

# Platelet-associated hypercoagulability in patients with Essential Thrombocythemia and Polycythemia Vera

## Citation for published version (APA):

Panova-Noeva, M. (2012). *Platelet-associated hypercoagulability in patients with Essential Thrombocythemia and Polycythemia Vera*. Maastricht University.  
<https://doi.org/10.26481/dis.20121129mp>

## Document status and date:

Published: 01/01/2012

## DOI:

[10.26481/dis.20121129mp](https://doi.org/10.26481/dis.20121129mp)

## Document Version:

Publisher's PDF, also known as Version of record

## Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

## General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

[www.umlib.nl/taverne-license](http://www.umlib.nl/taverne-license)

## Take down policy

If you believe that this document breaches copyright please contact us at:

[repository@maastrichtuniversity.nl](mailto:repository@maastrichtuniversity.nl)

providing details and we will investigate your claim.

## **CHAPTER 10**

### Summary, General Discussion and Conclusions



## **Summary and General Discussion**

Thrombosis is a frequent clinical complication in cancer patients. Since its first recognition in the 19th century by Armand Trousseau, many investigators contributed to further enlighten the link between cancer and thrombosis. While now it is a common knowledge that thromboembolic disease is often an early manifestation and frequent cause of morbidity and mortality in cancer patients, the specific pathogenetic mechanisms are still under investigation. Whereas much is known about the role of platelets in hemostasis, in recent years it is more and more recognized that platelets play a more complex role in cancer. As presented in Chapter 1, the interaction between platelets and tumor cells supports the growth and dissemination of malignant cells. In addition, platelets participate in tumor progression by generating thrombin, a central enzyme of the coagulation cascade. Thrombin generation has been linked to several steps of tumor progression, most likely acting through protease activated receptor (PAR) mediated cell signaling pathways.

In part one, Chapter 2, a review on tumor cell-induced procoagulant mechanisms summarizes important current concepts regarding the interactions between cancer cells and hemostasis, supporting a hypercoagulable state in patients resulting in a greater risk of thromboembolic complications. In Chapter 3, the current recommendations for prevention and treatment of venous thromboembolism (VTE) are discussed. Low molecular weight heparin (LMWH) remains the best treatment option for initial and long term treatment of VTE in cancer patients. In addition, numerous studies suggest a survival benefit for cancer patients treated with LMWH.[1-2]

The main objective of this thesis was to evaluate platelet-associated hypercoagulability in patients with Myeloproliferative Neoplasms (MPN), particularly in Essential Thrombocythemia (ET) and Polycythemia Vera (PV) patients. These particular clinical conditions have been chosen because their clinical course is strongly affected by the disease-related thrombosis. Also, management of these patients remains highly dependent on the patient's thrombotic risk (in Chapter 1).

The thrombotic tendency in this population has been evaluated using the new calibrated automated thrombogram (CAT) assay, measuring thrombin generation (TG) in platelet poor plasma, platelet rich plasma and in platelet lysates. Part two, Chapter 4, explores pre-analytical conditions that might influence in-vitro measurements of TG. Effects of blood collection method and addition of corn trypsin inhibitor to abolish in-vitro contact activation in TG measurements are investigated. Our study showed no need of corn trypsin inhibitor in TG assays with 1pM tissue factor initiation or higher.

## CHAPTER X

---

Part three of this thesis focuses on hypercoagulability in ET and PV patients, and particularly on platelet associated hypercoagulability. As described in Chapter 5 a study was performed in a group of 140 MPN patients (80 ET and 60 PV) in order to characterize for the first time the TG potential expressed by platelets from these subjects, as compared to platelets obtained from 72 healthy subjects and to identify what factors might be responsible for platelet TG. The TG parameters of interest (i.e., lag time, peak height, and slope) were evaluated in relation to platelet counts, platelet surface tissue factor (TF) and P-selectin levels, JAK2V617F mutational status, and patients' therapy. The analysis showed that patients positive for the JAK2V617F mutation were characterized by the highest TG potential, platelet surface TF and P-selectin levels. Patients on hydroxyurea (HU) were characterized by a significantly lower TG potential compared to non-HU treated patients, with the lowest values observed in HU treated JAK2V617F positive patients. Patients not receiving HU showed higher TG potential associated with JAK2V617F allele burden increment. This study suggests a platelet-dependent form of hypercoagulability in MPN patients, particularly in those positive for the JAK2V617F mutation. In addition, it shows a more beneficial effect of HU in subjects positive for JAK2V617F mutation, which significantly affects their prothrombotic phenotype.

While it is known that ET and PV patients are characterized by an increased number of circulating immature platelets, no information was available on whether this increase is influenced by pathogenetic factors including JAK2V617F mutational status, or by treatment regimen in these patients.[3] Therefore, we enrolled 46 ET and 38 PV patients to characterize the immature platelet parameters (IPF) measured by a new automated hematology analyzer (Chapter 6). Our results revealed two new elements regarding the association between MPN patients and IPF. The first finding is that the JAK2V617F mutation is linked to the quantity of IPF in patients with MPN, which might contribute to the prothrombotic phenotype in these patients. The second finding is that IPF is susceptible to HU treatment, which may additionally explain the favorable effect of this therapy on MPN as well as the associated thrombotic risk.

Antiplatelet therapy with low dose aspirin has been shown to reduce the risk of thrombosis (i.e. major venous thrombosis, pulmonary embolism, myocardial infarction and stroke) in PV patients.[4] Recently, low dose aspirin has been shown effective in reducing the incidence of venous thrombosis in JAK2V617F positive ET patients and to lower the rate of arterial thrombosis in patients with associated cardiovascular risk factors.[5] However, the large multicenter, controlled randomized clinical trial ECLAP, showed that low dose aspirin does not significantly reduce overall or cardiovascular mortality in PV patients.[4] So far, no study has investigated this issue in ET patients. Therefore, it remains uncertain whether low dose aspirin completely inhibits platelet function in MPN patients. In Chapter 7 we performed the PFA-100 assay in whole blood and the CAT assay in platelet rich plasma in a group of 46 ET and 38 PV patients to evaluate the effect of aspirin on platelet adhesive and procoagulant characte-

ristics. Our results showed higher TG in aspirin treated JAK2V617F positive compared to aspirin treated JAK2V617F negative patients. In the PFA-100 assay flow arrest was noted in 20% of MPN patients after collagen-adenosine diphosphate (CADP) and in 15% of these patients after collagen-epinephrine (CEPI) trigger, the majority taking aspirin. Higher immature platelet parameters from both ET and PV patients were significantly associated with shorter CADP closure time. In addition, in JAK2V617F positive patients, we found a significant and positive association between immature platelet count and TG. Our study suggests that elevated immature platelet parameters are important factors influencing increased platelet adhesive and procoagulant properties. Despite taking aspirin, patients with higher immature platelets had shorter CADP and CEPI closure time and higher TG. Recent observations have highlighted a wide biological variability in the interindividual response to aspirin's antiplatelet effects. 10-20% of patients experienced recurrent vascular events despite treatment with aspirin, referred to as treatment failure.[6] Recent studies suggest a possible role of immature platelets in so called resistance to antiplatelet therapy by expressing more COX-2 resulting in an increased thromboxane production.[7] In addition, hyperreactivity of younger platelets and incompletely inhibited COX-1 and COX-2 have been advocated for reduced antiplatelet effect of aspirin and aspirin resistance in patients with increased levels of immature platelets.[8] A recent study by Dragani et al. showed that low dose aspirin is unable to fully inhibit thromboxane A<sub>2</sub> biosynthesis in ET patients. The residual thromboxane A<sub>2</sub> in these patients is likely due to expression of unacetylated COX-1 and COX-2 in newly formed platelets [9].

Platelet adhesion and aggregation *in vivo* are influenced by a number of different agonists acting also through other than thromboxane dependent pathways. As described in Chapter 8 a study was performed in a group of 65 ET and 51 PV patients to evaluate platelet reactivity to different agonists using the multiple electrode platelet aggregometry and platelet reactivity to ADP in terms of procoagulant responses by using the CAT assay. We demonstrated that platelets from ET and PV patients are more reactive to ADP, not only in terms of increased platelet aggregation in a whole blood system, but also enhanced TG, particularly in those positive for the JAK2V617F mutation. We also observed evidence of aspirin failure, chronically administered to MPN patients, to fully inhibit platelet reactivity as shown by increased ADP and thrombin receptor activating peptide induced platelet aggregation, as well as ADP induced TG.

While the platelet's phospholipid contribution to TG in healthy subjects is well recognized, the potential platelet phospholipid contribution to TG in patients at risk of thrombosis, including MPN patients, is unknown. We demonstrated a powerful inhibitory effect of Annexin V on platelet associated TG indicating the critical participation of phosphatidylserine on the activated platelet surface in the thrombin formation process.

## CHAPTER X

---

Hydroxyurea (HU) is a recommended treatment approach in high risk patients.[10] It has been demonstrated efficient in reducing thrombotic events in high risk patients for thrombosis in a seminal randomized clinical trial.[11] Besides its myelosuppressive actions, additional mechanisms have been advocated to explain the anti-thrombotic effect of HU, including qualitative changes in circulating blood cells (i.e. decreased expression of tissue factor), decreased expression of endothelial adhesion molecules and enhanced nitric oxide production.[12] Nitric oxide is produced by vascular endothelium and platelets and it mediates vascular relaxation in response to vasoactive substances and shear stress. It provides anti-proliferative and antithrombotic functions by inhibiting vascular smooth muscle cell proliferation, monocyte adhesion, platelet aggregation, and thrombosis.[13] Our study found an increased level of circulating nitric oxide metabolites in MPN patients, particularly in ET patients, compared to healthy controls (Chapter 9). In addition, we observed increasing levels of nitric oxide metabolites upon increasing weakly dosage of HU.[14] Whether HU-induced nitric oxide production may be relevant in the treatment of MPN would deserve further studies.