Editorial

Showing one's true CALRs: spleen size and platelet count help refine the molecular diagnostic pathway for patients with splanchnic vein thrombosis

Karl Haslam, Stephen E. Langabeer

Cancer Molecular Diagnostics, St. James's Hospital, Dublin, Ireland

Correspondence to: Stephen E. Langabeer. Cancer Molecular Diagnostics, Central Pathology Laboratory, St. James's Hospital, Dublin 8, Ireland. Email: slangabeer@stjames.ie.

Comment on: Poisson J, Plessier A, Kiladjian JJ, *et al.* Selective testing for calreticulin gene mutations in patients with splanchnic vein thrombosis: a prospective cohort study. J Hepatol 2017. [Epub ahead of print].

Received: 26 June 2017; Accepted: 12 July 2017; Published: 09 August 2017.

doi: 10.21037/amj.2017.07.12

View this article at: http://dx.doi.org/10.21037/amj.2017.07.12

The classical Philadelphia chromosome-negative myeloproliferative neoplasms (MPN) of polycythaemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are myeloid disorders characterized by clonal expansion of mature hematopoietic cells, extra-medullary hematopoiesis and clinically by increased incidence of thrombosis, hemorrhage and the potential to transform to acute leukemia (1). The mainstay of MPN therapy has been phlebotomy, antiplatelet drugs and cytoreductive agents to control constitutional symptoms and prevent thrombotic events and leukemic transformation. More recently targeted therapies have shown considerable efficacy in these malignancies (2). Current MPN disease models envisage PV, ET and PMF as part of a continuum of phenotypes, reflected in the prevalence of the main MPN genotypic driver mutations which are generally found in a mutually exclusive fashion across subtypes and which contribute to phenotype through convergent activation of intracellular JAK-STAT signalling (3). The most common of these driver mutations is the *JAK2* V617F, present in more than 95% of PV patients and in 50–60% of ET and PMF patients. Up to 40% of patients with splanchnic vein thrombosis (SVT), which encompasses Budd Chiari syndrome, portal and mesenteric vein thrombosis, are subsequently diagnosed with either an overt or latent MPN (4,5). This high incidence compels molecular testing for the *JAK2* V617F mutation to be the primary investigation as to whether an MPN is the underlying cause of the SVT. Other risk factors for SVT including pregnancy, abdominal disorders, an inherited thrombophilic state, hormone therapy, autoimmune disorders or paroxysmal nocturnal hemoglobinuria with further investigations for acquired and hereditary thrombophilia warranted in those cases where the *JAK2* V617F mutation is absent. Other, less common driver mutations of MPN, namely those within *MPL* exon 10 and *JAK2* exon 12, are generally absent in patients with SVT (6,7). It should also be noted that ethnic differences in the etiology of diseases such as Budd-Chiari syndrome have been reported (8).

In 2013 insertion and/or deletion mutations of the *CALR* gene, that encodes the intracellular, calcium binding protein calreticulin, were discovered in 20–30% of patients with ET and PMF (9,10). As previously mentioned, *CALR* mutations occur in a virtually mutually exclusive manner of the *JAK2* V617F necessitating detection to be incorporated into the routine molecular diagnostic algorithm for MPN (11). Interestingly, those MPN patients with *CALR* mutations have a significantly lower risk of thrombotic events than their *JAK2* V617F-positive counterparts (12,13).

Initial investigations into the frequency of *CALR* mutations in patients with SVT revealed a low frequency (<2%) or absence in European cohorts (14,15). These findings were borne out by several subsequent studies with conflicting opinion as to whether *CALR* mutation screening should be part of the diagnostic algorithm for SVT: both clinical and laboratory-orientated arguments exist for screening all *JAK2* V617F-negative cases, not screening at all, or for screening only those cases with other clinical or hematological features of an MPN also present (16).

© AME Medical Journal. All rights reserved.

In an effort to resolve this screening issue, Poisson and colleagues have analysed an extended cohort of more than 500 SVT patients in order to identify those with the highest likelihood of harboring *CALR* mutations (17). From an initial test cohort of 312 SVT patients, five *CALR* positive patients (1.6%) were identified, all of whom had a spleen height of 16 cm or larger and a platelet count greater than 200×10^9/L. These indices, chosen on the basis of previous findings of low sensitivity but high specificity for detection of underlying MPN in SVT patients in the pre-*JAK2* era (18), were extended into a validation cohort of SVT patients and enabled identification of a further 2 of 6 (33.3%) *CALR* mutated cases. Despite resulting in a positive predictive value of only 46.6%, perhaps more importantly from a molecular diagnostic perspective, a negative predictive value of 99.7% for *CALR* mutation detection was obtained across both cohorts indicating who not to screen. Whilst the platelet count threshold appears arbitrary (the normal range for platelets in adults being 150×10^9–400×10^9/L) this threshold may account for the effects of portal hypertension such as hypersplenism and hemodilution which can lead to red cell and platelet counts below MPN diagnostic criteria (18). Poisson *et al.* (17) go on to propose an algorithm for MPN diagnosis in SVT based on these results and recommend *CALR* mutation screening should only be considered in those *JAK2* V617F-negative SVT patients with splenomegaly and a platelet count $>200\times10^9$/L thereby limiting subsequent further bone marrow biopsy analysis to those patients where a *JAK2* V617F or *CALR* mutation is not detected.

Inclusion of these basic hematological and clinical indices, in addition to molecular data, into an easily adopted model has the potential to rapidly exclude suspected MPN and to minimise invasive bone marrow evaluation to appropriate patients, thereby enhancing diagnosis and improving overall clinical management in SVT patients. As the prevalence of *CALR* mutations in SVT becomes more clearly defined, it will be interesting to speculate if *CALR* genotype (19) influences phenotype, outcome, or response to therapy as observed in overt MPN.

Acknowledgements

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *AME Medical Journal*. The article did not undergo external peer review.

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/amj.2017.07.12). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. Passamonti F, Mora B, Maffioli M. New molecular genetics in the diagnosis and treatment of myeloproliferative neoplasms. Curr Opin Hematol 2016;23:137-43.
2. Vannucchi AM, Harrison CN. Emerging treatments for classical myeloproliferative neoplasms. Blood 2017;129:693-703.
3. Grinfeld J, Nangalia J, Green AR. Molecular determinants of pathogenesis and clinical phenotype in myeloproliferative neoplasms. Haematologica 2017;102:7-17.
4. De Stefano V, Qi X, Betti S, et al. Splanchnic vein thrombosis and myeloproliferative neoplasms: molecular-driven diagnosis and long-term treatment. Thromb Haemost 2016;115:240-9.
5. How J, Zhou A, Oh ST. Splanchnic vein thrombosis in myeloproliferative neoplasms: pathophysiology and molecular mechanisms of disease. Ther Adv Hematol 2017;8:107-18.
6. Fiorini A, Chiusolo P, Rossi E, et al. Absence of the JAK2 exon 12 mutations in patients with splanchnic venous thrombosis and without overt myeloproliferative neoplasms. Am J Hematol 2009;84:126-7.

7. Kiladjian JJ, Cervantes F, Leebeek FW, et al. The impact of JAK2 and MPL mutations on diagnosis and prognosis of splanchnic vein thrombosis: a report on 241 cases. Blood 2008;111:4922-9.
8. Qi X, Han G, Guo X, et al. Review article: the aetiology of primary Budd-Chiari syndrome – differences between the West and China. Aliment Pharmacol Ther 2016;44:1152-67.
9. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med 2013;369:2379-90.
10. Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med 2013;369:2391-405.
11. Langabeer SE, Andrikovics H, Asp J, et al. Molecular diagnostics of myeloproliferative neoplasms. Eur J Haematol 2015;95:270-9.
12. Rotunno G, Mannarelli C, Guglielmelli P, et al. Impact of calreticulin mutations on clinical and hematological phenotype and outcome in essential thrombocythemia. Blood 2014;123:1552-5.
13. Rumi E, Pietra D, Pascutto C, et al. Clinical effect of driver mutations of JAK2, CALR or MPL in primary myelofibrosis. Blood 2014;124:1062-9.
14. Turon F, Cervantes F, Colomer D, et al. Role of calreticulin mutations in the aetiological diagnosis of splanchnic vein thrombosis. J Hepatol 2015;62:72-4.
15. Haslam K, Langabeer SE. Incidence of CALR mutations in patients with splanchnic vein thrombosis. Br J Haematol 2015;168:459-60.
16. Langabeer SE. CALR mutation analysis is not indicated in patients with splanchnic vein thrombosis without evidence of a myeloproliferative neoplasm: a micro-review. Ann Gastroenterol 2016;29:557-8.
17. Poisson J, Plessier A, Kiladjian JJ, et al. Selective testing for calreticulin gene mutations in patients with splanchnic vein thrombosis: a prospective cohort study. J Hepatol 2017. [Epub ahead of print].
18. Chait Y, Condat B, Cazals-Hatem D, et al. Relevance of the criteria commonly used to diagnose myeloproliferative disorder in patients with splanchnic vein thrombosis. Br J Haematol 2005;129:553-60.
19. Pietra D, Rumi E, Ferretti V, et al. Differential clinical effects of different mutation subtypes in CALR-mutant myeloproliferative neoplasms. Leukemia 2016;30:431-8.

doi: 10.21037/amj.2017.07.12
Cite this article as: Haslam K, Langabeer SE. Showing one's true CALRs: spleen size and platelet count help refine the molecular diagnostic pathway for patients with splanchnic vein thrombosis. AME Med J 2017;2:107.

© AME Medical Journal. All rights reserved.