The platelet aIIbβIII integrin has no role in thrombosis in myeloproliferative neoplasm

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Abstract

We present a patient with congenital Glanzmann Thrombasthenia, who developed recurrent venous thrombosis. Over time, she developed the clinical picture of a myeloproliferative neoplasm, being JAK2 positive. This case clearly indicates that the platelet aIIbβIII integrin (lacking in Glanzmann thrombasthenia) does not have a role in thrombosis in MPN

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Key Clinical message

- Glanzmann Thrombasthenia does not protect from venous thrombosis in MPN

- we hypothesize on other mechanisms of thrombosis in MPN

- dabigatran can be given in a patient with Glanzmann Thrombasthenia

To the editor,

Glanzmann thrombasthenia (GT) is a rare autosomal recessive platelet disorder characterized by a lack of functional integrins αIIb or β3. The clinical phenotype is dominated by an increased mucocutaneous bleeding tendency.[1] The occurrence of venous thrombosis in these patients is very rare, but a total of 12 case reports have been published.[2,3]

We describe a 74-year old woman with GT caused by compound heterozygosity for a pathogenic missense mutation (c.1787T>C p.(Ile596Thr)) and a donor splice site mutation (c.2841+1G>T p.(?)) in the ITGA2B gene. Her medical history is extensive, and her bleeding score (ISTH-BAT) was 22 in 2016. She suffered from hypertension for several years and smoked 5 cigarettes a day for over 20 years, but stopped smoking in

2011. She has had multiple episodes of mild microcytic anemia since 2014, for which she undergoes regular colonoscopies for surveillance of adenomas. In March 2016, she developed a superficial thrombophlebitis of her right calve after a long car drive, which was treated with 200mg celecoxib once daily for 4 weeks. In April 2016 she had angina pectoris with ischemic abnormalities on the electrocardiogram. An MRI-heart with adenosine showed baso-inferoseptal and baso-anteroseptal perfusion defects. She was treated with statins, metoprolol and amlodipine, but platelet inhibitors were contraindicated due to her GT. Treatment was successful, and she remains free of cardiac complaints until today. In October 2017, she developed another thrombophlebitis in her left calve, for which treatment with celecoxib for 4 weeks was started again. In February 2018, after a mild pneumonia, a deep vein thrombosis (DVT) was diagnosed in the right leg, extending from the popliteal vein to the distal external iliac vein. Her blood count at that time showed a mild microcytic anemia (hemoglobin 9.0 g/dL) with normal leucocyte and platelet counts. CT-thorax/abdomen showed no malignancies; antiphospholipid antibodies were negative. Treatment with 110mg dabigatran twice daily was started for 3 months. Two weeks after stopping dabigatran, an exacerbation of her microcytic anemia was detected that resolved after iron suppletion. In September 2018, a new DVT occurred in the left leg, for which dabigatran was restarted. After 3 months, dabigatran was reduced to once daily 110mg for long term use. She experienced no increase in bleeding tendency and is on dabigatran since. A year later, in October 2019, she had higher hemoglobin values than she was used to (hemoglobin 14.7 g/dL), her platelet count, which was always in the normal range, increased to $599x10^9$/L and a mild leucocytosis developed ($11.9x10^9$/L). A JAK2 mutation was found (c.1849 G>T; p.Val617Phe) and the diagnosis polycythemia vera was made. To prevent further thromboembolic disease, low dose hydroxyurea treatment (500mg daily) was started, and her blood count normalized within two weeks. Meanwhile, she showed slight progression of DVT in her legs, after which dabigatran was increased to twice daily 110mg. A year later, in Dec 2020, she had progression of DVT, after which dabigatran was increased from twice daily 110mg to twice daily 150mg. To date, she is without complaints and tolerates both dabigatran and hydroxyurea very well.

Philadelphia chromosome negative myeloproliferative neoplasms (MPNs) include polycythemia vera, essential thrombocytosis (ET) and myelofibrosis. The hallmark of MPN is a dysregulation of cellular processes in bone marrow precursors. Thrombosis frequently occurs in MPNs. Well known risk factors are age and prior thrombosis. In addition, hematocrit, leucocytosis, smoking and hypertension all independently contribute to thrombotic risk in MPNs,[4, 5] although they are not incorporated in the current thrombotic risk stratification model. There is a clear link between both leucocytes and red blood cells and activation of the coagulation system in MPNs. Activated monocytes in MPN show increased surface tissue factor expression, which is the principal component in the extrinsic pathway of coagulation and erythrocytosis causes blood flow stasis, leading to endothelial injury and hypercoagulability. Erythrocytosis also leads to high shear stress condition within the vessel, leading to platelet activation and microparticle release.

As the key finding in ET is thrombocytosis, it is plausible that platelets contribute to thrombotic risk in MPNs. The efficacy of platelet inhibitors for primary prevention of thrombosis in MPNs further supports an important role for platelets in MPN-associated venous thrombosis.

Nevertheless, there is no clear correlation between platelet count and thrombotic risk in ET,[5] although there are reports of altered platelet reactivity.[6-10] This case report clearly demonstrates that platelet aggregation through integrin αIIbβ3 does not contribute to venous thrombosis in MPNs. This is supported by the observation that platelet aggregates are not dominantly present in venous thrombi. Instead, platelets adhere either directly to the activated endothelium or to adherent leukocytes, forming small heterotypic aggregates. Platelet recruitment to the venous thrombus depends on the interaction between GPIbα and exposed VWF.[11]

How then, would platelets contribute to thrombosis in MPNs?

The interplay between platelets and the coagulation system in thrombus formation appears to be pivotal. It has been demonstrated that αIIbβ3 deficient platelets aggregate if fibrin formation is allowed.[12] This fibrin-dependent platelet aggregation is thought to be mediated by glycoprotein VI (GPVI).[13]Indeed, GPVI has been identified as a promising antithrombotic target as GPVI deficiency protects against thrombosis while not interfering with normal hemostasis.[14] Activated platelets subsequently provide a surface on which the

coagulation reaction can propagate. Moreover, they secrete both activated factor V and polyphosphates, greatly enhancing the coagulation reaction.

Platelet-leucocytes complexes also play a significant role in venous thrombosis. New evidence shows that CTL2 (choline transporter-like protein 2, also known as SLC44A2) on the surface of neutrophils drives neutrophil recruitment, activation and NETosis *in vivo* , and that CTL2 is directly bound by activated $\alpha_{IIb}\beta_3$ integrins on platelets in a flow-dependent manner *in vitro*. [15] Especially in MPN, exacerbated response to Toll Like Receptor stimulation is suggested to promote platelet/leukocyte/endothelial interactions and secretion of inflammatory mediators, reinforcing the thromboinflammatory state.[16] However, the lack of $\alpha_{IIb}\beta_3$ integrins in GT suggests that platelet-leucocyte complexes do not play an important role in MPN related VTE.

In this case, MPN was diagnosed after the occurrence of VTE. Hence, primary prevention was not an issue. However, if we would have found MPN before she developed VTE, we still would not have started antiplatelet therapy, in the assumption that her M. Glanzmann would provide a natural protection against VTE. We decided to treat the VTE with a DOAC, as the superiority of DOACs over vitamin K antagonists in terms of safety is demonstrated.[17] We used a dose of 110mg twice daily, as also recommended by the manufacturer in patients with an increased bleeding risk. Unfortunately, our patient had progression of VTE for which the dose of dabigatran was increased twice. She did not experience any bleeding episodes in 2 years of follow up. We are not aware of other patients with GT currently treated with a DOAC. We do like to point out that this is a unique case, where the procoagulant activity is pronounced, perhaps counterbalancing the bleeding risks with a DOAC here.

In summary, this case shows that the platelet $\alpha_{IIb}\beta_3$ integrin is not involved in the pathogenetic mechanism of relapsing venous thrombosis in MPN. This might argue for further interest in the therapeutic role of GPVI inhibition.

Conflict of interest

RS and RU have no conflicts of interest to declare

Contributions

RS and RU both wrote the manuscript

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Perez Botero J, Lee K, Branchford BR, et al. Platelet Disorder Variant Curation Expert Panel. Glanzmann thrombasthenia: genetic basis and clinical correlates. Haematologica 2020;105:888-894.
2. Ragsdell B, Thachil J. Lessons from recurrent deep vein thrombosis in Glanzmann thrombasthenia. Haemophilia 2013;19:e391-393.
3. Girolami A, Sambado L, Bonamigo E, Vettore S, Lombardi AM. Occurrence of thrombosis in congenital thrombocytopenic disorders: a critical annotation of the literature. Blood Coagul Fibrinolysis 2013;24:18-22.
4. Grinfeld J. Prognostic Models in the Myeloproliferative Neoplasms. Blood Rev 2020;42:100713.
5. Campbell PJ, MacLean C, Beer PA, et al. Correlation of blood counts with vascular complications in essential thrombocythemia: analysis of the prospective PT1 cohort. Blood 2012;120:1409-1411.
6. Connor DE, Ma DDF, Joseph JE. Flow cytometry demonstrates differences in platelet reactivity and microparticle formation in subjects with thrombocytopenia or thrombocytosis due to primary haematological disorders. Thromb Res 2013;132:572-577.
7. Griesshammer M, Beneke H, Nussbaumer B, Grünewald M, Bangerter M, Bergmann L. Increased platelet surface expression of P-selectin and thrombospondin as markers of platelet activation in essential

thrombocythaemia. Thromb Res 1996;96:191-196.
8. Cesar JM, de Miguel D, García Avello A, Burgaleta C. Platelet dysfunction in primary thrombocythemia using the platelet function analyzer, PFA-100. Am J Clin Pathol 2005;123:772-777.
9. Panova-Noeva M, Marchetti M, Russo L, et al. ADP-induced platelet aggregation and thrombin generation are increased in Essential Thrombocythemia and Polycythemia Vera. Thromb Res 2013;132:88-93.
10. Małecki R, Gacka M, Kuliszkiewicz-Janus M, et al. Altered plasma fibrin clot properties in essential thrombocythemia. Platelets 2015;7104:1-7.
11. Sang Y, Roest M, de Laat B, de Groot PG, Huskens D. Interplay between platelets and coagulation [published online ahead of print 12 Jun 2020]. Blood Rev doi.org/10.1016/j.blre.2020.100733
12. Lisman T, Adelmeijer J, Heijnen HF, de Groot PG. Recombinant factor VIIa restores aggregation of alphaIIbbeta3-deficient platelets via tissue factor-independent fibrin generation. Blood 2004;103:1720-7.
13. Onselaer MB, Hardy AT, Wilson C, Sanchez X, Babar AK, Miller JLC, Watson CN, Watson SK, Bonna A, Philippou H, Herr AB, Mezzano D, Ariëns RAS, Watson SP. Fibrin and D-dimer bind to monomeric GPVI. Blood Adv 2017;1:1495-1504.
14. Andrews RK, Arthur JF, Gardiner EE. Targeting GPVI as a novel antithrombotic strategy. J Blood Med 2014;5:59-68.
15. Kenny M, Schoen I. A handshake between platelets and neutrophils might fuel deep vein thrombosis. Platelets 2020;31:624-626.
16. Marín Oyarzún CP, Glembotsky AC, Goette NP et al. Platelet Toll-Like Receptors Mediate Thromboinflammatory Responses in Patients With Essential Thrombocythemia. Front Immunol 2020;11:705.
17. Mai V, Bertoletti L, Cucherat M, et al. Extended anticoagulation for the secondary prevention of venous thromboembolic events: An updated network meta-analysis. PLoS One 2019;14:e0214134.