Editorial

Detecting *CALR* mutations in splanchnic vein thrombosis: Who and how?

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The mainstay of therapy for the myeloproliferative neoplasms (MPN) has been phlebotomy, antiplatelet drugs and cytoreductive agents to control constitutional symptoms and to prevent leukemic transformation and thrombotic events. There exists an increased incidence of both arterial and venous thrombosis in MPN patients, of which the latter may manifest in the microcirculation or as deep vein thrombosis, pulmonary embolism, cerebral venous thrombosis or splanchnic vein thrombosis (SVT).[1] SVT can be the first presenting feature of either a hematologically evident or latent MPN with the molecular identification of the *JAK2* V617F MPN driver mutation an essential component of the molecular diagnostic work up in such cases in order to assign appropriate clinical management.[2,3] Subsequently identified low frequency MPN-driving mutations in *JAK2* exon 12 and *MPL* exon 10 are generally considered to be absent in SVT,[4,5] although rare case reports exist.[6,7] The landmark discovery in 2013 of insertion and/or deletion (indel) mutations of *CALR* (the gene that encodes the endoplasmic reticulum-associated, calcium binding protein Calreticulin) in up to 80% of *JAK2* V617F-negative essential thrombocythemia and primary myelofibrosis patients[8,9] prompted several groups to investigate whether these mutations were also prevalent in patients presenting with SVT, enabling a prompt diagnosis of MPN.

To date, multiple studies have reported the incidence of *CALR* mutations in patient cohorts presenting with SVT and are summarized in Table1.[10-23] Of more than 1500 SVT patients now analyzed, *CALR* mutations were present in only 1.3% of patients, roughly equating to one patient in seventy-five. Screening all SVT patients for *CALR* mutations would therefore appear to be an inappropriate use of resources; however, this issue has been recently addressed. Incorporating a spleen size of greater than 16 cm and a platelet count of more than 200×10^9/L into the diagnostic algorithm allows for the positive identification of the majority of *CALR* mutation-positive cases, sparing the remainder of patients from an invasive bone marrow biopsy required to assess morphological appearances.[20] As marked geographical differences exist in the etiology of SVT and Budd-Chiari syndrome, [24,25] the above algorithm requires verification in regional-specific cohorts.

From a laboratory perspective, a further two issues require consideration. Some recent evidence suggests that MPN patients with SVT have a significantly lower *JAK2* V617F allele burden (< 10%) than those MPN patients without SVT.[26] Whether this phenomenon is also observed in patients with *CALR*+ SVT remains to be answered. Given the number of methodological approaches available for the detection of *CALR* mutations,[27,28] careful validation and selection of a sensitive technique is required so as not to under-diagnose the potential underlying MPN. Secondly, all the pathologically annotated, MPN-associated *CALR* indel mutations result in a +1 alteration of the reading frame leading to a loss of the terminal calreticulin localization domain. *CALR* mutations in two SVT patients have been reported as being in-frame[19,21]: caution in interpretation is required as these mutations may be of germ-line origin and of uncertain pathogenicity.[29] Sequencing of *CALR* in

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Table 1: Studies reporting the incidence of C*ALR* mutations in patients with splanchnic vein thrombosis.

Reference No.	SVT patients (*n*)	CALR+	SVT site	MPN diagnosis
[10]	209	4 (1.9%)	BCS (*n* = 2), PVT (*n* = 2)	ET (*n* = 3), PMF (*n* = 1)
[11]	144	0 (0%)	-	-
[12]	29	0 (0%)	-	-
[13]	66	1 (1.5%)	Not specified	Not specified
[14]	40	0 (0%)	-	-
[15]	141	1 (0.7%)	PVT	PMF
[16]	132	0 (0%)	-	-
[17]	83	2 (2.4%)	PVT (*n* = 2)	ET (*n* = 1), MPN-U (*n* = 1)
[18]	100	0 (0%)	-	-
[19]	41	2 (4.9%)	BCS (*n* = 2)	ET (*n* = 1), PMF (*n* = 1)
[20]	312*	5 (1.6%)	BCS (*n* = 1), PVT (*n* = 4)	ET (*n* = 1), PMF (*n* = 4)
[21]	210	2 (1.0%)	BCS (*n* = 2)	Not specified
[22]	1	1 (100%)	PVT	ET
[23]	24	2 (8.3%)	BCS (*n* = 2)	Not specified
Total	**1532**	**20 (1.3%)**		

SVT: splanchnic vein thrombosis; MPN: myeloproliferative neoplasm; BCS: Budd Chiari syndrome; PVT: portal vein thrombosis; ET: essential thrombocythemia; PMF: primary myelofibrosis; MPN-U: myeloproliferative neoplasm-unclassified. *Test cohort: J. Poisson personal communication.

patient constitutional material is consequently needed for clarification.

Screening for *CALR* mutations in SVT patients remains a worthwhile endeavor as diagnosis of the underlying MPN is critical for fitting patient treatment. Future work on *CALR* mutation type and allele burden may reveal insights into the pathogenetic mechanisms at play in patients with SVT.

Conflicts of Interest

The author has no conflicts of interest to declare.

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