

Clonal hematopoiesis and inflammation: a link with thrombosis and malignancy

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INTRODUCTION

The discovery of driver somatic mutations in JAK2 (V617F), CALR, or MPL in patients with polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF) has opened a new era in the understanding of the pathobiology of these myeloproliferative neoplasms (MPN) and has contributed to the knowledge on MPN-associated arterial and venous thrombosis.^{1,2} Several additional mutations, most of them affecting genes involved in epigenetic regulation (TET2, ASXL1, EZH2, IDH1, DNMT3A) and RNA splicing (SRSF2) have also been identified in MPNs. These mutations can either precede, or accumulate on, a JAK2V617F mutated clone and cooperate with JAK2V617F to favor clonal dominance, modify disease phenotype, or promote leukemic transformation.³

Thrombotic complications are the major cause of morbidity and mortality in PV and ET and are mechanistically associated with the mutation status;³ in around 20% of patients, thrombosis may occur years before the MPN diagnosis.^{4,5} After diagnosis, the thrombotic risk persists and accounts for an incident rate of 3.5% person-year in PV while it is slightly lower in patients with ET and primary myelofibrosis (PMF) (2.5 and 2% person-year, respectively).^{4,5} These estimates are three or more-fold greater than in the normal population with or without generic risk factors for cardiovascular events such as diabetes, hypertension, smoking obesity.^{4,5}

In a population-based study carried out in Sweden, recruiting 9429 patients with MPNs and 35,820 matched controls from 1987 to 2009, the hazard ratios (HRs) for arterial thrombosis in MPNs compared with controls were 3.0, 2.0, and 1.5 at 3 months, 1 and 5 years, respectively. The corresponding HRs for venous thrombosis were 9.7, 4.7, and 3.2, respectively.⁶

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CLONAL HEMATOPOIESIS OF INDETERMINATE POTENTIAL

In 2014, seminal studies identified somatic mutations of TET2, ASXL1, EZH2, IDH1, DNMT3A e JAK2V617F, in individuals without hematological abnormality.⁷⁻⁹ These genetic events allow to identify a new entity called “clonal hematopoiesis of indeterminate potential” (CHIP) as it was uncertain whether it could evolve towards hematological malignancies. The frequency of CHIP was age-related and found in up to 50% of elderly persons and associated with an excess of mortality.¹⁰ Interestingly, arterial thrombosis (myocardial infarction, stroke) and heart failure were frequently reported in these individuals and attributed to systemic chronic inflammation driven by the genetic mutation particularly.^{11,12} The link between the JAK2V617F-mutation and chronic inflammation, revealed by elevation of inflammatory biomarkers, is now explained by the fact that the JAK2617F-mutation *per se* is a generator of reactive oxygen species (ROS)¹³⁻¹⁵ activating cells carrying this mutation (*e.g.*, granulocytes, monocytes, B-cells, T cells, platelets) and explaining the increased risk of atherosclerosis and thrombosis.¹⁶ In addition, the same mechanism involving inflammation in the bone

marrow compartment is considered a driving force of clonal evolution and transition to more advanced phases of MPN.^{17,18}

CHIP, THROMBOSIS AND RISK OF EVOLUTION TO MYELOPROLIFERATIVE NEOPLASMS

In the general population, CHIP-associated mortality is due to an increased risk of atherosclerotic cardiovascular disease (CVD), particularly in subjects with CHIP clones characterized by a high variant allele fraction (VAF).¹³⁻¹⁶ Conversely, the risk of developing a hematological neoplasm including MPN, from CHIP status, is relatively modest, 0.5% to 1% per year, and most individuals will remain asymptomatic with normal blood counts.^{17,18}

However, the transition towards clonal myeloid disorders can be higher in some subgroups of patients with arterial thrombosis who subsequently may develop MPN. As alluded to before, 20% of MPN patients do experience thrombosis before the onset of this disease, particularly in arterial sites. This finding suggests that PV, ET and PMF have a long period which, with current diagnostic criteria, escapes the diagnosis of MPN. However, the pathogenesis of both CHIP and overt MPN is similar and involves genetic mutations and inflammation; in addition, the characteristics that mark the risk of vascular events and evolution to MPN, could be operating in the CHIP stage, as well. Besides these genetic alterations, further factors, including cytokine-driven inflammation by the malignant clone and non-malignant cells of the bone marrow microenvironment, may deteriorate the hematological disease course.^{19,20} Moreover, CHIP is likely to contribute to the whole thrombotic risk together with other generic risk factors for cardiovascular events.^{18,21}

It follows that recognizing which of these patients may be at greater risk of future hematological developments in MPN and thrombotic events, could help with appropriate monitoring and early intervention proposals. Future studies should address the question of screening for CHIP in patients with premature or unexplained coronary artery diseases or stroke in the absence of traditional risk factors. Moreover, obtaining results from NGS mutation may be appropriate in young people with thrombotic events occurring in unusual sites, such as splanchnic or cerebral vein thrombosis, who often present blood counts within the normal range and no other signs of myeloproliferative disease such as splenomegaly. In addition to evaluating the VAF for CHIP mutations, in particular for JAK2V617F it could also be useful to search for biomarkers of inflammation that are linked to both the cardiovascular complications and the risk of hematological transformation. Furthermore, it should be highlighted that thrombosis is a multifactorial event, in which CHIP

should be considered an additional contributor to enhance the inflammatory status.^{22,23}

Two inflammatory biomarkers, high-sensitivity (hs)-CRP and PTX3 belonging to the superfamily of pentraxins were found markers of thrombosis in ET and PV and were correlated with JAK2V617F allele burden ($p=0.003$).²⁴ Interestingly, hs-CRP was confirmed as a marker of inflammation in CHIP supporting the link between inflammation and coronary artery disease in the CHIP stage as well. The relationship between leukocytosis and thrombosis has been extensively investigated in several experimental studies,^{25,26} based on the notion that in MPNs, chronic and subclinical systemic inflammation has a critical role in the pathogenesis of vascular events. However, strong evidence in support of leukocytosis as an inflammatory biomarker potentially contributing to differentiate prognostic categories in PV is still missing. In a very recent meta-analysis,²⁷ the association of leukocytosis with thrombosis was stronger in ET than in PV and exclusively related to arterial events, as shown in our previous analysis of ECLAP patients where time-dependent and not baseline leukocytosis was associated with myocardial infarction.²⁸ Other Authors found that the persistent leukocytosis in PV is linked to hematologic evolution rather than thrombosis.²⁹

While by definition there is no pathological elevation of blood counts in CHIP, in a large population study, a significant increase of white cell, hemoglobin and platelet counts compared to the controls, was documented to be JAK2V617F VAF dependent and associated with an increased venous thrombosis risk.³⁰

Interestingly, new data are emerging on the role of non-myeloid inflammatory cells, such as T lymphocytes and monocytes in the process of immune thrombosis; in particular, experimental work has consistently shown that T-reg lymphocytes are involved in the regulation of the prothrombotic action of activated neutrophils in the process of fibrin formation and dissolution.^{31,32} Based on this knowledge, the ratio between neutrophils-to-lymphocytes (NLR) was considered to represent a synthesis of these two opposed actions in the thrombotic events and was tested in PV and ET. It was found that elevated value of NLR was a prognostic factor of venous and not arterial thrombosis as it was the JAK2 V617F VAF superior to 50%.³³⁻³⁵ This is a new area of research that deserves new investigations also in the stage of CHIP.

In conclusion, the tight association between clonal hematopoiesis and chronic inflammation has brought out the notion that CHIP and MPNs are unique models to elucidate common mechanisms of genetic damage initially not associated with obvious diseases, but which represent a risk of developing frank diseases. Thrombosis, in the setting of either CHIP and MPN, may reflect the effects of inflammation and mark a transition state from latency to more frank disease.

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