Thrombosis and hemostasis at the University of Padua: a reappraisal on the occasion of its 800th year of history

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ABSTRACT

The year 1222 has traditionally been accepted as the University of Padua's founding date. The University of Padua is a prestigious center for learning and research, and over the centuries, it has produced luminaries in the most significant disciplines, including medicine, law, philosophy, theology, literature, engineering, astronomy, physics, politics, and religion. The *Studium* of the teaching of Medicine began around 1250 with the establishment of the Collegium of Medical and Arts Doctors. The history of Medicine at Padua University is extraordinarily rich and counts on the contribution of masters such as Vesalius, Falloppia, Girolamo Fabrici d'Acquapendente, William Harvey, Vallisneri, Ramazzini, Morgagni and many others including Galileo Galilei himself. This year marks the 800th anniversary of the University of Padua, and to commemorate this historic event, the Editor has asked the three of us to summarize the university's most significant contributions to the fields of hemostasis and thrombosis over the past eight decades. Among all, it should be mentioned the relevant contribution of Prof. Antonio Girolami, who was the founder of the group of Thrombosis and Hemostasis in Padua and one of the Italian and international leaders in the field of the diagnosis and treatment of congenital bleeding disorders. However, due to the large number of outstanding scientists and significant research conducted in these fields at Padua University, it was extremely difficult for us to provide a concise summary of the university's numerous contributions. Eventually, we concluded that it would be more useful to share with the Readers the experiences we have had over the past several decades, focusing on specific aspects of our research, work, and life at Padua University, and attempting to highlight the aspects that we believe have contributed most to the advancement of knowledge in the fields of thrombosis and hemostasis. Therefore, three topics have been selected and presented separately in a narrative format as pieces of our lives and of the history of our university.

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Key words: Padua University, history, hemostasis, thrombosis.

Contributions: The authors contributed equally.

Conflict of interest: The authors declare no potential conflict of interest.

Funding: None.

Received for publication: 5 September 2022.
Revision received: 20 October 2022.
Accepted for publication: 20 October 2022.

Bleeding, Thrombosis and Vascular Biology 2022; 1:52
doi:10.4081/btvb.2022.52

DIAGNOSTIC AND THERAPEUTIC MANAGEMENT OF DEEP VEIN THROMBOSIS: FROM THE MID '80 TO THE PRESENT DAY

(by Paolo Prandoni)

Diagnosis of deep vein thrombosis

Following the demonstration that the clinical diagnosis of deep vein thrombosis (DVT) is inaccurate, for many years ascending phlebography has constituted the only reliable method of DVT detection. Because of its invasiveness, however, in the '70s phlebography was replaced by a non-invasive method, impedance plethysmography. When I started addressing the diagnosis of DVT in collaboration with a group of young, strongly motivated, collaborators I decided to test the accuracy of a computerized tool (computerized impedance plethysmography), produced by an Italian engineer, using as reference standard ascending phlebography. At the end of a study encompassing the recruitment of several hundred patients with suspected DVT, we concluded that this procedure is largely inaccurate.[1] Although our results were criticized, they eventually ended up causing the abandonment of this procedure everywhere in the world in favor of an emerging strategy, *i.e,* ultrasonography of the leg vein system. In

1989, in collaboration with a group of Dutch researchers from the University of Amsterdam, guided by Jan Wouter ten Cate, I published the results of a prospective cohort study, in which the diagnostic accuracy of compression ultrasonography (CUS) for detection of proximal DVT was tested.[2] The results were impressive, as this simple and highly reproducible method of DVT diagnosis was found to possess an accuracy that was comparable to that of phlebography. In a subsequent prospective cohort study, we showed that it is safe to label patients as being free from DVT if CUS is negative and remains negative in two further determinations to be performed in the subsequent days;[3] and that this approach can be further simplified by repeating the CUS in the only patients in whom the D-dimer is positive despite a baseline negative CUS.[4] In addition, in a more recent prospective controlled study we showed that excluding the presence of DVT in symptomatic patients with the combination of proximal CUS with D-dimer is as effective as and safer than performing a whole leg color-Doppler assessment, as long as it obviates the risks of unnecessary treatment of isolated calf DVT.[5] Finally, according to the results of an Italian prospective cohort study, designed in collaboration with Walter Ageno from the University of Insubria, the extensive procedure was found to be a reasonable diagnostic approach in patients who present with a negative CUS and a negative D-dimer in spite of a painful calf, as it has the potential to identify those isolated calf-vein thromboses that can benefit from anticoagulation, while offering alternative explanations for patient's complaints in those who are free from thrombosis.[6]

Initial treatment of deep vein thrombosis

At the beginning of my activity, intravenous unfractionated heparin (UFH) in doses adjusted to the aPTT was the standard of treatment of DVT. Because of the need for intravenous infusion and laboratory monitoring, however, it was progressively replaced by fixed-dose subcutaneous low-molecular-weight heparin (LMWH) in doses adjusted to the body weight. In collaboration with the Dutch group, we decided to embark on a randomized clinical trial addressing the comparison between the two strategies. In both groups, the heparin treatment was overlapped with and followed by vitamin K antagonists (VKA) to be administered for at least three months. All patients underwent phlebography and perfusion lung scanning at baseline and after 10 days and were followed up for up to six months. Surprisingly enough, there was a trend favoring LMWH in terms of recurrent venous thromboembolism (VTE), major bleeding complications, earlier vein recanalization and improvement of lung scanning.[7] Based on these findings and those from additional, subsequent studies LMWH has become the standard of treatment of DVT. Because of the administration of fixed doses, without the need for laboratory monitoring, home treatment of DVT was hypothesized and then persuasively demonstrated by a multicenter randomized clinical trial, which involved my Institution.[8]

Clinical course of deep vein thrombosis

Since the mid '80s, I decided to follow-up prospectively over time patients after their episode of DVT. In a prospective cohort study published in 1996 in collaboration with the Dutch group, we showed for the first time that the prognosis of proximal DVT in terms of recurrent VTE after discontinuing anticoagulation is less benign than previously thought.[9] The rate of recurrent VTE after three months of anticoagulation was indeed found to be around 30% over a follow-up period of 5-8 years, this incidence being much higher in patients with unprovoked DVT that in those with thrombosis triggered by removable risk factors. These findings inaugurated the search for individually tailored approaches to the length of anticoagulation after a first DVT episode. The long-term follow-up of patients with DVT, conducted at our Institution, led to three additional outstanding findings: the demonstration that regardless of the type of thrombosis, the persistence of residual vein thrombosis, as shown by ultrasonography three months after the index episode, increases the rate of recurrent VTE;[10] the demonstration that patients with unprovoked DVT exhibit a significantly higher rate of subsequent overt malignancies than those with secondary thrombosis;[11] and the demonstration of an association between DVT and atherosclerosis.[12] In addition, we showed that under VKA treatment cancer patients exhibit a remarkably higher risk of both recurrent VTE and major bleeding complications than cancer-free patients:[13] this demonstration offered the background for all subsequent investigations addressing the value of LMWHs in place of VKA for the treatment of cancer-associated VTE.

Prevention of recurrent venous thromboembolism

In an attempt to identify the optimal duration of anticoagulant therapy after a first episode of VTE, two multicenter Italian studies were performed in the '90s in patients presenting with primary DVT and primary pulmonary embolism (PE), respectively, in collaboration with Giancarlo Agnelli from the University of Perugia. In each of the two randomized clinical trials, we showed that extending to one year the three-month course of VKA treatment – which was the dominant strategy at the end of last century – in patients with unprovoked proximal DVT and PE, respectively, is not associated with long-standing clinical benefit.[14,15] Indeed, prolonging anticoagulation simply delayed the timing of VTE recurrences. The results of these two studies revolutionized the ap-

proach to patients with unprovoked VTE. Indeed, an impressive series of subsequent studies have conclusively demonstrated the advantage of an indefinite anticoagulation in patients with unprovoked VTE, provided they are not perceived as being at a high risk of bleeding. The replacement of VKA with the current direct oral anticoagulants, characterized by a more favorable therapeutic profile, has streamlined this therapeutic conduct. Among the several investigations conducted in this field, I cannot help mentioning the Einstein Choice study I had the privilege to coordinate in collaboration with Jeff Weitz from the McMaster University in Canada.[16] In a randomized controlled clinical trial, involving hundred centers worldwide we showed that preventive doses of rivaroxaban are as effective as and safer than therapeutic doses for protection against recurrent VTE in patients with a first episode of unprovoked or weakly provoked VTE, both doses being definitely more effective than aspirin.[16]

THE ESTABLISHMENT OF A THROMBOSIS GROUP AND THROMBOSIS SERVICE FOR PATIENTS ON ANTICOAGULATION THERAPY AT THE UNIVERSITY OF PADUA

(by Vittorio Pengo)

The idea of setting up specialized care for patients on oral anticoagulant therapy (OAT) in Padua was born in the Institute of Cardiology directed by Professor Sergio Dalla Volta in the early 1970s. In those years, patients on OAT were predominantly heart patients suffering from rheumatic mitral stenosis or wearers of mechanical ball or swing disc valve prostheses.

Patients with prosthetic valves for out-of-hospital monitoring of OAT often had to rely on doctors without adequate training, laboratory technicians, or were forced to resort to self-medication. Patients with mitral stenosis, many of them young women with previous rheumatic disease, often did not receive anticoagulant treatment despite the fact that this valvular heart disease carried a high thromboembolic risk. Professor Luciano Schivazappa, then consultant cardiologist in cardiac surgery and responsible for the follow-up of patients with valvular heart diseases and cardiac valve prosthesis wearers, was fully aware of these problems.

The solution was obvious: it was necessary for us cardiologists to learn the theory and practice of how to handle anticoagulant drugs properly, and then set up an outpatient clinic to monitor the OAT of so many patients left dangerously to their own devices.

This was not an easy task, as it involved, in an ultraspecialist era, going beyond the strictly cardiological field and acquiring skills considered to belong to other disciplines such as hematology or internal medicine. We could run the risk of being considered 'Martians' within our clinic. In fact, for those of us who wanted to take an interest in platelets and coagulation factors considered extraneous to cardiology, someone affectionately but with a hint of derision immediately coined the term 'tilers'.

In 1973 the first nucleus was formed under the leadership of Professor Antonio Girolami to establish a service that would provide qualified assistance to patients undergoing anticoagulant therapy. Patients received an OAT control booklet where we recorded the date, percentage of prothrombin activity and dose of anticoagulant at each check-up. Soon our interest expanded to patients with venous thromboembolic disease.

In the meantime, I was attending postgraduate school in Internal Medicine (1975-1980) and had been assigned to a laboratory on the fourth floor of the polyclinic that dealt with coagulation. The subject did not excite me also because it had seemed complicated to me during my degree course. However, by attending that laboratory I began to appreciate the subject, and this turned into pure passion when I happened to catch a congenital alteration of antithrombin III which led to a publication in an international journal.[17] A further turning point in my history as a researcher occurred when I studied the plasma of a patient who had an unexplained lengthening of the activated partial prothrombin time (aPTT). It was 'Lupus Anticoagulant'. I became interested in the subject and during a visit to Padua by Prof Sandor Shapiro of Jefferson Medical College in Philadelphia I was offered to go to the United States to study the subject. The visit was very fruitful[18, 19] and I have not left this topic since.

After returning from the US, I founded a journal entitled Thrombosis and Atherosclerosis, published by Piccin in Padua, which had little luck and ceased publication after a few years due to a lack of sponsors following the drug scandals of the early 1990s in Italy.

The 1980s were characterized by a further expansion of Prof. Schivazappa's initial group, which was joined by Prof. Paolo Prandoni and me.

The first resource was the acquisition of a room in the kitchen wing of the hospital, which we converted into a laboratory, equipping it with a counter and the minimum necessary for routine coagulation tests. The method used for measuring Quick's time was manual and the value was expressed as a percentage of prothrombin activity. In the absence of technicians to carry out the tests and maintain the laboratory, help was sought from students and cardiology residents. In the meantime, Prof Prandoni was working on instrumental diagnostics of venous thromboembolism.

The first step for me was to put order in the cramped kitchen room used as a laboratory and to plan the necessary equipment for proper laboratory control of anticoagulated patients and for research activities. An archive of

patients was created by numbering the booklets for the OAT control. Checks were only carried out one afternoon per week: blood samples and therapy prescriptions were taken in the cardiology outpatient clinic on the ground floor of the polyclinic, coagulation tests were performed in the laboratory attached to the kitchens.

By this time, the group had found its autonomy as there was both clinical and laboratory expertise within it. One thousand nine hundred eighty-four was an important year because the first results concerning the activity of our laboratory were published and the first attempt was made to express the level of anticoagulation more correctly by introducing the Corrected Prothrombin Ratio (CPR). This innovation was the result of my visit to the Thrombosis Service in Leiden headed by Prof Broekman. The CPR value had to be kept between 1.8 and 3 in all patients.

In 1986, a further decisive step was taken in the standardization of laboratory tests for monitoring OAT with the introduction of the INR (International Normalized Ratio). The INR makes it possible to standardize the levels of anticoagulation obtained in laboratories using different reagents and thus to compare the quality of therapy and the risk of hemorrhage in different centers based on the actual level of anticoagulation.

A further development for the monitoring of our OAT patients came from the introduction of a computer program whose name is PARMA, an acronym for Anticoagulated Monitoring Reporting Archiving Program. The program was purchased by me in Modena while the computer, an Olivetti M24 (20MB!), had been obtained on loan as compensation for a study on thrombosis.

At the suggestion of a patient who had a mechanical valve prosthesis, and at my suggestion, an association was founded in 1987 to deal specifically with patients on OAT. At first, it was called APA (Associazione Pazienti Anticoagulati) but when it was founded, in order to give the initiative a national scope, I preferred to call it A.I.P.A. (Associazione Italiana Pazienti Anticoagulati).

The official foundation of the A.I.P.A. took place in December 1987. Writing the statute and finding a notary and the funds to pay for it was no easy task. In 1989, the center was included in the 1989-91 regional health and social plan (annex to Regional Law No. 21 of 20 July 1989) as a Thrombosis Prevention and Therapy Service, a name it still retains today. In the same year, at the proposal of our center and eight other Italian centers, the Federation of Anticoagulated Surveillance Centers (FCSA) was founded.

From that moment on, an uninterrupted growth in skills and in technical and human resources began, leading to the establishment of the Thrombosis Prevention and Therapy Service, which over time reached a peak of around 5,000 users being treated with warfarin or acenocumarol.

The systematic follow up of patients with pulmonary embolism led to an important publication in 2004 on the course of disease ending in Chronic Thromboembolic Pulmonary Hypertension (CTEPH).[20]

Despite the introduction of new anticoagulants, the Service continues to be the point of reference for treatment with oral anticoagulants, which always pose problems that can only be dealt with by a Service with personnel competent in the issue.

FROM INHERITED HYPERCOAGULABLE STATES TO FACTOR IX PADUA: A FASCINATING STORY ON HOW THROMBOPHILIA HAS HELPED HEMOPHILIA

(by Paolo Simioni)

In the early ‘80s when I defended my thesis in Medicine and Surgery entitled “The hypercoagulable states: laboratory diagnosis and clinical implications” at Padua University Medical School, the concept of inherited hypercoagulability was still associated with a limited number of families with a predisposition to thrombosis whose members were carriers of heterozygous antithrombin (AT) defects. Actually, some of these patients with the so-called “heparin binding defects” were not at all prone to thrombosis or only mildly prone to it even though they showed similar activity levels as those found in patients with true AT defects and thrombosis.[21] I thought then that there was no end to the strange things that may happen in the world of inherited coagulation defects and thrombosis. In those years protein C (PC) and protein S (PS) were discovered, and inherited defects of these physiological clotting inhibitors were associated with familial thrombosis. Dr Antonio Girolami, a scientist in the field of hemostasis and thrombosis mainly involved in the characterization of rare bleeding defects (*i.e.*, hemophilia, Factor X Friuli and others), was the chief of a coagulation lab at Padua University Hospital. He was my mentor and suggested that I focus on patients with inherited PC and PS defects. My work dealt with young patients, with personal and family history of thrombosis often resulting from minor risk factors such as surgery, oral contraceptive treatment, or pregnancy. Again, my curiosity was aroused by the discrepancies between antigen and activity PC levels (to my surprise, chromogenic was normal and coagulometric activity reduced!) found in one family. This led me in 1993 to intern at Dr Rogier Bertina’s coagulation lab in Leiden, where I learnt how to isolate PC from plasma and study its properties. Actually, the above-mentioned variant, named “Protein C Padua 2” because of a Cys substitution at position Arg-1 was bound to alpha-1-microglobulin, which strongly impaired its functional activity (dysfunction in the GLA-domain) and

caused thrombosis in carriers.[22] I was strongly attracted to both the perfection of nature as well as it flaws in matters related to coagulation. In the same lab in Leiden, I had the pleasure of meeting Dr Valder Arruda from Brazil and discuss with him a number of topics concerning hemostasis. Valder was actively involved in research on hemophilia but very open to discussing any other topics. When I came back to Padua in early 1994, something had become absolutely clear in my mind: I was keenly interested in discovering new "mistakes" in nature causing inherited thrombophilia and, as a physician, I proposed to take care of these patients. I was positively surprised when late in 1994 and then in 1996, respectively, Factor V Leiden (Activated Protein C, APC, resistance) [23] and prothrombin G20210A mutations [24] were identified in many patients with thrombosis in Dr Bertina's laboratory in Leiden, where I had worked.

The contribution to thrombophilia from Padua Thrombosis and Haemostasias Center during the following years was mainly related to several large cohort studies in thrombophilic families for the assessment of the risk of first (and recurrent) VTE in carriers of inherited defects.[25-29] In addition, peculiar and rare coagulation factor variants such as pseudo-homozygous APC resistance [30-31] were characterized and some unique PC defects were identified (for example, in thrombosis patients with only the beta-form of PC in blood).[32] Due to my personal feeling and the previous positive cooperation experiences, many of these studies were performed thanks to many scientists from different countries around the world. At the same time thousands of inherited patients with thrombophilia belonging to hundreds of families were - and still are! - identified and followed on a clinical and laboratory ground at Padua University Hospital.

Let me move on to a few years later, when I was in charge of the lab and clinical research on thrombophilia at Padua University Hospital looking for new inherited thrombophilic conditions based on clinical manifestations of patients and their coagulation lab phenotypes. Surprisingly enough, most of the information available for clinical management of thrombophilic patients was based on the results of old studies only dealing with thrombophilia defects discovered years before. In contrast, it was commonly seen that in a large number of families (almost 50%) symptomatic for thrombophilia, none of those defects could be identified.

Within this context, the year 2007 saw the casual and fortunate finding of a young boy who had experienced deep vein thrombosis and presented such a high level of coagulation FIX activity (772%!) that my first thought was that it could only be a lab mistake or a typo error in reporting the data. The test was repeated several times and the data confirmed, but the real surprise came when we also detected a normal FIX antigen level (92%) in the proband's plasma. Sequencing of the F9 gene led to the discovery of Factor IX Padua, a gain-of-function mutation in the F9 gene (R338L) responsible for a hyper-functional FIX molecule. The expression in vitro of recombinant Factor IX Padua confirmed the properties of the hyperfunctional molecule and, particularly, an 8 to 10-fold increased functional procoagulant activity. This discovery also sparked off the idea of a potential use of this variant for the gene therapy of hemophilia B. It was at that time in 2008 that I contacted my old friend Dr Valder Arruda, who was involved in the field of the gene therapy of hemophilia B in Philadelphia. He fully confirmed the characteristics of the FIX Padua variant and contributed to the publication of the data.[33] This discovery had the potential to help the development of the gene therapy of hemophilia B in humans. As a matter of fact, many already terminated or still ongoing clinical trials of gene therapy for hemophilia B using the FIX Padua provide a clear demonstration of a successful and stable cure for many hemophilia B patients.[34] "Nature does nothing uselessly" (Aristotle, 384-322 A.C.), which seems to be perfectly true of the FIX Padua. I have no words to express my deep sorrow for the recent news that Valder has prematurely passed away. It was a great privilege for me to work with him.

In 2012 a novel gain-of-function polymorphism in the prothrombin molecule leading to resistance to AT was identified in Japan (Prothrombin Yukuhashi, Arg596Leu).[35] The molecular basis of this defect is a missense mutation of the prothrombin Arg596 residue (exon 14) resulting in impaired thrombin–AT binding and defective inhibition of the mutated thrombin by AT. A unique prothrombin variant Arg596Trp, named prothrombin Padua 2, with the same mechanism of resistance to AT was identified in two families from the Veneto area.[36]

The story of inherited thrombophilic defects found in Padua has recently been enriched by the discovery of the first defect in the F8 gene (*FVIII Padua*) associated with markedly elevated FVIII levels and severe thrombophilia, the so-called Factor VIII Padua.[37] Genetic analysis revealed a 23.4-kb tandem duplication of the proximal portion of the F8 gene (promoter, exon 1 and a large part of intron 1), which co-segregated with high FVIII levels across the family. The identification and characterization of this gene defect in two families from the North-East of Italy was the result of a fruitful and prolonged cooperation with a Dutch group of scientists at Maastricht University and, in particular, with Dr Jan Rosing, Dr Tilman Hackeng and Dr Elisabetta Castoldi. Whether also the mechanism underlying the expression of FVIII Padua can be helpful for the gene therapy of hemophilia A is currently under investigation.

The large number of newly discovered inherited defects in the last decades both in Padua and all over the world [38] and their clinical impact seems to justify why

testing for thrombophilia patients belonging to families with VTE should not be abandoned.

I have no doubt that the University of Padua will continue to make contribution to the research fields of thrombophilia and hemophilia gene therapy in the years to come.

REFERENCES

1. Prandoni P, Lensing AWA, Büller HR, et al. Failure of computerized impedance plethysmography in the management of clinically suspected deep-vein thrombosis. Thromb Haemost 1991;65:233-6.
2. Lensing AWA, Prandoni P, Brandjes D, et al. Detection of deep-vein thrombosis by real-time B-mode ultrasonography. N Engl J Med 1989;320:342-5.
3. Cogo A, Lensing AWA, Koopman MMW, et al. Compression ultrasonography for diagnostic management of patients with clinically suspected deep vein thrombosis: prospective cohort study. Br Med J 1998;316:17-20.
4. Bernardi E, Prandoni P, Lensing AWA, et al. D-Dimer testing as an adjunct to ultrasonography in patients with clinically suspected deep vein thrombosis: prospective cohort study. Br Med J 1998;317:1037-40.
5. Bernardi E, Camporese G, Büller HR, et al. Serial 2-point ultrasonography plus D-dimer vs whole-leg color-coded Doppler ultrasonography for diagnosing suspected symptomatic deep vein thrombosis: a randomized controlled trial. JAMA 2008;300:1653-9.
6. Ageno W, Camporese G, Riva N, et al. Analysis of an algorithm incorporating limited and whole-leg assessment of the deep venous system in symptomatic outpatients with suspected deep-vein thrombosis (PALLADIO): a prospective, multicentre, cohort study. Lancet Haematol 2015;2: e474–80.
7. Prandoni P, Lensing AWA, Büller HR, et al. Comparison of subcutaneous low-molecular-weight heparin with intravenous standard heparin in proximal-vein thrombosis. The Lancet 1992;339:441-5.
8. Koopman MMW, Prandoni P, Piovella F, et al. Treatment of venous thrombosis with intravenous unfractionated heparin administered in hospital as compared with subcutaneous low-molecular-weight heparin administered at home. N Engl J Med 1996;334:682-7.
9. Prandoni P, Lensing AWA, Cogo A, et al. The long-term clinical course of acute deep venous thrombosis. Ann Intern Med 1996;125:1-7.
10. Prandoni P, Lensing AWA, Prins MH, et al. Residual venous thrombosis as a predictive factor of recurrent venous thromboembolism. Ann Intern Med 2002;137:955-60.
11. Prandoni P, Lensing AWA, Büller HR, et al. Deep-vein thrombosis and the incidence of subsequent symptomatic cancer. N Engl J Med 1992;327:1128-33.
12. Prandoni P, Bilora F, Marchiori A, et al. An association between atherosclerosis and venous thrombosis. N Engl J Med 2003;348:1435-41.
13. Prandoni P, Lensing AWA, Piccioli A, et al. Recurrent venous thromboembolism and bleeding complications during anticoagulant treatment in patients with cancer and venous thrombosis. Blood 2002;100:3484-8.
14. Agnelli G, Prandoni P, Santamaria MG, et al. Three months versus one year of oral anticoagulant therapy for idiopathic deep venous thrombosis. N Engl J Med 2001;345:165-9.
15. Agnelli G, Prandoni P, Becattini C, et al. Extended oral anticoagulant therapy after a first episode of pulmonary embolism. Ann Intern Med 2003;139:19-25.
16. Weitz JI, Lensing AWA, Prins MH, et al. Rivaroxaban or aspirin for extended treatment of venous thromboembolism. N Engl J Med 2017;376:1211-22.
17. Girolami A, Pengo V, Cappellato G, et al. Antithrombin III Padua: a "new" congenital antithrombin III abnormality with normal or near normal activity, normal antigen, abnormal migration and no thrombotic disease. Folia Haematol Int Mag Klin Morphol Blutforsch 1983;110:98-111.
18. Thiagarajan P, Pengo V, Shapiro SS. The use of the dilute Russell viper venom time for the diagnosis of lupus anticoagulants. Blood 1986;68:869-74.
19. Pengo V, Thiagarajan P, Shapiro SS, Heine MJ. Immunological specificity and mechanism of action of IgG lupus anticoagulants. Blood 1987;70:69-76.
20. Pengo V, Lensing AW, Prins MH, et al. Incidence of chronic thromboembolic pulmonary hypertension after pulmonary embolism. N Engl J Med 2004;350:2257-64.
21. Campello E, Spiezia L, Adamo A, Simioni P. Thrombophilia, risk factors and prevention. Expert Rev Hematol 2019;12:147-58.
22. Wojcik EG, Simioni P, d Berg M, et al. Mutations which introduce free cysteine residues in the Gla-domain of vitamin K dependent proteins result in the formation of complexes with alpha 1-microglobulin. Thromb Haemost 1996;75:70-5.
23. Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64-7.
24. Poort SR, Rosendaal FR, Reitsma PH, et al. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996;88:3698-703.
25. Simioni P, Prandoni P, Lensing AW, et al. The risk of recurrent venous thromboembolism in patients with an Arg506—>Gln mutation in the gene for factor V (factor V Leiden). N Eng J Med 1997;336:399-403.
26. Sanson BJ, Simioni P, Tormene D, et al. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. Blood 1999;94:3702-6.
27. Simioni P, Sanson BJ, Prandoni P, et al. Incidence of venous thromboembolism in families with inherited thrombophilia. Thromb Haemost 1999;81:198-202.
28. Simioni P, Tormene D, Prandoni P, et al. Incidence of venous thromboembolism in asymptomatic family members who are carriers of factor V Leiden: a prospective cohort study. Blood 2002;99:1938-42.
29. Tormene D, Simioni P, Prandoni P, et al. The incidence of venous thromboembolism in thrombophilic children: a prospective cohort study. Blood 2002;100:2403-5.
30. Simioni P, Scudeller A, Radossi P, et al. "Pseudo homozygous" activated protein C resistance due to double heterozygous factor V defects (factor V Leiden mutation and type I quantitative factor V defect) associated with thrombosis: re-

port of two cases belonging to two unrelated kindreds. Thromb Haemost 1996;75:422-6.
31. Simioni P, Castoldi E, Lunghi B, et al. An underestimated combination of opposites resulting in enhanced thrombotic tendency. Blood 2005;106:2363-5.
32. Simioni P, Kalafatis M, Millar DS, et al. Compound heterozygous protein C deficiency resulting in the presence of only the beta-form of protein C in plasma. Blood 1996;88:2101-8.
33. Simioni P, Tormene D, Tognin G, et al. X-linked thrombophilia with a mutant factor IX (factor IX Padua). N Engl J Med 2009;361:1671-5.
34. George LA. Hemophilia gene therapy: ushering in a new treatment paradigm? Hematology Am Soc Hematol Educ Program 2021;2021:226-33.
35. Miyawaki Y, Suzuki A, Fujita J, et al. Thrombosis from a prothrombin mutation conveying antithrombin resistance. N Engl J Med 2012;366:2390-6.
36. Bulato C, Radu CM, Campello E, et al. New Prothrombin Mutation (Arg596Trp, Prothrombin Padua 2) Associated With Venous Thromboembolism. Arterioscler Thromb Vasc Biol 2016;36:1022-9.
37. Simioni P, Cagnin S, Sartorello F, et al. Partial F8 gene duplication (factor VIII Padua) associated with high factor VIII levels and familial thrombophilia. Blood 2021;137:2383-93.
38. de la Morena-Barrio ME, Suchon P, Jacobsen EM et al. Two SERPINC1 variants affecting N-glycosylation of Asn224 cause severe thrombophilia not detected by functional assays. Blood 2022;140:140-51.