sheng *et al.* 2022[23]	USA	Retrospective	279	Urothelial	5.6 mo	13 Total thromboembolism: 6 mo: 9.1 (6.0-13.0) 12 mo: 13.6 (9.6-18.4)	2
Moik *et al.* 2021[19]**	Denmark	Population cohort	3259	All cancers	6 12 24	3.9 (3.3-4.7) 5.7 (4.9-6.6) 7.3 (6.2-8.4)	1.3 (0.9-1.8) 2.2 (1.7-2.8) 3.1 (2.4-3.8)
Overvad *et al.* 2022[20]**	Denmark	Population cohort	3946	All cancers	6 12	2.6 3.8	1.3 1.9

*Outcomes identified by ICD codes; **Outcomes were identified by ICD10 codes +/− imaging codes. CI, confidence interval; MI, myocardial infarction; mo, months; N/A, not available; USA, United States of America. IQR, interquartile range; VTE, venous thromboembolism; ATE, arterial thromboembolism; AVE, acute vascular event; PE, pulmonary embolism; DVT, deep vein thrombosis; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; ICI, immune checkpoint inhibitor.

ing chemotherapy.[17,22] Since ICIs can substantially prolong survival, their use in cancer therapy may increase the risk of thrombosis as a function of exposure time.[17] A study found that metastatic renal cell carcinoma and urothelial carcinoma patients' thrombosis rates plateaued after 30 and 36 months, respectively.[23,24] This shows that thrombosis risk increases with ICIs therapy duration.

Further, a recent meta-analysis found that combination ICIs increased the incidence of myocardial infarction and VTE in non-small cell lung cancer patients.[13] A different study found that combined-ICIs did not enhance risk.[25] Other trials found similar risk of ICI-chemotherapy combination compared to chemotherapy alone,[26,27] and comparable thrombosis rates.[17,28] Given the frequent significant baseline differences in these patients, comparing the risks of thrombosis linked with chemotherapy plus ICIs to ICI alone or chemotherapy alone may be problematic.

Arterial thromboembolism incidence

ATE incidence data are scarce. Some studies suggest rates within 1-2% over 6-17 months (Table 2). In a study of various malignancies, ATE incidence at 6 months, 12 months, and 24 months was 1.3 (95% CI 0.9-1.8), 2.2 (95% CI 1.7-2.8), 3.1 (95% CI 2.4-3.8), respectively.[19] In contrast, in a Vienna cohort study, 9 ATE events occurred in 672 patients [cumulative incidence 1.8% (95% CI, 0.7-3.6)].[1] ICIs were associated with threefold more cardiovascular (CV) events than other anticancer therapies in a matched cohort analysis of 2842 patients by Drobni *et al*. In the same matched cohort study, the comparison of risk increases in before and after ICI use were also similar.[29] A recent meta-analysis of 61 studies found that ICI regimens increased ATE risk [odds ratio (OR) 1.58, 95% CI 1.21-2.06].[16] More research is needed to evaluate if reducing and managing CV risk factors can help this population.

Candidate mechanisms and biomarkers

The exact mechanisms of ICI-related thrombosis are not fully understood. ICIs' principal immune-related toxicity mechanisms involve T-cell-mediated autoimmune-like reactions.[12] Impaired immunological checkpoints are connected to pro-inflammatory conditions and elevated cytokine levels.[9] By boosting pro-inflammatory T cells and macrophages in atherosclerotic plaques, PD-1 blockade accelerates atherogenesis, vascular inflammation, and atherosclerosis.[30,31] Pre-clinical animal models demonstrate that ICI use promotes atherosclerotic disease, which may increase arterial thrombotic events.[9] Activated T cells can also increase tissue factor production by macrophages and monocytes, increasing hypercoagulability.[9,32,33] In a pilot translational study, blood samples from 25 patients (15 of whom developed VTE after starting ICIs, and 10 of whom did not) at the time of starting the treatment (ICIs) were analyzed. Results showed pretreatment levels of myeloid-derived suppressor cells (5.382±0.873 *vs*. 3.341±0.3402, mean±SEM; P=0.0045), IL-8 (221.2±37.53 *vs*. 111.6±25.36, mean±SEM; P=0.016), and soluble vascular cell adhesion molecule 1 (1210±120.6 *vs*. 895.5±53.34, mean±SEM; P=0.0385) were significantly higher in patients that developed VTE. These indicators suggest a role for immune-mediated inflammation and shed light on thrombus development in ICI patients.[17,25]

To maximize patient benefit, limit toxicity, and direct combination therapy, predictive biomarkers are needed. An important study evaluated whether early C-reactive protein (CRP) dynamics could predict ICI-associated VTE.[34] In 405 patients, CRP was measured at baseline and every 4 weeks for the first 3 months of ICI therapy. A 2.5-fold spike in CRP indicated a flare, whereas a 50% decline was defined as a response. In a multivariable analysis that included death, an early CRP flare was associated with VTE [HR 3.58 (95% CI 1.07-11.94)]. Patients with CRP response had the lowest VTE risk. In a follow-up study, early CRP kinetics were also found to serve as a tumor-agnostic predictor of treatment response, progression risk, and mortality.[35]

A small study of 30 patients receiving ICIs found that a baseline high sensitivity (hs)-troponin T (TnT) ≥14 ng/L was associated with a higher risk of CV outcomes/primary endpoints, including death, stroke, transient ischemic attack (TIA), PE, and/or heart failure (HF).[17,36] Therefore, only individuals with hs-TnT ≥14 ng/L before the first cycle died from stroke/TIA or new-onset HF. Additionally, nine out of thirteen patients with progression of cardiac disease exhibited hs-TnT ≥14 ng/L before the first cycle (P=.012).[36] In another retrospective study of 135 patients receiving first-line pembrolizumab for metastatic cancer, hs-troponin I (TnI) >50 ng/L before the first and second doses was an independent predictor of major adverse cardiac events, such as myocarditis, acute coronary syndrome, HF, VTE, CV hospitalization, and/or mortality (HR 8.1, 95% CI 1.67-37.4) after a mean follow-up of 490 days.[37] Before the first pembrolizumab dose, hs-TnI >50 nl/L increased all-cause death risk. In view of these findings, the European Society of Cardiology 2022 cardio-oncology guidelines recommend monitoring cardiac troponin before each ICI cycle.[17, 38] Table 3 lists candidate biomarkers with their rationales.

Risk factors

Understanding ICI cancer patients' thrombosis risk variables may help identify high-risk patients and target thromboprophylaxis. Multiple cohort studies have attempted to identify risk factors associated with ICI-related VTE (Table 4). In the Vienna

Table 3. Candidate biomarkers of immune checkpoint inhibitor-associated venous thromboembolism or arterial thromboembolism.

Biomarker	Outcome
MDSC[25]	VTE development after ICI initiation
IL-8[25]	VTE development after ICI initiation
sVCAM-1[25]	VTE development after ICI initiation
Early CRP flare*[34]	Risk of ICI-associated VTE
CRP response**[34]	Lower risk of ICI-associated VTE
hs-TnT ≥14 ng/L***[36]	Higher risk of CV outcomes
hs-TnI >50 ng/L[37]	Independent predictor of major cardiac events^

MDSCs, myeloid-derived suppressor cells; VTE, venous thromboembolism; ICI, immune checkpoint inhibitor; IL-8, interleukin-8; sVCAM-1, soluble vascular cell adhesion molecule-1; CRP, C-reactive protein; hs-TnT, high sensitivity troponin T; hs-TnI, high sensitivity troponin I. *CRP flare is when it increases 2.5 times; **CRP response is when it is decreased by 50%; ***baseline hs-TnT ≥14 ng/L; ^major cardiac events including myocarditis, acute coronary syndrome, heart failure, VTE, cardiovascular hospitalizations and cardiac death.

cohort study, a prior VTE history predicted future VTE [sub-distribution HR (SHR), 3.69; 95% CI, 2.00-6.81]. Patient-related factors like: female gender,[39,40] history of thromboembolism,[1,18,28,41-45] younger age,[18,25,46] smoking,[46,47] and poor Eastern cooperative oncology group status,[44,48] history of hypertension) were among the potential risk factors identified.[18] Cancer-related factors like lung cancer,[28,29] metastasis,[25,42,49] were also identified. Interestingly, history of melanoma showed a decreased chance of VTE.[18] For treatment-related factors: combined ICI use (16.7% *vs*. 5.0% at 6 months and 21.3% *vs*. 9.5% at 12 months, respectively; P=0.02),[45] combined chemotherapy-ICI use have been identified as risk factors for VTE.[17,26,27] However, it is still an area of discussion whether ICI combination regimens or ICI chemotherapy regimens increase VTE risk compared to any one medication alone.[17] Also, Sheng *et al*. concluded that in a cohort of urothelial cancer patients on ICIs are associated with a higher risk of thromboembolism.[23] Drobni *et al*. studied the use of steroids or statins and found that statins or corticosteroids can attenuate ICI-induced total aortic plaque volume growth by more than threefold.[17,29] Although arterial thrombosis data are scarce, studies showed risk variables such as age, diabetes, hypertension, smoking, and a history of CV illness like traditional athrosclerosis.

The Khorana score (KS) has previously been validated to predict risk in heterogenous cancer population receiving systemic therapy. Its value in prediction in an immunotherapy-only cohort has not been fully evaluated. Some studies found KS to predict for risk of VTE[18,20,45] whereas others did not.[1,24,26,42,47-51]

Immune checkpoint inhibitors-related thromboembolism and survival

While some studies have found no correlation between thrombosis and poor survival in patients receiving ICIs,[40,46,48]

Table 4. Select risk factors for thrombosis identified in cancer patients receiving immune checkpoint inhibitors.

Study	Risk factors for thrombosis (multivariable)[17]	Result highlights
Hegde *et al.*, 2017[39]	Female	In the multivariate analysis, gender was the only covariate that significantly correlated with thromboembolic events (Female *vs.* Male HR 3.1, 95% CI 1.02-9.5, P=0.045)
Bar *et al.*, 2019[28]	NSCLC	Whether using chemotherapy or ICIs, the AVE rate for patients with adenocarcinoma of NSCLC was comparable
	H/o AVE	Two percent (2.6%) of patients experienced AVEs within six months of the start of their ICIs (95% CI 1.8-3.6).
	Hypertension Dyslipidemia	Lung adenocarcinoma, prior AVE, hypertension and dyslipidemia were correlated with AVEs
Nichetti *et al.*, 2019[47]	Current smoker PD-L1 >50%	Smokers (42.9% *vs.* 23.3%, p = 0.05) [compared to no TE event group] PD-L1 expression >50% (43.3 *vs.* 18.8%, P=0.01) [compared to no TE event group]
Ando *et al.*, 2020[41]	h/o thromboembolism	One risk factor for the incidence of CAT was a history of arterial or venous thromboembolism (ATE or VTE) (odds ratio: 6.36, P=0.039)
Drobni *et al.*, 2020[29]	Overall study: ICIs, age, h/o stroke, diabetes, hypertension, NSCLC, male, h/o radiation	A matched cohort analysis showed a three-fold increase in CV events after ICI beginning (hazard ratio, 3.3 [95% CI, 2.0-5.5]; P<0.001). At 2 years, CV events increased from 1.37 to 6.55 per 100 person-years in the case-crossover (adjusted hazard ratio, 4.8 [95% CI, 3.5-6.5]; P<0.001). ICIs increased overall aortic plaque volume progression >3-fold (from 2.1%/y before to 6.7%/y after) in the imaging research
Deschênes-Simard *et al.*, 2021[46]	Age <65 Higher PD-L1 level Smoking <12 mo from diagnosis to ICIs	Patients aged <65 (HR =2.00; 95% CI =1.11-3.59) Tumors with PD-L1 between 1-49% (HR =3.36; 95% CI = 1.19-9.50) or >50% (HR =3.22; 95% CI =1.21-8.57) Active smoking (HR =2.00; 95% CI =1.12-3.58) A time lag of less than 12 months between diagnosis and first ICI treatment (HR =2.06; 95% CI =1.09-3.89)
Gong *et al.*, 2021[18]	Age ≤65 Khorana score ≥2 h/o hypertension Strong trend: h/o VTE (HR 1.42, 95% CI 0.99-2.06) (melanoma is associated with decreased risks)	After initiating an ICI, the risk of VTE was 7.4% at six months and 13.8% at a year After initiating an ICI, the rate of VTE increased by a factor of four (HR 4.98, 95% CI 3.65-8.59, P<0.001) Deep vein thrombosis (HR 5.70, 95% CI 3.79-8.59, P<0.001) and pulmonary embolism (HR 4.75, 95% CI 3.20-7.10, P<0.001) were associated with 5.7- and 4.75-fold greater risks, respectively A history of melanoma and advanced age predicted a decreased risk of VTE, but a higher Khorana risk score, a history of hypertension, and a history of VTE suggested a higher risk when comparing individuals with and without a VTE incident

To be continued on next page

others have.[1,25,28,45,47] In a large study, Roopkumar *et al*. found that patients on ICIs who developed VTE had decreased OS [HR=1.22 (95% CI 1.06-1.41), P<0.008].[25] In the Vienna cohort study, VTE was associated with shorter OS as well (transition HR for death, 3.09; 95% CI, 2.07-4.60).[1] Similarly, another retrospective study showed that VTE was linked to a shorter OS in 219 immunotherapy-treated melanoma patients without brain metastases (median OS 1.3 years *vs*. not reached;P<0.001; HR 3.47 [95% CI, 1.66-7.24]).[52] Bar J. *et al*. observed a significant link between VTE and shorter survival.[28] On the other hand, a study found no correlation between VTE and poor OS [HR 1.33 (95% CI 0.63-2.80), P=0.44],[40] and another found no correlation between VTE and OS in thrombosis patients [HR 1.18 (95% CI 0.83-1.70), P=0.335.[46] In a cohort specifically studying urothelial cancer patients on ICIs, showed that the thromboembolism was associated with lower OS (HR 2.296, P=0.0004) with Bajorin score 1 or 2 (HR 1.490, P=0.0315), and Bajorin score 2 (HR 3.50, P<0.0001).[23] It is unclear whether this association with OS represents a biologic correlation (*i.e.*, VTE is a surrogate for aggressive tumor biology or tumor immune escape mechanisms) or simply an association with higher tumor burden.

Conclusions

ICIs represent a paradigm shift in treatment of malignancy, and their use is only expected to grow in the near future. Results

Table 4. Continued from previous page.

Study	Risk factors for thrombosis (multivariable)[17]	Result highlights
Gutierrez-Sainz *et al.*, 2021[40]	Female Melanoma	Melanoma and female sex were found to be independently l inked to a higher incidence of VTE Melanoma was also independently associated with [HR 2.42 (1.20-4.86), P=0.01] shorter OS
Guven *et al.*, 2021[48]	ECOG ≥1	A higher incidence of venous thrombosis was observed in patients (29.3% of patients) with a baseline ECOG performance level of 1 or higher (ECOG ≥1 *vs.* 0, HR: 3.023, 95% CI: 1.011-9.039, P=0.048)
Hill *et al.*, 2021[26]	Cancer treatment types (ICI-chemotherapy, targeted therapies) Smoking	Treatment type (P=0.034) Nicotine dependency (P=0.048)
Kewan *et al.*, 2021[50]	Anticoagulation at the time of ICI (univariate)	Incidence rate ratio: 2.23a
Moik *et al.*, 202[11]	h/o VTE	SHR, 3.69; 95% CI, 2.00-6.81
Roopkumar *et al.*, 2021[25]	Younger age Metastasis Biomarkers	Pretreatment levels of myeloid-derived suppressor cells (5.382 G 0.873 *vs.* 3.341 G 0.3402, mean G SEM, P=0.0045), interleukin 8 (221.2 G 37.53 *vs.* 111.6 G 25.36, mean G SEM, P=0.016), and soluble vascular cell adhesion protein 1 (1,210 G 120.6 *vs.* 895.5 G 53.34, mean G SEM, P=0.0385) were significantly higher in those who developed venous thromboembolism
Sussman *et al.*, 2021[45]	Combination ICI Khorana score ≥1 h/o CAD Anticoagulation at treatment start	Combination ICI (HR 2.70; 95%CI: 1.28 to 5.70; P=0.009) Khorana score ≥1 (HR 2.24; 95%CI: 1.06 to 4.74; P=0.03) History of coronary artery disease HR 2.71; 95%CI: 1.16 to 6.29; P=0.02) Anticoagulation at treatment start (HR 4.14; 95% CI: 1.60 to 10.7; p=0.003)
Alma *et al.*, 2022[49]	Metastasis BMI	Metastatic patients (11.1% *vs.* 1.5%, P=0.015) [univariate analysis] BMI (OR 1.07; 1.01-1.14, P=0.028) [logistic regression]
Bjornhart *et al.*, 2022[42]	h/o VTE ICI as first-line treatment Other mets (non-brain, liver, bone)	VTE was substantially linked to a lower OS in a multivariate analysis (HR 2.12 CI 95% [1.49-3.03], P<0.0001)
Canovas *et al.*, 2022[43] Lung cancer cohort	Hgb <10.9 g/dL at the start of ICI NLR <4.55 h/o thrombosis	HR 2.05; 95% CI: 1.14 to 3.69; P=0.008 [multivariate analysis] HR 2.14; 95% CI: 1.24 to 3.67; P=0.010 [multivariate analysis] HR 2.45; 95% CI: 1.2 to 5.01; P=0.010 [multivariate analysis]
Canovas *et al.*, 2022[43] Melanoma cohort	LDH >198 U/L NLR >3.01	HR 4.51; 95% CI: 1.01 to 20.24; P=0.049 [multivariate analysis] HR 3.65; 95% CI: 1.25 to 10.62; P=0.018 [multivariate analysis]
Endo *et al.*, 2022[44]	ECOG ≥2 h/o of thromboembolism	OR 3.84; 95% CI: 1.34 to 11.00; P=0.01 OR 6.03; 95% CI: 2.09 to 17.40; P<0.001
Khorana *et al.*, 2023[21]	History of radiation BMI ≥40 kg/m^2	Baseline radiation: HR, 1.25; P=0.03 Severe obesity (BMI ≥40 kg/m2): HR, 1.77; P=0.06
Sanfilippo *et al.*, 2022[27]	ICI-chemotherapy (*vs.* ICI alone)	ICI-chemotherapy HR =1.75 (95% CI: 1.07-2.83)

HR, hazard ratio; CI, confidence interval; ICI, immune checkpoint inhibitor; AVE, acute vascular event; NSCLC, non-small cell lung cancer; PD-L1, programmed cell death protein ligand-1; TE, thromboembolism; ATE, arterial thromboembolism; VTE, venous thromboembolism; CV, cardiovascular; OS, overall survival; ECOG, eastern cooperative oncology group; CAD, coronary artery disease; Hgb, hemoglobin; h/o, history of; IMDC: International Metastatic Renal Cell Carcinoma Database Consortium; BMI; body mass index; IQR, interquartile range; KS, Khorana score; OR, odds ratio; LDH, lactate dehydrogenase; NLR, neutrophil/lymphocyte ratio.

of multiple recent cohort and population studies indicate a high incidence and/or prevalence of both VTE and ATE in association with ICI therapy, although it is unclear whether this is higher than observed with chemotherapy and whether it is a function of increased exposure time given substantial prolongation in survival with ICI therapy. Ongoing studies are evaluating mechanisms and candidate biomarkers have been identified. The association of ICI-related VTE/ATE with worsened survival is of particular concern and deserves further investigation as does the benefit of primary thromboprophylaxis.

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Factor XI inhibitors: a new option for the prevention and treatment of cancer-associated thrombosis

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ABSTRACT

Venous thromboembolism (VTE) is a relatively common complication in cancer patients with potentially dire consequences. Anticoagulants are the mainstay of treatment of cancer-associated VTE. The anticoagulants most often used are low-molecular-weight heparin (LMWH) and direct oral factor (F) Xa inhibitors, which include apixaban, edoxaban, and rivaroxaban. Most guidelines recommend primary VTE prophylaxis with LMWH, apixaban, or rivaroxaban after abdominal or pelvic cancer surgery, or in high-risk ambulatory cancer patients. Both oral FXa inhibitors and LMWH have limitations. LMWH requires daily subcutaneous injections, and because of its renal clearance, its use may be problematic in patients with severe kidney disease. The risk of bleeding with oral FXa inhibitors may be higher than with LMWH in patients with intraluminal gastrointestinal or genitourinary cancers. Other problems with oral FXa inhibitors include potential drug-drug interactions and dosing issues in patients with thrombocytopenia or severe kidney or liver disease. Therefore, there remains a need for convenient and safer anticoagulants for VTE treatment in cancer patients. FXI has emerged as a potentially safer target for anticoagulants than FXa because FXI is essential for thrombosis, but mostly dispensable for hemostasis. This review summarizes the currently available therapeutic options for cancer-associated VTE, highlights knowledge gaps, and discusses the potential of FXI inhibitors to address key unmet clinical needs in this vulnerable patient population.

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Introduction

Venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) and pulmonary embolism, is relatively common in patients with cancer.[1] Over the last few decades, the incidence of VTE has remained relatively stable in the general population, whereas it has progressively increased in cancer patients.[2] In a large population-based study, the 6-month VTE risk in patients with cancer was 12-fold higher than that in subjects without cancer, and up to 23-fold higher among those receiving chemotherapy or targeted treatments.[2]

Cancer-associated thrombosis (CAT) represents the second most common cause of death in cancer patients after tumor progression,[1] and it's associated with substantial morbidity including repeated hospitalizations and delays or interruptions in potentially curative cancer-directed treatments.[3,4] CAT is a distressing experience for patients and their relatives, increasing the psychological burden on vulnerable individuals already overwhelmed by the cancer diagnosis and its treatment.[4,5] Treatment of VTE in cancer patients is challenging because of their heightened risk of recurrence and bleeding compared with patients without cancer.[6,7] Both recurrent VTE and bleeding are associated with poorer quality of life, deferral or disruption of cancer treatments, and increased healthcare costs.[8]

The purpose of this review is to summarize the current therapeutic options for CAT, highlight current knowledge gaps, and explain how the new generation of factor (F) XI inhibitors may address unmet clinical needs.

Current treatment of cancer-associated thrombosis

Low molecular-weight heparin (LMWH) and direct oral FXa inhibitors, which include apixaban, edoxaban, and rivaroxaban are the current standards of care for CAT. In patients with CAT, studies have shown that oral FXa inhibitors are at least as effective as LMWH, and associated with a non-significant increase in major bleeding.[9-14] Recent clinical guidelines suggest oral FXa inhibitors as an alternative to LMWH for most patients with CAT.[15-18] Oral FXa inhibitors are more convenient to administer than LMWH, but their use may be problematic in patients with impaired gastrointestinal absorption secondary to vomiting, diarrhea, or upper gastrointestinal surgery. Patients with gastroesophageal or genitourinary cancers, especially those with unresected luminal tumors, had a higher risk of bleeding with edoxaban or rivaroxaban than with dalteparin.[9,10,19,20] Although the risk of bleeding with apixaban was similar to that with dalteparin,[13] recent guidelines suggest the use of LMWH rather than oral FXa inhibitors in patients with unresected gastrointestinal or genitourinary cancers.[15-18] All oral FXa inhibitors are substrates of P-glycoprotein, and apixaban and rivaroxaban are metabolized by cytochrome (CYP) 3A4. Consequently, the concomitant use of drugs that affect these pathways could influence drug concentrations and increase the risk of bleeding or thrombotic complications.[21,22] Most guidelines endorse the preferential use of LMWH in cancer patients receiving strong inducers or inhibitors of P-glycoprotein or CYP3A4.[21] Oral FXa inhibitors are cleared in part by the kidneys and are metabolized in the liver, which limits their utility in patients with severe kidney or liver disease. In addition, since patients with brain cancer, hematological malignancies, or severe thrombocytopenia were excluded or underrepresented in the trials comparing the oral FXa inhibitors with dalteparin for the treatment of CAT, adjusted-dose LMWH is often the preferred option in these patients.

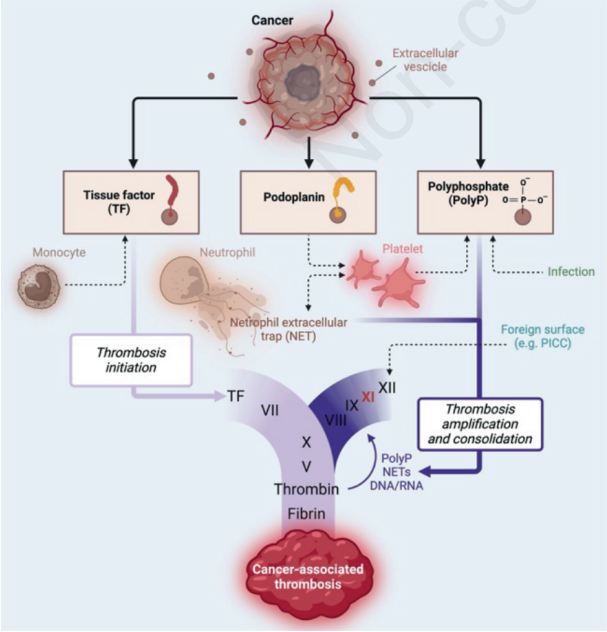

Figure 1. Role of factor XI in the pathophysiology of cancer-associated thrombosis.

Limitations of current cancer-associated thrombosis treatments

Bleeding remains the major concern with both oral FXa inhibitors and LMWH.[20] The risk of bleeding with current anticoagulants is related to their inhibitory effects on both thrombosis and hemostasis because they target FXa and thrombin, which are implicated in the common coagulation pathway.[23] Preclinical and clinical evidence suggests that thrombosis can be uncoupled from hemostasis by targeting FXI, which is situated upstream to FXa in the intrinsic pathway of coagulation and is essential for thrombosis, but mostly dispensable for hemostasis.[23] Therefore, anticoagulants that target FXI have the potential to be safer than the currently available agents.

Role of factor XI in cancer-associated thrombosis

FXI can be activated by FXIIa or by thrombin. Regardless of the activator, FXIa is likely to play a central role in the pathophysiology of CAT (Figure 1).[24] The extrinsic coagulation pathway is initiated by tissue factor (TF) exposed at the site of blood vessel injury, or expressed on leukocytes or microvesicles that become tethered to activated endothelial cells. This pathway is particularly important in patients with cancer because some cancer cell types constitutively express TF and release extracellular vesicles bearing TF.[25,26] In addition, increased expression of TF by monocytes has been reported in cancer patients.[1] TF binds FVII or FVIIa to form the TF-FVIIa complex, which activates FX and FIX. FIXa together with FVIIIa assemble on the surface of activated platelets to form intrinsic tenase, which amplifies and sustains FXa and thrombin generation. Since the capability of the TF-FVIIa complex to propagate coagulation may be limited once the thrombus extends beyond the TF source, feedback activation of FXI by thrombin is thought to be essential for thrombus growth.[23]

Activation of FXI by FXIIa may also contribute to CAT. FXII activation can be triggered by negatively charged polyanions including neutrophil extracellular traps released by activated neutrophils, DNA or RNA released from cancer cells, or by polyphosphate released from activated platelets or from bacteria during infections.[27-31] Central venous catheters (CVCs) are often used in patients with cancer for the administration of chemotherapy or other medications, or for blood product transfusion. Contact of the blood with CVCs triggers FXII activation (Figure 1).[32] The relevance of FXII and FXI to catheter thrombosis is highlighted by studies in rabbits that revealed attenuation of catheter-related thrombosis with reduction in the levels of FXII or FXI with target-specific antisense oligonucleotides (ASOs).[33]

Preclinical and epidemiological evidence supporting factor XI as a safer target than factor Xa

Mice deficient in FXI or FXII exhibited defective thrombus formation at sites of arterial or venous injury without evidence of increased bleeding.[34-41] Likewise, antibodies against FXI inhibited thrombosis in rodent models without affecting bleeding, and a reduction in the level of FXI with ASOs reduced shunt thrombosis in baboons.[41] Studies comparing the effects of antibodies against factor FXI or FXII in non-human primates suggested more potent antithrombotic effects with FXI-directed antibodies.[24,42-44]

Epidemiological data support the relevance of FXI in thrombosis. Elevated FXI levels were associated with increased VTE risk,[45-47] and congenital deficiency protected against VTE and ischemic stroke with little or no bleeding.[48-52] The evidence that FXI deficiency is protective against myocardial infarction is less consistent.[48,49] In Mendelian randomization studies, lower FXI levels were associated with reduced risks of VTE and ischemic stroke without an increased risk of major bleeding,[47] whereas high FXI levels were associated with a higher risk of VTE and ischemic stroke.[52,53] These findings align with the observations that subjects with congenital FXI deficiency rarely have spontaneous bleeding and do not experience the muscle, joint, or intracranial bleeding that often occurs in persons with hemophilia A or B.[54,55] Although spontaneous bleeding is rare in patients with congenital FXI deficiency, bleeding can occur after trauma or surgery, often at anatomic regions with increased fibrinolytic activity such as the gastrointestinal and genitourinary tract, and the nasopharynx.[48-50,54-56]

In contrast to FXI, the epidemiological evidence linking FXII with thrombosis is weaker. Despite robust data showing that FXII deficiency or inhibition attenuates arterial and venous thrombosis in animal models, epidemiologic studies failed to demonstrate protection from thrombosis in patients with FXII deficiency and reported an inconsistent association between higher FXII levels and thrombotic risk.[46,57,58] FXII inhibition may also potentially be of limited benefit in settings such as cancer where TF is the major driver of thrombin generation because feedback activation of FXI by thrombin can bypass FXII inhibition.[37,59] Because of the uncertain role of FXII in thrombosis, FXI has gained attention as the more attractive target.[52]

Pharmacological strategies targeting factor XI

As shown in Table 1, multiple pharmacologic strategies to inhibit FXI are under clinical development. These include: i) ASOs (*e.g.*, fesomersen) that reduce the hepatic synthesis of FXI; ii) monoclonal antibodies (*e.g.*, gruticibart, osocimab, abelacimab) that inhibit FXI activation, FXIa activity, or both; iii) small molecules (*e.g.*, asundexian, milvexian) that block the active site of FXIa (Figure 2). Each strategy has its strengths and weaknesses. ASOs and monoclonal antibodies require parenteral administration, while small molecules are given orally. ASOs have a slow onset of action requiring 3-4 weeks of subcutaneous administration to lower FXI levels within therapeutic ranges, which limits their usefulness for the initial treatment of thrombosis or immediate thromboprophylaxis.[52] Although second-generation ligand-conjugated ASOs like fesomersen have a more rapid onset of action of 1-2 weeks, this is still too slow to enable their use for acute VTE treatment. Small molecules have a rapid onset of action as do monoclonal antibodies if they are given intravenously, achieving maximum plasma concentrations 2-4 hours after administration, and thus enabling their use for acute

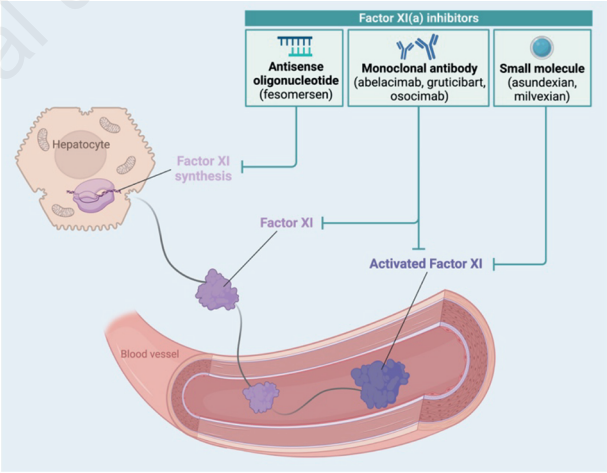

Figure 2. Pharmacologic strategies to inhibit factor XI.

Table 1. Pharmacological features of factor XI-directed strategies.

	Antisense oligonucleotides (fesomersen)	Monoclonal antibodies (abelacimab, gruticibart, osocimab)	Small molecules (asundexian, milvexian)
Mechanism of action	Block synthesis	Bind target protein	Bind target protein
Administration route	Subcutaneous	Intravenous or subcutaneous	Oral
Administration frequency	Weekly to monthly	Monthly	Daily
Onset of action	Slow (weeks)	Rapid (hours to days)	Rapid (1 to 4 hours)
Offset of action	Slow (weeks)	Slow (weeks)	Rapid (12 to 24 hours)
Renal clearance	No	No	Yes
CYP450 metabolism	No	No	Yes*
Potential for drug-drug interactions	No	No	Yes*

*Asundexian is not metabolized via the CYP system. CYP, cytochrome.

management. Small molecules have a short half-life and require once- or twice-daily administration, whereas ASOs and monoclonal antibodies have a long half-life allowing once-monthly subcutaneous dosing. While more convenient, the long half-life of ASOs and monoclonal antibodies could be problematic in case of serious bleeding or trauma, or in patients requiring urgent surgery. Small molecules are partly cleared by the kidneys, and milvexian is metabolized to a small extent by CYP3A4, thus there is a potential for accumulation of asundexian and milvexian in patients with kidney failure and for drug-drug interactions with milvexian.[60-62]

Clinical studies with factor XI inhibitors

The clinical evaluation of new anticoagulants usually starts in patients undergoing major orthopedic surgery because such patients are at risk for postoperative DVT that can be efficiently detected by venography. Although DVT is often asymptomatic in such patients, its presence or absence can help to inform dose selection. Following this drug development pathway, fesomersen, osocimab, abelacimab, and milvexian were compared with enoxaparin for VTE thromboprophylaxis after elective knee replacement surgery.[63-66] A meta-analysis of these studies showed a 40-50% reduction in post-operative VTE and a 59% reduction in clinically relevant bleeding with FXI inhibitors compared with enoxaparin.[67]

The safety of long-term FXI inhibition with abelacimab was highlighted by the results of the phase II AZALEA study that compared monthly subcutaneous abelacimab in doses of 90 mg or 150 mg with rivaroxaban (20 mg once daily) in 1282 patients with atrial fibrillation and a median CHA_2DS_2-VASc score of 5 (https://www.acc.org/Latest-in-Cardiology/Clinical-Trials/2023/11/10/22/46/azalea-timi-71). Abelacimab at the 150 mg dose was associated with a 67% reduction in major and clinically relevant non-major bleeding, a 74% reduction in major bleeding, and a 93% reduction in major gastrointestinal bleeding compared with rivaroxaban. The incidence of stroke and systemic embolism was low (~1%) and comparable between the two groups. Although the results of the AZALEA study have yet to be published, these preliminary data support the safety of long-term potent FXI inhibition with 150 mg of abelacimab compared with rivaroxaban.

Abelacimab is the only FXI inhibitor that has advanced to phase III evaluation in CAT. Abelacimab is currently under investigation in two multicenter, randomized, open-label phase III studies for the treatment of CAT (Table 2). In the ASTER trial (NCT05171049), 1655 patients with cancer and acute VTE, including symptomatic or incidental lower limb acute DVT and/or symptomatic or incidental PE involving a segmental or more proximal pulmonary artery will be randomized to abelacimab or apixaban for 6 months. The MAGNOLIA trial (NCT05171075) will include approximately 1020 patients with unresectable, locally advanced, metastatic, or non-metastatic gastrointestinal or genitourinary cancer and acute VTE. Since edoxaban and rivaroxaban were associated with more bleeding than with dalteparin in patients with these types of cancer and guidelines give preference to LMWH,[15-18] abelacimab is compared with dalteparin in the MAGNOLIA study. In both phase III trials, abelacimab is given at a dose of 150 mg once monthly with the first dose administered intravenously to ensure rapid FXI inhibition and subsequent doses given subcutaneously. Abelacimab has potential limitations including the lack of a specific antidote. However, strategies to prevent or treat bleeding include the administration of tranexamic acid, low-dose FVIIa, or activated prothrombin complex concentrates.[68] Concern has also been raised about the possibility that the high level of TF expression by some tumors may result in such explosive thrombin generation that feedback activation of FXI by thrombin could be potentially bypassed.[37,59] Although this phenomenon has been observed in some animal models, the fact that patient recruitment in the ASTER and MAGNOLIA trials is continuing suggests that this is unlikely to be a major issue in humans.

Gruticibart, an antibody that binds FXI and blocks its activation by FXIIa, was evaluated for the prevention of catheter-related thrombosis in a small, non-randomized phase II trial.[69] In this study, 22 ambulatory cancer patients undergoing central line placement received a single dose (2 mg/kg, through the catheter within 24 of placement) of gruticibart, and underwent ultrasound evaluation on day 14. Compared with no intervention, gruticibart reduced the incidence of catheter-related thrombosis on surveillance ultrasound from 40% to 12.5% (Table 2).[69]

Conclusions and future directions

Robust preclinical and clinical data in patients with atrial fibrillation and patients undergoing orthopedic surgery support FXI inhibition as a potential paradigm shift in the prevention and treatment of CAT. Abelacimab, a monoclonal antibody tar-

Table 2. Completed and ongoing studies of factor XI inhibitors in patients with cancer.

Drug	Mechanism	Route	Study (NCT number)	Indication	N	Comparator
Abelacimab	Monoclonal antibody against FXI and FXIa	Intravenous followed by subcutaneous	ASTER NCT05171049	CAT	1655	Apixaban
		Intravenous followed by subcutaneous	MAGNOLIA NCT05171075	CAT, GI/GU	1020	Dalteparin
Gruticibart	Monoclonal antibody that blocks FXI activation	Intravenous	NCT04465760	Prophylaxis for CVC-related thrombosis in cancer	22	None (single arm)

CAT, cancer-associated thrombosis; CVC, central venous catheter; GI, gastrointestinal; GU, genitourinary; IV, intravenous; SC, subcutaneous.

geting both FXI and FXIa, holds promise for reducing bleeding risk compared with current anticoagulants and overcoming some of the limitations of the oral FXa inhibitors by eliminating the potential for drug-drug interactions and concerns about kidney or hepatic dysfunction. The reduction in gastrointestinal bleeding with abelacimab compared with rivaroxaban observed in the AZALEA trial suggests that eliminating active drugs in the gut may reduce local bleeding, which could provide abelacimab with an advantage over oral FXa inhibitors for CAT treatment in patients with gastrointestinal cancers.

With its long half-life, abelacimab may be an ideal agent for primary prophylaxis in high-risk cancer patients receiving chemotherapy or for postoperative thromboprophylaxis after major cancer surgery. FXI inhibitors may also have a role in the prevention of catheter-related thrombosis, as suggested by a small phase 2 study with gruticibart.[69] The results of the ASTER and MAGNOLIA trials, whose completion is expected in 2025, will establish the safety and efficacy of abelacimab in this highly challenging clinical scenario and may prompt investigation into the utility of the oral FXIa for the prevention and treatment of CAT.

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Identifying novel biomarkers using proteomics to predict cancer-associated thrombosis

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ABSTRACT

Comprehensive protein analyses of plasma are made possible by high-throughput proteomic screens, which may help find new therapeutic targets and diagnostic biomarkers. Patients with cancer are frequently affected by venous thromboembolism (VTE). The limited predictive accuracy of current VTE risk assessment tools highlights the need for new, more targeted biomarkers. Although coagulation biomarkers for the diagnosis, prognosis, and treatment of VTE have been investigated, none of them have the necessary clinical validation or diagnostic accuracy. Proteomics holds the potential to uncover new biomarkers and thrombotic pathways that impact the risk of thrombosis. This review explores the fundamental methods used in proteomics and focuses on particular biomarkers found in VTE and cancer-associated thrombosis.

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Introduction

Venous thromboembolism (VTE) represents a significant global public health issue, impacting roughly 10 million individuals annually worldwide and contributing to over 3 million annual fatalities.[1] Among patients with cancer, thrombotic events are highly prevalent, with active cancer accounting for 20% of the overall incidence of VTE.[1] The annual incidence of VTE in patients with cancer is 5-20% in higher-risk malignancies, compared to 0.1% in the general population.[2] Clinical biomarkers for the diagnosis, risk prediction, recurrence estimation, and response to treatment in cancer-associated thrombosis (CAT) are limited.[3]

D-dimer as a marker of endogenous fibrinolysis, has found utility as a valuable biomarker in clinical practice for the diagnosis of VTE. Nevertheless, it lacks specificity, such that is largely used as a diagnostic assay to exclude VTE, primarily due to its strong negative predictive value.[4] Although various additional biomarkers, including P-selectin, tissue factor (TF), microRNAs, among others, have been investigated, none have been validated sufficiently for routine application in clinical practice.[5,6]

Predicting VTE recurrences continues to pose challenges. D-Dimer in combination with clinical and genetic risk factors has been applied to help predict which patients will develop recurrent VTE following a course of therapeutic anticoagulation. Various studies have indicated that, following an initial spontaneous VTE, patients with low D-Dimer levels have a low risk of VTE recurrence upon discontinuation of anticoagulation.[7,8] Conversely, patients experiencing a provoked VTE with elevated D-Dimer levels after discontinuing anticoagulation therapy, have an increased risk for VTE recurrence.[9,10]

Biomarkers for thrombosis prediction in cancer

Standard cutoffs for D-Dimer have limited specificity, particularly in cancer patients in which D-Dimer levels are often increased at baseline. In cancer patients, higher levels of D-Dimer,

above the 75th percentile, have been found to correlate with an increased risk of VTE.[11,12] A rising D-Dimer level over time has also been found to be predictive of VTE in the cancer population.[13] The Khorana score is useful for VTE prediction in ambulatory cancer patients, with a high negative predictive value (>80%). The score incorporates pre-treatment platelet count and leukocyte count, hemoglobin level, cancer type, and body mass index (BMI). The positive predictive value of a higher-risk Khorana score is approximately 10%.[14]

Different models have been developed to improve the accuracy of thrombosis prediction with mixed results, using various cutoffs for D-Dimer, and the addition of biomarkers to the Khorana score.[15-17] The Vienna Cancer and Thrombosis study score added D-Dimer and soluble p-selectin to the Khorana score factors, with an accurate prediction of VTE.[18] The PROTECHT removed BMI from the prediction model and included chemotherapy.[19] Additional prediction models, including ONKOTEV, COMPASS-CAT, Tic-ONCO, and IMPEDE, among others, have attempted to enhance the diagnostic accuracy of VTE in patients with malignancies. These models integrate various factors such as various types of malignancies, cancer stages, genetic risk factors, and D-Dimer levels.[14]

Considering D-Dimer's low positive predictive value, limited specificity, and modest discriminatory ability in cancer patients, there is a need for novel specific biomarkers to more effectively exclude VTE in this population.[20-24] Additionally, traditional clinical VTE diagnostic assessment tools, such as the Wells' or Geneva scores, show limited efficacy in ruling out VTE in cancer patients. The mere presence of a comorbid malignancy elevates the clinical probability of VTE, requiring imaging for the majority of cancer patients to effectively exclude thrombosis.[15]

Measuring the proteome

Based on the modest diagnostic and predictive accuracy of available coagulation biomarkers in VTE, the question remains whether measurement of other circulating plasma proteins offers clinical benefit. Proteomic screens are promising not only for discovering novel biomarkers for VTE in cancer but also for enhancing our understanding of the underlying pathophysiology of thrombus formation and the complex interplay among various prothrombotic factors, such as chemotherapy, immune response, and underlying malignancies.[25] Various technologies are utilized to measure proteins in tissues, serum, or plasma, including highly optimized single protein assays, mass spectrometry (MS), and affinity-based assays. Below is a summary of proteomic methodologies and observations to date pertaining to VTE.

Mass spectrometry proteomics

MS is a technique used for the identification and quantification of proteins within a sample, allowing for customized measurements of specific targets of interest. It provides valuable insights into the structure, function, and composition of the proteome across diverse biological systems. The process involves ionizing peptides generated by proteolysis, a step accomplished through methods like electrospray ionization, surface-enhanced laser desorption ionization, and matrix-assisted laser desorption ionization. Subsequently, an electric or magnetic field is used to separate the ionized peptides in a mass analyzer, based on their mass-to-charge ratios (m/z). Tandem MS enhances the confidence of peptide identification by using molecules that have undergone prior fragmentation, performing further fragmentation, and isolation in a secondary mass analyzer.[26]

Common combinations of mass analyzers and ionization methods include matrix-assisted laser desorption ionization, and liquid chromatography (LC) coupled with tandem MS. To aid peptide identification, various processes are typically employed, including sample enrichment, fractionation, depletion, and labeling. These methods promote protein separation, enhance detection sensitivity, and facilitate the identification of less abundant plasma proteins. Following peptide identification, specialized software tools and search algorithms are used to identify the parent protein in online databases. To enhance result confidence, statistical methods and secondary proteomics techniques are employed for validation. Furthermore, multiplexed MS allows for the simultaneous analysis of multiple samples in a single measurement, employing various methods such as isobaric labeling or label-free quantification.[27,28]

Various methods deploy MS techniques for proteomic screening, including shotgun proteomics and targeted strategies. Shotgun proteomics, frequently employed in discovery studies, indirectly measures entire proteomes by analyzing peptides produced through the enzymatic breakdown of intact proteins. Analyzing complex samples such as plasma, poses a particular challenge in shotgun proteomics due to the differential abundance of proteins. In contrast, targeted proteomics approaches provide an alternative to the shotgun method, employing specific ions to identify a predefined set of peptides.[29] Targeted approaches demonstrate high sensitivity, specificity, and reproducibility, proving effective for protein identification in diverse samples, and excluding the need for affinity reagents.[30-32]

MS proteomics offers some advantages in comparison to conventional affinity-based assays (Table 1), such as its high specificity and ability to process large sample volumes. In addition, MS allows for the examination of post-translational modifications and characterization of isoforms, enhancing its versatility in protein analysis.[33,34] On the contrary, limitations of the technique include a constrained ability to detect low abundance proteins in plasma; a limited dynamic range potentially hindering a comprehensive assessment of the proteome. MS encounters difficulties analyzing large, hydrophobic proteins and complex samples. It is also expensive and requires skilled operators and advanced instrumentation. Notably, the throughput capacity of conventional MS is comparatively lower than that of affinity-based assays.[35] This disparity contributes to the weak correlation observed between MS and affinity-based platforms.[30]

Mass spectrometry in venous thromboembolism proteomics

The literature on proteomics techniques for identifying clinical biomarkers in thrombosis is heterogeneous and limited, ranging from the analysis of complex plasma samples to specific protein analyses within distinct cellular subsets.[36] Table 2 summarizes studies focusing on the analysis of plasma samples using proteomics in VTE.

Zhang *et al.* used matrix-assisted laser desorption ionization MS analysis in patients with acute pulmonary embolism (PE) and healthy controls. After validation with ELISA, only haptoglobin was associated with PE.[37] Various studies have associated hapto-

globin levels with VTE. It appears that acute PE without pulmonary hypertension induces haptoglobin, but severe PE causes the haptoglobin concentration to decrease in proportion to the severity of the pulmonary hypertension.[38,39]

Han *et al.* profiled proteins from 13 plasma samples using MS and 32 plasma samples using antibody-based-assay proteomics. Samples were obtained from two independent case-control studies of patients with high-risk PE, non-high-risk PE, and healthy controls. Serum amyloid A-1 (SAA1), calprotectin (S100A8), tenascin- C (TNC), gelsolin (GSN), and histidine-rich glycoprotein (HRG), were differentially expressed in patients with PE and or in high-risk PE, in comparison to healthy controls.[40]

Tandem MS has also been used to analyze plasma samples from patients with VTE. Jensen *et al*. found that Transthyretin, vitamin K-dependent protein Z, and protein/nucleic acid deglycase, were associated with incident VTE in a study comparing patients with VTE and healthy controls.[41]

Affinity proteomics

Affinity proteomics employs binding agents to serve as probes for the targeted detection of proteins. Binding agents include antibodies or aptamers, single-stranded DNA or RNA molecules designed to selectively bind specific targets.[42] Diverse platforms use affinity proteomics for the large-scale study of biomarkers, including antibody-based suspension bead arrays, proximity extension assays, surface arrays, and the aptamer-based Soma Scan assay.[27,43,44] All platforms enable multiplexed profiling of proteins, enabling simultaneous analysis of samples within a single experiment.[45]

Affinity-based assays offer several notable advantages, including a wide dynamic range, multiplexing capability, high specificity that minimizes cross-reactivity with non-target proteins, and versatility across various sample types. Limitations of affinity-based proteomics include alterations between targets and binding agents. For instance, structural and conformational changes in proteins, nonspecific protein binding, missense mutations, single nucleotide polymorphisms, and differential splicing can disturb the interaction between binding epitopes and targets.[43,44,46] Mutations within a gene's coding regions induce alterations in the amino acid sequence of the associated protein. Changes that occur within the epitope target region may significantly impact the protein's binding affinity.[46] To address this issue, various methods have been developed to validate the target of the binding agent.[47]

Antibody-based assays

Proximity extension assays

Proximity extension assays (PEA) use antibodies conjugated with DNA strands, engineered to hybridize after binding to a particular target molecule. This process generates a unique DNA template that can be amplified, detected, and quantified using polymerase chain reaction, enabling the quantification of the target molecule. Olink proteomics offers multiple commercially available PEA panels.[34] PEA methods have also been paired with different genomic technologies such as next-generation sequencing, to increase throughput capacity for proteomic screening.[48]

Table 1. Comparison between mass-spectrometry and affinity-based assays.

Characteristic	Proteomics technologies	
	Mass-spectrometry	Affinity-based assays
Protein detection	Measures m/Q of peptide fragments	Uses antibodies or aptamers that bind to proteins
Protein quantification	Provides absolute quantification of proteins, with an average of 10 peptides per protein	Provides relative quantification but not absolute quantification of proteins
Dynamic range	Lower sensitivity for the detection of low abundance proteins (<10 ng/mL) Detects high and medium abundance proteins. Targeted labelling is required for the detection of low abundance proteins	Wide dynamic range. Higher sensitivity for the detection of low abundance proteins
Specificity	High degree of specificity. Not restricted to a predefined set of targets Suitable for use in diverse species and across a wide range of sample types	Specificity varies according to the assay type and degree of cross-reactivity Restricted to a predefined set of targets Alteration of binding sites leads to a decrease in specificity
Characterization of structural modifications, post-translational modifications, and isoforms	Detects conformational changes in proteins, diverse protein isoforms, and post-translational modifications	Unable to detect protein isoforms, post-translational modifications, and other proteoforms
Throughput capacity	Traditional MS methods have limited throughput capacity (low to moderate). Affinity enriched or affinity selection MS methods are used to overcome this challenge	High throughput capacity using various affinity reagents
Reproducibility	Lower reproducibilityModest intra-assay variation	Higher reproducibilityLow intra-assay variation
Sample volume	Large samples (30-100 µL)	Immunoaffinity arrays (PEA) 1-100 µL Aptamer-based arrays: 65 µL
Multiplexing	All proteins in the sample (10->5000)	Immunoaffinity arrays (PEA) 100 proteins Aptamer-based arrays: >1300 proteins

m/Q, mass-to-charge ratio; MS, mass spectrometry; PEA, proximity extension assays.

Table 2. Proteomic studies in venous thromboembolism.

Author, year	Sample	Cohort	Comparative groups	Methods	Results	Parameters (cutoff, sensitivity, specificity, significance, correlation, HR)
Bruzelius *et al.*,[33] 2016	Plasma	Patients from the VEBIOS and FARIVE studies	VEBIOS VTE (n=88) Healthy controls (n=85) Replication study FARIVE VTE (n=580) Healthy controls (n=589)	IC-MS ELISA Bead arrays 755 antibodies targeting 408 proteins	VWF and PDGFB levels were significantly higher in patients with VTE *Results were verified using patients from the FARIVE study	VWF (P<0.001) PDGFB (P=0.002) Pearson's correlation between studies: VEBIOS:0.42 FARIVE:0.26
Jensen *et al.*,[34] 2018	Plasma	Patients from the Tromsø Study	VTE (n=100) Healthy controls (n=100)	TMT LC-MS	Strongest biomarkers for the development of VTE: -Transthyretin -Protein Z (ProZ) -Protein/nucleic acid deglycase (DJ-1)	Transthyretin P=0.00015 ProZ P=0.0018 DJ-1 P=0.0055
Razzaq *et al.*,[59] 2021	Plasma	1388 Patients with DVT with or without PE from the MARTHA and EOVT studies	MARTHA PE (n=95) DVT (n= 1105) DVT+PE (n=188) Verification study EOVT PE (n=143) DVT (n=196)	1. SBA in combination with Machine learning methods-ANN model 2. Application of the LIME algorithm 3. GWAS conducted on the LIME estimate	PLXNA4 was identified as a susceptibility locus for isolated PE phenotype. Homozygote carriers for the rs1424597-A allele were more frequently observed in PE than in DVT patients *Results were verified using patients from the EOVT study	GWAS on the LIME estimate (rs1424597): (P=5.3×10-7) at the PLXNA4 locus Homozygote carriers -isolated PE phenotype vs DVT MARTHA (2% vs. 0.4%) P=0.005 EOVT (3% vs. 0%) P=0.013
Ten Cate *et al.*[49] 2021	Plasma	532 Patients from the GMP-VTE study	GMP-VTE PE (n=96) DVT (n=160) DVT+PE (n=276) Verification study Gutenberg Health study (n=5778)	PEA 96-plex Olink panels (cardiometabolic, cardiovascular II and III, inflammation, and immune response) Proteomics in combination with machine learning: LASSO-regularized regression models	Prognostic proteins for the development of primary isolated PE in comparison to DVT or DVT+PE: - Interferon-γ - GDNF -Interleukin-15Rα *Results were verified using patients from the Gutenberg health study	HR per SD increase - Interferon-γ HR (1.34 95% CI, 1.23-1.45; P<0.0001 -GDNF HR (0.40 5% CI, 0.29-0.55; P<0.0001) -Interleukin 15Rα HR (0.55 95% CI, 0.43-0.71; P<0.0001)
Han *et al.*,[43] 2021	Plasma	Patients with PE and healthy controls from two case control studies	Discovery MS analysis: high-risk PE (n=3) non-high-risk PE (n=6) healthy controls (n=4) Antibody array analysis high-risk PE (n=10) non-high-risk PE (n=10) healthy controls (n=12) Verification study High-risk PE (n=25) Non-high-risk PE (n=25) Healthy controls (n=26)	MS Antibody array proteomic technology ELISA	Differentially expressed proteins in patients with PE/High-risk PE: -SAA1 -S100A8 -Tenascin-C(TNC) -Gelsolin -HRG *Results were verified using an independent cohort of 76 patients	AUC for PE diagnosis: P<0.05 -SAA1 Cut-off :1.26 µg/ml (AUC 0.882) -S100A8 Cut-off :1.19 µg/ml (AUC 0.788) -TNC Cut-off :12.62 µg/ml (AUC 0.795) AUC for High-risk PE diagnosis: -S100A8 Cut-off :1.7 µg/ml (AUC 0.773) -TNC Cut-off :17 µg/ml (AUC 0.720)

To be continued on next page

Proximity extension assays in venous thromboembolism proteomics

The literature on PEA studies in VTE proteomics represents a limited yet evolving landscape, marked by significant variability in methodologies across studies. While some investigations identify specific protein associations with VTE phenotypes, the overall heterogeneity in approaches emphasizes the need for further standardization and larger-scale studies. Below are a few highlighted key studies.

Ten Cate *et al*. identified 5 proteins specifically associated with an isolated PE phenotype, compared with deep vein throm-

Table 2. Continued from previous page.

Author, year	Sample	Cohort	Comparative groups	Methods	Results	Parameters (cutoff, sensitivity, specificity, significance, correlation, HR
Zhang *et al.*[44]	Plasma	Patients with PE and matched healthy controls	18 patients PE (n=9) Healthy controls: (n=9) Verification study 48 patients PE (n=24) Healthy controls (n=24)	2DE MALDI-TOF MS ELISA	Haptoglobin was overexpressed in the serum of PE patients. *Results were verified using an independent cohort of 48 patients	Haptoglobin cut-off: 256.74 mg/l AUC 0.764 (95% CI, 0.622- 0.906) P<0.01
Memon *et al.*,[52] 2018	Plasma	357 patients with suspected DVT from a prospective multicenter (7 centers) management study in southern Sweden	90 patients included Confirmed acute DVT (n=45) Healthy matched controls (n=45)	PEA Olink Panel (Cardiovascular III)	Proteins significantly associated with VTE: -P-Selectin -TF pathway inhibitor TFPI) -VWF -Transferrin receptor protein 1(TR) -Osteopontin -Bleomycin hydrolase -ST2 protein	-P-Selectin AUC 0.84 (95% CI 0.76-0.92) P=0.000001 -TFPI AUC 0.74 (95% CI 0.64-0.85) P=0.00001 -VWF AUC 0.77 (95% CI 0.67-0.87) P=0.00001 -TR AUC 0.78 (95% CI 0.69-0.88) P=0.000001 -Osteopontin AUC 0.72 (95% CI 0.61-0.82) P=0.0004 -Bleomycin hydrolase AUC 0.72 (95% CI 0.62-0.83) P=0.0003 -ST2 protein AUC 0.71 (95% CI 0.60-0.83) P=0.0007
Iglesias *et al.*,[64] 2023	Plasma	Patients from the VEBIOS study	VTE (n=144) Healthy controls (n=140) Verification studies: -DFW-VTE -FARIVE -RETROVE -MARTHA	SBA LC-MS/MS	Complement factor H related 5 protein (CFHR5) was independently associated with VTE. *Results were verified with 4 independent cohorts from 4 large studies	Diagnosis of acute VTE associated with 1 SD increase of CFHR5 concentration: OR 2.54 (95% CI 1.52-4.66) P=1.05E-03

2DE, two-dimensional gel electrophoresis; ANN, artificial neural networks; DVT, deep venous thrombosis; DFW-VTE, Swedish Karolinska age-adjusted D-dimer study; FARIVE, French multicenter case-control study; GDNF, glial cell-line derived neurotrophic factor; GMP-VTE, genotyping and molecular phenotyping in venous thromboembolism study; GWAS, genome-wide association study; HR, hazard ratio; HRG, histidine-rich glycoprotein; IC, immunocapture; LC, liquid chromatography; LIME, local interpretable model-agnostic explanations; MARTHA, Marseille Thrombosis Association study; MS, mass spectroscopy; RETROVE, *Riesgo de Enfermedad Tromboembolica Venosa* study; SAA1, serum amyloid A-1; SBA, suspension bead array; SD, standard deviation; PE, pulmonary embolism; TF, tissue factor; TMT, tandem mass tag; VEBIOS, venous thromboembolism biomarker study; VTE, venous thromboembolism; VWF, Von Willebrand Factor; DJ-1, deglycase; PDGFB; platelet-derived growth factor subunit B.

bosis (DVT) or DVT-associated PE phenotypes. Using 5 PEA panels, 3 proteins (interferon-γ, glial cell-line derived neurotrophic factor, and interleukin-15Rα) were found to be differentially expressed in VTE patients.[49] Ligation of the inferior vena cava to induce DVT in mice, demonstrated that intrathrombotic levels of interferon-γ were progressively elevated as the post ligation interval extended.[50] In addition, Interleukin15 complexes have a well-established role in cardiovascular disease, participating in inflammatory pathways and coronary thrombosis.[51]

In the context of DVT, Memon *et al*. employed a single PEA panel to profile proteins in patients with acute DVT and matched controls. The study identified 7 proteins significantly associated with VTE, including p-Selectin, TF pathway inhibitor, Von Willebrand factor (VWF), transferrin receptor protein 1, osteopontin, bleomycin hydrolase, and ST2.[52] P-selectin increases leukocyte and platelet rolling and adhesion, enhances TF expression in monocytes, and instigates the release of procoagulant substances (53). The role of transferrin receptor protein 1, osteopontin, bleomycin hydrolase, and ST2 in thrombosis remains under investigation (54–56).

Bead-based assays

Bead-based assays are antibody-based methods for proteomic screening, involving the immobilization of antibodies into microscopic beads. Each bead is conjugated with an antibody that interacts with proteins from a biological sample and creates complexes that can be quantified. Unique fluorescent labels are often attached to allow the identification of the complexes. Bead-based assays have a high throughput capacity and high multiplexing ability. They have been applied for various purposes, including the detection of cytokines, auto-antibodies, the analysis of monoclonal antibodies, and biological warfare agents.[34]

Bead-based assays in venous thromboembolism proteomics

The VEREMA affinity proteomics study assessed plasma samples using bead arrays obtained from patients with VTE and matched healthy controls. A set of 408 proteins, selected for their known involvement in the coagulation cascade, expression in endothelial cells, and associations with cardiovascular disease and inflammation pathways, served as targets. The findings were then compared to plasma samples from the French FARIVE study for replication, ultimately confirming the independent associations of VWF and platelet-derived growth factor subunit B (PDGFB) with VTE.[57] PDGF is expressed in endothelial cells and platelets, and elevated levels are associated with an increased risk of thrombosis.[58]

Various studies have used bead-based assays to identify biomarkers that are able to distinguish between PE and DVT.

Razzaq *et al.* analyzed plasma samples of patients with VTE from the Marseille Thrombosis Association study (MARTHA) study using a machine learning framework employing an artificial neural network approach to integrate plasma proteomics with genetic data. The MARTHA study involved targeted affinity proteomics using suspension bead assay technologies. PLXNA4 was identified as a new susceptibility locus for PE.[59] PLXNA4 plays an important role in pathways related to thrombosis, stimulating TNF-α and IL-6 production in macrophages.[60] Its ligand SEMA3, is known to promote vascular remodeling and regulate platelet aggregation.[61,62] It has been strongly associated with various lung function markers but its precise association with PE is still under study.[63]

Complement factor H-related 5 (CFHR5) protein represents a potential diagnostic and or risk predictive biomarker for VTE. Suspension bead arrays were used to analyze plasma samples obtained from patients in the VEBIOS study. Elevated levels of CFHR5 were associated with increased thrombin generation and platelet activation *in vitro*.[64] Notably, the association between CFHR5 and VTE was also reported in a cohort of patients with COVID-19 infection.[65]

Aptamer-based assays

Aptamers are nucleotide-based agents with protein affinity. Large nucleotide sequences are mixed with target peptides or proteins for binding. A commercial platform based on a large library of synthetic oligonucleotide ligands was developed by Somascan. The specificity of the technique can be limited by cross-reactivity among agents. Somascan aptamers are modified with aromatic benzyl side chains to reduce cross-reactivity.[34] Aptamer-based assays have a high sample throughput and multiplexing capacity, with a wide dynamic range, and no toxic or immunogenic potential.[66]

There are limited studies to date evaluating proteins through the Somascan platform and the development of thrombosis.

In a study of 59 critically ill adolescents using data obtained from the Somascan platform, 9 patients developed incident DVT. Higher levels of thrombin-antithrombin complexes and lower levels of factor XIII were associated with DVT. In addition, CD36, macrophage inhibitory cytokine-1, and erythropoietin receptor were marginally associated with DVT.[67]

Comparative analysis of proteomics techniques

Comparing different proteomic platforms such as MS, antibody, and aptamer, affinity-based assays have generally demonstrated limited correlation. Although consistent and comparable outcomes across different platforms are lacking.

The analysis of 173 human blood plasma samples using both MS-based platforms and PEA (Olink), identified 35 proteins common to both techniques. The two MS platforms demonstrated a strong correlation coefficient exceeding 0.5 for 23 of these 35 proteins. However, across all three platforms, including PEA and MS, only 6 out of the 35 proteins exhibited a correlation coefficient exceeding 0.5.[68]

Various investigations have found a weak correlation between PEA (Olink) and the SomaScan platforms.[46,69,70] However, studies have been constrained by a limited number of analyzed proteins and a small sample size. For instance, in a comparative study of 27 healthy individuals and 27 with acute VTE, there was a poor agreement for 8 common coagulation proteins including D-dimer and fibrinogen.[71] In addition, a large-scale plasma proteomics study comparing the United Kingdom Biobank Olink (PEA) and Iceland Somascan platforms, revealed a modest Spearman correlation between both techniques.[72]

Proteomics in cancer-associated thrombosis

CAT exhibits distinctive features that set it apart from other types of VTE, including differences in risk factors, pathophysiology, and management strategies. Central to its pathogenesis is the pivotal role of TF, a key player in cancer progression and CAT.[15] TF induces the activation of platelets and the coagulation cascade. Its release into circulation occurs within TF-positive extracellular vesicles. Notably, certain tumor types, including pancreatic, ovarian, brain, and cervical cancers, manifest elevated levels of TF, with potential correlations to specific oncogenic gene mutations, angiogenesis, and tumor histological grade.[73,74] Procoagulant proteins such as plasminogen activator inhibitor 1, podoplanin, and protein disulfide isomerase have also been implicated in CAT. Table 3 provides a summary of proteomic plasma biomarkers evaluated in CAT.

Differential proteomic expression in various malignancies

Proteomic investigations include differential protein expression across various malignancies as they relate to CAT. In a study of patients with lung (N=30, 15 with VTE) and pancreatic cancer (N=30, 15 with VTE) using LC-MS, there were distinct differential expression patterns of immunoglobulin-derived proteins and tetranectin in cancer patients with and without VTE. Particularly noteworthy was the absence of overlap between lung and pancreatic cancer, emphasizing the nuanced variations in mechanisms and proteins based on the primary malignancy site.[75] Cancer-derived immunoglobulins are highly expressed in cancer cells and mediate multiple processes in cancer progression, coagulation, and inflammation, including activation of platelet aggregation.[76] Furthermore, the analysis of plasma samples from 20 patients with non-small cell lung cancer (NSCLC) and VTE, and 15 NSCLC patients without VTE, demonstrated differential expression of 5 proteins (SAA1, S100A8, lipopolysaccharide binding protein, haptoglobin, and lactate dehydrogenase B) in VTE patients.[77]

The platelet proteome

Platelets play a crucial role in cancer biology and CAT. Research has indicated that the platelet proteome exhibits variations based on the primary site of malignancy. For example, in a MS proteomics study involving patients with brain cancer, lung cancer, and healthy controls, while the platelet proteome remained unaltered in brain cancer, distinctive modifications and differential expression of proteins were observed in patients with lung cancer when compared to the healthy control group.[78] Furthermore, a separate study involving the platelet proteome of 9 individuals with diverse malignancies found that the platelet proteome was affected not only by the type of primary malignancy but also by the oncological treatment.[79]

Extracellular vesicles

Extracellular vesicles (EVs) facilitate the interaction between cancer cells, platelets, and the vascular system. In the context of CAT, cancer cells release EVs containing diverse bioactive substances, such as proteins, nucleic acids, and lipids. These EVs contribute to the hypercoagulability observed in cancer patients. Specifically, EVs released by cancer cells can activate platelets, inducing platelet aggregation and the formation of microthrombi. Furthermore, EVs have the potential to activate the coagulation cascade and hinder fibrinolysis, thereby amplifying the risk of thrombosis.[1,74]

Understanding the proteomic composition of these EVs is crucial for unraveling the molecular mechanisms underlying CAT. MS proteomics was applied to analyze EVs released from

Table 3. Potential biomarkers in cancer-associated thrombosis.

Lung cancer	
Proteins increased in VTE patients	IgV kappa light chain (76)
Proteins increased in non-VTE patients	Tetranectin (76)
Non-small cell lung cancer	
Proteins increased in VTE patients	SAA1, S100A8, LBP, HP and LDHB (78)
Pancreas cancer	
Proteins increased in VTE patients	IgM Fc, immunoglobulin kappa chain variable region, Ig kappa chainVKIII-JK3, immunoglobulin heavy chain variable region, immunoglobulin kappa light chain variable region (76)
Proteins increased in non-VTE patients	Immunoglobulin kappa light chain variable region of different sequence (MW 8 kDa), phospholipase D (76)
Colorectal cancer	
Proteins associated with increased risk of cancer associated thrombosis	Angiotensinogen, apolipoprotein B100, CD5 antigen-like, and immunoglobulin heavy constant mu (85)
Platelet proteome in cancer patients	
Upregulated proteins	Lung cancer: Accelerated F13A1, Endoplasmic reticulum proteins (CALR, HSPA5, P4HB) (79) Patients with cancer *vs* healthy controls:FXIII, CALR (82)
Downregulated proteins	Patients with cancer *vs* healthy controls: Integrin alpha-IIb, albumin, gamma-enolase, and integrin beta 3 (82)

CALR, calreticulin; F13A1, factor XIII 55 kDa fragment; FXIII, coagulation factor XIII; HP, haptoglobin; HSPA5, heat shock protein family A; LBP, lipopolysaccharide binding protein; LDHB, lactate dehydrogenase B; P4HB, prolyl 4-hydroxylase subunit beta; SAA1, serum amyloid A1; S100A8, calprotectin; VTE, venous thromboembolism.

platelets in various triple-negative breast cancer (TNBC) cell lines. Results revealed that TNBC cell lines induced platelet aggregation, and the subsequent protein profiling of extracellular vesicles released by platelets highlighted their active participation in this process. Notably, uPAR and PDGFRβ were identified as crucial contributors to the complex mechanism of extracellular vesicle-induced platelet aggregation.[80]

Comparing proteomic screening techniques in cancer-associated thrombosis

There is scant literature comparing proteomics screening techniques in CAT. A study compared multiple reaction monitoring (MRM) MS proteomics with conventional assays to evaluate levels of coagulation factors and fibrinolysis-related proteins. LC-MS was used to profile 31 proteins related to coagulation and fibrinolysis in 75 patients (25 with VTE, 25 with cancer and VTE, and 25 with healthy controls). All samples also underwent traditional antibody or activity-based assays. Both methods had a Pearson correlation of 0.77, indicating a good correlation, but MRM MS had a higher sensitivity, multiplicity, and performance.[81]

Limitations of proteomics in cancer-associated thrombosis

Proteomics has been applied in the context of thrombosis, revealing several promising biomarkers. However, despite these findings, markers have not been globally incorporated into clinical practice. The challenge in using these biomarkers can be attributed to several factors, including limited congruence among study outcomes, substantial variations in methods, protein sample preparation, sample types, and study populations. Proteomics studies in CAT exhibit significant heterogeneity, rendering direct comparisons between investigations challenging. Furthermore, the absence of external study validation adds complexity to the interpretation of results. The majority of these studies had a small sample size, impacting the statistical significance and general applicability of the findings. Subsequent analyses are warranted, with an emphasis on achieving greater methodological similarity across studies.

Conclusions

Proteomics enables the comprehensive analysis of protein alterations on a large scale, offering valuable insights for the timely diagnosis, accurate risk assessment, and effective treatment of VTE and CAT. The effective application of biomarkers to clinical practice requires the validation of studies using independent diverse cohorts. Artificial intelligence and machine learning methods are currently under investigation and represent promising tools in combination with proteomics for the identification of biomarkers in thrombosis.

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Risk of recurrent venous thromboembolism in cancer patients after discontinuation of anticoagulant therapy

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ABSTRACT

Anticoagulant therapy is recommended for cancer-related venous thromboembolism (VTE). Recurrent VTE prevention is the main goal of this treatment. The majority of evidence-based practice guidelines recommend anticoagulant treatment for at least 6 months. Based on individual assessment of potential benefits and risks, tolerability, drug availability, patient preference, and cancer activity, active cancer patients should continue anticoagulant treatment beyond the 6-month course. When cancer is no longer active or the risk outweighs the benefit, anticoagulant therapy is usually stopped after 3-6 months. Until recently, there was little data on the risk of recurrent VTE in cancer-associated VTE patients after stopping anticoagulants. New results and evidence synthesis have emerged in the last 3 years. Recurring VTE occurs in over 30% in the 5 years after treatment discontinuation. In the first six months, recurrence rates are 10-15%. Recurrences reach 31% at 2 years and stabilize between 2 and 5. Duration of prior anticoagulation does not affect cumulative recurrence. The high risk of recurrent VTE after discontinuing treatment supports guidelines to continue anticoagulant treatment if cancer is active. Stopping anticoagulants after 3-6 months may not be ideal, so randomized clinical trials should be conducted quickly. This review highlights the need to improve cancer patients' primary VTE prevention efforts.

Introduction

Venous thromboembolism (VTE) is a common complication in cancer patients that increases mortality and results in morbidity from recurrent thromboembolism.[1-3] The yearly incidence of VTE among patients with cancer has increased by approximately 3-fold between 1997 and 2017.[1] As the treatment of cancer continues to improve, reducing morbidity from complications such as VTE among cancer survivors is an increasingly important goal. Although primary prevention is the most effective strategy to achieve this goal, reducing the incidence of recurrent VTE can also have an important impact on reducing morbidity and maintaining quality of life among cancer survivors who have experienced VTE complicating their diagnosis of cancer.

Anticoagulant therapy is the treatment of choice for cancer-associated VTE.[1,4-9] The primary goal of this treatment is to prevent recurrent VTE. Current approaches for anticoagulant therapy, including low-molecular-weight heparin and direct oral anticoagulants, are very effective for preventing recurrent VTE while treatment is continued. Evidence-based practice guidelines recommend continuing anticoagulant treatment for at least 3 to 6 months, with most guidelines recommending treatment for at least 6 months.[4-8] There is a consensus from guideline panels and expert narrative reviews that the optimal duration of anticoagulant treatment for patients with cancer-associated VTE remains uncertain due to a lack of definitive data from randomized clinical trials.[1,4-9] In general, extended or indefinite anticoagulant treatment beyond the initial 6-month course is recommended for patients with active cancer,[4-9] based on individual assessment of the potential benefit and risk of continued treatment, tolerability, drug availability, patient preference, and cancer activity. It is common practice to discontinue anticoagulant therapy after 3 to 6 months in patients in whom cancer is no longer considered to be active, or in whom the risk of continued treatment is assessed to outweigh the potential benefit.

A starting point or foundation for assessing the risk-benefit of extended anticoagulation is to have valid and sufficiently precise data on the risk of recurrent VTE over time after discontinuing anticoagulant treatment. This data is critical for the clinician and patient to assess the potential benefit that extended anticoagulant treatment can provide, and to consider this in the context of the risk of bleeding with contemporary long-term anticoagulant treatment. Rigorous data on the risk of recurrent

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VTE in the months to years after discontinuing anticoagulant treatment in patients with cancer-associated VTE has been sparse until relatively recently.

The purpose of this narrative review is to summarize the available evidence quantifying the risk of recurrent VTE after discontinuing anticoagulant treatment in patients with cancer-associated VTE. New results and synthesis of evidence have become available during the last 3 years,[2,10-12] and these reports are the focus of this review.

Evidence from systematic reviews

Moik *et al.*[10] performed a systematic review of the evidence on the incidence of recurrent VTE and bleeding between 6 and 12 months after a diagnosis of cancer-associated VTE. The authors identified 11 studies, which were either randomized trials or cohort studies, that included 3,019 patients 18 years of age or older with active cancer at the time of diagnosis of VTE, and appropriate follow-up to document the outcomes of recurrent VTE and bleeding during the period of 6 to 12 months after diagnosis. There was substantial heterogeneity in the reported rates of recurrent VTE during this period, ranging from 1% to 12%.[10] The studies varied in the anticoagulation strategies, and the authors were not able to determine an aggregate rate of recurrence for patients on or off anticoagulation. In general, the reported risk of recurrent VTE was highest (13-15%) for patients not receiving anticoagulation in whom there was evidence of residual vein thrombosis by imaging at 6 months. The rates of recurrence were 1% to 4% for patients receiving anticoagulation, except for one prospective observational study which reported a rate of recurrence of 12%.[10] The authors suggested the latter result was due to the very high thrombotic risk of the cohort due to both advanced stage of disease and a preponderance of very prothrombotic tumor types.[10] The rates of major bleeding were 1-4% among patients receiving continued anticoagulation.[10]

A recent systematic review and meta-analysis by van Hylckama Vlieg *et al.* [11] provides important new data on the risk of recurrent VTE after discontinuing anticoagulation in patients with cancer-associated VTE. These authors assessed the rate of recurrent VTE and the cumulative rate of VTE recurrence in patients with a first cancer-associated VTE who completed at least 3 months of anticoagulant treatment and were followed up after discontinuation of this treatment. The analysis was performed using the data from 14 studies involving 1,922 patients. The methodology was rigorous and followed current best practices for meta-analysis. The pooled rates of recurrent VTE for the time intervals of 0 to 3, 3 to 6, 6 to 12, 12 to 24, 24 to 36, and 3 to 5 years after discontinuing treatment are summarized in Table 1. The rate of VTE recurrence for these intervals ranged from 14.6% to 1.1%, with the highest risk during the early periods after discontinuing therapy.[11] The cumulative rates of recurrent VTE for up to 5 years after discontinuing treatment are summarized in Table 2. The results document high cumulative rates of recurrent VTE, ranging from 23% at 6 months after discontinuation of anticoagulant treatment to more than one-third of patients having a recurrence within 5 years.[11] The authors discussed several limitations of the study, several of which would likely cause the results to be an underestimate of the recurrence rate after discontinuing therapy. The cumulative rates of recurrent VTE are so high that the limitations of the study would be unlikely to lead to different conclusions.

Table 1. Rate of recurrent venous thromboembolism after discontinuation of anticoagulant therapy in patients with cancer-associated venous thromboembolism. Reproduced with permission from van Hylckama Vlieg *et al.*[11]

Time	No. of studies	No. of patients at risk	Recurrent VTE events	Event rate per 100 person-years
0-3 months	15	1922	63	14.6
3-6 months	15	1375	69	10.3
6-12 months	13	888	57	6.4
12-24 months	11	615	60	4.0
24-36 months	9	366	10	1.1
3-5 years	5	128	10	2.2

VTE, venous thromboembolism.

Table 2. Cumulative rate of recurrent venous thromboembolism after discontinuation of anticoagulant therapy. Reproduced with permission from van Hylckama Vlieg *et al.*[11]

Time after discontinuation	Cumulative rate of VTE recurrence, %	95% CI
6 months	23.4	12.9-33.3
1 year	28.3	15.6-39.6
2 years	31.1	16.5-43.8
3 years	31.9	16.8-45.0
5 years	35.0	16.8-47.4

VTE, venous thromboembolism; CI, confidence interval.

Recent evidence from registries or population-based studies

Using the *Registro Informatizado de la Enfermedad ThromboEmbolica* cohort, Lapebie *et al*.[12] evaluated the rate of recurrent VTE and predictors of recurrence during the 1 year after discontinuation of anticoagulation among patients with an index VTE associated with active cancer, and who completed a course of at least 3 months of anticoagulant therapy. From a total of 14, 318 patients with cancer-associated VTE, 3,414 patients had anticoagulant treatment discontinued after at least 3 months of therapy. The length of anticoagulant treatment was 3 to 6 months in 1699 patients (49.8%), 6 to 12 months in 1146 patients (33.6%), and >1 year among 569 patients (16.7%). The cumulative incidence of recurrent VTE after discontinuation was 10.2% [95% confidence interval (CI), 9.1 to 11.5 %] at 1 year, 19.7% (95% CI, 17.0 to 22.5%) at 5 years, and 27.6% (95% CI, 22.1 to 33.3%) at 10 years.[11] The cumulative incidence of recurrence after discontinuing anticoagulant therapy did not change according to the length of previous treatment. In a cohort comparison of 6,532 patients with cancer-associated VTE who did not have a recurrence during the first 3 months and were receiving continued anticoagulant treatment, the corresponding cumulative rates of recurrent VTE at 1, 5 and 10 years were 3.2% (95% CI, 2.6 to 4.0%), 6.0% (95% CI, 4.2 to 8.3%) and 13.5% (95% CI, 5.9 to 9.5%).[12]

Several potential predictors of recurrent VTE were evaluated using multivariable analysis. The features most strongly associated [hazard ratio (HR) >2] with recurrent VTE were the type of cancer, the post-thrombotic syndrome, residual pulmonary artery obstruction, and the presence of an inferior vena cava filter.[12] Surgery in the 2 months prior to the diagnosis of VTE was associated with a lower hazard for recurrent VTE (HR 0.60).[12] The HRs for recurrent VTE for different groups of the type of cancer are shown in Table 3; the 95% CIs for the HRs overlap widely across these groups.[12]

A recent population-based prospective study provides new data on the incidence and burden of cancer-associated VTE,[2] and the rate of recurrent VTE up to 2 years after diagnosis among patients with active cancer and among those with a history of cancer >6 months prior to diagnosis of VTE. The age-adjusted incidence of cancer-associated VTE among adults aged 18 years or more was 70.0 (95% CI, 65.1 to 75.3) per 100,000 general population.[2] Recurrent VTE documented by imaging during the 2 years after the initial diagnosis occurred in 38 of 304 patients (12.5%) with active cancer (of whom 37% were receiving anticoagulant therapy at the time of recurrence), and in 34 of 424 patients (8.0%) with a history of cancer >6 months prior to their index diagnosis (of whom 38% were receiving anticoagulant treatment).[2] Among this latter group, the majority of recurrences occurred within the first 6 months, but approximately one-third of the recurrent events accumulated later throughout the 2-years follow-up period.[2] The time course of the recurrent VTE among patients with active cancer, and in patient with a history of cancer >6 months previously is shown in Figure 1; the cohorts of patients without cancer, stratified by the presence or absence of transient or persistent provoking risk factors are also shown for comparison.[2]

Discussion and Conclusions

This review of the data on the risk of recurrent VTE following discontinuation of anticoagulant therapy leads to several inferences. First, there is a high cumulative incidence of recurrent VTE, more than 30%, during the 5 years following discontinuation of treatment.[11] The risk of recurrence is highest during the initial 6 months, with reported rates of approximately 10% to 15% (Table 1). Recurrences continue to accumulate significantly to 31% at 2 years, with the cumulative incidence stabilizing between years 2 and 5 (Table 2). The cumulative recurrence rate appears to not be influenced by the duration of prior anticoagulation.[12] This pattern is similar to that observed in patients without cancer who have unprovoked VTE.[13]

Second, the high risk of recurrent VTE after discontinuing treatment provides support for the recommendation from guidelines that anticoagulant treatment be continued if the cancer is active unless the risk of bleeding is too great.[4-8] Among the 14 studies in the systematic review by van Hylckama Vlieg,[11] 10 studies included information on the stage of cancer, although the precise number of patients with active versus non-active cancer was not available. The proportion of patients with metastatic disease, indicating active cancer, ranged from approximately 18% to 76% of patients in these studies. More granular information is needed on the risk of recurrence in the groups with active versus non-active cancer at the time of discontinuing treatment. This is particularly important since it is common practice to discontinue treatment after 6 months if the cancer is no longer active, a practice which may need to be revisited. The relative benefit and risk of continuing versus stopping anticoagulant treatment among patients whose cancer is no longer considered active should be evaluated by a randomized trial.

Third, the available data support the concept that tumor type influences the risk of recurrent VTE (Table 3),[12] with some tumors being especially strong in promoting recurrent thromboembolism. Further studies are needed; however, to determine precisely how information on tumor type should be incorporated

Table 3. Effect of the type of cancer on the risk of recurrent venous thromboembolism after discontinuation of anticoagulant therapy in patients with cancer-associated venous thromboembolism. Adapted with permission from Lapebie *et al.*[12]

Site of the cancer	sHR	95% CI
Oropharynx, larynx, melanoma	Reference	-
Others, hematological, colorectal, uterus, bladder, kidney, prostate, breast, vulva	2.94	0.91-9.52
Lung, cerebral, stomach, esophagus, liver, ovary	3.56	1.07-11.80
Pancreas, biliary system, carcinoma of unknown origin	6.86	1.89-24.85

sHR, sub-hazard ratio; CI, confidence interval.

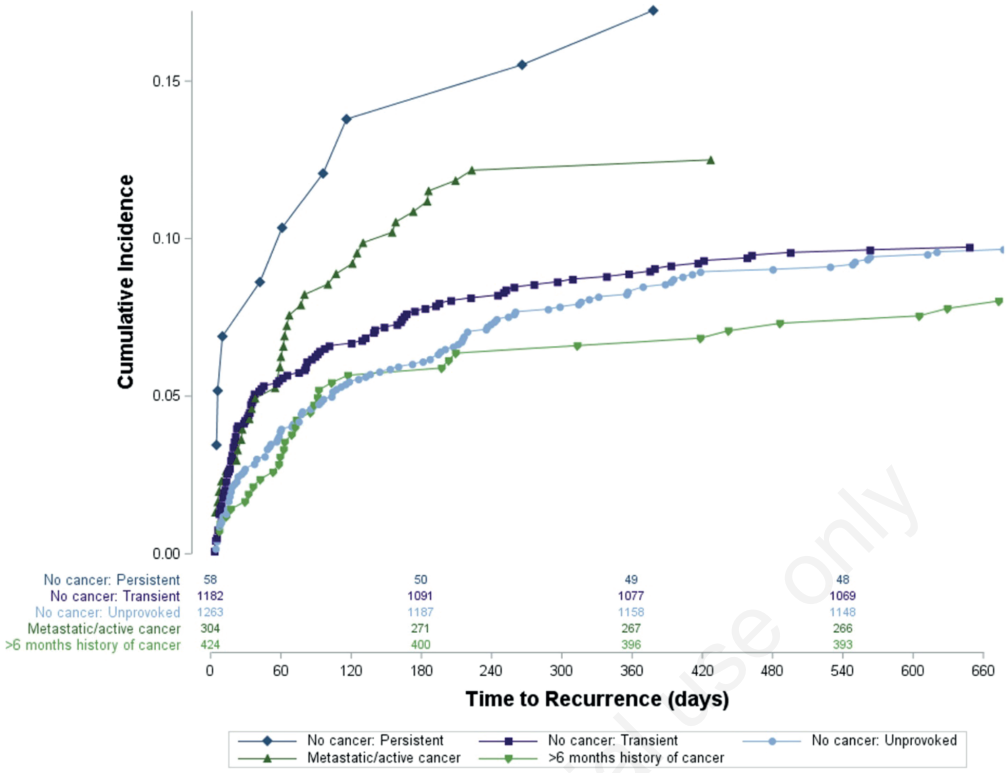

Figure 1. Cumulative incidence of the first recurrent venous thromboembolism stratified by status of cancer and provoking risk factor status. Reproduced with permission from Raskob *et al.*[2]

into the individual patient's decision to continue or discontinue anticoagulant therapy. Because contemporary oncology, clinical decision-making, and patient care are "tumor specific", future clinical trials of anticoagulant treatment should also focus on specific tumors, or stratify by tumor type, to be most helpful to the practicing oncologist.

In conclusion, the available literature indicates there is a major burden of morbidity from recurrent VTE after discontinuing anticoagulant treatment in cancer patients with VTE. The optimal duration of anticoagulant treatment in cancer patients with VTE continues to be unresolved. The practice of discontinuing anticoagulants after 3 to 6 months of treatment may not be optimal, and randomized clinical trials to address this issue should be performed expeditiously. The safety of extended anticoagulation is also an important consideration, and the ongoing, larger study of treatment using a lower dose of a factor Xa inhibitor, with the hope of reduced bleeding risk, is awaited with interest.[14] Patients with cancer-associated VTE are also an attractive target patient group for evaluating merging new anticoagulants which are potentially safer, such as the factor XI inhibitors.[15] Finally, while reducing the risk of recurrent VTE can have an important impact on the disease burden of VTE in the cancer patient population, the most effective approach with the greatest potential impact is to prevent VTE in the first place. The results of this review further underscore the importance of strengthened efforts for primary prevention of VTE in cancer patients.

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PLENARY SESSION 1
EMERGING TRENDS IN CANCER-ASSOCIATED THROMBOSIS (PART I)

OC-01

IMMUNE CHECKPOINT BLOCKADE PROMOTES THROMBOSIS VIA T-CELL AND NEUTROPHIL ACTIVATION, AND TUMOR-CELL ASSOCIATED TISSUE FACTOR IN A MURINE MODEL OF COLORECTAL CANCER

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Introduction: The use of immune checkpoint inhibitors (ICI) to treat cancer is associated with several immune-related adverse events (irAE), including venous thrombosis.
Aim: To define mechanisms of ICI-associated thrombosis (IAT), we used a mouse model of colorectal cancer in which ICI stimulates the development of venous thrombi.
Materials and Methods: Mice bearing CT26 mouse colorectal tumors were treated with ICI (aPD-1+aCTLA4). Levels of circulating nucleosomes (Nu.Q H3.1), neutrophil extracellular traps (NETs), neutrophil-platelet aggregates, thrombin-antithrombin (TAT) complexes, and tumor and extracellular vesicle (EV) tissue factor (TF) were measured before and after ICI. Tumor cytokines were also profiled. The role of tumor-derived TF in IAT was determined using mice bearing CT26 cells in which TF was deleted using CRISPR/Cas9 (TFKO cells).
Results: ICI treated tumor-bearing mice developed larger thrombi than mice treated with control IgG and demonstrated elevated levels of circulating nucleosomes (114 *vs* 82 ng/ml), NETs (15.8 *vs* 6.8%), platelet-neutrophil aggregates (44.8 *vs* 22.3%), and TAT complexes (12.6 *vs* 7.9 ng/ml). TF expression was increased in tumor extracts from ICI-treated mice. Tumors from ICI-treated mice expressed increased levels of IFNɣ (2-fold) and TNFα (5-fold) and CXCL11 (6.8-fold). To assess the role of IFNɣ on tumor cell TF expression, CT26 cells were incubated with IFNɣ, which increased TF expression in a concentration dependent manner. Increased TF expression was associated with phosphorylation of STAT1 at Tyr701 and STAT3 at Tyr705, along with increased IRF-1 expression, a downstream target of STAT1, that was blocked by a JAK1/2 inhibitor, baricitinib. While the quantity of large EV remained constant, treatment with IFN-γ enhanced release of small EVs, accompanied by upregulation of Rab27a, a small GTPase that initiates release of small EVs.
Conclusions: IFN-γ, potentially originating from activated T cells induced by ICI, contributes to increased TF expression in tumor cells via the JAK-STAT pathway. Through upregulation of Rab27a, IFNɣ may also contribute to the release of TF+ EV. TF KO in CT26 cells resulted in reduced tumor and EV-associated TF and was associated with a decrease in IVC thrombus size after ICI treatment (24.2 *vs* 20.5 mg; P=0.047). Neutrophil and platelet activation, and tumor-associated TF may contribute to ICI-associated thrombosis.

OC-02

NAVIGATING THE INTERPLAY OF CANCER, HEMOSTASIS, AND THROMBOSIS: INVESTIGATING TISSUE FACTOR IN COLORECTAL CANCER (CALGB/SWOG 80405)

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Introduction: Cancer induces hypercoagulability. Tissue factor (TF) plays a crucial role in the coagulation cascade as a transmembrane receptor and cofactor for factor VII/VIIa. The TF-VIIa complex activates PAR2, leading to intracellular signaling. Elevated TF expression (exp) in malignancy correlates with cell proliferation, angiogenesis, and metastasis. In colorectal cancer (CRC), TF holds a pivotal role and is associated with oncogenic alterations (KRAS, BRAF, HER2).
Aim: This study investigates TF's prognostic and predictive relevance in CRC. TF-targeted antibody-drug conjugates are under investigation in solid tumors.
Materials and Methods: 433 metastatic CRC patients (pts) treated with bevacizumab (VEGFRi, n=226) or cetuximab (EGFRi, n=207) in combination with first-line chemotherapy were analyzed from the CALGB/SWOG 80405 trial. TF and PAR2 RNA from formalin-fixed, paraffin-embedded (FFPE) tumor samples were sequenced on the HiSeq 2500 (Illumina). Overall survival (OS) was compared by tertiles of TF exp (high *vs* mid *vs* low). Logrank p-values describe differences without pt characteristic adjustment. Transcriptome-wide gene association analysis used linear regression, adjusting for multiple factors. Gene Ontology enrichment analysis used the top 100 TF-associated genes.
Results: TF exp correlates with genes maintaining epithelial integrity, cell adhesion, migration, extracellular matrix structure, antigen processing, glycosylation, Wnt pathway regulation, cytokine production, and MAP kinase pathways. High TF exp associates with shorter median OS in the entire cohort (25.2 *vs* 30.9 *vs* 35.4 months, p=0.0051), FOLFOX-treated (22.4 *vs* 30.9 *vs* 33.4, p=0.0044), and EGFRi-treated pts (22.4 *vs* 30.9 *vs* 33.4, p=0.0044). This impact is notable in EGFRi-treated pts with liver metastases (23.6 *vs* 29.9 *vs* 35.1 months, p=0.016). TF exp lacks predictive value for OS in FOLFIRI or VEGFRi-treated pts. PAR2 exp levels do not correlate with survival outcomes.
Conclusions: TF exp is a prognostic marker in CRC and is predictive of OS in FOLFOX and EGFRi-treated pts, especially

those with liver metastases, possibly through PAR2-independent mechanisms. Further investigation into its association with EGFR is crucial. These findings underscore the significance of exploring TF and related thrombosis-associated genes in CRC.
Support: U10CA180821; U10CA180888, UG1CA180830, U24CA196175 (SWOG); https://acknowledgments.alliancefound.org.Lilly; Genentech; Pfizer; Clinicaltrials.gov Id#: NCT00265850.

PLENARY SESSION 2
EMERGING TRENDS IN CANCER-ASSOCIATED THROMBOSIS (PART II)

OC-03

PREDICTION OF CLINICALLY SIGNIFICANT BLEEDING IN PATIENTS ANTICOAGULATED FOR CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM: VALIDATION OF THE B-CAT SCORE IN A COHORT OF PATIENTS FROM THE TESEO STUDY

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Introduction: No validated score is currently available for prediction of clinically significant bleeding in patients anticoagulated for cancer-associated venous thromboembolism (Ca-VTE).
Aim: The objective of this study was to validate the B-CAT score, a new tool designed to classify the risk of bleeding in oncology patients receiving anticoagulation.
Materials and Methods: Data came from the TESEO study, a national, multicenter and prospective registry that documents patients with Ca-VTE. Patients anticoagulated for any type of VTE were included and observed over a period of 180 days for major or clinically relevant bleeding. The variables of the B-CAT score (tumor location, presence of metastasis, history of major or clinically relevant bleeding, anaemia, coagulopathies, and cerebrovascular and gastrointestinal disease) were selected, except for minor trauma, and minor surgery and clinically relevant bleeding not requiring hospitalization after Ca-VTE, as these were not available. Patients were classified according to bleeding risk into three categories, and a multivariate logistic regression model was developed using these variables to estimate the risk of bleeding.
Results: The study cohort comprised 2301 patients with Ca-VTE receiving anticoagulation. After an observation period equivalent to 848 person-years, 157 significant bleeding events were identified (6.8%; 18.5 per 100 person-years): 63 major bleeding events (40.1%; 7.4 per 100 person-years) and 94 clinically relevant bleeding events (59.9%, 11.1 per 100 person-years). Patients classified as low (47.8%), medium (59.5%), and high (1.7%) risk as determinated by B-CAT score had different 6-month significant bleeding rates: 11.4, 24.4, and 100 per 100 person-years, respectively ($p<0.001$). The predictive model showed adequate calibration (Hosmer-Lemeshow test: $p=0.886$) and discrimination, evidenced by C-statistic index for significant bleeding, major bleeding, and clinically relevant bleeding of 0.63 (95% confidence interval: 0.58-0.67), 0.61 (0.53-0.69), and 0.63 (0.57-0.69), respectively, as shown in Figure 1.
Conclusions: We have validated the bleeding risk score B-CAT in patients with Ca-VTE receiving anticoagulation. This model can contribute to standardizing decision-making in a context where quality evidence is limited.

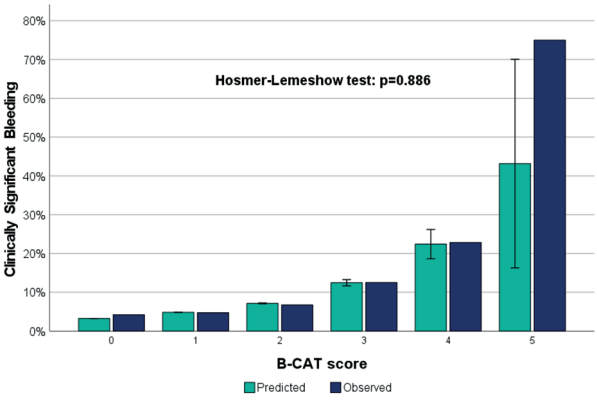

Figure 1. Observed and predicted rates of clinically significant bleeding for several values of the B-CAT score.

OC-04

TISSUE FACTOR PATHWAY-RELATED BIOMARKERS IN PANCREATIC CANCER: PLASMA LEVELS OF ACTIVATED FACTOR VII-ANTITHROMBIN COMPLEX MAY PREDICT VENOUS THROMBOEMBOLISM

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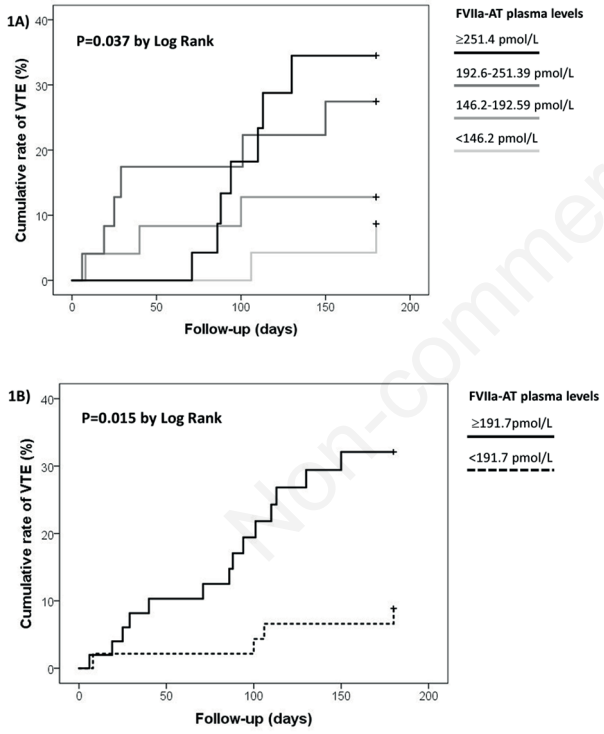
Introduction: Tissue factor (TF), the main initiator of the coagulation cascade, is well recognized to play a key role in pancreatic cancer-associated thrombosis. However, the laboratory evaluation of the transmembrane protein TF is hampered by multiple factors. The soluble activated factor VII–antithrombin complex (FVIIa-AT) is considered an indirect marker of TF exposure by reflecting TF-FVIIa interaction.

Aim: To evaluate plasma levels of FVIIa-AT and other TF pathway-related biomarkers, like tissue factor pathway inhibitor (TFPI), in a cohort of patients with pancreatic cancer and to analyze their association with subsequent VTE risk during a 6-month follow-up.

Materials and Methods: Patients with advanced pancreatic cancer planned for a new chemotherapy regimen were prospectively enrolled in 4 centers in The Netherlands and Italy between January 2019 and September 2021. Blood was drawn at baseline and patients were followed for 6 months for the occurrence of venous thromboembolism (VTE), including splanchnic vein thrombosis. FVIIa-AT, FVII Antigen (FVII Ag), and total TFPI were determined by ELISA.



Cumulative rate of venous thromboembolism (VTE) during the follow-up in the pancreatic cancer cohort (n=98) stratified on the basis of FVII-AT plasma concentration: **A)** according to the levels of quartile distribution and **B)** according to the threshold value defined by ROC curve analysis, i.e. 191.7 pmol/L (at level of 48° percentile).

Figure 1.

Results: Ninety-eight patients with pancreatic cancer (50% females, mean age 66.5±9.0) were included. During a 6-month follow-up, 24 subjects (24.5%) died and 19 subjects (19.4%) developed VTE. Subjects with VTE had a higher baseline plasma concentration of FVIIa-AT as compared to those without VTE (240.3 [188.0-309.1] pmol/L *versus* 183.6 [166.1-202.9 pmol/L], P=0.023), while no significant difference was found for either FVII Ag or TFPI levels. Stratifying the study population on the basis of FVIIa-AT plasma levels, Kaplan-Meier curves showed a progressively increased rate of VTE from the lowest to the highest quartile (8.3%, 12.0%, 24.0%, 33.3%, respectively, log-rank P=0.037, Figure 1A). The ROC curve analysis defined a cut-point value at 191.7 pmol/L (48th percentile, Figure 1B). Subjects with high FVIIa-AT levels above this threshold value (≥191.7 pmol/L) had a more than three-fold increased risk of VTE as compared to those with low FVIIa-AT levels (HR 3.63 with 95%CI 1.20-11.04). This association was confirmed after adjustment for sex, age, BMI, FVII Ag, and TFPI by Cox regression models (HR 3.44 with 95%CI 1.08-10.98).

Conclusions: High plasma levels of FVIIa-AT predict an increased risk of VTE in the setting of advanced pancreatic cancer, thereby demonstrating the potential clinically meaningful role of TF pathway-related biomarkers to include identify high-risk patients.

PLENARY SESSION 3
ANTICOAGULATION IN HEMATOLOGICAL CANCER PATIENTS

OC-05

THE RISK OF THROMBOEMBOLIC RECURRENCE OUTWEIGHS THE RISK OF MAJOR BLEEDING IN CANCER PATIENTS TREATED WITH TINZAPARIN, EVEN IN PATIENTS WITH FRAGILITY CRITERIA. META-ANALYSIS OF PROSPECTIVE STUDIES INVOLVING 1413 INDIVIDUAL PATIENTS' DATA

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Introduction: On anticoagulant therapy, patients treated for Cancer Associated Thrombosis (CAT) remain at high risk of both venous thromboembolic recurrence (rVTE) and major bleeding (MB). In practice, fragile patients are particularly concerned by the risk of bleeding. Whether the risk of recurrence and the risk

of bleeding under anticoagulant therapy are higher in patients with criteria of fragility remains poorly assessed, as these patients are under-represented in randomized clinical trials.

Aim: We estimated the rate of rVTE and MB at 6 months according to patient characteristics from prospective cohorts and randomized studies involving CAT patients on tinzaparin, using a meta-analysis on individual patient data.

Materials and Methods: Eligible studies for this meta-analysis (PROSPERO registration CRD42019119907) had to include a central adjudication committee for study outcomes. Main outcomes were cumulative incidences of rVTE and MB at 6 months. The cumulative incidences were estimated using the Kalbfleisch and Prentice method considering the competing risk of death for rVTE and MB. Patients were considered "with fragility characteristics" when they had at least one of the following: age ≥75, body weight (BW) ≤50 kg, creatinine clearance (CrCl) <50 ml/min or ECOG ≥2.

Results: Three prospective cohort studies (AXA - NCT02898051, N=308; PREDICARE - N=409 (1); TICAT - N=247 (2) and the tinzaparin arm of the CATCH study (N=449 (3)) were included. The 6-months cumulative incidences of rVTE and MB of the entire population of 1413 patients were 6.2% [95% CI: 5.0%; 7.7%], and 3.4% [2.7%; 4.5%] respectively. Among these patients, 21.3% were over 75 years, 9.3% had a BW ≤50 kg, 13.9% a CrCl <50 ml/min and 30.2% an ECOG ≥2. In all situations (presence or absence of each fragility criterion), the risk of rVTE under treatment was found to be higher than the risk of MB (Figure1).

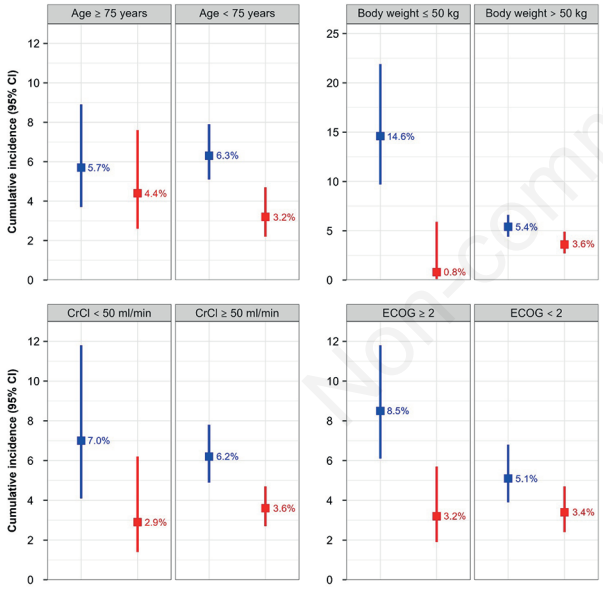

Figure 1. Risk of recurrent venous thromboembolism and major bleeding by 6 months in CAT patients treated with tinzaparin,according to patients' fragility characteristics Recurrent VTE (Blue), Major Bleeding (Red).

Conclusions: In CAT patients receiving tinzaparin for up to 6 months, the risk of rVTE is always greater than the risk of MB, but the rVTE to MB risk ratio is increased in fragile patients, often exceeding 2. This finding supports maintaining the recommended tinzaparin dose in fragile patients with CAT.

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OC-06

RAPID EXCLUSION OF CLINICALLY RELEVANT PLASMA LEVELS OF DIRECT ORAL ANTICOAGULANTS IN PATIENTS USING THE DOAC DIPSTICK IN VARIOUS INDICATIONS

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Introduction: Accurate and rapid detection of DOACs in the circulation remains a major challenge in patients presenting with major bleeding or with thrombotic events during treatment, or requiring urgent surgery or an invasive procedure. Rapid methods for assessing plasma concentrations of DOACs such as hemostatic assays, rotational thrombelastography using global or specific activators for blood clotting, require blood sampling, transportation of sample to the laboratory, centrifugation, coagulation platforms and coagulation specific reagents. A urine dipstick method contains one in all reagents and can be readily used as near patient test.

Aim: DOAC Dipstick (DOASENSE, Heidelberg, Germany) is a point-of-care test that uses a disposable test strip to detect DOACs in urine and to determines qualitatively for presence or absence dabigatran and factor Xa inhibitor (FXa) DOACs.

Methods and Results: Some recent investigations and studies have demonstrated the performance of DOAC Dipstick on urine at a threshold of >30 ng/mL plasma. A pooled analysis of 5 published studies calculated the following performance values (Thromb Haemost 2024). The proposed algorithm enhances medical decision-making in acute care indications useful primarily in hospitals not having readily available quantitative tests and 24/7. Another recently published pilot study, a plasma threshold of 100 and 120 ng/mL was compared to the results of the dipstick test for deciding on fibrinolytic or mechanical treatment in patients with acute ischaemic stroke or transient ischaemic attack. The sensitivity and specificity for FXA inhibitors were 83% and 93% of the still ongoing study (Front Neurol. 2023). The high sensitivity and NPV of the dipstick were also confirmed in a study in preoperative medicine using

a plasma threshold of >30 ng/mL determined by liquid mass spectrometry (Table 1). The receiver operating curve for the dipstick was 0.92 (95%CI 0.85 - 1.00) (submitted for publication).
Conclusions: The data confirm the ability of the DOAC Dipstick to exclude clinically significant levels at a plasma threshold of >30ng/mL of DOACs. An algorithm suggests quantitative method if dipstick results are positive if available within am adequality short time frame. As plasma threshold values have not yet been established for the various indications, further studies are performed.

Table 1.

	FXa DOACs Mean (95% CI)	Dabigatran Mean (95% CI)
Sensitivity	97.8 (95.6 – 99.0)	98.3 (91.0 – 100)
NPV	86.6 (76.0 – 93.7)	99.6 (97.7 – 100)
PPV	87.2 (83.7 – 90.1)	73.4 (63.7 – 83.2)
Specificity	50.0 (40.2 – 59.0)	91.8 (87.7 – 94 .9)

PLENARY SESSION 4
EXPLORING THE RELATIONSHIP BETWEEN HEMOSTASIS AND CANCER: NEW INSIGHTS

OC-07

INVESTIGATING A PROPOSED ANTI-CANCER IMMUNITY EFFECT OF RIVAROXABAN IN BREAST CANCER

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Introduction: Immune Checkpoint Inhibitors (ICI) are now licensed in the early and metastatic breast cancer setting. However, most cancer patients do not derive long-term benefit attributed to an intrinsic or acquired resistance. Myeloid cell-synthesized Factor Xa impedes anti-tumour immunity in the tumour microenvironment via the activation of PAR2, promoting tumour progression independent of coagulation. The Factor Xa inhibitor Rivaroxaban abrogates this tumour stimulatory effect. Improved response and survival is seen in melanoma patients on Factor Xa inhibitors (*e.g.*, for VTE prophylaxis) receiving ICI. We have recently completed a multi-centre phase II pre-operative 'Window-of Opportunity' randomised controlled trial of the oral Factor Xa inhibitor Rivaroxaban compared to no treatment in ER negative, stage I-III early breast cancer patients, the TIP Trial (n=88 patients). Patients were randomised 1:1 (Rivaroxaban 20mg od: no treatment) and received 14 (+/-3) days of treatment in the window between diagnosis and surgery or commencement of neoadjuvant chemotherapy.
Aim: The Factor Xa inhibitor Rivaroxaban promotes an anti-cancer tumour microenvironment in early breast cancer patients.
Materials and Methods: Using the PhenoCycler technology we shall comprehensively profile the immune microenvironment of TIP Trial FFPE tissue samples following transcriptome analysis of breast tissue cores collected into RNAlater. In work up experiments, we cultured the monocyte cell lines THP-1 and U937 and differentiated them into macrophages using PMA. We assessed expression of the macrophage marker CD68 and Factor X by Western Blot and tested Rivaroxaban-treated macrophage conditioned media by cytokine array.
Results: PMA-treated THP-1 and U937 expressed CD68 indicating successful differentiation into macrophages and expressed Factor X albeit at low levels, indicating they were suitable models for FXa-producing myeloid cells. In response to Rivaroxaban, both models showed a decrease in immune cell chemotactic cytokines such as CCL7 and CCL20. The highest increasing cytokines for THP-1 and U937 macrophages were FGF-7 and TGF-B2 respectively that have established roles in cancer cell migration.
Conclusions: The decrease in CCL7 and CCL20 cytokine levels may provide another mechanism by which FXa-producing myeloid cells effect the immune microenvironment. This provides preliminary data for the TIP Trial tissue analysis that should be complete by the 12th ICTHIC conference.

OC-08

TUMOR PLATELET TRANSCRIPTOME CHANGES AFFECTED BY MICRO METASTASIS

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Introduction: It is known that tumors induce profound alterations in platelet transcriptomes but modifiers and specific underlying mechanisms of platelet tumor education are incompletely understood. Thrombin is the major platelet activator through protease activated receptor (PAR) 4 in mice; and coagulation activation and thrombin generation is a hallmark of various cancers. We hypothesized that thrombin-PAR4 induced platelet hyperreactivity contributes to platelet transcriptome changes in tumor bearing mice.
Aim: We determined platelet transcriptomes of tumor free and tumor bearing WT and hyper-thrombotic thrombomodulin mutated TM^{Pro} mice. In hyper-thrombotic mice, we also deleted PAR4 to assess the role of thrombin signaling in platelets. We also prevented other thrombin signaling effects by crossing TM^{Pro} mice with a thrombin insensitive PAR1 point mutation ($PAR1^{R41Q}$) mouse.

Materials and Methods: We used the B16F10 transplantable tumor model, characterized tumor cell expressed and exosome released genes as well as platelet transcriptomes by RNA-seq.
Results: TM^{Pro} mice showed increased tumor growth, dependent on PAR signaling, and showed markedly lower platelet counts relative to tumor-bearing WT mice, indicating a profound intravascular prothrombotic state in this tumor model. Increased consumption of platelets in tumor-bearing hyper-thrombotic TM^{Pro} mice was reversed by PAR4, but not PAR1 signaling deficiency. Despite these variations in platelet counts, platelets from all genotypes showed very similar platelet transcriptome changes that overlapped to >80% with transcripts expressed in tumor cells and tumor cell-derived exosomes. Although hyper-thrombotic TM^{Pro} mice display increased metastasis to the lungs in various tumor models, spontaneous micro metastases were very low and not different between the different strains carrying B16F10 tumors. In contrast, bone marrow metastasis was indicated by increased abundance of tumor cell transcripts; and these were also highly enriched in the platelet transcriptomes of the tumor bearing mice.
Conclusions: Platelet transcriptome changes can be traced to tumor cell transcripts, as well as tumor-cell derived exosomes, and occur independent of thrombin-induced alterations of platelet hyperreactivity and platelet half-life in tumor bearing mice. In contrast, tumor platelet education is closely correlated with the degree of micro metastasis in bone marrow, indicating a transfer of tumor derived RNA and exosomes to megakaryocytes.

PLENARY SESSION 6
NOVEL BIOMARKERS FOR PREDICTING CLINICAL OUTCOMES IN CANCER PATIENTS

OC-09

GROWTH DIFFERENTIATION FACTOR-15 IS ASSOCIATED WITH RISK OF MAJOR BLEEDING IN CANCER PATIENTS WITHOUT ANTICOAGULATION: RESULTS FROM A PROSPECTIVE COHORT STUDY

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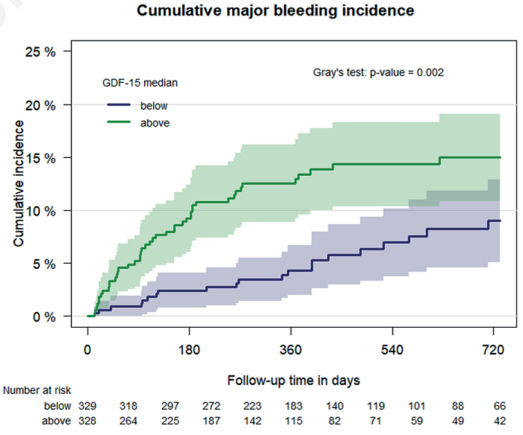
Introduction: Hemostatic imbalances are frequent in patients with cancer. Despite extensive knowledge of venous thromboembolism risk, there is limited understanding of bleeding risk, risk factors, and biomarkers predictive for bleeding in cancer patients without anticoagulation. Prior research indicates that growth differentiation factor-15 (GDF-15), a stress-response protein of the transforming growth factor-ß superfamily, holds promise as a predictive biomarker for bleeding risk in various patient populations, including a previous analysis in patients with cancer receiving anticoagulation.
Aim: We aimed to investigate the association between GDF-15 and bleeding risk in a novel cohort of patients with cancer initiating systemic anti-cancer therapies who did not receive anticoagulation.
Materials and Methods: Major bleeding (MB) was defined according to the ISTH recommendation. Measurements were performed in serum samples drawn before initiation of anti-cancer treatments with the Elecsys® GDF-15 assay (Roche Diagnostics, Rotkreuz, Switzerland). The association between GDF-15 and MB was analyzed in a Fine and Gray model accounting for all cause-mortality as competing risk.
Results: In total, 670 patients (49% women) were included in this analysis (median age: 61, interquartile range [IQR]: 53-69). During a median follow-up of 18 months (IQR: 11-28), 67 patients (10.0%) experienced a MB (12-month cumulative incidence: 8.3%, 95% confidence interval [CI]: 6.1-10.4). The median GDF-15 level was 1739.5 ng/L (IQR: 996.5-3437). Elevated GDF-15 levels were significantly associated with an increased risk of MB (SHR per doubling: 1.41 [95% CI: 1.20-1.66]), also when adjusting for sex, age, BMI, tumor type and stage, albumin, and hemoglobin (SHR: 1.28, 95% CI: 1.02-1.61). The cumulative incidence of MB was higher in patients with GDF-15 levels above the median (>1739.5 ng/L) than in those with levels below the median (≤1739.5 ng/L) (12-month cumulative incidence [95% CI]: 12.5% [8.9-16.2] *versus* 4.3% [2.0-6.7], p=0.002, Figure 1).
Conclusions: In patients with cancer without anticoagulation, elevated GDF-15 levels were significantly associated with an increased risk of major bleeding. Therefore, GDF-15 is a promising candidate biomarker for bleeding risk prediction in patients with cancer without anticoagulation.



Cumulative incidence of major bleeding in patients with GDF-15 levels below (n=335, blue line) (≤1739.5 ng/L) versus above the median (n=335, green line) (>1739.5 ng/L). Patients were divided according to their GDF-15 level and the group with levels below 1739.5 ng/L (≤median) was compared to the group with levels above 1739.5 ng/L (>median) within a Fine and Gray subdistribution hazard model (p=0.002).

Figure 1.

OC-10

PREDICTIVE VALUE OF ACTIVATED FXI-ANTITHROMBIN COMPLEX IN CANCER-ASSOCIATED THROMBOSIS (CAT): A PROSPECTIVE COHORT STUDY IN LUNG CANCER PATIENTS

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G. Masci[6], C. Tondini[7], F. Petrelli[8], F. Giuliani[9], A. D'Alessio[10], A. Santoro[6], G. Gasparini[4], R. Labianca[11], H. ten Cate[2], A. Falanga[3], on behalf of the Hypercan Investigators

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Introduction: Patients with lung cancer are particularly vulnerable to thrombosis, especially when undergoing chemotherapy. Activated factor XI (FXIa) plays a significant role in the contact system, which contributes to the pathophysiology of CAT. However, further extensive research is still necessary to fully understand the role of the contact system in lung cancer-related thrombosis.
Aim: In a prospective cohort of newly diagnosed non-small cell lung cancer (NSCLC) patients enrolled in the HYPERCAN study, we measured the levels of biomarkers of contact activation to assess whether they can help predict venous thromboembolism (VTE) within 6 months after starting chemotherapy.
Materials and Methods: Prechemotherapy plasma samples were tested by ELISA for *in vivo* complexes of contact pathway activation (*i.e.*, plasma kallikrein:C1-esterase inhibitor [PKa:C1Inh], FXIa:antithrombin [FXIa:AT], FXIa:C1Inh, FIXa:AT, and thrombin generation (*i.e.*, prothrombin fragment 1+2 [F1+2], thrombin-antithrombin complex [TAT]). Clinical data and VTE were recorded prospectively.
Results: A total of 719 (489M/230F, median age: 66 years) NSCLC patients (568 metastatic and 151 locally advanced) were studied. The 6-month cumulative incidence of VTE was 10%, with a higher incidence in the metastatic group (12%) compared to the locally advanced group (4%). A total of 68 patients developed VTE, and they were found to have significantly higher ($p<0.001$) levels of FXIa:AT complex, F1+2, and TAT before receiving chemotherapy compared to those who did not develop VTE. This finding remained significant even after correcting for age and gender. The results of a multivariable analysis revealed that FXIa:AT [HR 1.18 (95%CI 1.02-1.39)] and TAT [HR 1.30 (95%CI 1.08-1.57)] are independent risk factors for VTE during chemotherapy. Additionally, patients with FXIa:AT and TAT values above the highest quartile had a significantly higher incidence of VTE than those with values below the 3rd quartile. The difference was significant, with the former group exhibiting 23% incidence as opposed to the latter group's 8% incidence (log-rank <0.001), as shown by KM analysis.
Conclusions Patients with NSCLC who developed VTE showed increased activation of their contact system pathway. Furthermore, a scoring system based on both FXIa:AT and TAT was developed to identify patients who have a higher chance of developing VTE. These findings support the use of FXIa inhibitors in the prevention and treatment of CAT.

PLENARY SESSION 7
ANTICOAGULATION IN CANCER PATIENTS

OC-11

MANAGING A CANCER-ASSOCIATED THROMBOSIS CLINIC - OPPORTUNITIES AND CHALLENGES

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Introduction: Cancer-associated thrombosis (CAT) remains the number one cause of death during chemotherapy and the second-leading cause of all cancer deaths (1, 2). CAT is associated with a high risk of recurrent thrombosis, bleeding, and mortality (3). Cancer patients are estimated to have a 2-20-fold higher risk of developing venous thromboembolism (VTE) (4). Treatment of CAT is challenging, and the introduction of direct oral anticoagulants (DOACs) has made treatment decisions complex. Expert groups of clinicians across the world have defined various consensus guidelines (5). Despite evidence based guidelines, implementation remains unpredictable (6). A dedicated CAT service may improve overall standards of care in this setting. This is viewed positively both among patients and clinicians (1). Establishing a dedicated CAT clinic model for cancer patients with or at risk of VTE would help to reduce mortality and cut down financial cost (2).
Aim: To address these challenges, a dedicated CAT service has the potential to improve patient care by addressing various aspects of unmet needs.
Materials and Methods: A new dedicated thrombosis service was launched for cancer patients at University Hospitals of North Midlands (UHNM) in the UK. This presentation encapsulates recent developments in this area in light of the experience gained in running the CAT service for the last two years.
Results: A total of 2266 new patients were referred to anticoagulant management service within a year, from Dec. 2022 to Dec 2023, of which 282 were CAT patients. A significant number of them continued as follow-up cases but some of them were discharged back to the primary care. CAT service carries a multidisciplinary team that meets weekly. The comprehensive management of CAT requires a multidisciplinary approach that integrates anticoagulant therapy, cancer treatment, prophylaxis, bleeding management, and supportive care. This approach involves a diverse panel of specialists, including oncologists, haematologists, pharmacists, and clinical nurse specialists. The overarching goal is to minimize the risk of recurrent thrombosis, mitigate bleeding risks, and ultimately improve patient outcomes and satisfaction. The other aspects include addressing prophylaxis, supporting patients, research, education, and training.
Conclusions: In the pursuit of enhancing clinic access for patients grappling with CAT, seeking consultation with healthcare providers well-versed in thrombosis in cancer patients is crucial. Proposing a CAT service, aims to provide a specialized care, improve communication, offer support, and foster research and training in this field.

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OC-12

PROGNOSTIC FACTORS ASSOCIATED WITH RECURRENT VENOUS THROMBOEMBOLISM IN PATIENTS WITH CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM: SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction: Recurrent venous thromboembolism (VTE) is frequent in cancer patients. Understanding the factors associated with an increased or decreased rate of recurrent VTE is essential for developing evidence-based tools to guide decisions regarding optimal duration and intensity of anticoagulation in this patient population.

Aim: To determine the association between prognostic factors and recurrent VTE in patients with cancer-associated VTE.

Materials and Methods: We searched MEDLINE, Embase, and the Cochrane Library (from inception to February 2024) for randomized controlled trials and cohort studies that examined factors associated with recurrent VTE in patients with cancer-associated VTE. We also obtained additional data from the Hokusai VTE Cancer, CLOT, CATCH, and SELECT-D randomized trials. For the primary analysis, we only pooled prognostic factors that were derived from a multivariable model which included at least age, sex, cancer site or cancer stage, and reported in at least two studies.

Results: Of 4587 citations, 26 studies (51,594 patients) met inclusion criteria for this review. Most of the studies were observational cohorts of cancer patients with VTE receiving anticoagulation for six months. Factors associated with an increased rate of recurrent VTE included a history of VTE (adjusted hazard ratio [aHR] 1.50 [95% confidence interval [CI] 1.08 to 2.09]), Eastern Cooperative Oncology Group performance status ≥1 (aHR 1.88 [1.44 to 2.46]), advanced cancer (aHR 1.43 [1.17 to 1.75]), lung cancer (aHR 2.19 [1.29 to 3.74]), genitourinary cancers (aHR 1.38 [1.10 to 1.74]), pancreatic cancer (aHR 6.06 [2.04 to 12.08]), elevated C-reactive protein (aHR 3.62 [1.27 to 9.58]), elevated D-dimer (aHR 2.93 [1.70 to 5.03]), and elevated soluble P-selectin (aHR 4.98 [2.00 to 12.40] (Table 1). Conversely, female sex (aHR 0.89 [0.79 to 0.99]) and recent surgery (aHR 0.56 [0.40 to 0.76]) were associated with a decreased rate of recurrent VTE (Table 1).

Conclusions: This systematic review and meta-analysis summarizes the association between several prognostic factors and recurrent VTE in patients with cancer-associated VTE. These factors should be carefully considered in risk stratification frameworks to help make clinical decisions regarding management of patients with cancer-associated VTE.

Table 1.

Prognostic Factors Associated with Recurrent Venous Thromboembolism.

Prognostic Factors	Studies	Adjusted Hazard Ratio (95% CI)	P	I^2(%)
Patient Factors				
Female sex	12	0.89 (0.79 to 0.99)	0.038	4
Older age	9	0.97 (0.95 to 1.00)	0.017	71
History of VTE	6	1.50 (1.08 to 2.09)	0.015	15
Renal insufficiency	4	1.09 (1.00 to 1.18)	0.061	72
Recent surgery	2	0.56 (0.40 to 0.76)	0.0003	0
Initial VTE				
DVT alone	9	1.25 (0.82 to 1.91)	0.292	70
Proximal DVT	3	0.98 (0.56 to 1.74)	0.956	22
Symptomatic VTE	3	1.36 (0.56 to 3.31)	0.502	52
Residual vein obstruction[a]	2	3.68 (0.69 to 19.7)	0.127	83
ECOG performance status ≥1	6	1.88 (1.44 to 2.46)	<0.0001	0
Cancer Factors				
Advanced cancer	13	1.43 (1.17 to 1.75)	0.0006	46
Chemotherapy	5	1.08 (0.86 to 1.37)	0.499	50
Cancer site				
Brain	3	1.38 (0.62 to 3.08)	0.429	84
Breast	5	0.46 (0.19 to 1.11)	0.084	87
Lung	6	2.19 (1.29 to 3.74)	0.004	83
Gastrointestinal	3	1.01 (0.46 to 2.22)	0.977	73
Genitourinary	3	1.38 (1.10 to 1.74)	0.006	0
Hepatobiliary	2	2.03 (0.19 to 21.27)	0.555	75
Pancreas	2	6.06 (2.04 to 12.08)	<0.0001	0
Biomarkers				
Elevated C-reactive protein	3	3.62 (1.27 to 9.58)	0.010	51
Elevated D-dimer	4	2.93 (1.70 to 5.03)	0.0001	57
Elevated P-selectin	2	4.98 (2.00 to 12.40)	0.0006	0

ECOG=Eastern cooperative oncology group; DVT= deep vein thrombosis; VTE=venous thromboembolism
[a]In patients who discontinued anticoagulation.

POSTER SESSION 1
BIOMARKERS/ HYPERCOAGULABILITY I

PO-01

ALTERED WHOLE BLOOD THROMBIN GENERATION AND HYPERRESPONSIVE PLATELETS ASSOCIATE WITH THROMBOEMBOLIC EVENTS IN PATIENTS WITH PANCREATIC DUCTAL ADENOCARCINOMA

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Introduction: Thromboembolic disease is a major complication in pancreatic ductal adenocarcinoma (PDAC) patients. Patients with PDAC often have altered blood cell counts, which associate with incident thrombosis. The high thrombotic risk in patients with PDAC may be partially explained by the effects of pro-coagulant blood cells.
Aim: 1. To compare blood cell dependent coagulation and fibrinolysis between PDAC patients and controls matched for age and sex. 2. To explore whether blood cell dependent coagulation associates with incident thrombosis in PDAC patients
Materials and Methods: Patients with locally advanced and metastatic PDAC and controls matched for age and sex were included. Thrombin generation (TG) was measured in whole blood (WB) and plasma. Platelet granule release capacity (PGRC) was measured in WB. Patients were followed for the occurrence of thromboembolic events during 6-months.
Results: At first, we tested differences in TG between patients (n=18) and controls (n=18). Patients (n=18) showed an increased endogenous thrombin potential (ETP) in WB, compared to controls (n=18; 2714 *vs* 2905, p=0.003). This was in contrast to plasma TG, as no difference in ETP was found in plasma when comparing patients to controls. For both plasma and WB the lag time was longer in patients compared to controls, respectively 10.5 *vs* 8.9 minutes (p=0.013) for WB and 7.6 *vs* 6.2 (p=0.006) for plasma. Secondly, the capacity of platelets to release granules was tested. Patients had hyperresponsive platelets, with a shorter time to maximum platelet granule release (43 *vs* 62 seconds, p=0.008). Of the 18 patients with PDAC, five patients developed thromboembolic events (28%). A shorter lag time in WB (HR=0.475, 95%-CI=(0.228-0.988)), not in plasma, and an increased PGRC (HR=1.148, 95%-CI=(1.007- 1.309)) were associated with thromboembolic events.
Conclusions: Patients with PDAC have an increased and delayed WB-TG coagulation profile compared to controls. The increase in coagulation was not found in plasma, implying blood-cell dependent procoagulant effects. Blood cell dependent coagulation seems to associate with incident thromboembolic events in patients with PDAC and platelets appear to play a key role. Hemostasis measurement in WB is likely to further improve thrombosis risk estimation in PDAC patients.

PO-02

THROMBO-HEMORRHAGIC EVENTS AND TISSUE FACTOR EXPRESSION IN NEWLY DIAGNOSED PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA RECEIVING INDUCTION THERAPY

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Introduction: Acute promyelocytic leukemia (APL) is characterized by a life-threatening coagulopathy, secondary to TF-mediated clotting activation. Current protocols including arsenic trioxide (ATO) and all trans retinoic acid (ATRA) have exhibited beneficial effects on the hemostatic derangement, particularly downregulating cellular TF expression. Given the still relevant rate of lethal thrombo-hemorrhagic events (THE) in APL, characterizing the coagulopathy and identifying predictive markers remains a critical issue.
Aim: We prospectively recorded THE occurrence in the first month after APL diagnosis, and monitored the circulating hypercoagulation markers and the molecular expression of TF, before and during induction therapy.
Materials and Methods: Sixty-five consecutive APL patients receiving ATRA+Idarubicin (n=60, GIMEMA AIDA2000) or ATRA+ATO (n=5, GIMEMA APL0406) for remission induction were enrolled in 2 Italian Centers (2005-2015). Blood samples were obtained from 35 patients at diagnosis before therapy (D0), and during induction on days (D)7, 14 and 28, and tested for Tissue Factor mRNA (TF mRNA) expression by peripheral mononuclear cells and for plasma levels of FVII-Antithrombin Complex (FVIIa-AT), a parameter of TF activity, together with markers of thrombin generation (TAT) and fibrinolysis (D-dimer).
Results: At D0, 12 patients (18%) presented with THE: 8 major bleeding (3 fatal intracranial and 5 non-fatal major bleedings (MB)) and 4 thrombosis (1 fatal). Within 3 days of ATRA, 2 additional fatal intracranial bleedings occurred, ac-

counting for 9% early deaths. In the next 20 days, 3 non-fatal MB and 2 non-fatal thrombosis developed. Laboratory study showed APL TF mRNA significantly higher than controls at D0, which progressively decreased by 68%, 70%, and 90%, at D7, D14 and D28, respectively. TAT and D-dimer levels, initially elevated, significantly decreased at D7, and were lowest at D28, while FVIIa-AT dropped significantly only at D28. Statistically significant correlations were found between the decrease in TF mRNA and the decrease in FVIIa-AT levels during induction therapy.
Conclusions: Our data show a significant rate of severe thrombo-hemorrhagic events in our cohort of APL patients (19/65, 29%), including 6 early fatal events. Laboratory data demonstrate the TF mRNA downregulation under induction therapy, which parallels hypercoagulation markers decrease. Persistent high TF-dependent clotting activation (FVIIa-AT) might explain post-ATRA THE.

PO-03

PLASMA MARKERS OF HEMOSTATIC ACTIVATION AND FIBRINOLYSIS IN PATIENTS WITH NON-METASTATIC CASTRATION-RESISTANT PROSTATE CANCER BEFORE AND AFTER SECONDARY HORMONAL THERAPY AND ANTI-PSMA RADIOIMMUNOTHERAPY

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Introduction: The number of venous and arterial thrombotic events in patients with prostate cancer (PCa) is amongst the most common across cancers, likely due to the prevalence of PCa. Events are related to stage as well as treatment. Multiple classes of therapeutic agents have improved outcomes for patients with PCa. While hormonal therapy has been utilized long-term, targeted radionuclides are newer. The effect of hormonal manipulation and radionuclides on the hemostatic and fibrinolytic system is under-studied. We previously described differences in plasma markers related to stage.
Aim: Here we report preliminarily results in a prospective, randomized study, for the first time assessing the effect of therapeutic radionuclides as well as hormonal therapy.
Materials and Methods: Patients with high-risk "non-metastatic" (CT and bone scan negative) castration-resistant prostate cancer (M0 CRPC) were enrolled in a multicenter study (NCT00859781). Treatment included a 1-month run-in period of open-label secondary hormonal therapy (ketoconazole and hydrocortisone) followed by addition of radioimmunotherapy (RIT) with radiolabeled anti-PSMA antibody J591 (blinded to receive the therapeutic beta/gamma emitter 177Lu *vs* the diagnostic gamma/auger emitter 111In in 2:1 ratio). Plasma was collected for analysis of markers of hemostatic activation, fibrinolysis, and angiogenesis at baseline, after 1 month of hormonal therapy, and 1 month after radionuclide. ELISA was performed for D-dimer, thrombin-antithrombin complex (TAT), tissue factor (TF), IL-6, IL-8, and VEGF.
Results: As previously reported, baseline pre-treatment levels of plasma markers appear to be overall higher in this M0 CRPC population compared to historical controls of untreated clinically localized disease. Median levels of plasma markers were not significantly different after 1 month of secondary hormonal therapy. However, after radiolabeled J591, D-dimer increased (median 4-fold) while TAT appeared to decrease. Full analysis of unblinded data is ongoing; it appears that the D-dimer increase is driven by changes after 177Lu as opposed to 111In and this may be similar for TAT.
Conclusions: Plasma markers of hemostatic activation and fibrinolysis appear to be affected by radioimmunotherapy. As there may be a relationship to cancer status in addition to treatment effects, analysis with relationship to PSA response, the development of metastatic disease, and thrombotic/bleeding events is ongoing.

PO-04

ALTERED PLASMIN GENERATION IN PATIENTS WITH PANCREATIC DUCTAL ADENOCARCINOMA

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Introduction: Thromboembolic disease is an important complication in pancreatic ductal adenocarcinoma (PDAC) patients. Fibrin degradation plays a role in the occurrence of a thrombus. However, assays studying fibrinolysis kinetics are unexplored. A recently developed assay to measure plasmin generation kinetics offers promising avenues for exploring potential alterations in the fibrinolytic system.
Aim: To study fibrinolysis phenotype in PDAC patients, using a case-control approach.
Materials and Methods: 18 patients with locally advanced and metastatic PDAC, before and 8 weeks after chemotherapy, and 18 controls with the same age and sex distribution, were included. Plasmin generation (PG), thrombin generation (TG) and clot lysis time (CLT) were measured in platelet-poor plasma (PPP). The relation between fibrinolysis parameters and PDAC prevalence was studied using a case-control analysis.
Results: In PDAC patients before and after chemotherapy, we observed significant changes in endogenous plasmin potential (EPP) and plasmin peak levels compared to controls (Figure 1). Specifically, patients exhibited higher EPP (116.1% & 110% *vs* 95.08%, p=0.008 & p=0.004) and plasmin peak levels (98.96% & 105.9% *vs* 93.57%, p=0.1 & p=0.006), expressed as median % relative to normal pooled plasma. Additionally, patients showed prolonged lag time (2.50 & 2.67 *vs* 2.33 min, p=0.26 & p=0.021) and ttPeak (5.33 & 5.53 *vs* 4.67 min, p=0.002 & p=0.0002) compared to controls. In contrast, profiles of patients before and after chemotherapy and controls were similar in terms of TG and CLT, with the exception of prolonged TG lag

time (3.67 *vs* 2.9 min, p=0.009) observed in patients before chemotherapy. In the presence of thrombomodulin (TM), patients showed less inhibition of EPP by TM (41.93% & 37.80% *vs* 55.14%, p=0.017 & p=0.001) and less inhibition of plasmin peak levels (26.14% & 31.61% *vs* 39.82%, p=0.003 & p=0.028) compared to controls.

Conclusions: PDAC patients exhibit an elevated plasmin generation compared to controls. Additionally, both PG and TG are delayed in PDAC patients. The observed delay in thrombin formation and fibrinolysis among PDAC patients may contribute to thrombus formation and increase the risk of venous thromboembolism (VTE). Strikingly, PDAC patients exhibit reduced sensitivity to the inhibitory effect of TM on PG.

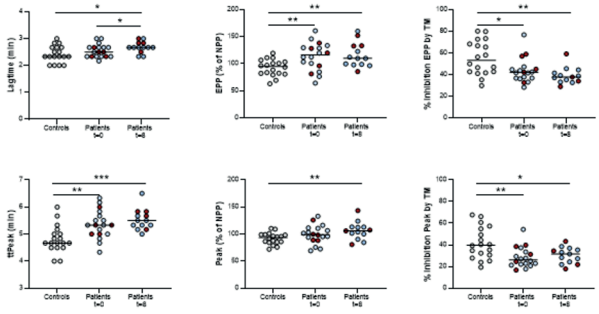

Figure 1. Plasmin generation in patients with PDAC and healthy controls.

Plasmin generation parameters in patients with PDAC before chemotherapy and 8 weeks after the start of chemotherapy and matched controls. Plasmin generation was triggered with 1.25 µg/mL tPA and 5 pM TF in the presence or absence of TM in platelet poor plasma. Medians are presented as error bar. Patients before chemotherapy (t=0) are indicated in light blue, patients during chemotherapy (t=8) are indicated in dark blue, healthy controls are indicated in grey. Patients that developed a thromboembolic event, either venous or arterial, are indicated in purple. Wilcoxon rank sum tests or Mann-Whitney U tests were performed to compare the groups. EPP: Endogenous plasmin potential, min: minutes; TF: Tissue Factor; TM: Thrombomodulin; t_0: first blood withdrawal before chemotherapy; t_8: second blood withdrawal 8 weeks after the start of chemotherapy. *P <0.05, **P<0.01, ***P<0.001.

PO-05

AN MRI RADIOMICS APPROACH TO PREDICT THE HYPERCOAGULABLE STATUS OF GLIOMAS

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Introduction: Venous thromboembolic events are frequent complications of Glioblastoma Multiforme (GBM) and Low-Grade Gliomas (LGG). The overexpression of the Tissue Factor (TF) plays an essential role in the local hypercoagulable phenotype that underlies these complications. Magnetic Resonance Imaging (MRI) plays a key role in the diagnosis and follow-up of LGG/GBM. It also allows for a powerful and non-invasive exploration of many facets of the biology of these tumors using a radiomics strategy, *i.e.* the extraction of features related to tumor morphology and texture. The possibility of using MRI radiomics to explore the local hypercoagulable status of LGG/GBM has not yet been tested.

Aim: Our aim was to build an MRI radiomics model for the non-invasive exploration of the hypercoagulable status of LGG/GBM.

Materials and Methods: Radiogenomics data available from two cohorts were used: TCGA (The Cancer Genome Atlas) and REMBRANDT (Repository for molecular BRAin Neoplasia DaTa) (n=136 and n=39 LGG/GBM patients, used as training and validation cohorts, respectively). We retrieved 120 tumor radiomics features and RNA expression levels of F3, encoding TF. The seven most contributive MRI radiomics features from LGG/GBM linked to high TF were identified in TCGA using Least Absolute Shrinkage and Selection Operator (LASSO) regression. A logistic regression model (Radscore) was built in order to identify the top-20% F3-expressing tumors, considered to be at high thromboembolic risk.

Results: This model had good performance in TCGA/training and REMBRANDT/validation cohorts: AUC=0.87 [CI95: 0.81-0.94, p<0.0001] and AUC=0.78 [CI95: 0.56-1.00, p=0.02], respectively. In agreement with the key role of the coagulation cascade in gliomas, LGG patients with a high Radscore had lower overall and disease-free survival. The Radscore was linked to the presence of specific genomic alterations, the composition of the tumor coagulome and the tumor immune infiltrate.

Conclusions: Our findings suggest that a non-invasive assessment of the hypercoagulable status of LGG/GBM is possible with MRI radiomics.

PO-06

ANTIPHOSPHOLIPID ANTIBODIES IN WOMEN WITH ENDOMETRIAL AND CERVICAL CANCER

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Introduction: Current generally accepted clinical and laboratory criteria for antiphospholipid syndrome are well known and include vascular thrombosis and pregnancy complications in patients with circulating antiphospholipid antibodies (aPLA). However, in the last few years, aPL have become a common finding in patients with malignancy.

Aim: The aim of our work was to understand the role of aPLA in patients with endometrial and cervical cancer.

Materials and Methods: The study included 96 cancer patients Grade 1 and Grade 2 aged 28 to 49 years with a verified histomorphological diagnosis of adenocarcinoma: endometrial cancer (group I, n=73) and cervical cancer (group II, n=23). The control group consisted of 60 healthy women. Plasma samples from all study participants were tested for the presence of lupus anticoagulant and IgG, IgM isotypes of antibodies to cardiolipin (aCL), β2-glycoprotein 1 (anti-β2-GP1), annexin V and anti-phos-

phatidylserine-prothrombin complex antibodies (anti-PS-PT) by enzyme immunoassay.

Results: Statistically significant differences were found in the assessment of aCL IgG/IgM, anti-b2-GPI IgG, anti- annexin V, IgM, anti- PS-PT, IgG, depending on the group of women with cancer and the control group (p=0.041, p=0.017, p=0.004, p=0.001, p=0.044, respectively). When comparing aPLA depending on Grade 1 or 2, we got the following results (Table 1). Statistically significant differences were revealed for aCL IgM (p <0.001 and p=0.008, respectively) for I and II groups. Anti-b2-GpI IgM prevailed in patients of both groups, IgG – in women with cervical cancer Grade 2. Antibodies to annexin V IgG, IgM prevailed in both groups in women with Grade 2 (p <0.001). Analyzing Grade 1/2, depending on the increased level of anti-PS-PT, IgM, it was not possible to establish statistically significant differences (p=0.597 for the IgG isotype, p=0.143 for IgM). Based on the data obtained, when evaluating antibodies to PS-PT, IgG, depending on Grade 1/2, we identified statistically significant differences (p <0.001).

Conclusions: We found a statistically significant increase in the aPLA titer in patients with endometrial and cervical cancer compared with the control group of healthy women. However, when comparing the antibody titer depending on Grade 1 or 2, we found a significant relationship between the high antibody titer in Grade 2 cancer patients compared to Grade 1. Further studies are needed to establish whether aPLA can be used as a diagnostic tool in oncogynecological cencer to identify patients at risk of disease progression and cancer recurrence.

Table 1. aPLA in cancer patients depending on Grade 1 and Grade 2.

Parameter	Category	**aCL, IgG** (IU/ml)			p
		Me	$Q_1 – Q_3$	n	
Endometrial cancer	Grade 1	4,60	2,23 – 5,68	41	0,829
	Grade 2	4,60	2,72 – 6,42	31	
Cervical cancer	Grade 1	5,01	4,62 – 5,99	13	0,750
	Grade 2	4,68	4,60 – 6,07	11	
Parameter	Category	**aCL, IgM** (IU/ml)			p
		Me	$Q_1 – Q_3$	n	
Endometrial cancer	Grade 1	2,10	1,80 – 3,17	41	< 0,001*
	Grade 2	5,50	3,51 – 6,27	31	
Cervical cancer	Grade 1	2,60	1,06 – 3,17	13	0,008*
	Grade 2	5,24	4,38 – 6,41	11	
Parameter	Category	anti-b2GpI, IgG (IU/ml)			p
		Me	$Q_1 – Q_3$	n	
Endometrial cancer	Grade 1	3,20	1,69 – 7,01	41	0,909
	Grade 2	4,74	1,66 – 6,76	31	
Cervical cancer	Grade 1	1,80	1,20 – 2,62	13	0,001*
	Grade 2	6,89	6,00 – 7,71	11	
Parameter	Category	anti-b2GpI, IgM (IU/ml)			p
		Me	$Q_1 – Q_3$	n	
Endometrial cancer	Grade 1	1,96	1,20 – 3,40	41	< 0,001*
	Grade 2	6,43	5,59 – 7,63	32	$p_{Ф3\ Grade\ 2 – Ф3\ Grade\ 1}$ < 0,001
Cervical cancer	Grade 1	0,73	0,43 – 2,10	12	$p_{ФIIIM\ Grade\ 2 – Ф3\ Grade\ 1}$ < 0,001
	Grade 2	7,20	6,49 – 25,95	11	$p_{ФIIIM\ Grade\ 1 – Ф3\ Grade\ 2}$ < 0,001
					$p_{ФIIIM\ Grade\ 2 – ФIIIM\ Grade\ 1}$ < 0,001
Parameter	Category	anti-ANX V, IgG (IU/ml)			p
		Me	$Q_1 – Q_3$	n	
Endometrial cancer	Grade 1	1,80	1,12 – 3,10	41	< 0,001*
	Grade 2	4,26	3,98 – 4,90	32	$p_{Ф3\ Grade\ 2 – Ф3\ Grade\ 1}$ < 0,001
					$p_{ФIIIM\ Grade\ 2 – Ф3\ Grade\ 1}$ = 0,001
Cervical cancer	Grade 1	1,55	0,87 – 2,64	12	$p_{ФIIIM\ Grade\ 1 – Ф3\ Grade\ 2}$ < 0,001
	Grade 2	4,12	4,00 – 8,54	11	$p_{ФIIIM\ Grade\ 2 – ФIIIM\ Grade\ 1}$ = 0,001
Parameter	Category	anti-ANX V, IgM (IU/ml)			p
		Me	$Q_1 – Q_3$	n	
Endometrial cancer	Grade 1	1,80	1,30 – 3,70	41	< 0,001*
	Grade 2	4,20	3,47 – 4,93	32	$p_{Ф3\ Grade\ 2 – Ф3\ Grade\ 1}$ = 0,001
					$p_{ФIIIM\ Grade\ 2 – Ф3\ Grade\ 1}$ = 0,004
Cervical cancer	Grade 1	1,64	0,08 – 2,19	12	$p_{ФIIIM\ Grade\ 1 – Ф3\ Grade\ 2}$ < 0,001
	Grade 2	4,70	4,17 – 4,83	11	$p_{ФIIIM\ Grade\ 2 – ФIIIM\ Grade\ 1}$ < 0,001
Parameter	Category	anti-PS-PT, IgG (IU/ml)			p
		Me	$Q_1 – Q_3$	n	
Endometrial cancer	Grade 1	2,90	2,10 – 4,80	41	< 0,001*
	Grade 2	8,06	2,55 – 8,90	32	$p_{Ф3\ Grade\ 2 – Ф3\ Grade\ 1}$ = 0,002
Cervical cancer	Grade 1	3,10	1,90 – 4,12	12	$p_{ФIIIM\ Grade\ 2 – Ф3\ Grade\ 1}$ = 0,026
	Grade 2	7,80	4,65 – 8,91	11	$p_{ФIIIM\ Grade\ 1 – Ф3\ Grade\ 2}$ = 0,026
					$p_{ФIIIM\ Grade\ 2 – ФIIIM\ Grade\ 1}$ = 0,036
Parameter	Category	anti-PS-PT, IgM (IU/ml)			p
		Me	$Q_1 – Q_3$	n	
Endometrial cancer	Grade 1	2,88	2,14 – 5,69	41	0,402
	Grade 2	3,25	2,61 – 5,69	32	
Cervical cancer	Grade 1	4,76	2,47 – 6,22	12	
	Grade 2	6,38	3,93 – 8,09	11	

* – differences are statistically significant (p <0.05).

PO-07

RAPID AND EFFECTIVE ISOLATION OF HUMAN PLATELETS FROM WHOLE BLOOD: MAXIMIZING PURITY FOR EVALUATING PROTEOMICS METHODS IN CLINICAL STUDIES FOR CANCER PATIENTS

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Introduction: Platelets are tiny, disc-shaped anucleate cells found in the blood that play a pivotal role in hemostasis and have emerged as key players in various physiological and pathological processes. To understand platelets function in cancer progression and their potential as biomarkers in cancer research and clinical studies, it is vital to gain insights into their proteomic profile. Isolating high-purity platelets from whole blood is crucial for accurate proteomic analysis. In this study, we present a rapid and effective protocol for the isolation of human platelets, maximizing purity.

Aim: We evaluate this protocol using various proteomic methods, aiming to determine the most suitable proteomics pipeline for analyzing platelets in cancer patients.

Materials and Methods: Blood samples were collected in ACD anticoagulant solution tubes to prevent platelet activation. The samples were then treated according to protocol and stored in -20 C for subsequent analysis. The platelets, red blood cells and white blood cells were counted in an automated cell counter at the laboratory clinic at Haukeland Hospital, Bergen. To validate the efficiency of the isolation protocol, we conducted proteomics analysis using Data Dependent Acquisition (DDA), Data Independent Acquisition (DIA), and Tandem Mass Tag (TMT) labeling.

Results: The protocol with our optimized centrifugation time exhibited minimal contamination from other blood components. The scatter plot controls showed positive correlation with no activation of platelets. The results from DDA, DIA and TMT demonstrated a notable and novel identification of platelet-specific proteins, facilitating a more accurate and detailed characterization of the platelet proteome.

Conclusions: Our rapid and effective platelet isolation protocol enhances the purity of isolated platelets and demonstrates its applicability for robust downstream proteomic analyses. DIA demonstrated slightly better coverage and sensitivity in identifying platelet proteins and would be the preferable choice.

PO-08

FACTOR VIIIC IMPROVES PREDICTION OF VTE BY THE THROMBOGYN SCORE

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Introduction: Venous thromboembolism (VTE) is a significant cause of morbidity and mortality in patients with gynaecological

cancer. Guidelines recommend prophylaxis following risk assessment with validated risk assessment tools. The Thrombogyn score is a risk model for gynaecological cancer patients developed and validated by our group which identifies patients at low and high risk for VTE[1]. Previous work has shown that Factor VIIIc (FVIIIC) is a predictive biomarker for VTE in cancer patients. Recent data has suggested that combining risk models with biomarkers for VTE can improve prediction of VTE in cancer patients.

Aim: To evaluate the ability of FVIIIC when combined with the Thrombogyn score and the Khorana score to predict VTE in a population of gynaecological cancer patients.

Materials and Methods: Gynaecological cancer patients who donated blood samples to the TCD gynaecological cancer bioresource between 2017-2020 were included in the study. All patients gave full and informed consent. Patients were followed up for a minimum of one year. All blood samples were collected before surgery. The Thrombogyn and Khorana scores were calculated for each patient and objectively diagnosed VTE was recorded during follow-up. FVIIIC levels were measured by chromogenic substrate assay. 1 point for FVIIIC above a prespecified cutoff (199.8%) was added to Thrombogyn and Khorana scores to create the extended Thrombogyn+FVIIIC and Khorana+FVIIIC scores respectively.

Results: 302 cancer patients were included in the study (Ovarian n=116, Endometrial n=124, Cervical n=37, Vulval n=25). The majority of patients were treatment naive (88.4%) at sampling. 22 patients developed VTE during follow-up. FVIIIC levels were significantly increased in patients who developed VTE compared with patients who were thrombosis free during follow-up(P=0.008). 2.6% of patients in the Thrombogyn low risk group (Thrombogyn score <1) developed VTE compared with 10.1% in the intermediate/high risk group(P=0.038). 6.6% of patients classified by the Khorana score as low risk (Khorana score<2) developed VTE during follow-up compared with 9.7% in the intermediate/high risk group(P=0.39). Cox regression analysis showed that the FVIIIC+Thrombogyn high risk group had a13.4 fold (95%CI1.47-117.8) increased risk of VTE and a cumulative incidence of VTE of 18.1% after 6 months compared with 1.5% in the low-risk group and 7.5% in the intermediate risk group. There was no significant difference in VTE risk between the risk groups with the FVIIIC+Khorana score. Overall survival was lower in the Thrombogyn+FVIIIC high risk group compared with the low-risk group (P=0.008).

Conclusions: Addition of FVIIIC data to the Thrombogyn score increases the ability of the score to predict VTE. In contrast, the Khorana score did not predict VTE in these patients either with or without FVIIIC. FVIIIC is an easily available assay in hospital laboratories and may be useful as an aid to prediction of VTE in gynaecological cancer patients. Further studies are required to determine the utility of the Thrombogyn+FVIIIC score to guide prophylaxis in gynaecological cancer patients post-surgery.

Reference

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POSTER SESSION 2
THROMBOHEMORRHAGIC COMPLICATIONS

PO-09

ASSOCIATION BETWEEN ANTICOAGULATION-RELATED BLEEDING AND MORTALITY IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM

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Introduction: Patients with hematological malignancies are at an increased risk of venous thromboembolism (VTE) requiring anticoagulation (AC), but they are also at higher risk of bleeding, both of which can be associated with morbidity and mortality. The association between AC-related bleeding and death in these patients is unclear.

Aim: To evaluate the association between AC-related bleeding and mortality in patients with hematological malignancies and cancer-associated VTE on AC.

Materials and Methods: In a nationwide cohort of US Veterans (2012-2020), we identified patients with active hematological malignancies and cancer-associated VTE who initiated AC within 30 days of VTE diagnosis. Patients were excluded if they had any outpatient AC prescriptions within 6 months prior to VTE. Bleeding events were identified by previously validated algorithms using ICD codes. We evaluated the association between bleeding and death within 12 months of AC initiation by multivariate Cox regression models, accounting into multiple potential confounders. The occurrence of bleeding events was analyzed as a time-variant variable.

Results: The cohort included 1825 patients, 123 (6.7%) had bleeding events within 12 months of starting AC (Table 1), while 162 (8.9%) patients died. Patients with bleeding events were more likely to have anemia, history of bleeding, aspirin use, chemotherapy use, and frailty (Table 1). Multivariable analysis showed that any episode of AC-related bleeding was associated with an increased risk of death (HR 3.1, 95% CI 1.9-5.2). In addition, increasing age, increasing frailty, and liver disease are other independent risk factors for death. Body mass index (BMI) was protective (*i.e.* higher BMI was associated with lower mortality). When further stratified by bleeding site, intracranial bleeding was associated with the highest risk of death (HR 17.0, 95% CI 5.9-49.5), followed by gastrointestinal bleeding (HR 4.03, 95% CI 2.2-7.4). Bleeding at other sites including the genitourinary track was not significantly associated with death.

Conclusions: In this cohort of patients with hematological malignancies and VTE initiated on AC, bleeding was associated

with a significantly increased risk of death within 12 months, with even higher risks when bleeding occurred in intracranial or gastrointestinal sites. AC-related bleeding events carry a substantial risk of mortality and future investigations focusing on strategies to reduce these complications are essential.

Table 1.

Variables	Yes (n = 123)	No (n = 1702)	p value
Age, median (Standard Deviation)	68 (10.1)	68 (9.8)	0.08
Alcohol Abuse % (n)	6.5 (8)	4.7 (80)	0.37
Anemia (HGB <10g/dL OR HCT <30%) % (n)	64.2 (79)	52.3 (890)	0.01
Anticoagulant Type			0.24
DOAC % (n)	37.4 (46)	39.1 (666)	
LMWH % (n)	43.1 (53)	36.1 (615)	
Warfarin % (n)	19.5 (24)	24.7 (421)	
AntiPLT Therapy			
Aspirin % (n)	24.4 (30)	15.6 (265)	0.01
Clopidogrel OR Ticagrelor % (n)	7.3 (9)	4.8 (82)	0.22
Aspirin + Clopidogrel or Ticagrelor % (n)	3.3 (4)	1.6 (27)	0.17
Bleeding History % (n)	17.9 (22)	8.0 (136)	0.0002
Cancer Type			
Leukemia % (n)	53.7 (7)	5.5 (94)	0.94
Lymphoma % (n)	49.6 (61)	53.8 (915)	0.38
MDS % (n)	17.1 (21)	13.7 (233)	0.3
Myeloma % (n)	27.6 (34)	26.1 (44)	0.7
Chemotherapy Tx % (n)	61.8 976)	49.2 (837)	0.007
Chemotherapy subtype			
BTK Inhibitor % (n)	1.6 (2)	3.9 (66)	0.21
Hydroxyurea % (n)	4.1 (5)	2.6 (45)	0.35
VEGF Inhibitor % (n)	0.8 (1)	0.2 (4)	0.24
VEGF TKI % (n)	0.8 (1)	0.3 (5)	0.33
eGFR Category			0.16
<30 % (n)	45.5 (56)	36.8 (627)	
30 to <60 % (n)	20.3 (25)	24.0 (409)	
>=60 % (n)	34.2 (42)	39.1 (666)	
Fall History/Predisposition % (n)	39.8 (49)	37.1 (632)	0.55
Liver Disease % (n)	2.4 (3)	3.4 (57)	0.58
Race Category			0.76
Black % (n)	25.2 (31)	22.6 (385)	
Other % (n)	2.4 (3)	2.1 (35)	
White % (n)	72.4 (89)	75.3 (1282)	
Stroke History % (n)	6.5 (8)	5.6 (95)	0.67
Thrombocytopenia <50,000 % (n)	8.1 (10)	9.6 (164)	0.58
Uncontrolled HTN % (n)	49.6 (61)	41.7 (710)	0.09
Frailty			0.001
Non-Frail % (n)	8.9 (11)	13.1 (223)	
Pre-Frail % (n)	3.3 (15)	25.6 (436)	
Mild Frail % (n)	28.5 (35)	26.8 (456)	
Moderate Frail % (n)	27.6 (34)	19.7 (335)	
Severe Frail % (n)	22.8 (28)	14.8 (252)	

PO-10

ASSOCIATION BETWEEN ANTICOAGULATION-RELATED BLEEDING AND MORTALITY IN PERSONS WITH SOLID TUMORS

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Introduction: Balancing the high risk of venous thromboembolism (VTE) recurrence against anticoagulant (AC)-related bleeding in patients with cancer-associated VTE presents a significant clinical challenge. Limited data exist regarding the risk that AC-related bleeding confers on survival in patients with cancer and delineating this risk could inform duration of AC therapy.

Aim: We aimed to quantify the association between AC-related bleeding and death in solid tumor patients with newly diagnosed cancer-associated VTE starting AC therapy.

Materials and Methods: Using a nationwide cohort of US Veterans (2012-2020), we identified solid tumor patients with cancer-associated VTE who initiated AC within 30 days of VTE diagnosis. Patients with outpatient AC prescriptions within 6 months preceding VTE were excluded. Utilizing Cox regression, we assessed the association between AC-related bleeding & death within 12 months of AC therapy initiation. Time-varying adjustment for AC-related bleeding events accounted for immortal time bias.

Results: We identified 9,326 patients with newly diagnosed VTE and active solid tumors starting AC therapy, of which 746 (8.0%) developed bleeding within 12 months. Patients with bleeding were more likely to have a history of alcohol abuse, anemia, previous bleeding, stroke, kidney or liver disease, metastatic disease, thrombocytopenia, uncontrolled hypertension & frailty. Bleeding occurred more often in gastrointestinal (GI) (22.1%), genitourinary (17.4%), & brain tumors (2.4%). Most patients received AC therapy with LMWH (52.8%), with lower DOAC use in the bleeding group (19.7% *vs* 24.4%). There was a total of 2,003 deaths at 12 months post-AC initiation. In the multivariable Cox regression, AC-related bleeding was associated with a 2.86-fold (95% CI 2.44-3.35) increased risk of mortality at 12 months. When stratified by bleeding sites, intracranial hemorrhage had the highest association with mortality (hazard ratio [HR] 5.68) followed by GI (HR 2.73), & other bleeding sites (HR 1.89) (Figure 1).

Conclusions: AC-related bleeding in patients with solid tumors and VTE is associated with increased mortality, with ICH & GI bleeding conferring the highest risk. These findings highlight the importance of careful risk assessment & monitoring in cancer-associated VTE patients receiving AC therapy.

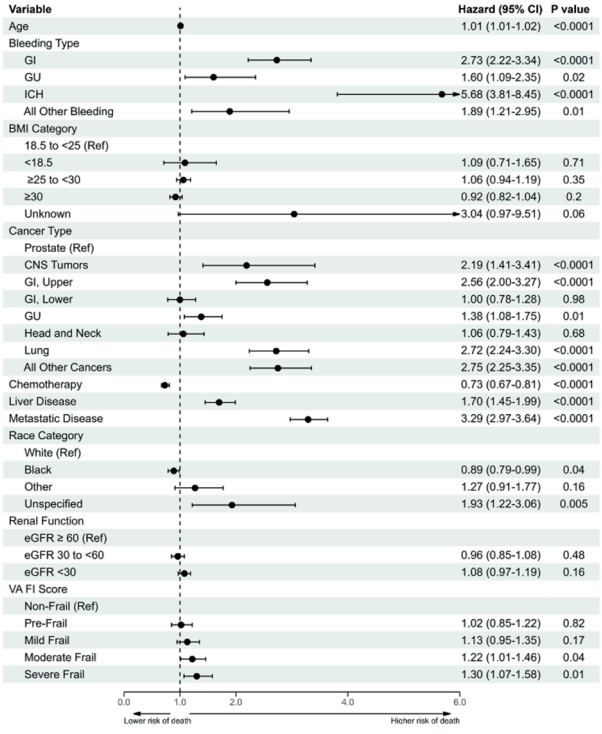

Figure 1.

PO-11

CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM: A CASE OF A POSSIBLE RESISTANCE TO DOAC

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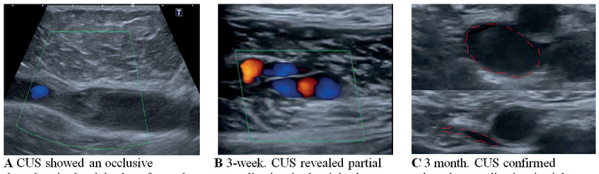
Introduction: Cancer-associated thrombosis is one of the major complications during cancer treatment, and the second most common cause of mortality in cancer patients. About 20% of patients develop a recurrence within 12 months despite optimal anticoagulation.
Aim: To discuss a real clinical practice case of a patient with VTE recurrence that was resistant to rivaroxaban.
Case presentation: A 56-year-old woman, with diffuse large B-cell lymphoma was referred to Cardio-Oncology Center. After 4 courses of R-CHOP chemotherapy, she complained of pain in the right hip that was ongoing for 1 week. She had a 1-point risk of VTE as per Khorana's score before the start of the cancer therapy. Lower-limb CUS showed an occlusive thrombus in the right femoral vein. Anticoagulation with rivaroxaban 15 mg BID was started. After a 3-week, the patient's condition continued to deteriorate: dyspnea, chest pain, and edema of the legs. ECG showed sinus tachycardia with HR 104 bpm. TTE demonstrated a slightly dilated right ventricular with mildly reduced systolic function and LVEF was 54%. Troponin I level was normal, while D-dimer elevated at 5800 ng/ml. Pneumonia signs and left pleural effusion were detected at the X-ray. PE was suspected. CTPA showed thrombi in the segmental and sub-segmental branches of the right and left pulmonary arteries. Lower-limbs CUS revealed partial recanalization in the right deep femoral vein. PE of intermediate-low risk and DVT were diagnosed. DOAC-resistant VTE was suspected, and enoxaparin 1 mg/kg BID was started. Non-compliance, interruption of therapy, inadequate dosing, cancer progression, and thrombophilia (protein C, protein S, antithrombin deficiency, and Factor V Leiden mutation) were excluded as the reasons for VTE recurrence. In 1-month FU no PE signs on CT, partial (70%) recanalization of DVT was confirmed by CUS, and the patient was switched to apixaban 5 mg BID. In 3 months CUS confirmed subtotal recanalization (Figure 1).
Conclusions: In this case, the patient had VTE recurrence and resistance to DOAC (rivaroxaban). After successful recanalization on LMWH, the patient was re-switched to another DOAC - apixaban. Future studies are needed to confirm our hypothesis.



A CUS showed an occlusive thrombus in the right deep femoral vein.

B 3-week. CUS revealed partial recanalization in the right deep femoral vein.

C 3 month. CUS confirmed subtotal recanalization in right deep femoral vein.

Figure 1.

PO-12

RISK OF RETHROMBOSIS AND MAJOR BLEEDING IN WOMEN WITH CANCER INCLUDED IN THE TESEO-SEOM REGISTRY

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Introduction: Patients with cancer have a higher risk of rethrombosis and bleeding during anticoagulantion than patients without cancer. Several factors including location of primary tumor, stage, oncological treatment, duration of anticoagulant therapy and sex may influence these risks.
Aim: We analyzed the risk of rethrombosis and major bleeding in women included in TESEO according to primary tumor location.
Materials and Methods: TESEO is an observational, non-interventional and prospective registry promoted by the Spanish Society of Medical Oncology (SEOM), with the collaboration of 52 Spanish 2 Portuguese centers, that recruit consecutive cases of cancer-associated thrombosis.
Results: A total of 2823 patients were recruited for the registry between July 2018 and December 2022, with 48% consisting of women (n=1351). Most common primary cancer in women were: breast cancer (BC) (n=282; 20.9%); colorectal (CRC) (223; 16.5%), lung (LC) (223; 16.5%) gynecological (GC) (200; 14.8%) and non-colorectal gastrointestinal cancer (non-CRC GI) (176; 13.0%). Pulmonary embolism was the most frequent thromboembolic event, regardless of the primary tumor location (BC 51.2%, CRC 57.4%, LC 65.9%, GC 56.5% and non-CRC GI 41.5%). Most of catheter-associated thromboses occurred in women with BC (59/156; 38%), followed by CRC (32/156; 21%). Median follow-up for all women was 7.4 months (IQR 2.1-16.7): 12.8 months (3.2-24.9) for BC patients, 9.5 (2.9-20.2) for CRC, 5.0 (1.4-11.8) for LC, 8.5 (2.6-19.5) for GC and 4.4 (1.3-10.8) non-CRC GI. The cumulative incidence of rethrombosis at 6 months, 12 months and end of follow-up was: 1.4%, 3.2% and 6.1% respectively for BC; 2.3%, 4.5% and 8.1% for CRC; 1.3%, 2.2% and 3.1% for LC; 3.0%, 4.0% and 6.1% for GC; 5.8%, 8.1% and 10.4% for non-CRC GI. The cumulative incidence of major bleeding at 6 months, 12 months and the end of follow up was: 2.1%, 3.2% and 3.9% respectively for BC; 1.4%, 1.4% and 1.8% for CRC; 0.9%, 1.3% and 1.3% for LC; 0%, 0% and 1.0% for GC; 0.6%, 0.6% and 1.2% for non-CRC GI. The percentage of patients with a duration of anticoagulant treatment greater than 12 months was: 32% (n=92) of BC, 28% (n=61) of CRC, 21% (n= 46) of LC, 28% of GC, 17% (n=30) of non-CRC GI.
Conclusions: In female cancer patients, the cumulative incidence of rethrombosis and bleeding varies depending on the location of the primary tumor. This information should be considered when deciding the duration of anticoagulant treatment.

PO-13

RISK OF CAR T-CELL THERAPY-RELATED THROMBOSIS AND BLEEDING: PRELIMINARY RESULTS OF THE MULTICENTER 'FOLLOW THAT CAR' REGISTRY

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Introduction: Chimeric antigen receptor T-cell (CAR-T) therapy has dramatically changed treatment and survival rates for patients with hematological malignancies. Reports on thrombosis and bleeding complications after CAR-T are emerging, but their relevance remains unclear because of large heterogeneity between studies.
Aim: To assess incidence of thrombosis and bleeding in a homogenous cohort of adults with lymphoma undergoing CAR-T therapy, and evaluate anticoagulation use in this population.
Materials and Methods: We used the 'Follow that CAR' registry, a retrospective multicenter cohort including adults with relapsed/refractory lymphoma receiving CAR-T therapy in the Netherlands between 2020-2022. Patients were monitored from start of lymphodepleting chemotherapy before CAR-T until 1 year after infusion. We recorded all venous and arterial thrombosis, major bleeding (according to ISTH definition) and death events during follow-up.
Results: 58 patients treated in Erasmus MC were included in this analysis (baseline characteristics; Table 1).

Table 1.

Patient characteristics	N = 58
Median age at baseline, years (IQR)	64 (55.8-69.0)
Sex, n (%)	
Male	39 (67.2%)
Female	19 (32.8%)
Diagnosis, n (%)	
Diffuse large B cell lymphoma (DLBCL)	33 (56.9%)
Transformed follicular lymphoma	14 (24.1%)
Mantle cell lymphoma	5 (8.6%)
T cell/histiocyte-rich large B cell lymphoma	3 (5.2%)
Other	3 (5.2%)
Previous lines of therapy, median (range)	2 (1-5)
History of autologous SCT, n (%)	18 (31.0%)
Bridging therapy, n (%)	39 (67.2%)
Lymphodepleting chemotherapy, n (%)	
Fludarabine/ Cyclophosphamide	58 (100.0%)
CAR-T cell product, n (%)	
Axicabtagene ciloleucel	41 (70.7%)
Lisocabtagene maraleucel	11 (19.0%)
Brexucabtagene autocel	5 (8.6%)
Zamtocabtagene autocel	1 (1.7%)
Median LDH levels at baseline, U/L (IQR)	246 (198.5-312.8)
Median platelet levels at baseline, x10^9/L (IQR)	188 (141.2-222.8)
History of thrombosis, n (%)	15 (25.9%)
Venous	12 (20.7%)
Arterial	3 (5.2%)
Cardiovascular comorbidity, n (%)	12 (20.7%)
Atrial fibrillation	8 (13.8%)
Antithrombotic therapy at baseline, n (%)	
No	27 (46.6%)
Yes	31 (53.4%)
LMWH	28 (48.3%)
Prophylactic dose	17 (29.3%)
Therapeutic dose	11 (19.0%)
Atrial fibrillation	3
Pulmonary embolism	3
Deep venous thromboembolism	3*
Cerebral vascular accident	1
Other VTE	1
DOAC	0 (0.0%)
Antiplatelet therapy	3 (5.2%)
Padua score at baseline, median (range)	4 (3-11)
Cytokine release syndrome (CRS) after infusion, n (%)	
Any	46 (79.3%)
Grade 1	16
Grade 2	24
Grade 3	6
Neurotoxicity (ICANS) after infusion, n (%)	
Any	28 (48.3%)
Grade 1	10
Grade 2	6
Grade 3	6
Grade 4	6

*Patient characteristics at baseline (upon admission before lymphodepleting chemotherapy) and during or after CAR-T infusion. CRS = cytokine release syndrome; DOAC = direct oral anticoagulant; ICANS = immune effector cell-associated neurotoxicity syndrome; IQR = interquartile range; LDH = lactate dehydrogenase; LMWH = low-molecular-weight heparin; SCT = stem cell transplantation; VTE = venous thromboembolism; * including 1 splanchnic vein thrombosis with concurrent atrial fibrillation.*

After median follow-up of 372 days, 5 patients experienced thrombosis (incidence rate 11.6% [95% CI, 3.76-27.02] per person-year); 3 had venous (all in upper extremity after central venous catheters) and 2 arterial thrombosis (1 myocardial infarction, 1 peripheral artery disease-related limb event). 4 patients experienced major bleeding (incidence rate 8.7% [95% CI, 2.38-22.37] per person-year). Median time to thrombosis and major bleeding was 55 and 22.5 days, respectively. 17 patients (29.3%) received thromboprophylaxis during CAR-T infusion, 12 (20.7%) therapeutic anticoagulation. One patient received therapeutic anticoagulation at time of major bleeding, all other thrombosis or bleeding occurred in patients without prophylactic or therapeutic anticoagulation. 17 (29.3%) patients had died after 1 year follow-up, mostly due to progression of underlying disease.
Conclusions: This homogeneous cohort confirms that patients are at considerable risk of thrombosis and bleeding in the first year after CAR-T therapy. These preliminary results will be completed with data from other Dutch centers, but justify further research on the relevance and prevention of these complications.

PO-14

ESTIMATING RISK OF BLEEDING AND THROMBOSIS FOR THROMBOCYTOPENIA IN CANCER-ASSOCIATED SPLANCHNIC VEIN THROMBOSIS: A TIME-VARYING ANALYSIS

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Introduction: Thrombosis and thrombocytopenia are common in patients with cancer, making decisions regarding anticoagulation challenging. We previously observed that thrombocytopenia at the time of diagnosis of cancer-associated splanchnic vein thrombosis (CA-SpVT) is not associated with risk of thrombosis recurrence and bleeding over the subsequent year. It is unclear, however, whether the risk of recurrence and bleeding varies with thrombocytopenia over time.
Aim: We analyzed risk of SpVT recurrence and bleeding with thrombocytopenia as a time-varying exposure in patients with CA-SpVT, adjusting for important covariates.
Materials and Methods: We performed a retrospective analysis of patients with CA-SpVT at our institution between 2010-2021. We excluded patients with myeloproliferative neoplasms and squamous and basal cell carcinoma and those without available platelet counts. We analyzed clinically-relevant bleeding (CRB; composite of major bleeding and clinically-relevant non-major bleeding) and SpVT progression or recurrence. Outcomes were analyzed using Cox proportional hazards models to calculate adjusted hazard ratios (aHR) and 95% confidence intervals (CI) and thrombocytopenia (<100x10^3/μL) and severe thrombocytopenia (<75x10^3/μL) as a time-varying exposure adjusting for age 65 or more years, sex, prior major bleed, comorbid chronic kidney disease, and use of antiplatelets or anticoagulants at baseline.

Results: We included 512 patients with CA- SpVT with median age 64.5 (IQR: 57.2-71.4); 62.3% male, and 57.8% with cirrhosis. Nearly 40% of patients (39.5%) had any thrombocytopenia, with 26.8% having severe thrombocytopenia. The most common cancer types were hepatobiliary (54.1%) and pancreatic (22.3%); 41.7% had metastatic disease. In adjusted time-varying analyses, periods of any thrombocytopenia were not associated with risk of CRB (aHR: 0.89, 95% CI: 0.55-1.45), but severe thrombocytopenia was associated with increased risk of CRB (aHR: 1.93, 95% CI: 1.19-3.15). Periods of any thrombocytopenia were associated with significantly increased risk of SpVT recurrence or progression (aHR: 1.96, 95% CI: 1.18-3.24). Severe thrombocytopenia was associated with a non-significantly increased risk (HR: 1.48, 95% CI: 0.81-2.70) (Table 1).
Conclusions: Thrombocytopenia, when analyzed as a time-varying exposure modulates risk of bleeding and SpVT recurrence/progression in patients with CA-SpVT. Clinicians should consider these competing risks when treating patients with anticoagulation.

Table 1. Risk of clinically-relevant bleeding (composite of major bleeding and clinically-relevant non-major bleeding) among those with varying degrees of thrombocytopenia at the most recent blood draw relative to those without thrombocytopenia in an adjusted time-varying analysis for patients with cancer associated splanchnic vein thrombosis.

Characteristic	Any thrombocytopenia (<100 x10^3/µL) HR (95% CI)	Severe thrombocytopenia (<75 x10^3/µL) HR (95% CI)	Most severe thrombocytopenia (<50 x10^3/µL) HR (95% CI)
Thrombocytopenia	0.85 (0.45-1.60)	2.06 (1.21-3.50)	1.79 (0.86-3.75)
Age <65 years vs. >65 years	1.31 (0.77-2.23)	1.04 (0.66-1.63)	1.06 (0.67-1.66)
Female vs. male sex	0.45 (0.24-0.86)	0.44 (0.25-0.76)	0.44 (0.25-0.77)
Cirrhosis vs. none	0.95 (0.53-1.68)	0.93 (0.58-1.50)	1.06 (0.68-1.67)
Abdominal surgery in past 3 months	1.43 (0.62-3.29)	1.32 (0.61-2.82)	1.26 (0.59-2.69)
Prior major bleed	1.43 (0.70-2.92)	1.24 (0.67-2.29)	1.29 (0.70-2.38)
Baseline creatinine >1.0	1.28 (0.79-2.08)	1.26 (0.78-2.04)	1.23 (0.76-2.00)
Recent systemic therapy	0.76 (0.35-1.63)	0.93 (0.52-1.68)	0.90 0.50-1.61)
Anticoagulation vs. none	1.85 (0.72-4.79)	1.01 (0.40-2.54)	1.04 (0.41-2.63)
Tumor vs. bland/mixed thrombus	0.97 (0.55-1.72)	0.98 (0.56-1.74)	0.99 (0.56-1.75)
Completely vs. partially-occlusive thrombus	1.41 (0.85-2.36)	1.51 (0.90-2.52)	1.49 (0.89-2.49)
Single vs. multiple vessels involved	1.38 (0.73-2.62)	1.29 (0.73-2.28)	1.27 (0.72-2.22)

PO-15

THROMBOTIC THROMBOCYTOPENIC PURPURA ASSOCIATED WITH NIVOLUMAB AND IPILIMUMAB IMMUNOTHERAPY IN METASTATIC MELANOMA: A CASE REPORT

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Introduction: Thrombotic Thrombocytopenic Purpura (TTP) is a rare auto-immune disorder characterized by thrombotic microangiopathy with hemolytic anemia, thrombocytopenia and organe failure. TTP is idiopathic or secondary to other conditions, for example systemic lupus or drugs. Anticancer immunotherapy is an emerging cause of TTP. We report the case of a patient presenting an acquired immune TTP 24 hours after the administration of a first cycle of Nivolumab and Ipilimumab in treatment of a metastatic melanoma.
Aim: The aim of this case report is to highlight the risk of TTP in patients receiving anticancer immunotherapy
Case Presentation: We report the case of a 51 year old female surgically treated for a melanoma in may 2021. Unfortunately, the patient experienced a relapse with brain metastasis in december 2022 treated by surgical excision and stereotaxic radiotherapy. An adjuvant immunotherapy with Nivolumab and Ipilimumab was started 03 march 2023. One day later, the patient was admitted in hospital for abdominal pain, vomiting and confusion. Blood test showed grade 4 thrombocytopenia, acute renal failure and elevated CRP. The first diagnosis was urinary sepsis and the patient was admitted in intensive care unit and treated by platelet transfusion and Piperacillin-Tazobactam. Despite this treatement, evolution was unfavourable and a TTP was suspected based on features of microangiopathye (hemolytic anemia, thrombocytopenia, organ failure, presence of schizocytes on blood semar) and confirmed by very low ADAMTS13 level (undosable activity inferior to 0.2% for a normal between 60.6 and 130.6%) and presence of anti-ADAMTS13 IgG antibodies. A diagnosis of acquired immune TTP was made and the patient received corticosteroids, Rituximab, Caplacizumab and plasma exchanges. Unfortunately, the TPP was refractory to this first line treatment and the patient died 2 days after a first dose of second line treament with Bortezomib.
Conclusions: TTP is a rare but fatal complication of anticancer immunotherapy and oncologists should be aware of this conditon. A prompt diagnosis can avoid platelet transfusion not recommended in TTP and permit the rapid initiation of a treatment by plasma exchange, corticosteroids, Rituximab and Caplacizumab. More data are needed to better understand and characterize TTP associated with anticancer immunotherapy (evolution, prognosis and safety of Caplacizumab).

PO-16

FURTHER PROOF THAT THE OTTAWA SCORE FAILS TO PREDICT RECURRENT VTE IN CANCER PATIENTS. META-ANALYSIS OF INDIVIDUAL PATIENT DATA

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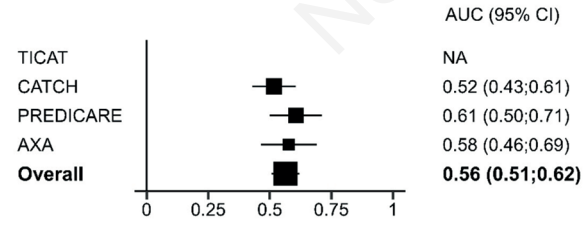
Introduction: The risk of venous thromboembolism (VTE) recurrence remains high in patients with cancer-associated thrombosis (CAT) despite therapeutic anticoagulation. The original Ottawa score, which was designed to stratify the risk of recurrent VTE in patients with CAT, has performed poorly. This may be due to the small sample sizes as well as the heterogeneity of statistical analyses and treatments used among the validation studies.
Aim: To overcome these sources of noise, we performed a meta-analysis using individual patient-level data to assess the performance of the Ottawa score in predicting VTE recurrence in CAT patients who were treated with the same therapy, tinzaparin, for at least 3 months.
Materials and Methods: Prospective studies of CAT patients treated with tinzaparin initially for at least 3 months and for which the clinical events of interest had been assessed by an independent central adjudication committee were eligible (PROSPERO: CRD42019119907). When eligible, the study sponsor was asked to provide individual patient data for each trial. The area under the receiver operating characteristic (ROC) curve, estimated risk and performance parameters were calculated with 95% confidence intervals (95% CI).
Results: Three prospective cohort studies and 1 randomised controlled trial were eligible (1413 patients) and the Ottawa score could be calculated for 1088 patients. For the patients considered at high risk of recurrence (Ottawa score ≥1, 59.4% of patients), the 6-month cumulative incidence of recurrent VTE was estimated to be 8.5% (95% CI, 6.6 to 10.8) compared with 5.0% (3.2 to 7.8) in the Ottawa low-risk group. The area under the ROC curve was 0.56 (0.51 to 0.62) with consistent results across studies (Figure 1). Using the recommended cut-off (score <or ≥1), the best parameter is the negative predictive value: the probability of a score <1 identifying patient without recurrent VTE is equal to 95.3% (93.3 to 97.4%). The other parameters were sensitivity 72.8% (62.6 to 83.0%), specificity 41.9% (37.8 to 45.9), and positive predictive value 8.6% (6.4 to 10.8).

Forest plot of area under the curve associated with the model including the Ottawa score variables in each study and in the overall meta-analysis population.



AUC (95% CI): area under the curve (95% confidence interval); NA: not available

Figure 1.

Conclusions: Despite the large number of patients and the standardisation of both treatment and dosage, the performance of the Ottawa score failed to accurately predict VTE recurrence in CAT patients treated with tinzaparin. In fact, the score mainly identifies low-risk patients.

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PO-17

USUAL-SITE VENOUS THROMBOEMBOLISM IN PATIENTS WITH CANCER-ASSOCIATED SPLANCHNIC VEIN THROMBOSIS

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Introduction: Venous thromboembolism in patients with cancer usually occurs in the deep veins (DVT)and pulmonary embolism (PE), but it may occur in atypical sites, such as the splanchnic veins (SpVT). It is unclear whether patients with cancer-associated (CA-SpVT) are at increased risk of usual-site VTE (Us-VTE), what the risk factors for US-VTE are, and how anticoagulation (AC) modulates this risk.
Aim: We analyzed US-VTE incidence and associated factors in patients diagnosed with CA-SpVT.
Materials and Methods: We performed a retrospective study of patients with CA-SpVT at our institution between 2010 and 2021. Outcomes included US-VTE (upper/lower-extremity DVT, PE) up to 1 year following initial SpVT. Medical records were manually reviewed to determine baseline clinical data, treatments, and outcomes. We performed log-binomial regression to identify independent risk factors for US-VTE including age (continuous), sex, cirrhosis, creatinine (continuous), recent systemic chemotherapy, tumor type (tumor *vs* mixed/bland), thrombocytopenia (platelet count <100x10^3/uL), and use of antiplatelets or anticoagulants.
Results: We identified 581 patients with CA-SpVT, with a mean age of 64 years, 36.4% male and 82.5% gastrointestinal malignancy; 39.2% were treated with AC. A total of 27 (4.6%) patients had a history of US-VTE prior to the diagnosis of SpVT and 23 (4.0%) presented with US-VTE concurrently with SpVT. The cumulative incidence of US-VTE at 1 year after diagnosis of SpVT in patients without prior or concomitant VTE was 5.4% (95% CI: 3.6-7.7) with death as a competing risk. Of these 27 US-VTE events in the follow up period, 14 were limb DVT and 13 were PE. Thrombocytopenia (<100x10^3/uL) occurred in 39.5% of patients and was not associated with US-VTE (P=0.70). There was no significant difference in US-VTE rates in patients that were treated with AC compared to those not receiving AC (6.3% *vs* 5.7%; P=0.70). Progression of SpVT was not associated with US-VTE (7.5% *vs* 5.4%, P=0.44) Multivariate regression did not identify any independent predictors of US-VTE (Table 1).

Conclusions: We observed US-VTE in patients with CA-SpVT concurrently and subsequent to SpVT, but was not associated with SpVT recurrence, thrombocytopenia or AC. More research is required to understand the interplay of SpVT and US-VTE in patients with cancer.

Table 1. Risk factors for usual-site venous thromboembolism in patients with cancer-associated splanchnic vein thrombosis.

	Unadjusted RR (95% CI)	Adjusted* RR (95% CI)
Age at time of SpVT >65 years	0.92 (0.47-1.79)	1.01 (0.93-1.09)
Male gender	1.15 (0.57-2.32)	0.98 (0.89-1.08)
Cirrhosis	0.13 (0.04-0.41)	1.06 (0.84-1.35)
Baseline creatinine >1.0	0.22 (0.05-0.92)	1.02 (0.88-1.19)
Recent systemic chemotherapy	1.08 (0.48-2.42)	1.00 (0.90-1.12)
Use of antiplatelets at baseline	1.55 (0.74-3.25)	0.97 (0.85-1.10)
Tumor vs. bland/mixed thrombus	1.50 (0.71-3.15)	0.99 (0.89-1.10)
Anticoagulation vs. no anticoagulation	1.11 (0.28-4.42)	0.99 (0.83-1.18)
Thrombocytopenia		
None	Ref	Ref
Platelets <100x10³/uL	0.32 (0.12 - 0.82)	1.0 0.86-1.17)

RR, risk ratio; CI, Confidence Interval

PO-18

DEEP VEIN THROMBOSIS AS AN INITIAL SYMPTOM OF PROSTATAE CANCER: A CASE REPORT

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Introduction: Prostate cancer does not belong to the high-risk entities for VTE as gastric and pancreas cancer. The initial incidence rate ratio (IRR) for prostate cancer is 3.25 (2.56 - 4.13). However, there have been reported cases of cancer that suffered VTE as an initial symptom of malignancy. The incidence of VTE has been shown to be the highest within the first few months after diagnosis of cancer.
Aim: In this abstract, we report the diagnosis of deep vein thrombosis that discovered prostate cancer in an 77-year-old man, presented for the first time with acute urinary retention and left leg swelling.
Case presentation: On clinical examination,there was a painful, hot and very swelling of the entire left leg with a positive sign of Homans. The diagnosis was confirmed by laboratory data (PT, aPTT, and D-Dimer test) and Doppler ultrasound.Because of the urinary retention he was sent to a urologist where, after the tests, he was diagnosed with adenocarcinoma of the prostate with metastasis in an inguinal lymph node. The coagulation tests shows very high results of D-Dimers (9500ng/ml), a sign of secondary activated fibrinolysis,other coagulation tests were normal.Dopller ultrasound showed the presence of an extensive acute deep venous thrombosis of the left sural vein extended to the popliteal and to the homolateral deep femora veinl. The patient was treated with effective anticoagulation therapy with LMWH and analgesics 7 days, and then he continue the treatment with 15 mg of Rivaroxaban twice daily for 21 days, followed by 20 mg of Rivaroxaban once daily. During the treatment laboratory values,clotting times, D-Dimer levels, and the Doppler ultrasound were repeated and showed signs of improvement. He was referred to urology for the surgical management of the prostate.
Conclusions: This case highlights the importance of screening for a cause of the thromboembolic event in patients. The existence of active cancer in a patient is a known risk factor for VTE and, conversely, the discovery of a first episode of deep vein thrombosis (DVT) may be the first clinical manifestation of cancer. Routine pelvic examination and an examination by a urologist especially in older patients with an unknown cause of urinary retention and deep vein thrombosis of the lower limbs can help in the early diagnosis of prostate cancer.

POSTER SESSION 3
EPIDEMIOLOGY

PO-19

PROGNOSTIC VALUE OF HULL SCORE 0 *VERSUS* SUBSEGMENTAL UNSUSPECTED PULMONARY EMBOLISM IN CANCER PATIENTS: A COMPARATIVE ANALYSIS

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Introduction: The significance of subsegmental pulmonary embolism (SSPE) and its impact on cancer patient outcomes is still under debate, with conflicting findings regarding its association with mortality risk or the existence of symptoms. The HULL score CPR (HS-CPR) stratifies ambulatory cancer patients with UPE and can identify truly asymptomatic, clinically unimpaired UPE patients with low-risk HULL Score 0 (HS 0) for proximate mortality (1-3).
Aim: This study aims to assess the anatomical distribution of PE among HULL Score 0 (HS 0) patients and to evaluate the predictive capacity of SSPE *vs* HS 0 for mortality outcomes.
Materials and Methods: 521 consecutive patients managed under the UPE-acute oncology service in HUTH NHS trust from February 2010 to April 2020 were included. These patients were evaluated and managed using the UPE pathway and prospectively categorised by the (HS-CPR) into low (HS 0), intermediate (HS 1-2), and high (HS 3-4) risk levels. 100% received anticoagulant treatment as per guidelines. CT reports were reviewed retrospectively to verify PE distribution. Survival outcomes were analysed using Kaplan Meier (univariate) methods and compared using the log-rank test.
Results: Among the cohort, 12.9% (67 patients) had only SSPE distribution, and 25.7% (134 patients) were classified as low-risk, HS 0. Over half of the SSPE patients (55.2%) fell into the intermediate or high-risk HS-CPR categories. The anatomical distribution of PE in the HS-0 patients was central (11.2%), lobar (26.1%), segmental (41%) and subsegmental (21.6%) PE. The median follow-up for the group was 12.1 months (ranging from 0.13 to 126.7 months). The median overall survival (OS) for UPE patients with SSPE was 14.7 months, with a 95% confidence interval (CI) of 10.8 to 18.6 months, compared to 26.3 months, with a 95% CI of 16.2 to 36.4 months, for those categorised as HS 0, p <.001 (Figure 1).
Conclusions: Hull Score 0, or truly asymptomatic UPE, does not correspond with the subsegmental distribution of PE. Indeed, several patients had central emboli (including one saddle embolus). Our study reveals that low-risk UPE patients, as identified by the HULL Score 0, demonstrate a significantly better survival

outcome than those with SSPE. Factors relating to the underlying malignancy likely have a greater impact on mortality.

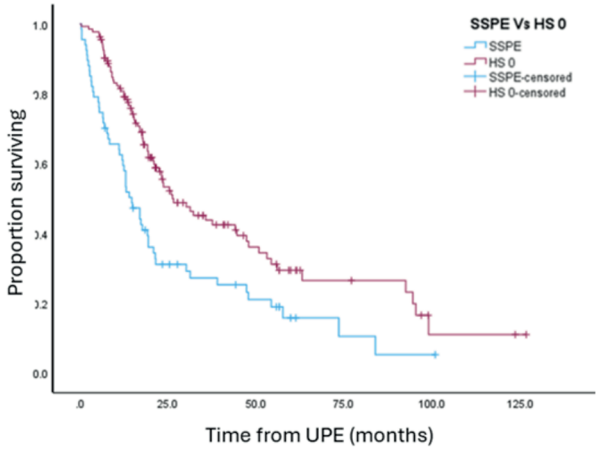

Figure 1. Survival (Kaplan Meier) for the HULL score 0 *vs* SSPE ($p<0.001$).

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PO-20

VENOUS THROMBOEMBOLISM AND RISK OF CANCER IN PATIENTS WITH STROKE: A POPULATION-BASED COHORT STUDY

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Introduction: Stroke is associated with an increased risk of venous thromboembolism (VTE), particularly in the initial months after stroke. It is a long-standing observation that VTE may be a marker of occult cancer. However, it is unclear whether VTE in patients with stroke is a marker of cancer.
Aim: To examine the risk of cancer after VTE in patients with stroke, compared to the expected risk of cancer, based on national cancer incidence rates.
Materials and Methods: We used Danish health registries to identify all patients with a first-time hospital diagnosis of VTE and a stroke prior to this date from 1996 to 2021. Follow-up started from the date of VTE diagnosis until either a cancer diagnosis, death, emigration, or end of study period, whichever came first. We calculated the absolute risk of cancer and standardized incidence ratios (SIRs) of cancer based on national cancer incidence for the first year after VTE and 1-15 years follow-up. Analyses were stratified based on stroke subtype and time from stroke to VTE.
Results: During the study period, we identified 9535 patients with stroke and a subsequent VTE, and within this group, 1085 cancer cases were observed. The median age at the time of the VTE was 76 years, with equal sex distribution. During the first year of follow-up, the absolute risk of cancer was 4.7%, with a SIR of 3.20 (95% confidence interval (CI): 2.91-3.52). Looking at the 1-15 years of follow-up, the overall SIR decreased to 1.15 (95% CI: 1.07-1.25). Within the first year, SIR according to stroke subtype were similar, with slightly higher SIR for subarachnoid hemorrhage [SIR 3.89 (95% CI: 2.51-5.74)], followed by ischemic stroke [SIR 3.19 (95% CI: 2.88-3.52)], and intracerebral hemorrhage [SIR 3.00 (95% CI: 2.08-4.20)]. For all three stroke subtypes, SIR decreased markedly for 1-15 years follow-up, but an increased risk remained among those with subarachnoid hemorrhage [SIR 1.32 (0.97-1.77)] and ischemic stroke [SIR 1.16 (1.07-1.26)]. The risk pattern varied minimally with the time from stroke to VTE when examining the first year for follow-up. However, for 1-15 years follow-up, increased risk notably persisted for VTEs more than twelve months after stroke with a SIR of 1.24 (95% CI: 1.13-1.35).
Conclusions: Venous thromboembolism may be a marker of undiagnosed cancer in patients with stroke.

PO-21

VENOUS THROMBOEMBOLISM AND RISK OF CANCER IN PATIENTS WITH A HISTORY OF MIGRAINE: A POPULATION-BASED COHORT STUDY

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Introduction: Migraine is associated with an elevated risk of venous thromboembolism (VTE). It is well established that VTE may be a marker of occult cancer, but it is unclear whether VTE in patients with migraine is a marker of cancer.
Aim: To examine cancer risk following VTE in patients with a history of migraine, compared to the expected cancer risk, based on national cancer incidence rates.
Materials and Methods: We used Danish health registries to identify patients with a first-time hospital diagnosis of VTE and a history of migraine from 1996 to 2021. Follow-up started from the date of VTE diagnosis until either a cancer diagnosis, death, emigration, or end of the study period, whichever came first. We calculated the absolute cancer risk and standardized incidence ratios (SIRs) based on national cancer incidence divided into the first year after VTE and 1-15 years follow-up to measure the relative cancer risk after VTE.
Results: We identified 9190 patients with VTE and a history of migraine and observed 1010 cancer cases. The median age at the time of the VTE diagnosis was 56 years, and 78% of the patients were females. During the first year after VTE diagnosis, absolute cancer risk was 3.6%, with a SIR of 4.08 (95% confidence interval (CI): 3.65-4.55). During 1-15 years of follow-up, the SIR of cancer remained elevated but decreased to 1.16 (95% CI: 1.07-1.25). During the first year, SIRs were 4.36 (95% CI: 3.50-5.36) for males and 3.99 (95% CI: 3.50-4.53) for females. For the 1-15 years follow-up, SIRs were 1.17 (95% CI: 1.00-1.36) for males

and 1.16 (95% CI: 1.06-1.26) for females. All cancer groups showed an increased SIR during the first year of follow-up. Further, a persistently increased risk during the 1-15 years follow-up was observed for cancers of neurological origin [1.46 (95% CI: 1.04-1.99)], hematologic cancers [1.41 (95% CI: 1.08-1.81)], hormone-related cancers [1.22 (95% CI: 1.07-1.38)], and smoking-related cancers 1.19 (95% CI: 1.02-1.38).
Conclusions: Venous thromboembolism is a marker of occult cancer in patients with a history of migraine.

PO-22

SENSITIVITY AND POSITIVE PREDICTIVE VALUE OF CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM DIAGNOSES IN THE DANISH NATIONAL PATIENT REGISTER

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Introduction: Hospital discharge diagnoses from administrative health registries are commonly used in epidemiological studies of cancer-associated venous thromboembolism. Yet, the validity of International Classification of Diseases (ICD) codes for identifying such events remains uncertain.
Aim: To explore the positive predictive value (PPV) and sensitivity of using ICD-10 discharge codes to identify cancer-associated venous thromboembolism within the Danish National Patient Register.
Materials and Methods: The PPV was estimated from a random sample of 370 ICD codes registered from 2017-2021 in the North Denmark Region. The PPV was calculated as the number of cases confirmed after manual search of the electronic health record divided by the total sample count. Sensitivity was determined by identifying of 100 patients with documented cancer-associated venous thromboembolism identified via use of therapeutic doses of low-molecular-weight heparin, who were sampled without knowledge of their ICD discharge diagnosis status. Sensitivity was calculated by dividing the number of patients with a concomitantly registered ICD code with the total number of patients with documented venous thromboembolism.
Results: The overall PPV of an ICD-10 diagnosis of cancer-associated venous thromboembolism was 75.9% (95% confidence interval: 71.3-80.0). Subgroup analysis (see Table 1) demonstrated particularly low PPVs for recurrent venous thromboembolism (44.2%), secondary position diagnosis (55.7%), outpatient diagnoses (65.3%), and for diagnoses given at surgical (66.7%), emergency wards (48.4%), or by palliative team or at hospices (0%). Overall sensitivity was 68.0% (95 CI: 58.3-76.3), meaning 32% of patients diagnosed in a hospital setting with cancer-associated venous thromboembolism were discharged without any registered ICD-code for venous thromboembolism.
Conclusions: The PPV of an ICD-10 discharge diagnosis of cancer-associated venous thromboembolism in the Danish Patient Register was 75.9%, but with notable variation across subgroups. The sensitivity of using ICD-codes to identify events was limited, as one in three patients with venous thromboembolism were discharged without any relevant ICD-code. Although the overall PPV may be adequate for research purposes, cautious interpretation of incidence of cancer-associated venous thromboembolism based on administrative register-based data is warranted.

Table 1.

Positive predictive values of ICD-10 discharge diagnoses for cancer-associated venous thromboembolism in the Danish National Patient Register in prespecified subgroups

	ICD-10 diagnoses, *n*	Positive predictive value (95% confidence interval)*
Venous thromboembolism subtype		
Deep vein thrombosis	147	71.4 (63.7-78.1)
Pulmonary embolism	223	78.9 (73.1-83.8)
Incident or recurrent event		
Incident	275	86.9 (82.4-90.4)
Recurrent	95	44.2 (34.6-54.2)
Underlying malignancy		
Haematological	172	80.8 (74.3-86.0)
Oncological	198	71.7 (65.1-77.5)
Diagnosis position		
Primary	239	87.0 (82.2-90.7)
Secondary	131	55.7 (47.2-63.9)
Inpatient or outpatient		
Inpatient	246	81.3 (76.0-85.7)
Outpatient	124	65.3 (56.6-73.1)
Registering department		
Oncology	52	88.5 (77.0-94.6)
Haematology	80	95.0 (87.8-98.0)
Other internal medicine	157	85.4 (79.0-90.0)
Surgical	15	66.7 (41.7-84.8)
Emergency ward	31	48.4 (32.0-65.2)
Hospice/palliative department	35	0.0 (N/A)

* Confidence intervals calculated using the Wilson score method.

PO-23

VENOUS THROMBOEMBOLISM IN CANCER PATIENTS AND SELF-RATED HEALTH: A CROSS-SECTIONAL STUDY

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Introduction: Cancer is a strong risk factor for venous thromboembolism (VTE), and cancer patients have a nine-fold higher VTE risk than the general population, as well as significantly higher rates of bleeding and recurrence during anticoagulant treatment than VTE patients without cancer. The occurrence of VTE is associated with interruption of cancer treatment, and increased morbidity and high mortality. Cancer patients with VTE have shorter survival than cancer patients without VTE. However, little is known about self-rated health (SRH) and quality of life in cancer patients with VTE.
Aim: Our objective was to assess the prevalence of poor SRH in cancer patients with VTE compared to two groups: patients with VTE only and patients with cancer only.
Materials and Methods: We conducted a cross-sectional study using data from the Better Health in Late Life cohort. Danish residents aged 50-65 years in the period 2021-2022 completed an online questionnaire covering lifestyle, stress, physical health, and mental health. The 12-Item Short Form Health Survey was used to measure SRH based on the question: "How do you find your

overall health?". Information regarding VTE, cancer, and comorbidities were retrieved from the Danish National Patient Registry. We estimated prevalence proportion ratios (PR) and 95% confidence intervals, adjusting for age, sex, and educational level, to compare poor SRH in patients with cancer and VTE to the comparison groups. Furthermore, we investigated this association across various levels of stress and comorbidity.

Results: We identified 231 persons with both cancer and VTE, 2236 persons with VTE only, and 9729 persons with cancer only. Overall, 46.3% of cancer patients with VTE reported poor SRH. The proportion of poor SRH in cancer patients and VTE patients was 30.9% and 24.3%, respectively. The difference in poor SRH was more pronounced in patients with low perceived stress, PR 2.14 (95% CI: 1.55-2.96) and PR 2.70 (95% CI: 2.01-3.64) compared to patients with VTE only and cancer only, respectively. The difference was smaller in participants with a high level of comorbidity, PR 1.11 (95% CI: 0.83-1.50) and PR 1.26 (95% CI: 0.95-1.68) compared to patients with VTE only and cancer only, respectively.

Conclusions: Cancer patients with VTE had a higher prevalence of poor SRH compared to patients with only cancer or VTE. However, the difference varied across levels of comorbidity and stress.

PO-24

POLY (ADP-RIBOSE) POLYMERASE INHIBITORS (PARPI) – ASSOCIATED THROMBOSIS IN PATIENTS WITH OVARIAN CANCER: A STUDY OF THE SPANISH SOCIETY OF MEDICAL ONCOLOGY (SEOM) THROMBOSIS AND CANCER GROUP

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Introduction: Although clinical trials with PARP inhibitors (PARPi) have shown a low incidence of venous and arterial thromboembolic disease (VTE/AT), we lack information on patients in routine clinical practice.

Aim: The objective was to evaluate the incidence and characterize VTE/AT in ovarian cancer patients treated with PARPi.

Materials and Methods: Retrospective, multicenter study promoted by the Spanish Society of Medical Oncology (SEOM). Individuals with ovarian cancer who initiated PARPi between 2015 and 2022 were recruited. Minimum follow-up was 6 months (except in cases of demise). We performed a descriptive analysis, analyzed the impact of VTE/AT on survival and determined predictor variables using multivariate logistic regression.

Results: 329 patients were recruited, whose baseline characteristics are shown in Table1.

Table 1. Baseline characteristics of the patients.

	Total (n = 329)	VTE/AT (n = 16)	No VTE/AT (n = 313)
Age – median (IQR)	62 (55-71)	60 (57-68)	62 (55-71)
ECOG PS – n (%)			
0	154 (46.8%)	6 (37.5%)	148 (47.3%)
1	147 (44.7%)	9 (56.3%)	138 (44.1%)
2	24 (7.3%)	1 (6.3%)	23 (7.3%)
3	4 (1.2%)	0 (0%)	4 (1.3%)
Stage – n (%)			
III	161 (49.2%)	5 (31.3%)	156 (50.2%)
IV	166 (50.8%)	11 (68.8%)	155 (49.8%)
Khorana Score – n (%)			
1	251 (76.3%)	10 (62.5%)	241 (77%)
2	71 (21.6%)	5 (31.3%)	66 (21.1%)
3	7 (2.1%)	1 (6.3%)	6 (1.9%)
Oncological situation at the start of PARPi - n (%)			
Complete response	92 (28.2%)	5 (31.3%)	87 (28.1%)
Partial response	149 (45.7%)	7 (43.8%)	142 (45.8%)
Stable disease	56 (17.2%)	4 (25%)	52 (16.8%)
Tumor progression	29 (8.9%)	0 (0%)	29 (9.4%)
Homologous Recombination Deficiency – n (%)			
No	151 (45.9%)	4 (25%)	147 (47%)
Yes	168 (51.1%)	12 (75%)	156 (49.8%)
Not determined/Unknown	10 (3%)	0 (0%)	10 (3.2%)
BRCA mutation – n (%)			
No	175 (53.2%)	6 (37.5%)	169 (54%)
Yes, BRCA1	74 (22.5%)	1 (6.3%)	73 (23.3%)
Yes, BRCA2	69 (21%)	9 (56.3%)	60 (19.2%)
Yes, BRCA 1 and 2	1 (0.3%)	0 (0%)	1 (0.3%)
Not determined/Unknown	10 (3%)	0 (0%)	10 (3.2%)
BRCA mutation type – n (%)			
Somatic	35 (24.3%)	1 (10%)	34 (25.4%)
Germinal	100 (69.4%)	8 (80%)	92 (68.7%)
Unknown	9 (6.3%)	1 (10%)	8 (6%)
Treatment modality – n (%)			
Maintenance after 1st line of mts disease	149 (45.4%)	8 (53.3%)	141 (45%)
Maintenance after 2nd line of mts disease	132 (40.2%)	5 (33.3%)	127 (40.6%)
Maintenance after 3rd or more lines of mts disease	41 (12.5%)	2 (13.3%)	39 (12.5%)
2nd line of mts disease	1 (0.3%)	0 (0%)	1 (0.3%)
3rd or later lines of mts disease	5 (1.5%)	0 (0%)	5 (1.6%)
PARPi type – n (%)			
Olaparib	160 (48.6%)	10 (62.5%)	150 (47.9%)
Niraparib	151 (45.9%)	6 (37.5%)	145 (46.3%)
Rucaparib	18 (5.5%)	0 (0%)	18 (5.8%)
Concomitant treatment with PARPi – n (%)			
No	315 (95.7%)	14 (87.5%)	301 (96.2%)
Bevacizumab	11 (3.3%)	0 (0%)	11 (3.5%)
Other	3 (0.9%)	2 (12.6%)	1 (0.3%)

ECOG PS: Eastern Cooperative Oncology Group Performance Status. IQR: interquartile range. Mts: metastatic. PARPi: poly ADP-ribose polymerase inhibitor. VTE/AT: venous and arterial thromboembolic disease.

After an observation period equivalent to 489 person-years, 16 thrombotic events were identified (4.9%; 3.3 events per 100 person-years). The form of presentation was: 31.3% deep vein thrombosis (DVT), 25% pulmonary embolism (PE), 18.8% visceral thrombosis, 12.5% catheter-associated thrombosis, 6.3% other forms of venous thrombosis, and 6.3% mixed event (venous and arterial). Concurrent with the diagnosis of thrombosis, 25% (n=4) were in progression. The median time between start of PARPi and VTE/AT was 4 months (interquartile range: 2–14.3 months). 62.5% of events were incidentally diagnosed and 75% in the outpatient setting. No patient experienced recurrence or bleeding as a complication. A higher proportion of thrombotic events was observed with olaparib (6.3%) compared to niraparib (4%) and rucaparib (0%), but the differences were not statistically significant (p=0.398). The most frequent presentation of VTE/AT associated with olaparib was DVT (40%), while in patients who received niraparib it was PE (50%), without a significant association being observed (p=0.2). Median overall survival was 33 months (95% CI 28.8-37.2) in the subgroup without VTE/AT, while in patients with VTE/AT it was 44 months (95% CI 22.5-65.5) (log-rank test=0.12). Multivariate

analysis revealed that combination treatment (PARPi+another agent) was associated with a lower risk of VTE/AT (OR 0.127, 95% CI 0.017-0.963) compared to PARPi alone.
Conclusions: The risk of VTE/AT associated with PARPi in patients with ovarian cancer is low, consistent with that has been described in clinical trials. VTE/AT associated with these drugs did not impact survival.

PO-25

IMPACT OF THROMBOEMBOLIC EVENTS ACROSS THE SPAN OF BREAST CANCER SURVIVORSHIP: DATA FROM A LARGE OBSERVATIONAL STUDY ON LONG-TERM BREAST CANCER SURVIVORS

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Introduction: Breast cancer (BC) patients comprise the main group of cancer survivors. There are several factors influencing the risk of venous thromboembolic events (VTE) in these patients, but we are unaware of previous studies that have evaluated the incidence of VTE in unselected series of long-term BC survivors.
Aim: Evaluation of the cumulated incidence and features of VTE in a large cohort of long-term BC survivors.
Materials and Methods: Ambispective observational study (ILL-CAR 2018-01). Approved by the Regional Ethics Board. All patients signed the informed consent for the study. Entry criteria included BC patients with a follow-up of at least 10 years from the time of their first therapy. Detailed clinical data were retrieved from a comprehensive electronic medical record, comprising all the information on hospital and primary care in our regional health system.
Results: 2,847 patients (p.) were included and are available for full analysis. Median time of follow-up from first therapy of BC: 18.7 years (10-55.3). 183 VTE were diagnosed in 152/2,847 p (5.3%). Median age at diagnosis of VTE: 71.7 years (37.5-97.5). Median interval from first therapy of BC to the diagnosis of VTE: 15.2 years (0.0-55.6). VTE were 96 deep-vein thromboses (DVT), 70 pulmonary embolisms (PE) and 17 PE with concurrent DVT. DVT (alone or with PE) were diagnosed in the lower limb (71), upper limb (23), splanchnic (8), cranial sinuses (2), other (9). VTE occurred with active metastatic BC (30 VTE), during adjuvant drug, surgical or radiation therapy of nonactive BC or during follow-up after adjuvant therapy (116 VTE), or associated to late second non BC neoplasms (37 VTE; 33 solid, 4 hematologic). Predisposing factors for VTE were active cancer and/or cancer therapy (117 VTE), medical conditions (40 VTE, including COVID-19 in 7), surgical procedures and/or traumatic lesions (13 VTE). No predisposing factors were found in 13 p. VTE evolved to chronic thromboembolism in 14 p.
Conclusions: The cumulated incidence of VTE remained low for BC survivors in this cohort with real world data, even after a long follow-up. This may be related to the generalized use of prophylaxis in medical and surgical settings and to advances in the clinical care of the patients. Secondary neoplasms are related to a substantial proportion of VTE in long-term survivors, and this may be a confounding factor for observational studies.

PO-26

VENOUS THROMBOEMBOLISM AND RISK OF CANCER IN PATIENTS WITH ATOPIC DERMATITIS

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Introduction: It is well established that venous thromboembolism (VTE) may be a marker of occult cancer in patients with unprovoked VTE. There is also increasing evidence that atopic dermatitis is a risk factor of VTE, but it is unclear whether VTE in atopic dermatitis patients is a marker of occult cancer.
Aim: To examine the risk of cancer after VTE in patients with a hospital diagnosis of atopic dermatitis in a nationwide cohort in Denmark
Materials and Methods: For 1980-2021 we used Danish health registries to identify all patients with and in- or outpatient clinic diagnosis of VTE, who were also recorded as having a previous or concurrent diagnosis of atopic dermatitis. Patients were followed from the date of VTE to first incident cancer, death, emigration, or December 31, 2021, whichever came first. To estimate possible increases in cancer risks we calculated age-, sex- and calendar period standardized incidence ratios (SIRs) using Danish national cancer rates to compare the observed cancer incidence among patients with atopic dermatitis and VTE to the one expected.
Results: In a cohort of 64,001 patients with a hospital-based diagnoses of atopic dermatitis, 435 patients (57% female) developed VTE. At the time of thromboembolic admission or during first year of follow-up, the cumulative incidence of all cancer types was 1.16% (95% confidence interval (CI): 0.44-2.57). The median age at VTE diagnosis was 46 years (interquartile range (IQR): 32-61) and the median follow-up time was 5.2 years (IQR: 2.0-11.2). A total of 27 cancers were observed during the study period. During the first year of follow-up five patients were diagnosed with cancer yielding a SIR of 1.8 (95% CI: 0.6-4.3). The overall SIR during the subsequent years of follow-up was 1.1 (95% CI: 0.7-1.7).
Conclusions: At the time of VTE or in the first year afterwards, we found an increased cancer risk in atopic dermatitis patients. In subsequent years a 10% increase in risk remained. These findings indicate that occult cancers promote thrombosis in atopic dermatitis patients. However, estimates were imprecise and diagnostic bias cannot be excluded.

PO-27

EPIDEMIOLOGIC STUDY OF PATIENTS WITH THROMBOTIC EVENTS REFERRED TO A TERTIARY HOSPITAL IN SOUTHERN IRAN

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Introduction: Thromboembolic events mainly occur in older age is related with high morbidity and mortality, and considerable health-care costs particularly in developing countries. Both

arterial and venous thromboembolism has known risk factors such as hyperlipidemia, obesity, diabetes, cancer, major surgery, central catheter.
Aim: We aimed to evaluate the occurrence of thrombotic events and related risk factors in a group of Iranian patients.
Materials and Methods: In this cross-sectional study, all patients (n=99) who were complicated by thrombotic events referred to the Hematology Research Center of Shiraz University of Medical Sciences were investigated from 2015 to 2017, in Shiraz, Southern Iran. Data were collected from their medical records by a designed data gathering form.
Results: The median age of the occurrence of thrombosis was 51 (IQR: 31) years. From all thrombotic events 52.5% occurred in females. Venous thrombosis was more prevalent than arterial (61.6% *vs* 38.4%). Hypertension, diabetes mellitus and ischemic heart disease were the most associated disease with thrombosis. Most of the patients (79.8%) had no episodes of relapse and the occurrence of relapse had no significant relationship with thrombophilia and underlying disease. Acceptable response rate for warfarin therapy was achieved in 46.5% with 5 mg and 43.4% with 5-7.5 mg.
Conclusions: Knowing the frequency and risk factors for thrombotic events lead to timely diagnosis and management of thrombosis. Atrial fibrillation and valvular rheumatic heart disease are the most common risk factors of thrombosis in our study showing prophylaxis is necessary in high-risk patients.

PO-28

RATES OF VENOUS THROMBOEMBOLISM DURING NEOADJUVANT CHEMOTHERAPY FOR OVARIAN CANCER: A NATIONAL STUDY OF UK GYNAECOLOGICAL CANCER CENTRES

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Introduction and Aim: This study aimed to determine the incidence of venous thromboembolism (VTE) in patients with advanced epithelial ovarian cancer undergoing neoadjuvant chemotherapy (NACT) before debulking surgery in gynaecological cancer centres across the UK. Patients generally receive 3 x 3 weekly cycles of combination chemotherapy before surgery. Secondary outcomes included overall incidence and timing of VTE since cancer presentation, VTE presentation (symptomatic/incidental), impact on cancer treatment and mortality.
Materials and Methods: All UK gynaecological cancer centres were invited, through the British Gynaecological Cancer Society network, to participate in this multicentre retrospective study. A bespoke data collection tool was developed, and peer reviewed. Data was captured on all patients undergoing NACT for FIGO stage III/IV epithelial ovarian cancer within a (centre-defined) 12-month period within 2021-2022. We excluded all patients on anticoagulation prior to ovarian cancer presentation. We excluded patients who developed VTE prior to commencing NACT from analysis of our primary outcome.
Results: Fourteen UK gynaecological cancer centres returned data on 660 eligible patients. The median age was 67.0 years. In total, 132/660 (20.0%) patients were diagnosed with a VTE from presentation with ovarian cancer until discharge following cytoreductive surgery. Excluding those who developed VTE prior to NACT, 66/594 (11.1%) patients developed VTE from start of NACT until discharge following cytoreductive surgery (median 11.3%, IQR 5.9-11.3), with no significant difference across centres (p=0.47). Of these 66, 45 (68%) developed pulmonary embolism and 30 (46%) developed deep vein thrombosis (9 had both), including in major abdominal/pelvic vessels. 37 (56%) presented symptomatically and 29 (44%) were incidentally diagnosed on imaging. VTE resulted in mortality in 3 patients (5%); delays/cancellation of treatment in 17 (26%); and need for inferior vena cava filter in 3 cases (4.5%).
Conclusions: Across a large, representative sample of UK gynaecological cancer centres, 1 in 9 patients undergoing NACT for advanced ovarian cancer developed a potentially preventable VTE. This led to adverse clinical consequences for one third. This unacceptably high VTE rate, and the limitations of existing risk-stratification tools in this cohort, justify consideration of a national protocol for thromboprophylaxis in this patient group.

POSTER SESSION 4
THROMBOPROPHYLAXIS IN CANCER

PO-29

ELECTRONIC HEALTH RECORD (EHR) INTEGRATION OF THROMBOEMBOLISM RISK STRATIFICATION MODEL IN PATIENTS WITH CANCER

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Introduction: Despite multiple risk models validated for venous thromboembolism (VTE) prediction in patients with cancer, the overall implementation rate is low.
Aim: We aimed to design and test a clinical decision support (CDS) tool to automatically calculate VTE risk at time of new treatment initiation in Epic, the most used electronic health record (EHR) in the US.
Materials and Methods: We designed a SQL algorithm in Epic Clarity data warehouse to 1) extract 11 variables to calculate the modified Khorana score (EHR-CAT, Li JCO 2023) at each new systemic therapy (cancer type, stage, treatment, body mass index,

blood count, history of VTE, paralysis, recent hospitalization, and Asian); and 2) identify clinical trial exclusions, including existing anticoagulation (AC), non-aspirin antiplatelet (AP), CYP3A4/P-glycoprotein inducer/inhibitor, abnormality in platelet, creatinine, bilirubin, alanine aminotransferase, weight <40 kg, acute leukemia, or primary/metastatic brain cancer. Finally, we designed a large language model natural language processing (NLP) algorithm to extract new VTE events. We tested the algorithm on patients with cancer and treatment plans at Harris Health System, TX in 1/2017-1/2023 and verified with chart reviews. Competing risk regressions were used to estimate the VTE incidence to account for cancer deaths.

Results: A total of 14,151 treatment plans from 7,640 patients with cancers were identified over 6 years. Clinical trial exclusion criteria removed 21.9% patients (26.6% plans). The most common reason for exclusion was AC for prior VTE (9.6%), brain metastasis (5.9%), and acute leukemia (4.4%). The final list included 10,264 treatment plans (73.7% chemotherapy) from 5,915 patients. After random sampling to create one plan per patient, the index plan count was 1st, 2nd, and 3rd+ in 67.3%, 22.2%, 10.5% of patients, respectively. The cumulative incidence of VTE at 6 months was 1.7%, 4.3%, 5.8%, 6.0%, 8.6%, and 14.7%, respectively, for scores 0-5 (time-dependent c index of 0.67). The original Khorana score resulted in c index of 0.61 (Figure 1).

Conclusions: We demonstrated the feasibility of a CDS design for VTE risk stratification in patients with cancer. Nearly 1 in 4 patients would be excluded based on trial exclusions due to potential bleeding risk. In the remaining eligible patients, EHR-CAT scores could be estimated using standard SQL algorithms and the resulting risk strata had similar observed VTE event rates as the original model. Future work on CDS implementation may lead to improved guideline-concordant thromboprophylaxis.

Structured Data SQL extraction for baseline variables & exclusion

7,640 patients (14,151 treatment plans) at HHS in 1/2017-1/2023 [EPIC BEACON]

- 533 patients (1355 plans) on anticoagulant (AC) prescription
- 113 patients (188 plans) on P2Y12 antiplatelet prescription
- 135 patients (277 plans) on CYP3A4/P-GP strong inducer/inhibitor prescription
- 235 patients (479 plans) with abnormal labs (Plt, Cr, Bili, ALT) or low weight
- 237 patients (629 plans) with acute leukemia diagnosis
- 417 patients (835 plans) with primary/metastatic brain cancer diagnosis

Free-Text Notes NLP transformer for VTE prediction

5,970 patients (10,388 treatment plans)

- 55 patients (124 plans) with recent VTE last 6 mo but not on AC

5,915 patients (10,264 treatment plans => randomly select 1 plan per patient)

Algorithm Performance Comparison

Khorana score	VTE at 6 mo	Cut-Off	EHR-CAT score	VTE at 6 mo	Cut-Off
		Cut-off 2+ treats **2,182** correct **10.1%** captures **52%** of all VTE events AUC = **0.61 (0.58-0.63)**	0- = 749 (12.7%)	12 (1.7%)	Cut-off 3+ treats **3,090** correct **9.7%** captures **72%** of all VTE events AUC = **0.67 (0.64-0.70)**
0 = 1,776 (30.0%)	85 (5.0%)		1 = 1,027 (17.4%)	42 (4.3%)	
1 = 1,957 (33.1%)	104 (5.6%)		2 = 1,049 (17.7%)	59 (5.8%)	
2 = 1,401 (23.7%)	108 (8.2%)		3 = 1,070 (18.1%)	61 (6.0%)	
3+ = 781 (13.2%)	100 (13.5%)		4 = 1,005 (17.0%)	80 (8.6%)	
			5+ = 1,015 (17.2%)	143 (14.7%)	

Figure 1.

PO-30

APIXABAN THROMBOPROPHYLAXIS IN MULTIPLE MYELOMA (MM) PATIENTS RECEIVING CHEMOTHERAPY: A COHORT STUDY

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Introduction: There are not yet sufficient data to recommend a particular myeloma VTE risk assessment model (RAM) or thromboprophylactic agent in clinical practice. We previously reported on low dose apixaban as thromboprophylaxis in high VTE risk MM patients on chemotherapy.

Aim: This cohort study aimed further to assess safety and efficacy of an apixaban-based thromboprophylactic strategy in MM patients in a regional cancer centre.

Materials and Methods: Data was systematically collected from electronic records for sequential MM patients receiving outpatient chemotherapy 1/11/2021-1/5/2023. Exclusion criteria included anticoagulation for other indications. Data collected included VTE history, thromboprophylaxis, 6 month VTE & bleeding event rate. Patients were risk assessed with an adapted International Myeloma Working Group (IMWG) RAM, received apixaban 2.5mg bd if high risk, and aspirin 75mg od or no thromboprophylaxis if low risk (Figure 1).

Results: 122 patients, 75M(61.5%) median age 63.9y & median BMI 26.6, were included. 4/122(3.3%) patients had prior VTE, all catheter-associated. Treatment regimens included lenalidomide in 68/122(55.7%), pomalidomide in 25(20.5%) & thalidomide in 9(7.4%). 15/122 (12.3%) had newly diagnosed MM. 57/122 (46.7%) patients received apixaban 2.5mg bd, 41 (33.6%) aspirin, 2 (1.6%) clopidogrel (1 aspirin allergy, 1 for cardiac ischaemia) & 22 (18%) did not receive thromboprophylaxis. 98/122 (80.3%) of thromboprophylaxis decisions were in accordance with local guideline. Newly diagnosed VTE occurred in 1/122 (0.8%), with lower limb DVT 1 month after starting DVRD. Patient received aspirin but prophylactic LMWH indicated (apixaban contraindicated as abnormal liver function). The patient was stratified as low thrombotic risk by SAVED & intermediate risk by IMPEDE RAM. 2/122 (1.6%) patients had clinically relevant non-major bleeding: 1 hematuria on aspirin–no cause found;1 rectal bleed during autograft–off anticoagulation as thrombocytopaenic. No major bleeding events occurred.

Conclusions: Use of apixaban 2.5mg bd in MM patients with high VTE risk, and aspirin in low VTE risk, had low thrombotic and bleeding rates in this cohort risk-stratified using modified IMWG criteria. The VTE rate compares favourably to published cohorts using IMWG RAM with LMWH for high risk patients eg Myeloma XI VTE rates >10%. Our study adds to the growing body of real world data supporting use of low dose apixaban as part of the thromboprophylactic strategy in MM.

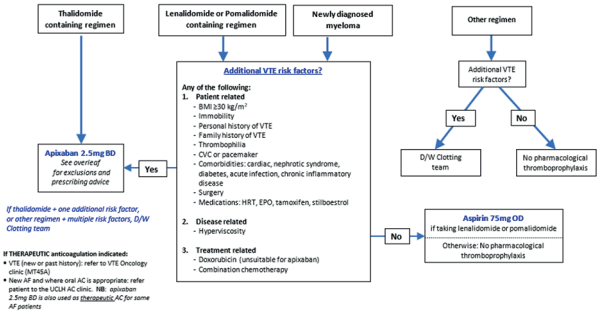

Figure 1. Thromboprophylactic strategy for MM patients introduced 2019 in our regional centre (Sayar *et al.*, 2022).

PO-31

TINZAPARIN PROPHYLAXIS IN PATIENTS WITH METASTATIC COLORECTAL CANCER (PROTINCOL)

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Introduction: Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide. CRC leads to increased activation of the clotting system. Since CRC patients present a higher rate of bleeding, careful evaluation of the risk/benefits of anticoagulant prophylaxis is necessary.
Aim: To evaluate low molecular weight heparin (LMWH) *versus* no treatment for primary thromboprophylaxis in metastatic CRC outpatients receiving first-line systemic cancer therapy
Materials and Methods: PROTINCOL (NCT05625932) is a randomised, open-label (PROBE), multicentre study (Figure 1). Patients will receive tinzaparin (75 IU/kg) or no treatment for 4 months and will be stratified based on: BRAF/RAS mutation, primary resection tumour and antiangiogenic therapy. Subsequently, two months of post-treatment follow-up will be carried out. The study outcomes will be assessed by a blinded central independent adjudication committee. The primary efficacy endpoints will include the cumulative incidence of any venous thromboembolism (VTE) event (symptomatic or incidental) including symptomatic central venous catheter VTE. Secondary variables will be clinically relevant bleedings, health-related quality of life and the predictive value of validated risk assessment scales of VTE, as well as a risk assessment of VTE based on a validated clinical-genetic model. A further 18 months of follow-up by telephone could be carried out at the end of the study to monitor for progression and survival. Our hypothesis is that prophylactic LMWH will reduce the 55% relative risk to an estimated VTE incidence of 13.5%. A total of 526 patients will be required.
Conclusions: Risk prediction of chemotherapy-associated VTE is a compelling challenge in oncology, as VTE may result in treatment delays, impaired quality of life, and increased mortality. Patients with a single type of metastatic cancer with a high risk of VTE will be selected for study inclusion. For the first time in ambulatory prophylaxis of cancer-associated thrombosis, a precision medicine approach will be used in a randomised clinical trial. If the individualization of antithrombotic prophylaxis can reduce the complications of outpatient cancer treatment and be cost effective, it would be of great value in the future care of patients with metastatic CRC.

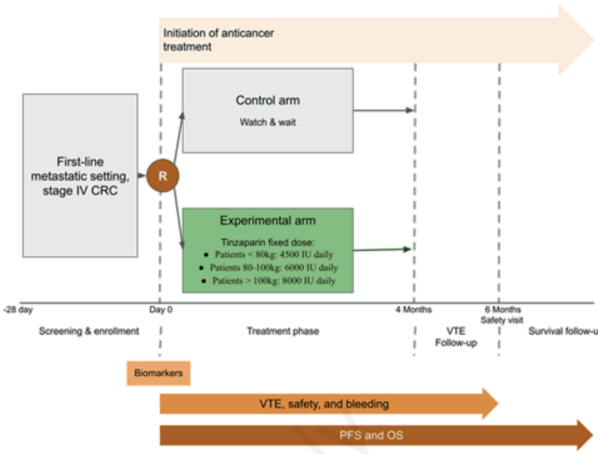

Figure 1. Study design.

PO-32

COMPARATIVE ANALYSIS OF RISK PREDICTION SCORES INCLUDING ALL TYPE OF CANCER ASSOCIATED THROMBOSIS.

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Introduction: Classically, only symptomatic deep vein thrombosis (DVT) and pulmonary embolism (PE) have been included in the predictive risk scores. However, visceral vein thrombosis (VVT) and central venous catheter–related thrombosis (CRT) as well as incidental events have clinical and economic implications in cancer patients.
Aim: The aim of the study is to conduct a comparative analysis of the different predictive risk scores in assessing all types of thrombotic events.
Materials and Methods: We evaluated and compared the performance of the Khorana, PROTECHT, CONKO, modified VIENA-CATS (without P-selectin value) and MICA-CATS risk scores in the second cohort of the multicenter, prospective ONCOTHROMB12-01 study. Data of 391 patients receiving ambulatory systemic therapy from 11 Spanish hospitals between 2018 to 2021 were analyzed. Clinical data associated with the risk of venous thromboembolism (VTE) were collected at the time of diagnosis. The primary outcome was confirmed incidental or symptomatic VTE including DVT, PE, VVT and CRT during a 12 month follow up period. Area under Receiver Operating Characteristics (AUROC) and area under Precision-Recall curve

(AUPRC) were used to compare the score's performance. The cutoff for considering high-risk patients in MICA-CATS score was 10%.

Results: Out of 391 patients (p), 229 men (58.6%) and 162 women (41.4%) with a median age of 64.7 years, 91 p (24.4%) developed a thrombotic event. The most common malignancy was pancreatic cancer (28.7%, n=112), followed by colorectal (26.4%, n=106) and lung cancer (19.2%, n=75). Most p were metastatic (53.3%, n=202), having 118 p locally advanced disease (31.3%) and 59p (15.5%) localized disease. The performance of the predictive scores is detailed in Table 1. Our findings show that MICA-CATS score demonstrates the best predictive capacity according to both AUROC (0.61, 95% CI 0.54-0.67) and AUPRC (0.38, 95% CI 0.30-0.46). For symptomatic events only MICA-CATS score seems to be predictive (0.60 [95% CI 0.5003-0.711]). Regarding incidental events, both PROTECHT and MICA-CATS score seems to be useful (HR 0.62 [95% CI 0.55-0.68] and HR 0.60 [95% CI 0.513-0.6813]).

Conclusions: The score that has the best predictive capacity for all types of events, including incidental and symptomatic VTE, is the MICA-CATS score. New models need to be developed in order to improve this outcome and consequently the patients who would benefit from thromboprophylaxis.

Table 1. Comparative AUROC and AUPRC among different scores.

Scores	AUROC	AUPRC
Khorana risk score	0.5453 [0.4768-0.6096]	0.2543 [0.2222-0.2973]
Incidental VTE	0.5522 [0.4813-0.6244]	0.1652 [0.1408-0.2008]
Symptomatic VTE	0.5191[0.4323-0.6188]	0.0902 [0.0734-0.1280]
PROTECHT	0.5819[0.5220-0.6395]	0.2651[0.2353-0.3049]
Incidental VTE	0.6242[0.5578-0.6878]	0.1855 [0.1591-0.2201]
Symptomatic VTE	0.4868[0.3870-0.5744]	0.0846 [0.0674-0.1290]
CONKO	0.5633 [0.5030-0.6236]	0.2617 [0.2307-0.3062]
Incidental VTE	0.5678 [0.4937-0.6405]	0.1721 [0.1455-0.2098]
Symptomatic VTE	0.5348 [0.4459-0.6162]	0.0914 [0.0746-0.1206]
VIENA-CATS	0.5870 [0.5309-0.6430]	0.2837 [0.2484-0.3361]
Incidental VTE	0.5687 [0.4914-0.6402]	0.1714 [0.1437-0.2116]
Symptomatic VTE	0.5869 [0.4928-0.6786]	0.1159 [0.086-0.1813]
VIENA MICA-CATS	0.6174 [0.5469-0.6767]	0.3803 [0.3055-0.4601]
Incidental VTE	0.6017 [0.5130-0.6813]	0.2044 [0.162-0.2819]
Symptomatic VTE	0.6029 [0.5003-0.7110]	0.2152 [0.1192-0.3651]

PO-33

DIRECT ORAL ANTICOAGULANTS FOR THE PREVENTION OF VENOUS THROMBOEMBOLISM AFTER CANCER SURGERY: A SYSTEMATIC REVIEW AND NETWORK META-ANALYSIS

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Introduction: Data on the role of direct oral anticoagulants (DOACs) for post-operative prophylaxis of venous thromboembolism (VTE) after cancer surgery are awaited.

Aim: We performed a systematic review and network meta-analysis (NMA) to evaluate the efficacy/effectiveness and safety of DOACs for VTE prophylaxis after cancer surgery.

Materials and Methods: PubMed, EMBASE and Cochrane Library were searched for eligible studies. Randomized controlled trials (RCTs) and non-randomized studies (NRSs) reporting on VTE events and/or bleeding complications in patient receiving DOACs for VTE prophylaxis after cancer surgery were included. A frequentist NMA using random-effects model was conducted to estimate the pooled odds ratios (ORs) and corresponding 95% confidence intervals (CIs), including direct and indirect evidence. P-scores were used to rank the treatment for all possible prophylactic strategies.

Results: Five RCTs (1694 patients) and 6 NRSs (2042 patients) were included in the analysis. When all the studies were considered regardless of the timing from surgery to the starting of DOACs, prophylaxis with apixaban (OR 0.12, 95% CI 0.02-0.73) or rivaroxaban (OR 0.26, 95% CI 0.07-1.04) and not with LMWH (OR 0.38, 95% CI 0.08-1.76) was associated with reduction in the risk of VTE at 30 days from surgery compared with placebo/no treatment. Prophylaxis with apixaban (OR 0.31, 95% CI 0.11-0.84) and not with rivaroxaban (OR 0.69, 95% CI 0.35-1.34) was associated with significant reduction in VTE at 30 days in comparison to LMWH. No significant difference in 30-day VTE was found with apixaban *vs* rivaroxaban (OR 0.45, 95% CI 0.13-1.49). Compared to placebo/no treatment, antithrombotic prophylaxis was associated with increased rates of clinically relevant bleeding at 30 days (apixaban OR 6.13, 95% CI 1.02-36.74; LMWH OR 7.29, 95% CI 1.18-44.94; dabigatran OR 3.95, 95% CI 0.10-151.4; rivaroxaban OR 2.62, 95% CI 0.58-11.91) (Table 1).

Conclusions: Our study in post-operative prophylaxis of VTE after cancer surgery support apixaban and rivaroxaban as promising alternative to LMWH, despite further high-quality data are needed in specific surgical settings.

Table 1.

Effect estimates and 95% confidence intervals table (random effect model) – VTE at 30 days from surgery

	DIRECT ESTIMATE			
NETWORK ESTIMATE	Api	.	0.31 (0.11; 0.84)	.
	0.45 (0.13; 1.49)	Riva	0.69 (0.35; 1.34)	0.26 (0.07; 1.04)
	0.31 (0.11; 0.84)	0.69 (0.35; 1.34)	LMWH	.
	0.12 (0.02; 0.73)	0.26 (0.07; 1.04)	0.38 (0.08; 1.76)	Placebo/no treat

PO-34

BARRIERS TO VTE PREVENTION IN AMBULATORY ONCOLOGY PRACTICE: A CLINICIAN-BASED SURVEY

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Introduction: Prior studies have shown underuse of evidence-based recommendations for venous thromboembolism (VTE) prevention in ambulatory oncology practice and have identified barriers such as clinicians' lack of knowledge and resource limitations.

Aim: We sought to evaluate use of and barriers to using VTE prevention by different clinician groups in a safety-net cancer clinic with limited healthcare resources.

Materials and Methods: From December, 2023 to February, 2024, we conducted an anonymous clinician survey at a safety-

net hospital system that primarily services uninsured and underinsured patients from low socioeconomic backgrounds. The survey assessed knowledge and awareness, current practice and attitudes towards, and barriers to using VTE prevention guidelines, and to solicit recommendations. We analyzed results based on years of medical experience (fellows <5 years *vs* senior clinicians >5 years) (Table 1).

Results: We received 35 responses from 46 distributed surveys (76% response rate) from 13 attending physicians, 15 fellows, and 3 NPs, where 55% of respondents had <5 years of oncology experience. There are similarities in both experience groups, and >80% of all clinicians would not prescribe anticoagulant (AC) prophylaxis in scenarios of high-risk cancer patients, despite 60% responding that it is "quite a bit" important to address VTE in oncology practice, and nearly all respondents (96%) felt "quite a bit" or "somewhat" comfortable prescribing/managing AC. Regarding knowledge, 54% responded "not at all" or "a little bit" familiar with guideline recommendations and VTE risk-assessment scores, and 50% were "not at all" or "a little bit" familiar with data from clinical trials. There are also notable differences. For example, 54% senior clinicians *vs* 80% fellows responded "never" or "rarely" use validated risk scores. The most frequent barriers identified by senior clinicians were not being convinced of evidence (38%), unfamiliarity with evidence (38%), and workflow limitations (38%); whereas fellows identified lack of unfamiliarity with evidence as the most significant barrier (90%) followed by workflow limitations (53%).

Conclusions: Recommended VTE prevention strategies are infrequently used in our resource-limited oncology practice. Similar barriers exist to those previously reported, including lack of knowledge and familiarity with evidence. We show that barriers may be different among senior clinicians *vs* fellows. While incorporating a clinical decision support tool addresses the workflow, we should focus on education of existing literature for fellows and generation of more convincing and targeted risk-stratified data for senior clinicians which may help to inform targeted implementation strategies.

Table 1.

Less experienced clinicians (0-5 years clinical experience) - Fellows and NPs		Experienced clinicians (5- >10 years clinical experience) - Attendings and NPs	
Areas assessed	**Comments/ opinions/ summary of responses**	**Areas assessed**	**Comments/ opinions/ summary of responses**
Knowledge Check	Apprehension in prescribing anticoagulants in demonstrated high-risk patients	Knowledge Check	Less apprehension in prescribing anticoagulants in demonstrated high-risk patients when compared to fellows
Awareness of Guidelines	Lack of familiarity with the guidelines (43.8% - not at all; 37.5% - a little bit)	Awareness of Guidelines	Some familiarity with the guidelines (57.1% - somewhat; 28.6% - a little bit; 14.3% not at all)
	Lack of familiarity with the RCTs conducted to demonstrate pro/cons of thromboprophylaxis (52.9% - not at all; 29.4% - a little bit)		Some familiarity with the RCTs conducted to demonstrate pro/cons of thromboprophylaxis (64.3% - somewhat; 14.3% - a little bit)
	Lack of familiarity with validated risk scores like Khorana score (50% - not at all, 31.3% a little bit)		Some familiarity with validate risk scores like Khorana score (21.4% quite a bit, 35.7% somewhat and 28.6% a little bit)
Importance of VTE assessment	Indicate importance of carrying out assessments (58.8%) but do not do so in practice (62.5%).	Importance of VTE assessment	Indicate importance of carrying out assessments (78.6%) and carry it out sometimes (57.1%) or usually (42.9%) depending on the specialty.
Barrier Assessment (Can choose multiple responses)	Not familiar with evidence behind prophylactic anticoagulation in cancer patients (94.1%)	Barrier Assessment (Can choose multiple responses)	Not familiar with evidence behind prophylactic anticoagulation in cancer patients (42.9%)
	Not able to due to workflow limitations (e.g. busy clinic) (52.9%)		Not able to due to workflow limitations (e.g. busy clinic) (35.7%)
	Not able to due to patient specific barrier (e.g. education/language) (29.4%)		Not convinced on the benefits of prophylactic anticoagulation in cancer patients (35.7%)
	Not convinced on the benefits of prophylactic anticoagulation in cancer patients (23.5%)		
	Not comfortable assessing VTE risk in cancer patients (e.g. high vs. low risk) (23.5%)		Not comfortable prescribing and managing anticoagulation in cancer patients (e.g. drug drug interaction, duration) (21.4%)
	Not comfortable prescribing and managing anticoagulation in cancer patients (e.g. drug drug interaction, duration) (17.6%)		
Recommendations (Free text response)	Multidisciplinary decision to prescribe anticoagulants	Recommendations	Risk assessment scores
	Built-in Epic tool/ optional EMR tool		Multidisciplinary action to prescribe anticoagulants/ Using tumor board presentations
	Evaluate bleeding risk before prescribing anticoagulant		Optional Epic tool with automated calculation

PO-35

EXPLORING THE IMPACT OF KRAS MUTATION TYPE ON THE THROMBOTIC RISK

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Introduction: Thrombotic complications are common in patients (pts) with cancer, and an important cause of morbidity and mortality. The management of this complication is particularly challenging and routine thromboprophylaxis is not recommended. Risk prediction scores have a disappointing clinical utility and the majority of these scores don't take in account specific genetic pathogenic variants, ignoring the presence of certain molecular aberrations in a variety of different cancers, namely lung tumors, that have been associated with an increased risk of venous thromboembolism (VTE) and arterial thrombotic events (ATE), such as ALK and ROS1 rearrangements and KRAS mutations, the latter with a 16.1% to 54% VTE incidence (a 2.6-fold increase).

Aim: Our study aims to determine VTE and ATE incidence in our population and to explore the impact different RAS mutations may have on the thrombotic risk.

Materials and Methods: Retrospective analysis of pts at a Portuguese tertiary center with histologically confirmed metastatic or locally advanced lung adenocarcinoma, who were tested for KRAS mutations with Next-generation sequencing between January 1st 2017 and December 31st 2022. Data cut-off was December 31st 2023. Data was obtained from pts clinical files, collected in an anonymous registry and analyzed with SPSS.

Results: 101 pts were identified, with a median age of 66 years old and 29 were female. 66 had metastatic disease and 35 had locally advanced disease. Concerning the mutational profile, the most frequent mutation was KRAS G12C (38 pts), followed by G12V (23 pts), G12D (14 PTS), G12A (7 pts) and G13C (5 patients). There were 12 events in 11 patients, corresponding to an overall incidence of VTE of 6.9% and ETA of 4.9%. Regarding the incidence according to specific mutations, the overall incidence in G12V was 17.4%, with VTE incidence of 13.1% and ETA 8.7%; G12D overall incidence was of 14.3%, VTE 7.1%, and ETA 7.1%. G12C overall incidence of 5.3%, VTE 2.6%, and ETA 2.6%. Noteworthy, G13C had a TVE incidence of 40% (2 events in 5 patients).

Conclusions: The use of thromboprophylaxis rests on suboptimal clinical models. Specific molecular aberrations in driver genes may drive the thrombotic risk, as we observed in our data that G12V and G13C KRAS mutations had higher incidence of VTE. The integration of this genetic information in future clinical models may improve its reliability. More research in expanded databases is required to validate these findings.

PO-36

ANTITHROMBOTIC AND ANTI-LEUKEMIC EFFECTS OF *RICINUS COMMUNIS* IN BENZENE–INDUCED LEUKEMIC WISTAR RATS

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Introduction: The use of medicinal plant is very wide spread in many parts of the world because it is commonly considered that herbal drugs are cheaper and safer as compared to synthetic drugs and may be used without or minimum side effects.

Aim: This study was designed to assess the efficacy of some herbal extract in the management of leukemia and their mode of actions.

Materials and Methods: Leukemia was experimentally induced in wistar rats by Benzene chromosolv at 0.2ml at 1:10 dilution water 1/2 – propanol 50/50 v/v in water daily via tail vein for 4 weeks. The Rats were divided into 6 main groups consisting of 6 rats per group. They were administered with the extracts of four different plants viz: *Ricinus communis, Rosy periwinkle,*

Psorospernum febrifugum, and Azadirachta indica separately after the determination of the LD50 for 4 weeks after induction. The LD50 of each of the extracts are *Ricinus communis* 340mg/kg, *Azadirachta indica* 40mg/kg, *Psorospermum febrifungum* 548mg/kg and *Rosy Periwinkle* 30mg/kg. The animal were thereafter weighed and sacrificed, blood samples were collected into appropriate containers for laboratory analysis of complete Blood Counts and coagulation profiles as well as BCL-2 gene expression using standard methodologies.
Results: We observed a statistically significant decrease in final weight in all groups (pre and post treated with the extracts and a statistically significant increase in WBC count in benzene induced rats ($p<0.05$, respectively) compared with non-induced controls. The induced leukaemia was the lymphocytic type. These values reduced significantly with the post treated animals especially with *Ricinus communis* ($P<0.05$, respectively). Also, there was a statistical significant increase in PTTK with concomitant decreases in the values of D-dimer, Protein C and S in the post-treated animals with all the 4 extracts ($P<0.05$, respectively) when compared with controls. Finally, the BCL-2 gene was significantly up-regulated in the animals treated *Ricinus communis* and *Psorospernum febrifugum* but with a higher value exhibited by the latter.
Conclusions: *Ricinus communis* exhibited a significant efficacy in the management of leukemia and its thrombotic complications over the other three extracts. A further pharmacologic potential of this extract is hereby indicated.

PO-37

CANCER ASSOCIATED THROMBOSIS (CAT): OPTIMIZING VTE PREVENTION AND IMPROVING PATIENT CARE EXPERIENCE

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Introduction: The University Health Network (UHN) Thrombosis and Hemostasis Program provides outpatient management for venous thrombosis (VTE). Currently treating more than 10,000 patients/year, greater than 5,000 CAT patients, this clinic is one of the largest in North America. The Clinic is notable for its innovative model of Nurse Practitioner (NP) led care delivery. VTE including CAT is associated with substantial morbidity, mortality, and health care expenditures. Cancer patients with higher Khorana score have an estimated risk of thrombosis of 10% during the first six months of diagnosis. The emotional distress caused by cancer associated thrombosis (CAT) and lack of understanding of CAT risk are well documented. Clinical practice guidelines underscorethe need to educate patients and prescribe VTE prophylaxis for patients at high risk for CAT. There is strong evidence-based data to direct best management for treatment and prophylaxis of CAT. While there is excellent data on the prevention of VTE with prophylaxis in the high-risk cancer population by more than 50% there is a substantial care gap in at risk cancer patients receiving this cost-effective intervention resulting in increased CAT and health care expenditures.
Aim: To evaluate a dedicated satellite Princess Margaret Hospital CAT clinic to expand CAT management to also manage CAT thromboprophylaxis better optimize and improve health care outcomes, patient well-being and decrease costs including emergency visits.
Materials and Methods: Develop and implement a QIRC approved quality improvement initiative to: 1. Establish program inclusion/exclusion criteria and standards of practice. 2. Develop patient decision and patient education tools. 3. Create database to enroll and track patient outcomes (bleeding, thrombosis, ER avoidance). 4. Promote patient awareness of CAT (how). 5. Evaluate impact of patient education and management for experience and satisfaction.
Results: Outcomes for 24 months since implementation in 2022: - ER avoidance:650 encounters (opportunity cost savings $250 K CDN). - Successful implementation of cancer prophylaxis program (60 high risk patients enrolled). - Metrics for provider and patient experience and satisfaction are very high.
Conclusions: The CAT clinic has resulted in ER avoidance, enhanced patient, and provider satisfaction with thrombosis management. We have established a VTE prophylaxis program that is meeting with growing interest and patient acceptance.

PO-38

CANCER ASSOCIATED THROMBOSIS: SURVEYING PATIENTS' AWARENESS AND EDUCATION NEEDS

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Introduction: Cancer-associated thrombosis (CAT) is a significant cause of morbidity and mortality. Despite guidelines suggesting the use of primary thromboprophylaxis in high-risk patients with cancer, patient awareness of risk and risk-reduction strategies are key to successful implementation.
Aim: Our goal was to understand gaps in patients' awareness of CAT and education about VTE prevention.
Materials and Methods: A questionnaire was designed for individuals with history of cancer or active cancer by a multidisciplinary expert group including patient advocates, pilot tested and electronically circulated through non-profit patient advocacy groups for thrombosis and cancer. Survey domains included: 1) risk factor awareness 2) clinical presentation/diagnosis 3) treatment 4) prevention. Response rate could not be calculated.
Results: There were 95 respondents, including 44% receiving active treatment for their disease (surgery, radiation, or chemotherapy within the last 3 months). Most respondents (89%) were women aged 50 or older. Breast cancer was the most common diagnosis (50%); 40% of respondents reported prior venous thromboembolism (VTE). Forty percent of respondents were unaware that cancer increased VTE risk. 57% of respondents were unaware that certain types of cancer can increase

VTE risk, 51% were unaware certain types of chemotherapy can increase VTE risk, and 41% were unaware that surgery increased VTE risk. One third of respondents had not received any information regarding the risk of VTE in patients with cancer. Only 30% reported hearing about this risk from their clinician, however the majority (80%) would like to receive additional information from their clinician. Sixty percent of respondents had not discussed thromboprophylaxis with their clinician, though 70% stated they would consider the use of thromboprophylaxis if discussed. Compared to patients with prior VTE, patients without prior VTE were more likely to perceive that a VTE diagnosis would affect coping with cancer and quality of life (Table 1).

Conclusions: Patients' awareness of CAT risk remains low. Clinicians are an important source of desired information about CAT. Discussion of thromboprophylaxis remains low, though patients are receptive to thromboprophylaxis as there is a high perceived impact on cancer treatment and survivorship. This study demonstrates patient education will be an important component of efforts to improve guideline implementation.

Table 1.

Domain	Prior VTE N = 35 n (%)	No Prior VTE N=56 n (%)	p-value[1]
Cancer Treatment			0.07
There was/would be an impact	14 (40)	34 (60)	
Coping			0.02*
There was/would be an impact	12 (35)	35 (63)	
Quality of Life			0.02*
There was/would be an impact	18 (51)	43 (76)	

[1]Chi-Square Test, (*) denotes significance

Real and Expected Impacts of VTE on patients with and without prior VTE. Patients with and without prior VTE responses to questions regarding the real or perceived effect on various domains of cancer treatment.

POSTER SESSION 5
TREATMENT OF THROMBOSIS IN CANCER

PO-39

USE OF TINZAPARIN IN THE TREATMENT OF PICC-RELATED THROMBOSIS IN CANCER PATIENTS

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Introduction: In recent years, the need for central venous catheters in oncology has increased. The ease of placement and management as well as the lower economic cost have given peripherally inserted central catheters (PICCs) absolute prominence. However, an increase in the rate of thrombosis associated with PICCs has been observed. Some studies have evaluated several risk factors for PICC-related thrombosis (PRT), but the results have been contradictory and are, thus, unclear. For the treatment of symptomatic catheter-related thrombosis in cancer patients, anticoagulant treatment with LMWH (low molecular weight heparin) is recommended for a minimum of 3 months.

Aim: To describe the characteristics and clinical variables of cancer patients with PRT and evaluate the effectiveness and safety of tinzaparin in the treatment of this complication.

Materials and Methods: This was a prospective, multicentre, observational study including cancer patients in whom a PICC was placed at the Catalan Institute of Oncology from November 2020 to February 2022. Patients were followed for 6 months and the incidence of PRT and associated variables were analysed. A sub-analysis of patients who presented PRT and were treated with tinzaparin was performed.

Results: 801 patients with PICC were included, 52 of whom presented symptomatic PRT (6.5%). All patients with PRT treated with tinzaparin were analysed (24); 62% were men with a mean body mass index of 26.3. 54% received onco-specific treatment with curative intention. Table 1 shows the distribution of patients according to the type of primary tumour. 16 (67%) patients had metastasis or locally advanced stages. Regarding the characteristics of the catheters, most were placed in the basilic vein (22-91.6%) and 2 in the brachial vein; 12 (50%) were inserted in the right arm; and in 14 (58%) patients the catheter began to be used on the same day of placement. For the treatment of PRT, patients received a dose of 175 IU/Kg/day of tinzaparin while the catheter was in placeand for 3 months after its removal. There were no cases of thrombosis recurrence or bleeding 6 months after initiating treatment.

Conclusions: PRT is a relatively frequent complication in cancer patients. Tinzaparin has shown to be effective and safe for preventing the recurrence of thrombosis in patients with PRT.

Table 1.

Patients distribution according to primary tumour

Primary Tumour	N
Breast	4
Germ cell	3
Pancreas	3
ENT	2
Ovary	2
Urothelial	2
Lung	1
Unknown origin	1
Colon	1
Endometrium	1
Gastroesophageal	1
Muscle	1
Bile	1
Other	1

PO-40

DIRECT ORAL ANTICOAGULANTS ARE ASSOCIATED WITH LOWER CIRCULATING LEVELS OF PROCOAGULANT EXTRACELLULAR VESICLES COMPARED TO LOW MOLECULAR WEIGHT HEPARIN TREATMENT IN PATIENTS WITH CANCER ASSOCIATED THROMBOSIS

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Introduction: Cancer Associated Thrombosis (CAT) affects up to 1 in 10 cancer patients and is a leading cause of death in this population. The mechanisms underlying thrombosis risk are varied but include the effects of pro-coagulant extracellular vesicles on plasma hypercoagulability. Low molecular weight heparins (LMWH) were previously considered to be the gold standard for anti-thrombotic therapy in cancer and are known to exhibit anti-inflammatory and other biological properties. Recently, Direct Oral Anticoagulants (DOAC) have emerged as alternatives to LMWH in this CAT cohort, however it remains unclear if these agents exhibit equivalent effects on EV pro-coagulant activity.
Aim: The EXPECT Study aims to characterise the small, large and procoagulant extracellular vesicles as well as plasma and EV cargo proteomic signatures upon treatment with DOAC compared to LMWH in CAT patients.
Materials and Methods: Patients with active cancer newly presenting with a DVT or PE to the Mater Misericordiae University Hospital treated with either DOAC or LMWH anticoagulation were recruited to the EXPECT Study with a baseline blood draw at point of VTE diagnosis and a follow up blood sample 2-9 weeks post-treatment. Small and large EVs were characterised using Nanoparticle Tracking Analysis (NTA) and flow cytometry respectively, quantifying the size, concentration and procoagulant profiles of EVs between treatment arms. Proteomic profiles of the soluble plasma proteins and EV cargo were quantified using tandem mass spectrometry and O-Link analysis.
Results: Small and large EV size and concentration were not significantly altered upon anticoagulant treatment, remaining unchanged between DOAC and LMWH treatment arms. Platelet-derived along with tissue factor and podoplanin expressing circulating EVs were attenuated in the DOAC arm to the same degree as LMWH anticoagulation, highlighting the comparable effects of these anticoagulants at reducing potent procoagulant circulating EVs. Proteomic signatures between the two treatment arms revealed intriguing insights into potential pleiotropic mechanisms at play, with a shift in inflammatory markers between groups.
Conclusions: No significant difference in procoagulant EV profiles were observed with DOAC therapy compared to LMWH, suggesting that both influence this pro-thrombotic mechanism in cancer to a similar extent.

PO-41

ANTITHROMBOTIC THERAPY DECISION-MAKING IN ADVANCED CANCER: KEY FACILITATORS AND BARRIERS

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Introduction: The decision to continue or stop antithrombotic therapy (ATT) in patients with cancer at the end of life is complex. SERENITY is a pan-European study aiming to develop and evaluate a shared decision-making support tool to facilitate ATT management; it is important to understand decisions for ATT.
Aim: To explore facilitators and barriers to ATT decision-making in advanced cancer.
Materials and Methods: Semi-structured interviews with patients and clinicians were conducted in from April 2023 to March 2024 across four countries (Denmark, France, Spain, UK). Data are being analysed using Framework Analysis, informed by concomitant SERENITY work packages, interview summaries and patient and public representatives.
Results: Sixty patients and 77 clinicians were interviewed (Table 1). The prioritisation of other medications and conditions is at the core of the barriers and facilitators for ATT decision-making. Patients had multiple comorbidities, with cancer taking precedence over other health concerns, relegating ATT to a low priority, which resulted in more passive decision-making. ATT indications were in the periphery when managing these patients. Clinicians described a culture of continuing and showed preference for exploring alternative options rather than deprescribing; this was coupled with clinicians not feeling it was their role to take on ATT decisions. Clinicians described the decision as complex, requiring knowledge and expertise from multiple specialties to guide it. However, clinicians also faced challenges with the lack of evidence to support the decision. Patient knowledge about their ATT, including the indication rationale, varied widely. Patients expressed more concern about the reason for being on ATT than that of its side effects, preferring alternative ATT options over deprescribing. Certain patients expressed a need for receiving additional information, indicating that the more information they received, the better. The importance of being informed about the decision and the options was emphasised by patients. This, alongside deferring to the clinicians' expertise, resulted in confidence in the decision that was made.
Conclusions: Barriers and facilitators were identified across various domains, spanning organisational, resource allocation, clinical practice, cultural considerations, and individual factors.

These must be taken into consideration in the development of the shared decision-making support tool.

Table 1.

Characteristic	Patients n=60	Characteristic	Clinicians n = 77
Male, n (%)	28 (47)	**Male, n (%)**	41 (53)
ATT indication		**Antithrombotic affiliated specialists, n (%)**	28 (36)
CAT	26 (44)	Cardiology	7 (9)
Atrial fibrillation	6 (10)	Neurology	4 (6)
Ischaemic heart disease	11 (18)	Vascular medicine/surgeon	8 (10)
Stroke (+/- AF)	3 (5)	Respiratory/Pneumologist	6 (8)
Heart Valve	2 (3)	Internal medicine	3 (4)
Multiple ATT indications	12 (20)		
Age		**Cancer specialists, n (%)**	14 (18)
45-54	6 (10)	Oncology	8 (10)
55-64	9 (15)	Haematology	6 (8)
65-74	24 (40)		
75-84	17 (28)		
85+	4 (7)	**Advanced disease care, n (%)**	35 (46)
		Palliative	10 (13)
ATT		Palliative nurse	8 (10)
DOAC	24 (40)	General Practitioner	10 (13)
LMWH	17 (28)	Geriatrician	7 (9)
Antiplatelets	16 (27)		
VKA	1(2)		
Dual antithrombotic therapy	2 (3)		

ATT: Antithrombotic therapy

PO-42

EFFECTS OF TINZAPARIN ON THE PRESENCE OF RESIDUAL THROMBUS IN PATIENTS WITH CANCER-ASSOCIATED THROMBOSIS

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Introduction: Residual thrombosis (RT) assessed by computed tomography (CT) in patients with venous thromboembolism (VTE) was reported to be 23% (C. Becattini *et al.* JTH 2019). However, information on RT is scarce in patients with cancer-associated thrombosis (CAT).
Aim: The objectives of this study were to assess the presence or absence of RT and to identify variables associated with RT in patients with CAT treated with tinzaparin.
Materials and Methods: Consecutive cohort of CAT patients from January 2008 to June 2022. During their neoplasm follow-up, all patients underwent follow-up CT, and the presence of RT was evaluated. Within VTE, we included patients with deep vein thrombosis (DVT), pulmonary embolism (PE), and unusual thrombosis locations.
Results: The study included 511 CAT patients treated with tinzaparin who underwent at least one follow-up CT. The mean age was 63.1 +/- 13.2 years, with a slight male predominance (52%). Regarding VTE location: PE (38.4%), DVT (35.6%), DVT and PE (16.4%), and atypical VTE (9.6%). During a median follow-up of 17.6 months (p25-75: 7.9-34) and a median anticoagulation duration of 5.7 months (p25-75: 3.1-12.9), 35.8% of patients (n=183) had RT. Multivariate analysis using Cox regression identified that variables associated with residual thrombosis were metastasis (HR: 1.9; 95% CI: 1.4-2.6), ECOG performance status >1 (HR: 2.4; 95% CI 1.6-3.6), and tumor locations (pancreatic and gynecological *vs* others) (HR: 1.6; 95% CI 1.1-2.3).
Conclusions: One-third of cancer-associated VTE patients treated with tinzaparin have residual thrombosis, with identified variables associated with residual thrombosis.

PO-43

A SINGLE-CENTER EXPERIENCE WITH THE USE OF TINZAPARIN FOR THE TREATMENT OF VENOUS THROMBOEMBOLIC EVENTS IN BREAST CANCER PATIENTS ON THERAPY WITH TARGETED DRUG AGENTS

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Introduction: Targeted therapy (TT) has a major impact on the care of patients with breast cancer (BC). Monoclonal antibodies (MoAbs), antibody-drug conjugates (ADC) and kinase inhibitors (KI) are the main agents for TT in BC. Some of these agents, like the cyclin-dependent kinase inhibitors (CDKI), increase the risk of venous thromboembolic events (VTE), and there are some concerns on potential interactions and adverse events of anticoagulant therapy in this context, but published evidence about this topic is limited.
Aims: Review of tinzaparin use for BC patients on TT in a single academic center (2016-2023).
Materials and Methods: Retrospective observational analysis on BC patients treated with TT who received treatment with tinzaparin (TZP). Full data were retrieved from the electronic medical record, covering all the information from hospital and primary care in our regional health service.
Results: 28 patients (p.) received TZP concurrently with TT with antiHer2 monoclonal antibodies, antibody-drug conjugates and/or kinase inhibitors. Median age at the start of TZP: 55.4 years (34.2-83.5). VTE were 11 pulmonary embolisms (PE), 3 PE plus concurrent deep vein thromboses (DVT) and 14 DVT (9 upper limb, 4 lower limb, 1 renal). Therapy setting was adjuvant for early disease in 7 p. and noncurative for metastatic disease in 21 p. Dominant sites of metastatic disease: 11 visceral, 5 bone, 3 soft tissues, 2 brain. TT included KI as follows: palbociclib (10 p.), palbociclib followed by alpelisib (1 p.), abemaciclib (1 p.) and everolimus (1 p). MoAbs included several schemes with trastuzumab (10 p.) and pertuzumab plus trastuzumab (2 p.). ADC included sacituzumab govitecan (2 p.) and trastuzumab deruxtecan (1 p.). Duration of TZP was dependent on the setting, and 17 p. received TZP for more than 6 months, with 9 p. reaching 1 year of TZP or even longer. 8/28 p. continued therapy with direct oral anticoagulants and 1 p. changed to other low-molecular- weight heparin. 7 p. continued TZP in palliative care. 21/28 p. had total resolution of the VTE during TZP, 2 p. had residual disease, and 5 were not fully evaluated. Major bleedings were absent and tolerance was good. 1 p. stopped TZP after developing an itching abdominal rash.
Conclusions: TZP had a good pattern of tolerance and displayed a high activity for event resolution and prevention of new episodes and complications from VTE in BC patients under therapy with KI, MoAbs and ADCs.

PO-44

TREATMENT OF CANCER ASSOCIATED VENOUS THROMBOSIS WITH EDOXABAN IN PATIENTS RECEIVING CONCOMITANT ENZALUTAMIDE

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Introduction: Enzalutamide is a second-generation androgen receptor inhibitor licensed for treatment of metastatic hormone sensitive prostate cancer and high risk non-metastatic castration-resistant prostate cancer. Primarily eliminated by hepatic metabolism, renal excretion provides an insignificant elimination pathway for enzalutamide and its active metabolite. Enzalutamide is a strong inducer of CYP 3A4 and is transported via P-glycoprotein (P-gp). The cytochrome P450 enzymes (CYP3A4 and 2D6) are responsible for the metabolism of the Anti-factor Xa Direct Oral Anticoagulants (DOAC), apixaban and rivaroxaban. Edoxaban is an oral direct factor Xa inhibitor not metabolised by the cytochrome p450 pathway but does undergo P-gp transportation.
Aim: Effect of enzalutamide treatment on anticoagulant efficacy and safety of edoxaban treatment of cancer associated venous thromboembolism (CAT) and atrial fibrillation (AF).
Materials and Methods: Consecutive patients referred to a regional CAT service (with an indication for anticoagulation) who were receiving enzalutamide and DOAC, apixaban or rivaroxaban, were switched to edoxaban. Steady state plasma trough level measurement (24hours after last dose) of edoxaban was completed for all patients. All patients were followed up for a period of 12 months (or until death, whichever occurred earlier) to describe any recurrent thrombotic and/or bleeding episodes.
Results: 13 patients (8 VTE and 5 AF) received concomitant edoxaban and enzalutamide and had 15 trough edoxaban levels taken. Patients were all male with a median age of 74yrs (range 64-84). The median edoxaban plasma level was 27ng/ml (range 22.5-43.4ng/ml). No major or clinically significant non-major bleeds were recorded. No patients in the cohort experienced on-treatment recurrence of VTE or stroke within the monitoring period (Table 1).
Conclusions: There was no evidence of clinically significant drug-drug interaction between enzalutamide and edoxaban. There were no edoxaban levels above the expected steady state level when administered with enzalutamide. Co-administration of enzalutamide and edoxaban patients treated for CAT / AF in this patient group did not result in on-treatment recurrence of VTE or stroke within the monitoring period.

Table 1.

Age	Weight (kg)	Crcl(ml/min)	Indication for anticoagulation	Edoxaban dose	Edoxaban Trough Level (ng/ml)*	Major bleeding	On treatment recurrent venous thrombosis / stroke
76	82	102	Popliteal DVT	60mg OD	34.9	No	No
71	102	93	Upper Limb DVT	60mg OD	30.7	No	No
72	123	99	Ilio Femoral DVT	60mg OD	24.9	No	No
84	73	61	Femoral DVT	60mg OD	22.7	No	No
77	91	89	Fem-pop DVT	60mg OD	37.6	No	No
77	96	94	PE	60mg OD	41.9	No	No
77	96	94	PE	60mg OD	26.6	No	No
69	104	107	PE	60mg OD	27	No	No
71	79	62	PE	60mg OD	43.4	No	No
76	102	66	AF	60mg OD	42.8	No	No
72	114	111	AF	60mg OD	24.1	No	No
78	100	93	AF	60mg OD	22.5	No	No
74	113	105	AF	60mg OD	32.7	No	No
72	117	174	AF	60mg OD	26	No	No
72	117	146	AF	60mg OD	23.3	No	No

PO-45

CHEMOTHERAPY ASSOCIATED CEREBRAL VENOUS SINUS THROMBOSIS (CVST)-EXPERIENCE FROM A TERTIARY CANCER CENTRE

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Introduction: Cancer patients area at increased risk of venous thromboembolism. The risk is further increased by chemotherapy. The diagnosis and management of CVST is challenging in cancer patients receiving chemotherapy.
Aim: To study the clinical profile and outcome of patients with CVST post chemotherapy
Materials and Methods: This is a retrospective study of cancer patients with chemotherapy associated CVST registered at the cancer thrombosis clinic of a tertiary referral cancer centre in the period 2018 to 2022. The details of cancer, chemotherapy, details of CVST, its treatment and related complications were recorded. The data was analysed using IBM SPSS statistics 25 software. The study is approved by the Institute ethics committee.
Results: 25 patients with chemotherapy associated CVST were registered in the study period. Females were predominant (60%). The median age was 31 years (range 16-71). 56% had hematolymphoid malignancy, all had received l asparaginase prior to CVST and further l asparaginase was discontinued. 44% patients had solid tumors of these 72% developed CVST after cisplatin based chemotherapy and cisplatin was discontinued in all except 1. Seizures was the commonest presentation (60%). The CVST was diagnosed by CECT brain in 72% and MRI brain in 28%. Cerebral Infarcts were seen in 8 patients (7 were hemorrhagic). All except one patient received therapeutic anticoagulation. All patients were started on Low molecular weight heparin (LMWH). After initial treatment with LMWH 5 patients were shifted to oral anticoagulation (Rivaroxaban-4, warfarin-1), in rest LMWH was continued. Chemotherapy induced thrombocytopenia (CIT) occurred in 13/14 patients of hematologic malignancy, requiring interruption of LMWH in 6 patients. In 8/11 patients with solid tumor, chemotherapy was continued of whom only 1 patients developed CIT. 18 patients received anti epileptic drugs. Bleeding complications (non neurologic) were seen in 2 patients and 2 developed recurrent CVST. The median duration of anticoagulation was 6 months (range 1-18 months).11/14 patients showed complete recanalization on follow up neuroimaging.
Conclusions: The commonest chemotherapeutic drugs associated with CVST are l asparaginase and cisplatin. LMWH was the anticoagulation of choice and was well tolerated with good clinical outcome. CIT is a common challenge in management of CVST patients with hematolymphoid malignancy.

PO-46

DURATION OF ANTICOAGULATION IN CANCER ASSOCIATED THROMBOSIS WHAT IS IDEAL

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Introduction: Cancer associated thrombosis (CAT) has been increasing in prevalence and is the second common cause of death after cancer itself. Both, deep venous thrombosis and pulmonary thromboembolism are prevalent, more common than in the general population. Despite this, the optimal duration and intensity of anticoagulation remain unclear.
Aim: We describe and analyze a case of cancer associated thrombosis and discuss the evidence (or the lack of it) in areas of management and then subsequently areas where further direction and guidelines are essential.
Materials and Methods: An elderly lady presented to the clinic for symptomatic right lower limb proximal deep vein thrombosis. She had 2 weeks ago, undergone surgery for adenocarcinoma of

the right colon, status post, hemicolectomy and was now awaiting neoadjuvant chemotherapy. In view of the proximal DVT, she was started on tablet Apixaban, after discussing the risk benefit ratio and other options available (Low molecular weight heparin). For the next 6 months, she continued her anticoagulation as well as her chemotherapy, often interrupting oral anticoagulation for minor, but clinically significant bleeding, which caused great distress to herself and her family. Further scans and colonoscopy revealed recurrence of tumor, for which she was advised to undergo further chemotherapy. In the interim, the deep vein thrombosis had resolved and a repeat scan of the right leg did not even show a residual thrombosis.

Results: In such an instance, what should be the strategy for anticoagulation? A) Continue therapeutic anticoagulation in view of tumor recurrence and the fact she had had no major bleeding while on anticoagulation. B) Continue prophylactic anticoagulation, at a reduced dose, in order to minimize the risk of bleeding but at the same time trying to offer some level of protection from recurrence of venous thromboembolism. C) Withhold further anticoagulation in view of the fact that she has no VTE. D) Consider changing the anticoagulant to low molecular weight heparin in view of better tolerability and lesser risk of bleeding.

Conclusions: We discuss in our presentation, the pros and cons as well as the evidence behind each of the options.

PO-47

WHAT IS THE IDEAL ANTICOAGULANT?

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Introduction: With the advent of Novel Oral Anticoagulants (NOAC), they remain the choice for anticoagulation in most clinical circumstances, except when there is renal impairment, significant valvular heart disease, pregnancy and lactation, mechanical heart valve and significant drug interaction. Despite safety and data for more than 10 years, cost remains a significant issue and some patients who are adjusted with Warfarin, are keen to continue rather than switch over to NOAC.

Aim: We describe a clinical scenario where it calls upon choosing the right anticoagulant for a patient with multiple comorbidities.

Materials and Methods: An elderly lady was admitted to the hospital for bleeding per rectal (PR). Amongst other comorbidities, she had atrial fibrillation, CHADS2 Vasc score of 4, on Warfarin with a target International Normalised Ratio (INR) of 2-3, and obesity (BMI 27). Due to the PR bleed, Warfarin was withheld and she underwent a colonoscopy, which showed a rectal adenocarcinoma, for which she was advised surgery. In view of PR bleeding and requirement of 2 pints of blood, her anticoagulation was withheld and she was given compression stockings as well as pneumatic compression devices for VTE prevention. While awaiting work up for the major surgery, she developed a right lower limb swelling, which was diagnosed to be a proximal deep vein thrombosis. Surgeons planned to do a hemicolectomy and since there was a localized cancer, did not offer any further chemo/radiotherapy.

Results: In such an instance, what should be the strategy for long term anticoagulation? A) Continue therapeutic anticoagulation with Warfarin, insert an Inferior vena cava (IVC) filter in the perioperative period, remove it post operatively and resume anticoagulation with Warfarin. B) Stop Warfarin, and change to NOAC in the long term. C) Continue Warfarin and there is no need to change to NOAC since the risk factors in this case were obesity, immobility and hospitalization; with no prophylactic anticoagulation.

Conclusions: We discuss in our presentation, the pros and cons of choosing between Warfarin and NOAC (and if so, the particular NOAC) in this scenario with multiple confounding factors, which play out in real life.

PO-48

A 44 KILOS UTERINE FIBROID RESECTION COMPLICATED BY EXTENDED VENOUS THROMBOEMBOLISM NOT SOLVED BY FULL DOSE EDOXABAN. A CLINICAL CASE

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Introduction: 44 y-o woman, without cardiovascular risk factors, in levothyroxine therapy after thyroidectomy due to cancer, reported a marked abdominal enlargement (Figure 1) in the last year. Thus, being addressed to bariatric surgery, she started further examinations. An abdominal ultrasound detected an exceptionally large pelvic mass severely compressing the great abdominal vessels, confirmed by a CT scan to be solid and non-homogeneous (60 x 50 cm), that was completely not suspected at the initial clinical evaluation of the bariatric surgeon.

Aim: At this time, she was admitted to our institution at the oncological gynecological unit. During her stay, a 44 Kg uterine fibroid was resected, but 1 day after surgery she complained with pain and swelling of the right leg without dyspnea or chest pain.

Materials and Methods: An ultrasound of the lower limbs showed an extended right superficial femoral deep vein thrombosis (DVT). A CT scan detected an incidental subsegmental bilateral pulmonary embolism (PE), bilateral ovarian DVT and right internal iliac DVT. D-dimers: 6081 ng/mL, troponin: 48.4 ng/L. The patient was hemodynamically stable. An echocardiogram was performed: no signs of right ventricular dysfunction with normal ejection fraction (58%). sPESI score was 0 (low-risk PE). She started enoxaparin 1 mg/Kgx2/die (weight: 67 Kg) replaced after 1 week with edoxaban 60 mg/die. After 3 months she came back to our institution to follow up and was completely asymptomatic. A CT scan showed resolution of PE but persistence of the ovarian and iliac DVT. An ultrasound detected a partial resolution of the femoral DVT.

Results: Due to subsequent complaints of the patient for epistaxis (Hemoglobin: 10 g/dL), to the absence of malignant disease and to the chronic nature of the venous thrombotic events mostly incidentally detected, we decided to proceed with the anticoagulant treatment reducing edoxaban dose (30 mg/die).

Conclusions: Abdominal and pelvic major surgery is strongly associated with venous thromboembolism. In this clinical case, an extremely large uterine fibroid (44 Kg), although not malignant,

heavily compressed the abdominal vessels and may have caused soon after surgery the development of incidental PE and symptomatic DVT. Due to DVT persistence and to the complaints of the patient for clinically relevant non-major bleedings, we decided to reduce the edoxaban dose and check the patient clinical conditions with frequent follow up visits.

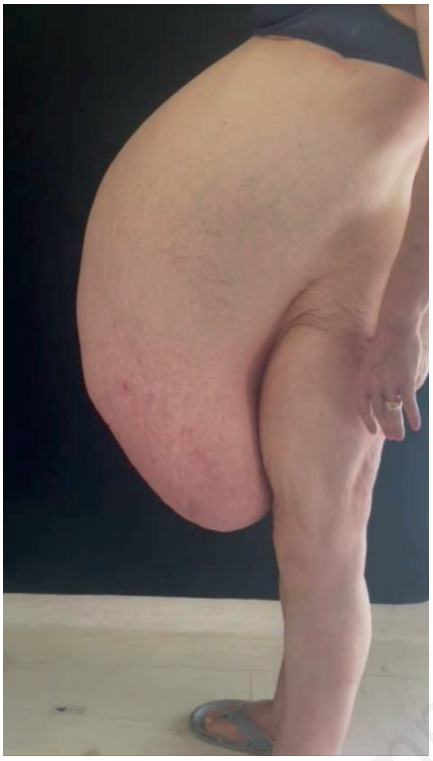

Figure 1.

POSTER SESSION 6
HEMOSTATIC PROTEINS AND CANCER BIOLOGY

PO-49

ANTI-PROLIFERATIVE, ANTI-ANGIOGENIC AND ANTI-INVASIVE EFFECT OF ANTITHROMBIN IN GLIOBLASTOMA MULTIFORME

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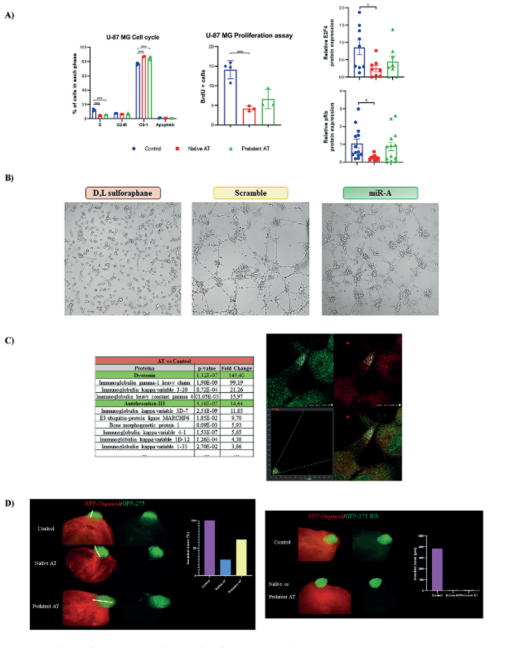
Introduction: Antithrombin (AT) has other functions beyond hemostasis. We have demonstrated that native and prelatent AT reduce migration and invasion as well as the expression and phosphorylation of VEGFA, STAT3, and ERK1/2 in U87 glioblastoma multiforme (GBM) cells. Regarding GBM, it is the most lethal primary malignant tumor of the central nervous system in adults, and the median survival is 12-15 months after diagnosis.

Aim: 1) Investigate the role of AT on other pro-tumor pathways using *in vitro* and preclinical models of GBM; 2) identify the potential receptor of AT in GBM.

Materials and Methods: Native and prelatent AT were purified from healthy donor's plasma. Prior to each experiment, U87 cells were treated with 2.16 µM of native, prelatent AT or PBS. A microarray analysis was performed on U87 (Human Clariom D). Differences were validated by western blot. Cell cycle and proliferation were assessed by flow cytometry. Three microRNAs (miR-A/B/C) with altered expression in GBM were transfected into U87 cells, and angiogenesis was evaluated by co-culture with endothelial cells. The receptor was determined by crosslinking and immunoprecipitation, followed by quantitative proteomics and confocal microscopy. Healthy human brain RFP-organoids were generated from iPSCs, and neurospheres from the biopsy of one GMB patient, at diagnosis (GFP-275) and at relapse (GFP-275-BIS). Anti-invasive AT effect was validated through organoids and neurospheres co-culture.

Results: AT treatment reduced the expression of cell cycle-related genes (FC, p-value): CDK4 (-1.64, 2.60-5), CCNE2 (-2.06, 2.65-6), RB1 (-1.58, 1.37-6) and E2F4 (-2.03, 1.09-7). Native AT reduced U87 S-phase and proliferation as well as E2F4 and pRb expression (Figure 1A). Moreover, AT increased miR-A expression (FC 1.61), and our results preliminarily suggest that miR-A reduces angiogenesis (Figure 1B). We identified dystonin as the receptor of AT on U87, and confocal microscopy images show small regions of colocalisation in U87 between dystonin and AT (Figure 1C). Interestingly, native and prelatent AT partially reduced invasion of 275 neurospheres (100% *vs* 28.89%, 100% *vs* 65.64%, respectively), and completely reduced 275-BIS neurospheres invasion of the brain-organoids (Figure 1D).

Conclusions: AT has surprising and versatile anti-tumor properties on GBM. Our results support its potential therapeutic usefulness in GBM, a tumor in which new treatments are urgently needed.



Antitumor role of AT on GBM. A) Antiproliferative effect. Cell cycle and proliferation were assessed by measuring 7-AAD and BrdU incorporation by flow cytometry (n=3/group) after treating U87 cells with 2.16 µM native or prelatent AT or PBS (control) for 12h. **B) MiRNA control of angiogenesis on endothelial cells.** Representative images of the angiogenesis assay after transfecting U87 cells with miR-A, and in comparison with a scramble and D,L sulforaphane controls. **C) Identification of the receptor: Dystonin.** Left panel: results from quantitative proteomic. Right panel: dystonin (green) and AT (red) co-localization by confocal microscopy. Potential areas of co-localization are visualized in white. **D) Anti-invasive effect on a 3D model of invasion.** GFP-275 (top) or GFP-275 BIS (bottom) neurospheres (green) were obtained from the biopsy at diagnosis or relapse of a GBM patient, respectively. Human brain organoids (red) were generated from induced pluripotent stem cells. Representative fluorescence images of the results are shown on the left. On the right is the percentage of invasion of each neurosphere. * AT: antithrombin; GBM: glioblastoma; GFP or RFP: Green or Red Fluorescence Protein, respectively.

Figure 1.

PO-50

RIVAROXABAN COMPARED TO NO TREATMENT IN EARLY BREAST CANCER PATIENTS (THE TIP TRIAL, EUDRACT 2014-004909-33): EFFECT ON EPCAM SERUM CONCENTRATIONS AS A SURROGATE FOR CIRCULATING TUMOUR CELLS (CTCS)

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Introduction: The TIP Trial is a multi-centre phase II pre-operative 'Window-of Opportunity' RCT of Rivaroxaban (Factor Xa inhibitor) *vs* no treatment in ER negative, stage I-III early breast cancer patients (n=88). Patients were randomised 1:1 (Rivaroxaban 20mg od: no treatment) and received 14 (+/-3) days of treatment in the window between diagnosis and surgery/start of neoadjuvant chemotherapy. We hypothesised that TF-FXa inhibition would reduce tumour growth and metastases. A secondary outcome was circulating tumour cell (CTC) enumeration in response to Rivaroxaban/control. Unfortunately, an Institute fire disrupted the time-critical CTC analysis. EpCAM (Epithelial cell adhesion molecule) is expressed by CTCs and is therefore a potential surrogate marker.

Aims and Methods: In early breast cancer, to determine if: 1. Serum EpCAM decreases in response to Rivaroxaban. 2. EpCAM correlates with plasma thrombin-antithrombin III (TAT), Tissue Factor (TF) and D-dimer, at baseline, and with tumour Ki67. 3. Change in EpCAM correlates with change in TAT, TF, D-dimer, and Ki67 in response to Rivaroxaban. EpCAM, TAT and TF were measured by ELISA, D-dimer by immunoturbidimetry and Ki67 by IHC 'pre' Rivaroxaban/Control and 'post' treatment.

Results: Of 77 patients with serum pre and post treatment, 21 (27%) had detectable EpCAM at baseline (8 of 40 controls; 13 of 37 Rivaroxaban). All 8 controls (+ 2 additional) had EpCAM at 'post' treatment. All 13 Rivaroxaban (+ 0) had EpCAM at 'post' treatment. When dichotomised as EpCAM+ (n=44) and EpCAM- (n=110), TAT (but not Ki67, D-dimer or TF) was higher in EpCAM+ (Table 1). Change in EpCAM from baseline to 'post' treatment was lower in Rivaroxaban *vs* controls (mean 0.95 (95% CI 0.86-1.03) *vs* 1.06 (0.98-1.14), p=0.04). However, (despite randomised allocation), pre-treatment EpCAM were lower in controls (mean 208 (95% CI 133-282) *vs* 374 (298-491) pg/ml, p=0.003). EpCAM did not correlate with Ki67, TAT, TF or D-dimer at baseline. In the Rivaroxaban (but not control) arm, changes in EpCAM and TF correlated (Pearson r=0.61, n=12, p=0.04). Change in EpCAM did not correlate with TAT, Ki67 or D-dimer change.

Conclusions: There appears to be a small but significant decrease in EpCAM in response to Rivaroxaban in early breast cancer. EpCAM+ patients have higher plasma TAT, which could indicate increased coagulation caused by CTCs. The correlation between EpCAM and TF in the Rivaroxaban group warrants further investigation.

Table 1.

Marker	EpCAM+ (Mean, 95% CI)	EpCAM- (Mean, 95% CI)	p-value (Mann-Whitney)
TAT (ng/ml)	8.5 (5.1-12.0)	6.3 (4.5-8.2)	0.01
Ki67 (%)	31 (24-39)	24 (21-28)	0.08
D-dimer (ng/ml FEU)	478 (352-604)	469 (377-560)	0.9
TF (pg/ml)	44 (40-49)	41 (39-42)	0.15

PO-51

CHARACTERISATION OF PROCOAGULANT FIBROBLASTS IN THE EARLY BREAST CANCER MICROENVIRONMENT THROUGH *EX VIVO* CULTURE

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Introduction: Mammographic density, reflecting increased fibroblast activity, is a strong independent risk factor for breast cancer. Both cancer-associated fibroblasts (CAFs) and high-density breast tissue have increased alpha smooth muscle actin (α-SMA) and secrete elevated levels of cytokine TGFβ1. Tissue factor (TF) is overexpressed in breast cancer and is correlated with a worse prognosis. We have previously found higher levels of TF expression in DCIS (pre-cancer) fibroblasts compared to normal breast fibroblasts, and even higher TF expression in invasive breast cancer (IBC) fibroblasts.

Aim: To test if: 1. Increased tumour fibroblast procoagulant marker expression and activity, promotes breast cancer progression. 2. High density breast reflects a wound-like stroma (more procoagulant fibroblasts) that promotes breast cancer development.

Materials and Methods: Primary fibroblasts were cultured from 144 fresh breast tissue samples from IBC (n=50), DCIS (n=12), risk reduction mastectomy (n=14) and reduction mammoplasty patients (n=10). Procoagulant (TF) and fibroblast activation marker (α-SMA) expression were assessed by immunocytochemistry. Procoagulant activity of fibroblasts and their conditioned media (CM) were quantified by modified prothrombin time. In fibroblast CM, TF activity and TGFβ1 levels by ELISA were quantified. Migration of MCF-7 breast cancer cells was measured by a migration scratch assay in the presence of fibroblast CM, with and without TF (10H10 antibody) and TGF-β Receptor (SB 431542) inhibitors. Breast density was assessed by BI-RADS.

Results: Fibroblasts were successfully cultured from 108 of 144 samples (75%). TF expression correlated with α-SMA (r=0.61, p=0.009) and procoagulant activity (r=-0.42, p=0.01). In CM, TF and TGFβ1 correlated with CM procoagulant activity (r=-0.51, p<0.0001; r=-0.41, p=0.0001). MCF-7 cell migration, in fibroblast CM, correlated with TF levels (r=0.52, p=0.01). Combined inhibition of both TF and TGF-β receptor inhibited migration and was more effective than either inhibitor alone. There was no difference in procoagulant or fibroblast activation markers between high (BI-RADS C/D) and low (A/B) density patients.

Conclusions: This study provides *ex vivo* functional results showing that fibroblast procoagulant phenotype correlates with fibroblast activation phenotype. Increased fibroblast TF and TGF-β secretion promotes breast cancer cell migration, with combined inhibition a potential therapeutic strategy.

PO-52

CANCER ASSOCIATED THROMBOSIS ALTERS NEUTROPHILS TO PROMOTE PANCREATIC DUCTAL ADENOCARCINOMA PROGRESSION

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Introduction: Cancer-associated thrombosis (CAT) is a marker of poor prognosis with disease progression and increased cancer-related morbidity and mortality. Neutrophils, the most abundant of the innate immune cells, are critical cellular determinants of venous thrombosis. Pancreatic ductal adenocarcinoma (PDAC) is a highly prothrombotic cancer with a poor prognosis.
Aim: Given the increasing appreciation for the role of the innate immune system in CAT as well as in tumor growth, we posited that the cells of the innate immune system, specifically neutrophils are altered in CAT and facilitate tumor growth.
Materials and Methods: C57BL/6J mice receive intra-pancreatic or subcutaneous injection with PAN02 cells to develop pancreatic tumors (PDAC). Venous thrombosis was achieved by (1) complete inferior vena cava (IVC) ligation for IVC thrombosis OR (2) pulmonary thrombosis by intravenous microbead (MB) injection. For cancer associated thrombosis or CAT, 48 hours after the injection of PAN02 cells, animals underwent either IVC ligation OR intravenous MB injection.
Results: 1. PDAC+CAT mice developed significantly larger tumors than only PDAC. 2. PDAC+CAT tumors showed decreased lymphocyte content (CD8 +T cells) in the TME. No difference was noted in neutrophil, macrophage content or angiogenesis. 3. Bone marrows showed decreased CD8+ T cells while spleen showed increase in neutrophils in PDAC+CAT as compared to PDAC mice. 4. Neutrophil depletion mitigated tumor growth in PDAC+CAT mice. Interestingly, tumors in the PDAC mice had increased tumor progression. Macrophage depletion did not alter tumor growth in either group. 5. Transcriptomic analysis of circulating neutrophils showed 210 DEG between PDAC only *versus* PDAC+CAT mice with significant alterations specifically in hypoxia and inflammatory pathways.
Conclusions: Our results are the first to demonstrate that CAT is associated with altered neutrophil activity that affects the TME and facilitates tumor growth. Additionally, CAT is associated with changes in the hematopoietic system evidenced by decreased CD8+ T cells in the bone marrow and increased neutrophil presence in the spleen. Tumor progression in CAT is neutrophil-dependent and is associated with an altered neutrophil transcriptome specifically in the inflammatory and hypoxia-mediated pathways. Ongoing studies are exploring the molecular mechanisms involved in CAT-directed neutrophil alterations and the cellular elements affecting the TME and tumor progression.

PO-53

GLUCOCORTICOIDS AS TRANSCRIPTIONAL REGULATORS OF THE TUMOR COAGULOME OF ORAL SQUAMOUS CELL CARCINOMAS

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Introduction: Oral Squamous Cell Carcinoma (OSCC) are the most frequent type of upper aerodigestive tumors. OSCC are characterized by a specific tumor coagulome, defined by the simultaneous high mRNA expression of the main regulators of coagulation (tissue factor, TF) and fibrinolysis (urokinase-type plasminogen activator, uPA). While the landscape of the human tumor coagulome has been rather well defined, studies adressing its regulation are lacking. Glucocorticoids are stress hormones that are used in clinics as anti-inflammatory drugs.
Aim: We explored the transcriptional regulation of the tumor coagulome of OSCC.
Materials and Methods: Two human OSCC cell lines (PE/CA PJ34 and PE/CA PJ41) were treated with dexamethasone and various agonists of the nuclear receptor family. Using immunoblotting and qPCR, we examined the expression of the core coagulome: TF, uPA and plasminogen activator inhibitor-1 (PAI-1). Data retrieved from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GSE159546, with chromatin immunoprecipitation and RNAseq data for lung cancer cell lines treated with hydrocortisone) were used to examine the effects of glucocorticoids. Functional assays measuring thrombin and uPA activation were also used *in vitro*.
Results: Dexamethasone, a potent agonist of the glucocorticoid receptor (GR), decreased uPA and TF expression, and activated PAI-1 expression. The decrease in uPA and TF expression is most likely explained by an anti-inflammatory signalling effect, as suggested in conditions of TNFα exposure. Conversely, PAI-1 induction was most likely the product of a direct, GR (*NR3C1*)-dependant transcriptional effect. Genomic data from GSE159546 allowed us to confirm and extend our conclusions to a larger array of coagulation-related genes (n=85 from KEGG). Our conclusions were independently validated with a functional analysis examining the activation of thrombin and uPA protease activity *in vitro* in OSCC cells. Finally, we examined the impact of direct PAI-1 regulation on the tumor microenvironment (TME) of OSCC. We observed a TME enriched in fibroblasts, endothelial cells and cells of the monocytic lineage, and a high TGF-β response.
Conclusions: Glucocorticoids exert potent, yet complex, regulatory effects on the expression of essential genes of the coagulome of OSCC. This regulation may be of importance for vascular complications in cancer patients, and it might also account for some of the effects of glucocorticoids on the TME.

PO-54

DESCRIPTION OF CLINICAL AND MOLECULAR FEATURES IN CANCER-ASSOCIATED VENOUS THROMBOEMBOLISM

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Introduction: Venous thromboembolism (VTE) stands as the second preventable cause of mortality in cancer patients and presents substantial challenges in the clinical management of cancer patients due to its impact on morbidity, mortality, and quality of life. Despite advances in understanding and preventing VTE in the cancer setting, gaps persist in our comprehension of this complication.

Aim: This study aims to describe the clinical, pathological, and molecular features of cancer patients with VTE, deepening our understanding of the cancer-thrombosis interplay. We aim to compare these features between VTE and non-VTE patients, identifying risk factors and predictive markers for this complication in oncology.
Matherials and Methods: We conducted a retrospective case-control study encompassing cancer patients diagnosed with VTE between 2022 and 2023 at Vall Hebron Hospital (cases) and cancer patients without VTE treated at the same center (controls). Clinical parameters, including cancer site, stage, pathological/ molecular profile, and treatment modalities, were documented. A univariate analysis was performed to compare cases and controls.
Results: A total of 123 cases and 100 controls were included in the analysis. The clinical characteristics described include age, sex, and Charlson and Khorana scores. According to the Khorana score, 75.41% of VTE cases were at intermediate or high risk for VTE. Female gender demonstrated an association with VTE. Cancer types are also described, with breast and pancreatic cancers exhibiting associations with VTE, while lung, colorectal, and gynecological cancers are not related. The distribution of cancer stages did not show differences between VTE cases and controls. Treatment modalities did not observe differences in cancer stage. Both chemotherapy and targeted therapies were associated with VTE. Regarding the molecular study, the most prevalent cancers among VTE patients were lung (predominantly adenocarcinomas; 13.6% EGFR mutated), breast (mostly invasive ductal carcinoma; 93.3% hormone receptor-positive and 40% HER2-positive), and colorectal cancer (all adenocarcinomas; 40% RAS/BRAF mutated, 13% with microsatellite instability).
Conclusions: The most prevalent cancers among VTE patients were lung (predominantly adenocarcinomas; 13.6% EGFR mutated), breast (mostly invasive ductal carcinoma; 93.3% hormone receptor-positive and 40% HER2-positive), and colorectal cancer (all adenocarcinomas; 40% RAS/BRAF mutated, 13% with microsatellite instability). Female gender, breast cancer, and pancreatic cancer were associated with VTE. Moreover, both chemotherapy and targeted therapies showed associations with VTE.

PO-55

MIR5683 PREDICTS VENOUS THROMBOEMBOLISM IN ADVANCED GASTRIC CANCER THROUGH REGULATION OF FIBRINOLYSIS AND ENDOTHELIAL TFPI EXPRESSION

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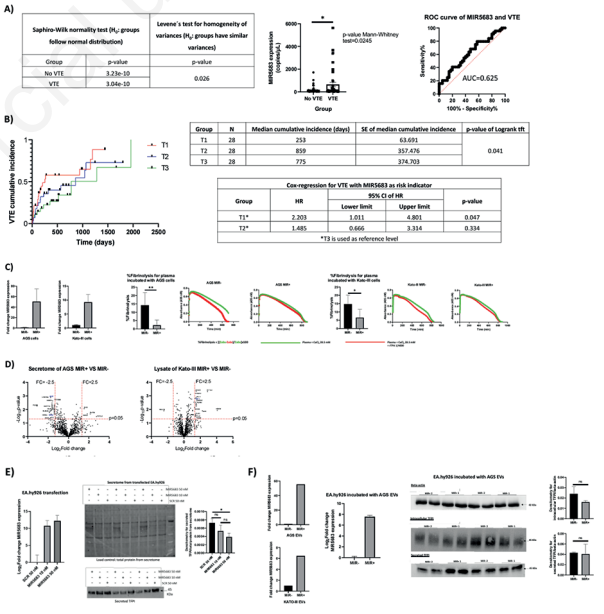
Introduction: Advanced gastric cancer (AGC) is one of the most thrombogenic neoplasms. Previously, we identified the microRNA MIR5683 overexpressed in AGC patients with venous thromboembolism (VTE) by transcriptomics (nested case-control [n=50 *vs* 50] study of patients selected from the AGAMENON registry [n=4000]).
Aim: To validate MIR5683 as a VTE biomarker in a new cohort and to explore the underlying mechanisms.
Materials and Methods: RNA purification from AGC biopsies (n=44 VTE patients *vs* 40 controls). Retrotranscription-preamplification-digital PCR for absolute quantification of MIR5683 expression. Correlation with VTE occurrence by Mann-Whitney test and ROC curve, and with VTE cumulative incidence by Cox-regression. Stable transfection of AGS and Kato-III cells with MIR5683/ empty vector (AGS-MIR+/MIR-, Kato-III-MIR+/MIR). Thrombin generation (TG) and fibrinolytic assays with platelet-poor plasma (PPP) previously incubated with cells. Quantitative proteomics of cell lines. Transient transfection of EA.hy926 cells with miR-5683 mimic and evaluation of TFPI (target according to TargetScanHuman) expression. Isolation of extracellular vesicles (EVs) from stably-transfected cells and incubation with EA.hy926.



Validation of MIR5683 expression as a biomarker for venous thromboembolism in advanced gastric cancer and study of the underlying mechanisms. A) Since the MIR5683 expression data of both patient groups did not follow a normal distribution and lacked homogeneity of variance, we compared MIR5683 expression between both groups of patients by Mann-Whitney test. We also checked the quality of MIR5683 expression as a venous thromboembolism biomarker by calculating the area under the ROC curve. **B)** To analyze the cumulative incidence of venous thromboembolism, we calculated tertiles according to MIR5683 expression data to divide patients into three groups: T1, or the 28 patients with the highest MIR5683 expression, T2, or the next 28 patients according to MIR5683 expression, and T3, or the 28 patients with the lowest MIR5683 expression. We also show the median cumulative incidence for the three groups and the p-value of Logrank test for trends. Finally, by Cox-regression, we obtained the hazard ratio expressing the differential risk of venous thromboembolism when comparing the T1 or T2 group with the T3 group, considered as the reference group for MIR5683 expression. **C)** Comparison of MIR5683 expression (U6 was used as loading control) between gastric cancer cells stably transfected with MIR5683 and empty vector. Fibrinolysis and thrombin generation in platelet-poor plasma incubated with these cells. In the fibrinolysis assay, for each plasma sample we analyze its coagulant or fibrinolytic activities by adding CaCl2 or CaCl2 and recombinant tissue plasminogen activator, respectively. Each graph with elapsed time corresponds to one representative replicate for each condition. **D)** Differentially expressed proteins between gastric cancer cells with MIR5683 and empty vector. From quantitative proteomics, we selected those proteins with |fold change|>2.5 and p-value<0.05. In blue we have highlighted the proteins related to fibrinolysis. We only show secretomes or lysates where we found differentially expressed proteins related to fibrinolysis. **E)** Effect of miR-5683 mimic on secreted TFPI from EA.hy296 cells. We performed transient transfection of two different concentrations of miR-5683 mimic in EA.hy926 cells and then measured secreted TFPI and total secreted protein as a loading control. **F)** Comparison of miR-5683 expression between extracellular vesicles from gastric cancer cells stably transfected with MIR5683 and empty vector. We also show effect of miR-5683 from extracellular vesicles derived from stably transfected AGS cells on TFPI from EA.hy926. We compared miR-5683 expression between EA.hy926 incubated with vesicles from AGS with MIR5683 vector and with vesicles from AGS with empty vector. We then measured secreted and intracellular TFPI from these endothelial cells, as well as beta-actin as a loading control. In all figure sections, the column graphs represent the mean and standard deviation of the different replicates. Significant statistical differences were calculated by unpaired t-test. H_0: null hypothesis; VTE: venous thromboembolism; copies/µL: unit for measuring quantitative expression by digital PCR; AUC: area under the curve; N: number of patients; SE: standard error; tft: test for trends; HR: hazard ratio; CI: confidence interval; MIR-:gastric cancer cells stably transfected with empty vector; MIR+:gastric cancer cells stably transfected with MIR5683 vector; ∑abs: sum of all absorbance points from time=0 to the time when the rTPA curve reaches absorbance=0; rTPA: recombinant tissue plasminogen activator; FC: fold change; p: p-value; SCR: scrambled mimic as negative control for transient transfection; EVs: extracellular vesicles; ns: not significant differences; *: p-value<0.05; **: p-value<0.01.

Figure 1.

Results: MIR5683 expression was significantly higher in VTE patients (p-value=0.025; ROC curve, AUC=0.625) (Figure 1A) and increased VTE risk with a significant hazard ratio (2.203, p-value=0.047) (Figure 1B). MIR5683 overexpression decreased fibrinolysis in PPP incubated with AGS and Kato-III (p-value<0.05) (Figure 1C). In AGS secretome, MIR5683 significantly downregulated MCP and SDC4, profibrinolytic proteins. In Kato-III lysate, MIR5683 significantly upregulated

TSP1, a plasmin inhibitor (Figure 1D). In EA.hy926, miR-5683 transfection reduced secreted TFPI (p-value=0.026) (Figure 1E). MIR-5683 levels were higher in EA.hy926 incubated with EVs from AGS-MIR+ *vs* EVs from AGS-MIR-, and increased miR-5683 levels reduced intracellular and secreted TFPI (Figure 1F). **Conclusions:** MIR5683 was validated as a novel VTE biomarker in AGC patients. These findings could be based on anti-fibrinolyitic effects of this miRNA, but also on its potential remote effect on endothelial TFPI.

PO-56

AAV-MOUSE DNASE I SUSTAINS LONG-TERM DNASE I EXPRESSION *IN VIVO* AND SUPPRESSES BREAST CANCER METASTASIS

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Introduction: Neutrophil extracellular traps (NETs) have been implicated in the pathology of various inflammatory conditions. In cancer, NETs have been demonstrated to induce systemic inflammation and thrombosis, impair peripheral vessel and organ function and promote metastasis. Administration of DNase I is one strategy to eliminate NETs but long-term treatment requires repeated injections and species-specific versions of the enzyme. In mouse models, this is currently limited by the availability of recombinant murine DNase I.
Aim: To enhance administration and therapeutic efficacy of DNase I and to enable long-term DNase I administration in murine cancer models to address potential effects on metastasis.
Materials and Methods: We have developed an adeno-associated virus (AAV) vector system for delivery of murine DNase I and addressed its potential to counteract cancer-associated pathology in the murine MMTV-PyMT model for metastatic mammary carcinoma. The AAV vector is comprised of capsid KP1 and an expression cassette encoding hyperactive murine DNase I (AAV-mDNase I) under the control of a liver-specific promotor.
Results: The AAV-mDNase I vector could support elevated expression and serum activity of murine DNase I over at least eight months. Neutrophil Gelatinase-Associated Lipocalin (NGAL), a biomarker for kidney hypoperfusion that is upregulated in urine from MMTV-PyMT mice, was suppressed in mice receiving AAV-mDNase I compared to an AAV-null control group. Furthermore, the proportion of mice that developed micro- and macro-metastasis was reduced in the AAV-mDNase I group. Moreover, we show that the plasma level of NETs is significantly higher in patients with metastatic breast cancer compared to those with local disease, or those that were considered cured at a 5-year follow-up, confirming NETs as interesting therapeutic targets in metastatic breast cancer.
Conclusions: Altogether, our data indicate that AAV-mDNase I has the potential to reduce cancer-associated impairment of renal function and development of metastasis. We conclude that AAV-mDNase I could represent a promising therapeutic strategy in metastatic breast cancer. We observed US-VTE in patients with CA-SpVT concurrently and subsequent to SpVT, but was not associated with SpVT recurrence, thrombocytopenia or AC. More research is required to understand the interplay of SpVT and US-VTE in patients with cancer.

POSTER SESSION 7
THERAPEUTIC CHALLENGES

PO-57

PERFORMANCE OF ESTABLISHED VTE RISK ASSESSMENT MODELS FOR THE PREDICTION OF ALL-CAUSE MORTALITY IN PATIENTS WITH CANCER – RESULTS FROM A PROSPECTIVE COHORT STUDY

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Introduction: Patients with cancer face a substantial risk of venous thromboembolism (VTE). VTE is also known to be associated with an increased mortality in patients with cancer. To identify patients with cancer at high risk of VTE and to implement effective thromboprophylaxis, several risk assessment models have been developed. Previously, some of them showed predictive ability for all-cause mortality in patients with cancer. However, this was not assessed in cohorts including patients with novel therapies, such as immune checkpoint inhibitors (ICI).
Aim: We aimed to assess the discriminatory performance of five established VTE risk assessment models in predicting all-cause mortality in a prospective observational cohort study including patients with cancer initiating systemic anti-cancer therapies, including ICI therapy.
Materials and Methods: The c-statistics for 6-months mortality risk discrimination of the Khorana, PROTECHT, CONKO, COMPASS-CAT, and the score by Ang Li *et al.* (J Clin Oncol. 2023;41(16):2926-2938.) were calculated.
Results: 625 patients (51% women) with a median age of 61 (interquartile range [IQR]: 52-69) years were included. The most common cancer types were lung (23.8%), breast (12.6%) and pancreatic (9.6%). Anti-cancer therapies initiated after study inclusion were chemotherapy (43.7%), combination of chemotherapy and ICI (17.6%), and ICI monotherapy (15%). At the time of inclusion, 390 (62.3%) patients had metastatic disease. During an observation period of 6 months, 64 patients died (6-month cumulative incidence: 8.9% 95% confidence interval [95% CI: 8.6-9.2]). The discriminatory performance of all five scores was moderate to poor, with the best c-statistic value seen with the Ang Li et al score, while the COMPASS-CAT score showed the lowest AUC value (c-statistics [95% CI]: Khorana: 0.58 [0.50-0.66], PROTECHT: 0.57 [0.49-0.65], CONKO: 0.60 [0.52-0.68], COMPASS-CAT: 0.54 [0.47-0.62], and Ang Li *et al.*: 0.64 [0.57-0.71]; Figure 1).

Conclusions: Five selected VTE risk assessment models showed a moderate to poor performance in predicting all-cause mortality in patients with cancer initiating systemic anti-cancer therapies.

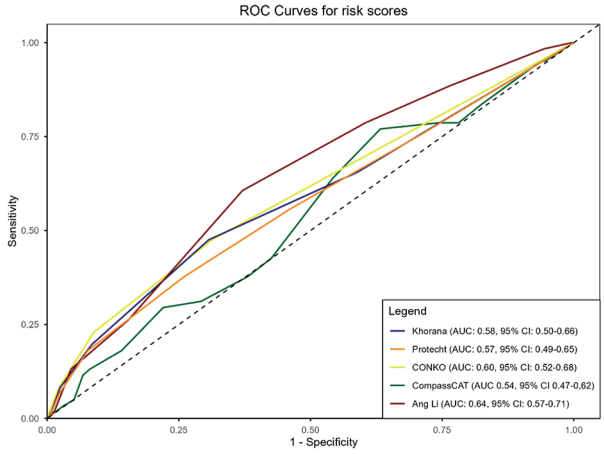

Figure 1.

PO-58

NOT PUBLISHED

PO-59

PATIENTS' EXPERIENCES, VALUES AND PERSPECTIVES ON ANTITHROMBOTIC THERAPY DECISION-MAKING IN ADVANCED CANCER

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Introduction: Patients' experiences, values and perspectives are essential to support decision-making on antithrombotic therapy (ATT) continuation or discontinuation in advanced cancer. However, patients' views remain largely unexplored. This study is a component of SERENITY, a pan-European project to develop a shared decision-making support tool (SDMST).

Aim: To explore patients with advanced cancers' experiences, values and perspectives of decisions about ATT continuation/discontinuation towards the end of life.

Materials and Methods: We conducted semi-structured interviews with patients with advanced cancer receiving ATT in the UK, Denmark, Spain and France. Data were analysed using Framework Analysis.

Results: Sixty patients were interviewed across the four countries (Table 1). Initial findings show that patient perspectives on their role in decision-making about their ATT differed. Some patients expressed a preference not to be involved or informed, while others expressed the decision should be shared, and placed importance on being informed about the decision the clinician has recommended; in addition, some felt they should have the ultimate authority over ATT decisions. Of note, there was little distinction between being informed about the decision and being involved in the decision, and patients had varying understandings of why they were on ATT, which could have affected their ability to engage in decision-making. Some patients expressed more concern about the reason for being on ATT over that of the medication itself, while others did not have a strong opinion about their ATT, deferring to their clinicians' expertise. For some there was a perception that there was no decision to make, either due to the complexities of the choice or that there was no circumstance in which ATT could be deprescribed, and they perceived their ATT as 'lifesaving'. Patients showed higher acceptance with continuing their ATT, either as normal, as a reduced dose, or changing to another ATT medication, over stopping ATT; they felt ATT medication was the "least of their troubles".

Conclusions: It is evident that patient views on decision-making about ATT varies and there are different influences on their ability to engage in the decision making. Development of an SDSMT could represent an opportunity to address patients' concerns about ATT indication and cater for the varied preferences and perspectives about involvement in decision-making.

Table 1.

Characteristic	Patients n=60
Male, n (%)	28 (47)
ATT, indication	
CAT	26 (44)
Atrial fibrillation	6 (10)
Ischaemic heart disease	11 (18)
Stroke (+/- AF)	3 (5)
Heart Valve	2 (3)
Multiple ATT indications	12 (20)
Age	
45-54	6 (10)
55-64	9 (15)
65-74	24 (40)
75-84	17 (28)
85+	4 (7)
Time on ATT	
Under 1 year	20 (33)
1-5 years	20 (33)
Over 5 years	20 (33)
ATT	
DOAC	24 (40)
LMWH	17 (28)
Antiplatelets	16 (27)
VKA	1(2)
Dual antithrombotic therapy	2 (3)

ATT: Antithrombotic therapy

PO-60

CLINICIANS' VIEWS AND EXPERIENCES OF ANTITHROMBOTIC THERAPY DECISION MAKING IN ADVANCED CANCER

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Introduction: The decision to continue or deprescribe antithrombotic therapy (ATT) in patients with advanced cancer is highly challenging, given the competing risks and benefits near the end of life. Clinicians' views and experiences of ATT decision making are essential for optimising ATT management. This study is a component of the pan-European SERENITY study, aimed at developing a shared decision-making support tool for ATT management.

Aim: To explore clinicians' experiences and perspectives of decisions about ATT continuation/deprescription in cancer patients near the end of life.

Materials and Methods: Semi-structured interviews were conducted with clinicians of varying specialties and fields of work, involved in ATT management across the UK, Denmark, Spain and France. Framework Analysis was used to analyse these data.

Results: Seventy-seven clinicians were interviewed across the 4 countries (Table 1). Clinicians' perceptions of roles (their own and others) in ATT decision making varied significantly. In the context of cancer and ATT near the end of life, participants revealed an extra layer of complexity in decision-making. This encompassed competing risk-benefit considerations and varying perceptions regarding responsibilities and appropriate timing for decision-making. Some medical specialties including palliative clinicians and general practitioners were more comfortable with taking on the decision of deprescribing ATT, while others were less prone to consider ATT deprescription as they were not the initial prescriber. Clinicians showed higher preference for considering medication adjustments to deprescribing ATT, including reducing doses and changing ATT medication. ATT deprescription was described as complex, with a variety of factors to consider, such as the specific ATT indication, the lack of evidence base to support the decision and difficulty establishing the optimal time for deprescription. Due to the complexity of the decision, clinicians placed significant value on the perspectives and preferences of patients in the decision-making process.

Conclusions: Clinicians' experiences and perspectives on ATT decision-making highlight the complex nature of ATT management. Understanding and clarifying roles and responsibilities is essential to ensuring active decisions about ATT management near the end of life. The multiple, competing factors influencing the decision in the context of cancer and ATT near the end of life is a significant challenge.

Table 1.

Characteristic	Clinicians n = 77
Male, n (%)	41 (53)
Antithrombotic affiliated specialists, n (%)	28 (36)
Cardiology	7 (9)
Neurology	4 (6)
Vascular medicine/surgeon	8 (10)
Respiratory/Pneumologist	6 (8)
Internal medicine	3 (4)
Cancer specialities, n (%)	14 (18)
Oncology	8 (10)
Haematology	6 (8)
Advanced disease care, n (%)	35 (46)
Palliative	10 (13)
Palliative nurse	8 (10)
General Practitioner (GP)	10 (13)
Geriatrician	7 (9)

ATT: Antithrombotic therapy

PO-61

LONG TERM VENOUS THROMBOEMBOLIC COMPLICATIONS IN CANCER PATIENTS WITH COVID-19 INFECTION

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Introduction: Several studies have highlighted the association between COVID-19 infection and venous thromboembolism (VTE). Nevertheless, limited research has been conducted on patients with active cancer and the impact of COVID infection on their long term venous thromboembolic risk.

Aim: Our objective was to identify clinical factors associated with VTE events following COVID-19 infection on patients with active cancer at MD Anderson Cancer Center.

Materials and Methods: A retrospective longitudinal study was conducted. The analyzed population included adults with active cancer and confirmed first COVID-19 infection (2020 through 2022) requiring hospital admission, and who were not taking any therapeutic anticoagulant therapy before. Demographic and clinical variables were reviewed: age, gender, ethnicity, race, body mass index (BMI), obesity status, COVID-19 vaccine status, cancer type, tumor stage, type of cancer therapy and its disease control before admission, Eastern Cooperative Oncology Group (ECOG) status, use of aspirin and remdesivir during hospital admission, prior lung and cardiovascular disease, and diabetes and

hypertension status. Additionally, admission to the Intensive Care Unit (ICU) and the use of mechanical ventilation during hospitalization were assessed. Clinical outcomes were deep venous thrombosis and pulmonary embolism within the following 6 months after admission for COVID-19 infection. Obtained data was further analyzed to determine the incidence of VTE and if there were correlations between VTE outcomes and clinical factors (Table 1).

Results: A total of 357 patients were included in the analysis. We found that the incidence of VTE within 6 months following admission was 6.7%. Amongst clinical factors, the history of hypertension showed significant association (p=0.02) with VTE outcome, however, it lost its significance (p=0.053) on multivariate analysis. Furthermore, the use of immune check point inhibitors for cancer (p=0.09) presented a tendency towards developing VTE.

Conclusions: Even though we only found hypertension as an associated factor to VTE, further investigation is needed to address the risk of VTE in cancer patients with severe illness from COVID-19 infection. Exploring other associated factors (*e.g.*, biomarkers) may help identify strategies to mitigate VTE risk in that population.

Table 1.

Characteristic	Categories	
Age (years)	Median, IQR	61, [52-72]
Sex	Female	158 (47.1)
Race	American Indian or Alaska Native	4 (1.1)
	Asian	17 (4.8)
	Black or African American	53 (14.8)
	White or Caucasian	220 (61.6)
	Other	59 (16.5)
	Unknown	4 (1.1)
Ethnicity	Hispanic or Latino	108 (30.3)
	Not Hispanic or Latino	241 (67.5)
	Unknown	8 (2.3)
Body mass index	Median, IQR	28.6, [25.2-38.9]
Obesity	Yes	137 (38.4)
COVID-19 Vaccine*	Yes	35 (9.8)
Chemotherapy before admission	Yes	137 (38.4)
Immunotherapy before admission	Yes	17 (4.8)
Tumor Type	Hematological	193 (54.1)
	Solid	163 (45.7)
	Both	1 (.3)
Metastatic disease¥	Yes	77 (21.6)
ECOG	0	83 (23.2)
	1	146 (40.9)
	2	63 (17.6)
	3	29 (8.1)
	4	6 (1.7)
Use of aspirin	Yes	113 (31.7)
Use of remdesivir	Yes	206 (57.7)
Prior Lung Disease	Yes	108 (30.3)
Prior Cardiovascular Disease	Yes	159 (44.5)
Diabetes	Yes	174 (48.7)
Hypertension	Yes	275 (77)
Mechanical ventilation	Yes	17 (4.8)
ICU	Yes	130 (36.4)

*COVID-19 vaccination received prior to date of hospital admission

PO-62

CATHETER-RELATED THROMBOSIS *VS* FIBROBLASTIC SLEEVE. INCIDENCE AND IMPACT IN ONCOLOGICAL AND HEMATOLOGICAL PATIENTS WITH PERIPHERALLY INSERTED CENTRAL CATHETER

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Introduction: Oncohematological patients often implant peripherally inserted central venous catheters (PICC). Associated complications are thrombosis and fibroblastic sleeve. Their correct discrimination can be difficult, as they are similar on ultrasound, but the management is completely different, with thrombosis alone requiring anticoagulant therapy. Few studies have investigated their incidence.

Aim: In a cohort of oncohematological patients with PICC, we evaluated the incidence of catheter-related thrombosis (CRT) and fibroblastic sleeve (FS) at 7-10 and 28-30 days.

Materials and Methods: We enrolled 45 patients. We also correlated the results with the type of oncological or hematologic disease.

Results: FS was identified in 11 patients (24.4%): 6 at 7-10 days (13.3%) and 5 at 28-30 days (11.1%); 5 patients (45.6%) had gastrointestinal cancer, 2 (18.1%) had airways cancer and 4 had breast cancer (36.3%). CRT was identified in 5 patients (11.1%): 3 at 7-10 days (60.0%) and 2 at 28-30 days (40.0%); 3 patients had gastrointestinal cancer (60.0%), 1 (20%) gynecological cancer and 1 (20%) onco-hematological disease. 3 thromboses were asymptomatic (60.0%). At the limits of significance (p-value 0.069) the relationship between vein diameter and development of fibroblastic sleeve/thrombosis (OR 5.29, 95% CI: 1.25 - 53.55). Statistically significant (p-value 0.039) the relationship between the timing of the complication and the platelets count (OR 1.03, 95% CI: 1.01 – 1.08).

Conclusions: FS is frequent (24.4%), but asymptomatic, in oncological and hematological patients. Less frequent (11.1%), but with significant consequences, is CRT. Discrimination between them is clinically relevant as almost one in four patients could undergo unnecessary anticoagulant therapy, with consequent waste of resources and potential serious side effects. Incidence of asymptomatic thrombosis (4.4%) leads us to underline how about one patient out of twenty may undergo an unacknowledged venous thrombosis with significant consequences.

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PO-63

HEMOSTASIS AND THROMBOSIS CONSULTATIONS AT A CANCER DEDICATED INSTITUTION: A SIX-MONTH EXPERIENCE

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Introduction: Thrombosis and bleeding are common and undesirable occurrences in cancer patients. Around 10% of people with both solid tumors and hematologic neoplasms die due to bleeding and up to 20% develop thrombosis during follow-up, which represents the second cause of mortality in this population. Precise diagnosis of hemostasis disturbances can be complex, as well as the management of thrombosis in cancer patients, who possess at

the same time bleeding and recurrence risks. To face these challenges sometimes a multiprofessional team with expertise in thrombosis and hemostasis is needed.
Aim: The aim of this study is to describe the reasons and analyze the impact of specialized consultations on thrombosis and hemostasis at an institution dedicated to cancer care during 6 months.
Materials and Methods: Retrospective longitudinal cohort study. Data were collected from medical records at an electronic platform (TASY®). All requests of a specialized opinion received by the Hematology - Thrombosis and Hemostasis team (H-TH) from December 1, 2017 to May 5, 2018 were included. Criteria applied to analyze the consultations were: relationship between the number of requests to H-TH *versus* the total amount of requests to the Hematology team, specialty of origin, reason for the request, number of solved requests, completed diagnosis, treatment and additional recommendations.
Results: During the study interval, a total of 130 consultations were performed by the Hematology team at the institution, 71 (54,6%) by H-TH. The majority of them came from Oncology (23), the Emergency Department (13) and the Gastrointestinal Surgery team (8). Other requests came from Gynecology, Intensive Care Unit and Head and Neck Surgery teams (4 requests each one), Vascular Surgery, Orthopedics, Urology and Mastology (3 requests each one), Neurosurgery (2) and the Pain Control team (1). From 24 consultations requested to clarify the diagnosis (17 due to altered coagulation tests and 7 due to thrombocytopenia), 71,8% were successful. Regarding requests for treatment doubts (23 on coagulation management, 12 on thrombosis, 7 on perioperative management and 5 due to bleeding), 98,5% were clarified during the hospitalization period. Follow-up time varied from 1 to 25 days, (mean of 6 days) and more than 50% of the patients were referred to the outpatient clinic on Thrombosis and Hemostasis after discharge.
Conclusions: The amount of requests on thrombosis and hemostasis issues in cancer patients showed to be relevant compared to general hematological requests as well as resolutive. This can justify the presence of hemostasis and thrombosis experts in cancer hospitals.

PO-64

PATTERNS OF VENOUS THROMBOEMBOLIC EVENTS AND THEIR CLINICAL IMPLICATIONS IN PATIENTS WITH NEUROENDOCRINE NEOPLASMS

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Introduction: Venous thromboembolism (VTE) is a significant concern in oncology, but research on its incidence and management in neuroendocrine neoplasms (NENs) is limited. While some studies estimate the incidence of VTE in this population to be around 7.5%, the lack of evidence from large and representative datasets has left gaps in our understanding of this phenomenon.
Aim: To address this knowledge gap by comprehensively analyzing the incidence and treatment of VTE in patients with NENs. We conducted a detailed analysis of a cohort of consecutive patients with gastroenteropancreatic (GEP) and thoracic NENs treated at our institution from 2017 to 2022.
Materials and Methods: Consecutive patients with GEP and thoracic NENs treated at our institution during the aforementioned study period were selected to assess the incidence of cancer-related VTE. Thrombotic events were classified as visceral (splenic, portal, and mesenteric thrombosis) and non-visceral (pulmonary embolism, deep vein thrombosis, catheter-associated thrombosis, and other etiologies). Information on patients' clinical characteristics, follow-up, and VTE treatment was collected.
Results: A total of 771 patients were included in the analysis, with 72 episodes of cancer-related VTE reported, accounting for 9.3% of the cohort. Of these episodes, 42 (58.3%) were classified as visceral VTE, and 30 (41.6%) as non-visceral VTE. Significant differences in clinical characteristics between the two groups were observed, as detailed in the provided univariate analysis in Table 1. In the univariate analysis, patients with non-visceral VTE presented more symptomatic episodes (53.3% *vs* 0%; p 0.001), and higher proportion of lung primary NEN (16.7% *vs* 2.4%; p 0.031). On the other hand, patients with visceral VTE were asymptomatic (100% *vs* 46.7%; p 0.001), younger (60 *vs* 69 years; p 0.01), pancreatic primary (76.2% *vs* 43.3%; p 0.005) and did not start anticoagulant therapy (71.4% *vs* 6.7%; p 0.001). Additionally, a subset of patients with visceral VTE developed portal hypertension as a complication, especially in pancreatic tumors, underscoring the severity of these events (19% *vs* 0%; p 0.02, compared with non-visceral VTE).
Conclusions: In conclusion, this study highlights the clinical significance of VTE in patients with neuroendocrine neoplasms, with an incidence of 9.3%. The findings underscore the predominance of visceral VTE (58%), especially in pancreatic tumors, and suggest the need to consider anticoagulant therapy in all cases. These findings provide crucial insights for the understanding and optimal management of VTE in patients with NENs.

Table 1.

	All VTE (n=72)	Visceral VTE (n=42)	Non-visceral VTE (n=30)	Visceral vs Non-Visceral
Sex (male)	49 (68,1%)	27 (64,3%)	22 (73,3%)	P 0,417
Age	64,32	60,93	69,07	P 0,01
VTE	20 (27,8%)	-	20 (66,7%)	
TEP	3 (4,1%)	-	3 (10%)	
TVP	2 (2,8%)	-	2 (6,7%)	
Catheter	23 (31,9%)	23 (54,8%)	-	
Splenic	6 (8,3%)	6 (14,3%)	-	
Mesenteric	13 (18,1%)	13 (30,9%)	-	
Portal				
Symptoms	56 (77,8%)	42% (100%)	14 (46,7%)	P 0,001
Incidental	16 (22,2%)	0	16 (53,3%)	P 0,001
Symptomatic				
Anticoagulant therapy	37 (51,4%)	11 (26,2%)	26 (86,7%)	P 0,072
LMWH*	3 (4,2%)	1 (2,4%)	2 (6,7%)	P 0,417
Other	32 (44,4%)	30 (71,4%)	2 (6,7%)	P 0,001
No treatment				
Primary NEN	6 (8,3%)	2 (4,8%)	4 (13,3%)	P 0,195
Unknown	6 (8,3%)	1 (2,4%)	5 (16,7%)	P 0,031
Pulmonary	10 (13,9%)	4 (9,5%)	6 (20%)	P 0,205
Small intestine	45 (62,5%)	32 (76,2%)	13 (43,3%)	P 0,005
Pancreatic				
NEN Grade	37 (56,9%)	18 (47,7%)	19 (70,4%)	P 0,065
G1-G2	28 (43,1%)	20 (52,3%)	8 (29,6%)	P 0,064
NET G3-NEC				

PO-65

CANCER-ASSOCIATED THROMBOEMBOLISM (CAT) RISK FACTORS AS WELL AS FRAILTY PREDICT IMMUNOTHERAPY-ASSOCIATED VENOUS THROMBOEMBOLISM (IAT) IN PATIENTS WITH NON-SMALL CELL LUNG CANCER (NSCLC)

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Introduction: There is limited knowledge about specific risk factors predisposing to immune checkpoint inhibitor (ICI)-associated VTE, also among patients with lung cancer despite a decade of ICI FDA approvals.
Aim: Therefore, we assessed CAT risk factors and frailty to predict ICI-associated VTE (IAT).
Materials and Methods: The association of VTE (PE or DVT) with detailed a priori selected known CAT risk factors, baseline patient characteristics, Khorana Score, other laboratory values, as well as VA Frailty Index assessed in NSCLC diagnosed between 2015-2019 and starting ICI therapy in a well-curated, retrospective, observational cohort study in the Veterans Affairs healthcare system (VA). A new VTE diagnosis was assessed starting 72 hours after ICI start (index date) until 6 months post index date. VTE was defined either PE, DVT, or splanchnic VTE requiring anticoagulation, while excluding superficial VTE. Cancer therapy is categorized into ICI-only therapy (single ICI N=1073; or dual ICI therapy N=11) *versus* ICI-chemotherapy combinations (N=373). The association between risk factors and VTE was assessed in a Fine-Gray competing risk model to adjust for the competing risk of death.
Results: 77 (5.3%) patients experienced a VTE by 6-months. For the total population, median age was 69 (range 36-97); 1,398 (96%) men; 257 (18%) Black race; 1,167 (80.1) Caucasian; 33 (2.2%) other race or ethnicity. The competing risk VTE multivariable modeling identified the following independent risk factors (adjustment included Khorana Score): ICI-chemotherapy HR=1.80 (95% CI: 1.10-2.94) and severe frailty HR= 2.62 (95% CI: 1.19-5.77). Khorana Score, recent hospitalization, as well as non-VTE conditions requiring aspirin or anticoagulation use (DOAC, warfarin) were associated with a limited increased risk for VTE without statistical significance. In the absence of frailty adjustment, comorbidities also predict for IAT risk, likely contributing to frailty's predictive ability.
Conclusions: We confirmed the following risk factors for ICI-associated VTE (IAT) that are independent from Khorana Score in a large NSCLC cohort: ICI-chemotherapy combination therapy, and newly identified severe frailty. Confirmed VTE risk factors and associated prediction models improve personalized thromboprophylaxis strategies and may enable IAT prevention trials in higher-risk populations.

POSTER SESSION 8
BIOMARKERS/ HYPERCOAGULABILITY II

PO-66

PLASMA KININOGEN LEVELS PREDICT DEVELOPMENT OF VENOUS THROMBOSIS IN CANCER PATIENTS: ANALYSIS OF SAMPLES FROM THE CASSINI STUDY

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Introduction: The contact activation system is not required for normal hemostasis but may contribute to pathologic thrombosis. Cleaved kininogen (cHK) is a useful biomarker for activation of the contact system in plasma, and we have shown that elevated levels of cHK are present in most patients with cancer as well as in tumor-bearing mice.
Aim: To determine whether levels of plasma kininogen (HK) or cleaved kininogen predict thrombosis in prospectively collected plasma samples from patients in the Cassini study, which included 818 patients undergoing cancer therapy randomized to rivaroxaban or placebo.
Materials and Methods: Plasma samples from Cassini patients with a negative compression ultrasound and Khorana score ≥2 were collected prior to randomization. Levels of HK and cHK from patients who developed VTE during the study (n=61) were analyzed using a nested case-control design in which each VTE samples was time-matched to two samples from patients without VTE. Samples were also matched by sex, age group, and pancreatic or non-pancreatic cancer. Seven plasma samples from normal individuals without cancer were used as controls. Results were divided into quartiles and the Wilcoxon rank-sum test was used to compare the first, median and third quartiles in the VTE and no VTE groups compared to the normal plasma. HK and cHK levels were measured using the Protein Simple WES immunoassay system, using calibration standards for each run to assure reproducibility.
Results: There were no significant differences in age, sex, BMI, Khorana score, or percent of patients with pancreatic cancer in the VTE *vs* matched no VTE groups. When compared to the normal plasma samples, levels of HK in cancer patients (N=168) were significantly lower (P=0.034). Though there was not a significant difference in levels of cHK (P=0.240), the ratio of cHK/HK was significantly higher in patients with cancer (P=0.016). However, when analyzing time to development of VTE using a conditional logit approach with strata by match group to estimate VTE hazard ratios, only levels of HK showed a significant negative association with VTE risk (0.69 per SD increase, Z statistic -2.26, P=0.024).
Conclusions: Despite increased levels of cHK and increased cHK/HK ratio in cancer patients *versus* controls, using the WES analytical method, only low levels of HK were found to be predictive of VTE in the Cassini study.

PO-67

ERYTROCYTE-RELATED PARAMETERS IN RELATION TO CANCER DIAGNOSIS: A CASE-COHORT STUDY OF HEALTHY SUBJECTS

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Introduction: Abnormal erythrocyte parameters, *i.e.* red blood cell distribution width (RDW) and mean corpuscular volume (MCV), are associated with various diseases (*i.e.* ineffective erythropoiesis, cardiovascular disease, venous thromboembolism, inflammation and cancer).

Aim: In healthy subjects' Italian study, we wanted to evaluate the predictive value of MCV and RDW of cancer diagnosis and understand the possible influence of lifestyle habits on these parameters.
Materials and Methods: In a large prospective cohort of 10,261 blood donors of the HYPERCAN Study (enrolled 2012-2022), a case-cohort study was designed comparing 286 cancer cases with 848 randomly selected controls. A lifestyle questionnaire was administered at study entry (*i.e.* alcohol intake, smoking habits, and sport practice). Clinical, hematological, and biochemical data were collected together with blood samples at baseline and after 6-12 months. Analyses were performed with the SPSS Statistics version 21.0 software.
Results: In the whole cohort, MCV and RDW were in the normal range values, (MCV=87.3fL, range 81-94; RDW=13%, range 12-14). A multivariate analysis, controlled for age, gender, and lifestyle habits showed a negative correlation between MCV and RDW values (beta=-0.356, p <0.001). In the group of males, higher MCV values were significantly associated with smoking habit. Among cancer cases, the most common tumor site was prostate (25%) in males, and breast cancer (37%) in females. By multivariate regression analysis corrected for age and gender, both MCV (OR 1.083; 95% CI:1.008-1.164; p=0.029) and RDW (OR 1.378; 95% CI:1.132-1.676; p=0.001) were significantly associated with subsequent cancer diagnosis. In particular, having RDW >13.45% and MCV >87.75fL significantly predicted cancer diagnosis (OR 1.839; 95% CI:1.153-2.931; p=0.011). A multivariate analysis according to gender displayed a significant positive association between MCV and prostate cancer diagnosis (p=0.027), and between RDW and breast cancer diagnosis (p=0.014).
Conclusions: Our data suggest a potential utility of erythrocyte-related parameters in early cancer diagnosis. Furthermore, the positive association between MCV and smoking habits emphasizes the importance of healthy lifestyle in cancer prevention.

PO-68

NETS BIOMARKERS IN WOMEN WITH ENDOMETRIAL AND CERVICAL CANCER

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Introduction: Initially discovered as a mechanism to protect the host from pathogens and prevent the spread of infection from the inflammatory site, neutrophil extracellular traps (NETs) have been implicated in the progression of other conditions such as autoimmune diseases, diabetes and cancer.
Aim: The aim of our work was to establish the role of NETs in cancer patients, and to determine their effect on tumor progression and the risk of thrombosis in patients with endometrial and cancer cancer.
Materials and Methods: The study included 96 patients with endometrial cancer and cervical cancer Grade 1 and Grade 2 aged 28 to 49 years (average age 45 years) with a verified histomorphological diagnosis of adenocarcinoma: endometrial cancer (group I, n=73) and cervical cancer (group II, n=23). The control group consisted of 60 healthy women without a complicated gynecological, oncological and thrombotic history. All patients were tested for level of myeloperoxidase (MPO) and citrullinated histone (CitH3), the neutrophil/lymphocyte ratio (NLR), as well as for interleukin-1β (IL-1β).
Results: When analyzing NETs markers depending on Grade 1 or 2, significant differences were revealed for MPO level, IL-1β and NLR in group I (p <0.001, p <0.001, p=0.002, respectively) (Figure 1). No differences were found for CitH3. When analyzing the content of the MRO, IL-1β level in blood plasma depending on Grade 1 or 2 cervical cancer, we found statistically significant differences (p=0.007, p=0.003, respectively) (Figure 1). No differences were found for CitH3 and NLR.
Conclusions: The results of the study show that NETs components such as MPO, citH3, IL-1β and NLR reflect the potential role of inflammation and NETs in many aspects of cancer. Laboratory biomarkers such as MRO, IL-1β and NLR were significantly more often elevated in patients with Grade 2 oncological pathology compared with Grade 1.

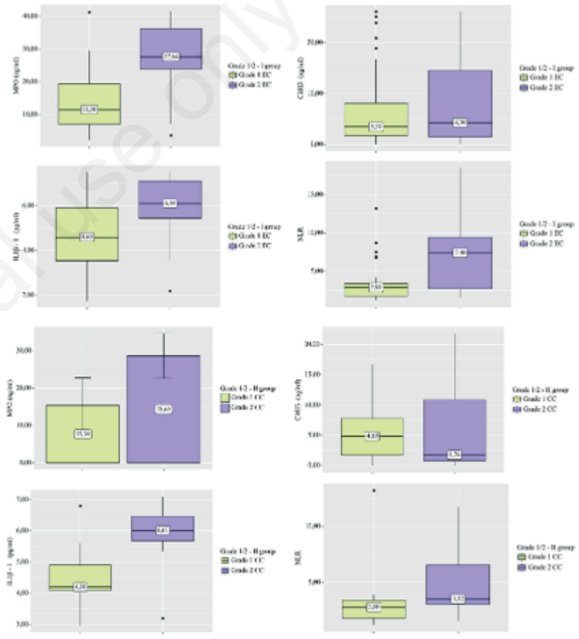

Figure 1. Boxplots for MPO, IL-1β, CitH3 and NLR in patients with endometrial cancer (EC) and cervical cancer (CC).

PO-69

MARKERS OF COAGULOPATHY IN MULTIPLE MYELOMA

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Introduction: Multiple myeloma (MM) is a plasma cell neoplasm characterized by clonal proliferation and accumulation of neoplastic cells and osteolytic skeletal involvement. Some of hemostasis disorders are attributed to M-Ig interactions with blood clotting factors (acquired von Willebrand's disease, acquired hemophilia A or deficits of other coagulation factors, circulating anticoagulant, hyperviscosity, amyloidosis and lupus anticoagulant) or with platelets (acquired thrombocytopathy), and also M-Ig-in-

dependent effects (thrombocytopenia, other thrombocytopathies, DIC, immobility and hypercalcaemia).
Aim: The aim of our work is to detect abnormalities of coagulation in patients in with newly diagnosed MM suitable for intensiv chemotherapy - depending on the activity of the disease, which predispose patients to thrombotic and bleeding complication, respectively, in MM. TGT is a global coagulation assai that measures the global capacity of blood plasma to form thrombin. Several clinical studies have shown that increased TG in platelet poor plasma (PPP) predicts an increased risk of (recurrent) VTE.
Materials and Methods: We included 189 patients with newly diagnosed multiple myeloma in this study. Patients with MM were examined by coagulation tests for detecting both bleeding and thrombotic tendency with following coagulation tests: PT, APTT, TT, fibrinogen, antithrombin, D-dimers, levels of coagulation factors (II, V, VII, X, VIII, IX, XI and XII), vWF, lupus anticoagulant, protein C, protein S, resistance to activated protein C and trombin generation assai modified with activated protein C. We also monitored plasma cell counts and serum M-Ig levels in these patients.
Results: We detected low level of vWF 25/189 (13,2%), high level of D-dimers 94/189 (49,7%), positive lupus antikoagulant 31/189 (16,2%), elevated level of FVIII 69/189 (36,5%). All markers were evaluated (average value, standard deviation) to the disease aktivity defined by the paraprotein level and a number of plasma cells (cytology analysis), respectively. A signifiant correlation was found between D-dimers and M-Ig quantity (p=0.0031), D-dimers and plasma cells number (p=0.0006), between vWF *vs* M-Ig quantity (p=0.0053). No correlation was found between vWF and plasma cells number (p=0.42), which is interesting. Correlations of vWF *vs* M-Ig quantity can predict bleeding conditions, however our ambition is to detect markers of thrombotic risk as well. For this purpose, we examined the modified TGT, which identified thrombotic pathology in eight cases (15%), while genetically determined thrombophilias were detected in only 3% of patients.
Conclusions: In newly diagnosed patients with MM, were commend increased attention to the level of D-dimers and vWF, especially in patients with higher disease activity according to M-Ig quantity in order to estimate possible bleeding or thrombotic complications and modified TGT for thrombotic complication, for which long term observation is needed.

PO-70

THROMBUS CHARACTERISTICS (COMPOSITION AND RESPONSE TO *IN VITRO* THROMBOLYSIS) AND PLASMA BIOMARKERS IN CANCER-RELATED ACUTE ISCHEMIC STROKE

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Introduction: Acute ischemic stroke (AIS) is a significant complication of cancer, often associated with a poor prognosis. AIS can also be the first manifestation of an occult cancer. The etiology of cancer-related AIS is frequently unknown, suggesting cancer-specific pathophysiological mechanisms that remain not clearly understood.
Aim: We aimed to identify the characteristics of thrombi from patients diagnosed with a cancer-related-AIS and to investigate plasma biomarkers associated with the presence of cancer during AIS.
Materials and Methods: AIS patients who underwent endovascular thrombectomy between January 2019 and December 2022 and who had both thrombus and citrate plasma samples available in the compoCLOT study were eligible. We retrospectively included patients with cancer-related-AIS (cancer group, n=11) with either nonbacterial thrombotic endocarditis (NBTE) or no other etiology identified. As a control group, we included patients without any history of cancer who experienced either cardio-embolic AIS (CE, n=23) or large artery atherosclerosis AIS (LAA, n=21), matched by age and sex to the cancer group.
Results: Thrombi were subjected to *ex vivo* thrombolysis in the presence of tissue-type plasminogen activator and plasminogen and analyzed by immunohistology or immunoassay to assess their composition. Thrombi from the cancer and CE groups were more resistant to lysis than thrombi from LAA group (median thrombus weight conservation 86 and 43 *vs* 8%, p=0.0006 and 0.006, respectively). Resistance to lysis was correlated positively with DNA content (r=0.75, p<0.0001) and negatively with red blood cell content (r=-0.66, p<0,0001). Within the cancer group, we identified a sub-group of white thrombi (n=5) that were poor in red blood cells but rich in platelets and Von Willebrand factor. Of note, all thrombi from NBTE (n=3) were in this sub-group. Plasma levels of D-dimer (D-Di), microvesicle-associated tissue factor (MV-TF) and myeloperoxidase (MPO) were higher in patients with cancer-related-AIS compared to patients without cancer, ie, patients pooled from CE and LAA groups (17350 *vs* 2040 ng/mL, 23 *vs* 9 fM and 48 *vs* 25pg/mL, p=0.02, 0.03 and 0.01, respectively). Levels of D-Di and MV-FT were especially elevated in plasma associated with white thrombi.
Conclusions: Thrombi from cancer-related AIS were more resistant to lysis, with an increased DNA content, when compared to LAA ones, but presented similar features (composition, sensitivity to lysis) to CE ones. The identification of a sub-group of white thrombi with similar characteristics, including all NBTE thrombi, suggests that the remaining thrombi within this group are associated with undiagnosed NBTE. Further prospective studies are required to assess the relevance of plasma biomarkers such as D-Di, MV-FT and MPO to identify patients with cancer- related AIS.

PO-71

CLINICAL SIGNIFICANCE OF IDENTIFYING ADAMTS13 AND VWF AS A HIGH-RISK FACTOR FOR THROMBOSIS IN GYNECOLOGICAL CANCER PATIENTS

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Introduction: A number of studies have shown that cancer patients have relatively higher levels of vWF and lower levels of ADAMTS13, and the dependence of this trend on the degree of cancer spread has been described. However, a clear relationship between the level and activity of ADAMTS13 and the risk of thrombotic complications has not been confirmed.
Aim: To substantiate the clinical significance of determining the level of ADAMTS13 and vWF in the blood in gynecological cancer patients with a high risk of developing thrombotic complications.
Materials and Methods: Group I consisted of 48 patients: 23

with ovarian cancer, 11 with adenocarcinoma of the cervical canal and 14 with breast cancer, who had a history of episodes of VTE. Group II consisted of 60 women: 20 with ovarian cancer, 20 with adenocarcinoma and 20 with breast cancer, in whom no clinically significant thrombotic complications were noted. The control group consisted of 25 women without malignant neoplasms. All patients had the level and activity of ADAMTS13 and vWF determined.

Results: In I group, the level of ADAMTS13 and its activity were significantly lower than those of II group and the control. During chemotherapy, there was a further decrease in both the level and activity of ADAMTS13. During polychemotherapy, the vWF level increased in both groups I and II and was significantly higher than the control group. In most patients, ADAMTS13 and vWF were within the reference values. For this purpose, an integral indicator was calculated - the ratio vWF/ADAMTS13. Noteworthy are the significant differences in the ratio of VWF and ADAMTS13 in groups I and II: 1.56 and 0.98, respectively, which significantly increased during chemotherapy: to 1.94 and 1.1, compared with the control group 0. 65.

Conclusions: The greatest prognostic significance for the development of thrombotic complications is the determination not of the activity of VWF or ADAMTS13 separately, but rather the ratio, which was confirmed in our study, VWF/ADAMTS13, which further increases during chemotherapy treatment. When vWF levels increase, even normal and subnormal ADAMTS13 levels and activity will not be able to sufficiently compensate for the increased vWF activity. It is the VWF/ADAMTS13 ratio, the imbalance of which occurs in cancer patients, that serves as one of the main prognostic factors for thrombotic complications.

PO-72

PLATELET PROTEOMIC PROFILING REVEALS MEDIATORS OF THROMBOSIS AND PROTEOSTASIS IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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Introduction: Myeloproliferative neoplasms (MPN) are characterised by myeloid proliferation and thrombocytosis. Patients with polycythemia vera (PV) and essential thrombocythemia (ET) have an increased risk of thrombosis and progression to myelofibrosis and/or acute leukaemia. While vascular risk is highest around the time of initial diagnosis, it remains elevated despite cytoreductive/anti-thrombotic therapy and represents the predominant source of early mortality and morbidity. There is emerging evidence that platelets are phenotypically distinct in multiple disease states, playing critical roles in a myriad of biological processes. However, the contribution of the platelet proteome to pathologic sequalae in MPN has yet to be fully elucidated.

Aim: We aimed to describe the untargeted platelet proteomic profile from a large clinical cohort of chronically treated ET and PV patients.

Materials and Methods: Platelet samples from patients with an established diagnosis of MPN (ET, n= 59; PV, n= 41) and healthy controls (n= 40) were recruited from the Mater Misericordiae University Hospital, Dublin, Ireland and the Papa Giovanni XXIII Hospital, Bergamo, Italy. Platelets were isolated from whole blood to generate platelet lysate. Differential proteomic signatures were established using label-free quantification (LFQ) mass spectrometry (MS). Identified peptides were searched using MaxQuant and bioinformatic analysis was performed using R.

Results: We evaluated the platelet proteome in 100 patients receiving treatment (anti-platelet/cytoreductive) for an established diagnosis of PV/ET and 40 healthy controls. 227 and 166 proteins significantly differentially expressed (false discovery rate <0.05; fold change >1.5) in ET & PV respectively. Mediators of inflammation were upregulated such as LGALS1 and MMP1. Effectors of platelet pro-coagulant activity were overexpressed in MPN including FcγRIIA and HSP47. Functional analysis of platelets using gene set enrichment demonstrated that proteins from the MTOR signalling pathway and unfolded protein response were enriched in PV & ET cohorts.

Conclusions: We describe the untargeted proteomic profile of platelets from a large clinical MPN cohort. In keeping with the observation that vascular risk remains elevated amongst chronically treated patients, we highlight the predominance of thromboinflammatory mediators in this group and demonstrate evidence of an altered platelet proteome despite standard therapy.

PO-73

NETOSIS IN GYNECOLOGICAL CANCER PATIENTS DURING ANTITUMOR THERAPY

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Introduction: Tumor cells secrete a large number of cytokines, which contribute to the development and maintenance of a chronic pro-inflammatory state and trigger the formation of extracellular neutrophil traps (NETs), which are part of the pathogenesis of both thrombosis and tumor growth. The dynamic changes in the formation of NETs during antitumor therapy, as well as the influence of anticoagulants and anti-inflammatory agents on them, have been poorly studied.

Aim: To determine the severity of NETosis reactions in gynecological cancer patients against the background of antitumor therapy, as well as the effect of LMWH and anti-inflammatory therapy (aspirin) on NETosis.

Materials and Methods: From 2019 to 2023, the study included 262 patients with neoplasms of the female reproductive system (uterine cancer (81), adenocarcinoma of the cervix (15), ovarian cancer (85) and breast cancer (81)), hospitalized for antitumor therapy. For all patients, blood was drawn four times: before the start of therapy, 14 days after surgery or the end of the 2nd course of chemotherapy, and also after the 4th and 6th courses. The studied parameters were NETosis markers (MPO antigen, Cit-H3 histone).
Results: The concentration of NETosis markers in cancer patients (citH3 1.78±1.03 ng/ml ($p<0.05$), MPO:Ag 15.97±11.83 ng/ml ($p<0.05$)) was initially significant increased compared to the control group. The severity of thromboinflammation before the start of therapy was higher, the higher the stage of the disease. 14 days after the 2nd course of chemotherapy, an increase in the concentration of both citH3 (2.46±1.24 ng/ml ($p=0.0001$)) and MPO:Ag (22.76±7.31 ng/ml ($p= 0.0001$)). 2 weeks after the 4th course of chemotherapy in the subgroup of patients (n=25) who used LMWH there was a significant decrease in the concentration of both markers of NETosis (CitH3 histone 1.35±0.36 ng/ml, MPO:Ag 17, 54±3.29 ng/ml, $p<0.05$). In the subgroup of patients taking LMWH+aspirin (n=28), a significant decrease in the concentration of NETosis markers was also noted (CitH3 1.15±0.36 ng/ml, MPO 15.12±4.28 ng/ml, p<0, 05).
Conclusions: Activation of NETosis occurs in all gynecological cancer patients at the start of antitumor therapy. Chemotherapy, compared to surgical treatment, leads to a more pronounced activation of NETosis. LMWH effectively reduces the severity of NETosis.

PO-74

AGEING AS SHARED RISK FACTOR FOR CANCER AND CARDIOVASCULAR DISEASE: THE IMPACT OF CANCER ON VASCULAR REMODELING

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Introduction: During (arterial) ageing, the risk of cancer and cardiovascular disease increases significantly. This is attributed to changes in vascular remodeling, *i.e.* changes in morphology, proliferation, and migration of vascular smooth muscle cells (VSMCs). This results in a reduction in elasticity of the vessel wall and an impaired ability to control blood flow and pressure. Furthermore, anti-cancer therapy is known to influence cardiovascular remodeling, while the direct effect of cancer itself is largely unknown.
Aim: To investigate the influence of human breast cancer cell conditioned media on ageing in iPSC induced-VSMCs (iVSMCs) phenotype.
Materials and Methods: iVSMCs were cultured as either young iVSMCs (P<13) or aged iVSMCs (P>30). For conditioned media, human breast cancer cells, BT474, were cultured in culture media (DMEM, 10% FBS, 1% P/S) for 24h. First, iVSMC phenotype was investigated by characterisation of several smooth muscle cells markers, including α-smooth muscle actin (α-SMA) and calponin. iVSMC proliferation and calcification was measured using impedance measurements (xCELLigence) and Biohybrid (Fetuin-A-AF546), respectively, after exposure to control or conditioned media (both at 1.8 and 4.8 mM Ca2+) over a period of 4 days.
Results: iVSMC phenotype was confirmed by characterization of smooth muscle markers α-SMA, p-myosin light chain (p-MLC), calponin, smooth muscle 22 α and S100A4. Aged iVSMCs show a significant decrease in α-SMA levels ($p=0.016$) and proliferation ($p=0.017$) compared to young iVSMCs. While young and aged iVSMCs exposed to conditioned media with 4.8 mM Ca2+ showed a significant decrease in proliferation (young iVSMCs $p=0.026$; aged iVSMCs $p=0.031$), vascular calcification was increased, as compared to control medium. Interestingly, aged iVSMCs exposed to conditioned media with 1.8 mM Ca2+ showed a lower proliferation rate ($p=0.001$) compared to young iVSMCs, while no difference could be detected for vascular calcification.
Conclusions: iVSMCs are a good model to investigate the effects of ageing on proliferation and vascular calcification *in vitro*. Furthermore, human breast cancer cells' conditioned media has a significant impact on ageing in iVSMCs, *i.e.* proliferation and vascular remodeling. Further research is needed to unravel the interactions between breast cancer and vascular remodeling.

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