Thrombin generation – a potentially useful biomarker of thrombotic risk in Philadelphia-negative myeloproliferative neoplasms

Romeo-Gabriel Mihaila

The diagnosis of essential thrombocythemia and polycythemia vera is often made during a thrombotic event which can be serious. Philadelphia-negative chronic myeloproliferative neoplasia patients have an increased thrombotic risk. This is assessed using various scoring systems but these are far from ideal and individual risk. The current trend to personalised medicine requires finding the most useful thrombotic risk biomarker in these patients. Routine tests for coagulation do not take account of both pro- and anti-coagulant factors which is why these tests are not useful in patients with Philadelphia-negative myeloproliferative neoplasms. Thrombin generation reflects more accurately the balance between pro- and anti-coagulant factors. Some parameters of thrombin generation such as the endogenous thrombin potential are higher in Philadelphia-negative myeloproliferative neoplasm patients, especially in JAK2 V617F carriers than in healthy controls. They are even higher in those with reactive thrombocytosis. The JAK2 V617F allele burden correlates more with a higher thrombin generation potential in patients who are not treated with hydroxycarbamidum. Instead, JAK2 V617F-positive patients with Philadelphia-negative myeloproliferative neoplasms were the most sensitive to hydroxycarbamidum, as was reflected in lower values of platelet thrombin generation potential. The use of thrombin generation examination in these patients would enable detection of imminent thrombosis and personalised prophylactic management.

**Key words:** essential thrombocythemia, JAK2 V617F, myeloproliferative neoplasms, personalized medicine, platelets, polycythemia vera, thrombin generation, thrombotic risk.

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Faculty of Medicine, "Lucian Blaga" University of Sibiu and Department of Hematology, Emergency County Clinical Hospital Sibiu, Romania
Corresponding author: Romeo Gabriel Mihaila, e-mail: romeomihaila@yahoo.com

**INTRODUCTION**

Cancer is a risk factor for thrombotic events. Black ethnicity and chemotherapy are also thrombotic risk factors for patients with cancer. Khorana et al developed a score to estimate the risk of venous thromboembolism in patients indicated for chemotherapy, which included the site of cancer, the platelet and white blood count before chemotherapy, the hemoglobin level or the use of erythropoietin, and the high body mass index. This score was validated in many clinical trials. Unfortunately, only a small number of patients had hematologic malignancies in the group studied by Khorana et al. Multiple myeloma, non-Hodgkin and Hodgkin lymphoma had the highest risk for venous thromboembolism in patients with hematologic malignancies in a study on hospitalized cancer patients. But patients with Philadelphia-negative myeloproliferative neoplasms (especially those with essential thrombocythemia or polycythemia vera) are rarely hospitalized. Essential thrombocythemia patients aged over 60 years, with white blood count over 11000/mm³, and thrombotic events in the history have higher thrombotic risk. The presence of cardiovascular risk factors and the mutation JAK2 V617F are other risk factors included in an international prognostic score of thrombosis of these patients. Patients with polycythemia vera have two main thrombotic risk factors: age over 60 years and thrombotic events in the medical history. Additional risk factors are: diabetes mellitus, arterial hypertension, congestive heart failure, hypercholesterolemia, and smoking. The presence of JAK2 V617F mutation in patients with myeloproliferative neoplasms increases the thrombotic risk (RR 2.94) compared to patients without this mutation; the risk is even higher in those who also have thrombophilia (especially in the presence of lupus anticoagulant and factor V Leiden) (ref.⁵).

But these risk factors are not ideal for thrombotic risk quantification in Philadelphia-negative myeloproliferative neoplasms patients. I decided to do a literature minireview on a potential useful biomarker: thrombin generation. I selected articles published in PubMed in the last 10 years, using the terms “thrombin generation”, “essential thrombocythemia”, “polycythemia vera”.

**THE THROMBOTIC RISK OF PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS**

Patients with Philadelphia-negative myeloproliferative neoplasms (Ph-negative MPN) are likely to develop venous or arterial thrombosis. About two-thirds of all these complications are represented by transient ischemic attacks and stroke. Those who are JAK2 V617-positive have higher risk of developing thrombotic complications. A ce-
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the lower values in patients who did not receive this medication and led to platelet thrombin generation potential compared to the with hydroxycarbamidum. This drug was able to decrease generation potential in patients who were not treated lele burden increase correlated with a higher thrombin present in factor and P-selectin found on the platelet surface were highest levels of thrombin generation potential and tissue subjects and was associated with platelet activation. The platelets was significantly higher than that of healthy eradation potential of platelet-rich plasma and isolated MPN patients is platelet-dependent. The thrombin gen

GENERATION AND PLATELETS

Adenosine diphosphate (ADP)-induced thrombin generation was significantly higher in polycythemia vera and essential thrombocythemia patients compared to control group. Those who were JAK2 V617F-positive had the highest values. It was observed that annexin V was less able to inhibit both basal and ADP-induced thrombin generation4. Indeed, the hypercoagulability of Ph-negative MPN patients is platelet-dependent. The thrombin generation potential of platelet-rich plasma and isolated platelets was significantly higher than that of healthy subjects and was associated with platelet activation. The highest levels of thrombin generation potential and tissue factor and P-selectin found on the platelet surface were present in JAK2 V617F-positive patients. JAK2 V617F allele burden increase correlated with a higher thrombin generation potential in patients who were not treated with hydroxycarbamidum. This drug was able to decrease platelet thrombin generation potential compared to the patients who did not receive this medication and led to the lower values in JAK2 V617F-positive patients treated with it12.

But cancer patients have high levels of circulating tissue factor, which is involved in thrombin generation - the most powerful platelet activator13. The mechanisms involved in the platelet energy production of essential thrombocythemia patients have an initial hyperactivity induced by adding thrombin in vitro: a higher quantity of lactate was generated by platelets at 5 minutes14. This can explain the platelet activation induced by thrombin generation in these patients. In addition, increased platelet activation is explained knowing that the generation of thrombin is also increased in essential thrombocythemia patients.

In a study on 111 patients with Ph-negative MPN and 89 control subjects the endogenous thrombin potential (ETP) was higher in the patients. There was a direct correlation between ETP and platelet counts and an inverse one between ETP and antithrombin, protein C, and free protein S levels11. ETP ratios were lower in patients treated with hydroxycarbamidum versus those treated with other drugs. In this study, thrombin generation was able to detect a procoagulant imbalance in patients with Ph-negative MPN11.

ACQUIRED RESISTANCE TO ACTIVATED PROTEIN C

Not only do clonal blood cells of patients with Ph-negative MPN manifest prothrombotic features, but vascular cells also have abnormalities and acquire a procoagulant role when they are exposed to inflammatory stimuli. The acquired resistance to activated protein C is involved in thrombin generation increase. This high level of thrombin generation and that of procoagulant microparticles released from platelets and vascular cells contribute to the prothrombotic feature of these patients8.

Compared to controls, endogenous thrombin potential was lower in the absence and higher in the presence of activated protein C in essential thrombocythemia and polycythemia vera patients, a fact explained by the presence of acquired activated protein C resistance, probably due to a reduction in plasma free protein S levels. Normalized activated protein C sensitivity ratio, which expresses the level of activated protein C resistance, had the highest values in JAK2 V617F-positive homozygous patients11. Essential thrombocythemia and polycythemia vera patients, in particular those who are JAK2 V617F-positive, also had reduced levels of thrombomin, factor V, and tissue factor pathway inhibitor11.

THE ROLE OF MICROPARTICLES IN HYPERCOAGULABILITY

Soluble tissue factor and microparticles are thrombotic risk factors in cancer patients16 and microparticles are also known to be biomarkers of a prothrombotic state17. Their activity can be assessed by circulating procoagulant activity (CPA). It was found that patients with Ph-negative
MPN have a higher CPA compared to healthy subjects. This increased CPA was associated with a lower inhibition of the thrombin generation in the presence of thrombomodulin, which suggests an acquired thrombomodulin resistance in patients with Ph-negative MPN, partly due to circulating microparticles, a pathophysiological component of their hypercoagulable state 17. Thrombomodulin is a thrombin cofactor in the process of protein C activation (a powerful anticoagulant factor), but the complex thrombin-thrombomodulin is also involved in fibrinolysis inhibition. The lowest inhibition rate of the thrombin generation was observed in homozygous JAK2 V617F-positive patients 17.

The number of microparticles with platelet and endothelial origin (positive for CD41, and respectively CD62E and CD144 antigens) found in the serum of patients with essential thrombocythemia was higher than in the control group and suggests a double cell activation (platelet and endothelial). The presence of microparticles which simultaneously express CD62E and CD41 was only discovered in patients with thrombotic risk factors and suggests a possible bilineage source 18. There was a correlation between the higher peak height of thrombin generation found in these patients and total number of microparticles, a fact that highlights the role of microparticles in thrombin generation and thrombosis 19.

The platelet-free plasma of patients with essential thrombocythemia had higher thrombin generation and procoagulant phospholipid activities compared to the control group. These two activities were associated with more elevated levels of FVIIa/antithrombin complex and microparticle-associated tissue factor antigen. JAK2 V617F-positive patients had higher thrombin generation versus JAK2 V617F-negative and normal subjects. Different parameters for thrombin generation were correlated with procoagulant phospholipid-assyay. No differences were found concerning thrombin generation or procoagulant activity between patients with and without thrombosis 19.

THE DIFFERENTIAL DIAGNOSIS BETWEEN REACTIVE THROMBOCYTOSIS AND ESSENTIAL THROMBOCYTHEMIA

Procoagulant phospholipids-assay and the use of calibrated automated thrombogram can contribute to the differential diagnosis between reactive thrombocytosis and essential thrombocythemia. Essential thrombocythemia patients had higher levels of circulating procoagulant phospholipids compared to those with reactive thrombocytoysis. Instead, those with reactive thrombocytosis had a longer lag time and more increased levels of endogenous thrombin potential, velocity index, and peak of thrombin generation 20.

CONCLUSION

The study of thrombin generation could be more useful for predicting the thrombotic risk in patients with thrombocytopathies than the classical tests used for coagulation investigation.

Extensive studies are needed on patients with Ph-negative MPN in order to establish if the level of thrombin generation correlates with thrombotic risk, and if this risk is not dependent on JAK2 V617F allele burden, as this mutation can influence leukocyte count, platelet and white blood cell activation 21 and interaction and some factors involved in plasma hypercoagulation 22.

Some thrombin generation parameters could be added to the thrombotic risk scores used in Ph-negative MPN patients, beside patient- and disease-related factors.

The association of high level of thrombin generation parameters with a high mean platelet volume, which is a recognized independent risk factor for stroke in patients with atrial fibrillation 23, or with the presence of microparticles which simultaneously express CD62E and CD41 in Ph-negative MPN patients could be other aims for future thrombotic risk studies.

Search strategy and selection criteria

Strategy research aimed at analysing the utility of thrombin generation as biomarker for thrombotic risk estimation. Scientific articles published in PubMed from 2007 to 2016 were searched, using the terms "thrombin generation", "essential thrombocythemia", "polycythaemia vera".

ABBREVIATIONS

ADP, adenosine diphosphate; Ph-negative MPN, Philadelphia-negative myeloproliferative neoplasms; ETP, endogenous thrombin potential; CPA, circulating procoagulant activity.

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REFERENCES


