Clonal hematopoiesis by *DNMT3A* mutations as a common finding in idiopathic splanchnic vein thrombosis

With an incidence of 0.5 cases per 100,000,[1] splanchnic vein thrombosis (SVT) belongs to the rare venous thromboses in unusual sites including portal, splenic and mesenteric veins and Budd-Chiari syndrome.[2] SVT is generally associated with cirrhosis and thrombophilia, or is a marker of occult cancers, in particular myeloproliferative neoplasms (MPN), liver and pancreatic cancers.[3] However, a significant portion of patients (14%) present with idiopathic SVT.[2]

Clonal hematopoiesis of indeterminate potential is a condition characterized by the presence of a clonal cell population, identified by a recognized hematologic neoplasm driver mutation at a variant allele frequency (VAF) above 2%, in the absence of a World Health Organization (WHO)-defined disorder.[4,5] Notably, beside the potential evolution into a hematologic cancer, clonal hematopoiesis (CH) is associated with an increased risk of cardiovascular disease.[6] While studying patients with SVT, we selected 15 consecutive patients with idiopathic SVT, defined as being negative for cirrhosis, abdominal infections or surgical procedures, and cancers (approved by the institutional ethics committee - code #275/2021). The median age of these 15 patients was 52 years (range, 31-75); eight patients were female. After at least 1 year of follow-up, no significant cytopenias or cellular abnormalities in the peripheral blood were observed. To rule out myeloproliferative disorders, a bone marrow biopsy was performed and no WHO-defined blood disorders were identified. However, it is worth noting that the overall cellularity of these samples was slightly increased in a few patients, with a more prominent expansion of the erythroid compartment. In three patients, a tendency to hyperplasia of megakaryocytes and, in one patient, reduced cellular dimension, were observed; these findings were, however, insufficient for a WHO classification as MPN.

A next-generation sequencing-based analysis of bone marrow specimens was performed using Myeloid Solution by Sophia Genetics. This analysis covered the coding regions, splicing junctions (± 25 bp) and internal tandem duplications of 30 genes, namely: *ABL1* (exons 4-9), *ASXL1* (exons 9, 11, 12, 14), *BRAF* (exon 15), *CALR* (exon 9), *CBL* (exons 8, 9), *CEBPA* (all exons), *CSF3R* (all exons), *DNMT3A* (all exons), *ETV6* (all exons), *EZH2* (all exons), *FLT3* (exons 13-15, 20), *HRAS* (exons 2, 3), *IDH1* (exon 4), *IDH2* (exon 4), *JAK2* (all exons), *KIT* (exons 2, 8-11, 13, 17, 18), *KRAS* (exons 2, 3), *MPL* (exon 10), *NPM1* (exons 10, 11), *NRAS* (exons 2, 3), *PTPN11* (exons 3, 7-13), *RUNX1* (all exons), *SETBP1* (exon 4), *SF3B1* (exons 10-16), *SRSF2* (exon 1), *TET2* (all exons), *TP53* (exons 2-11), *U2AF1* (exons 2, 6), *WT1* (exons 6-10), *ZRSR2* (all exons). Thirteen of the 15 patients with idiopathic SVT had mutations in the panel of studied genes (Figure 1A). In 7/15 (46%) patients, the mutational screening was coherent with CH (Table 1, upper part). Three (20%) of the 15 patients had a *JAK2* V617F mutation in accordance with the known association of SVT with MPN. In such patients, it could be postulated that SVT precedes the MPN disorder as a premalignant condition or is a phenotypic manifestation of the CH. In this respect, it is worth noting that *JAK2*, *CALR* and *MPL* are frequently mutated in SVT[7–9] and that *JAK2* V617F mutations are also observed years prior to a diagnosis of MPN, with different expansion kinetics, in relation to the observed VAF.[10] Three (20%) of the 15 patients had mutations in *DNMT3A* (Table 1, upper part). Interestingly, *DNMT3A* frameshift mutations were observed in two patients while in one patient the frameshift mutation was also associated with a missense mutation.

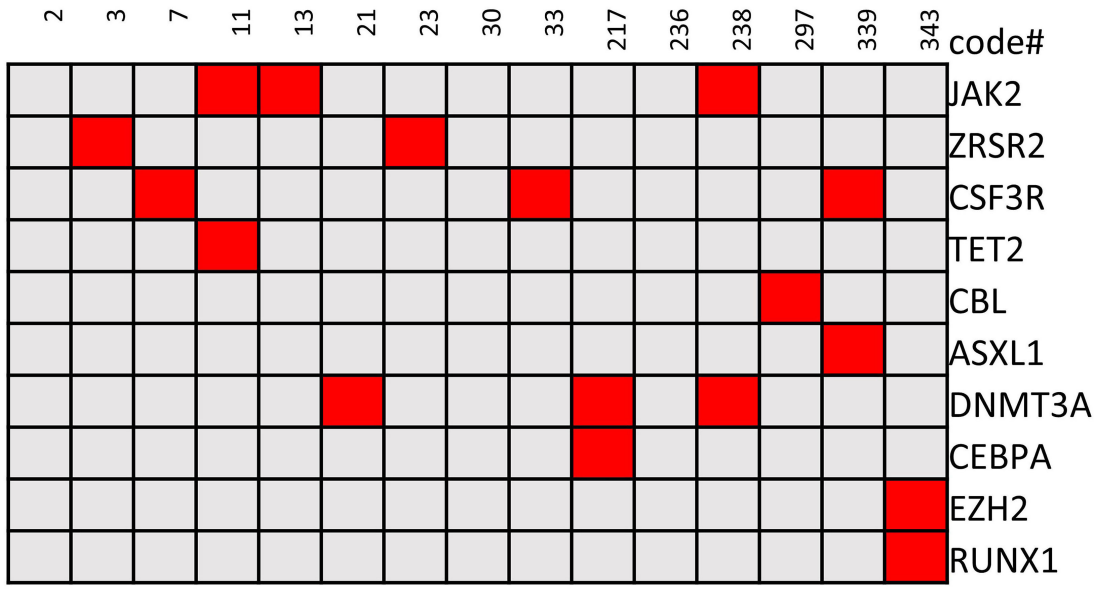

Figure 1. Distribution of mutations in patients with splanchnic vein thrombosis.

Table 1. Mutations and variant allele frequencies in patients with splanchnic vein thrombosis and clonal hematopoiesis.

Gene	Mutation	VAF %
CH-associated mutations		
JAK2	V617F	9, 7, 26
DNMT3A	Leu805 frameshift	2.8
	Tyr660Cys	2.1
	Thr691 frameshift	3
	Arg882Cys	6
EZH2	Glu404del	2
Additional mutations		
ZRSR2	Ser445_Arg451dup	100
	Ser447_Arg448dup	97
CSF3R	Glu405Lys	49
	Val674Met	49
	Glu808Lys	49
CBL	Ile393Thr	50
TET2	Asn488Metfs9	46
Asxl1	Arg302His	48
	Leu542Lysfs6	44
RUNX1	Asn248Thr	40
CEBPA	His195_Pro196dup	49

VAF: variant allele frequency; CH: clonal hematopoiesis.

The different VAF of these two mutations suggests the presence of compound heterozygosity or two distinct subclones, further highlighting the tendency to select for these aberrations in patients with SVT. While *DNMT3A* mutations are associated with MPN/myelodysplastic syndromes,[11] to our knowledge no data have linked *DNMT3A* mutations to thrombosis. Lastly, one patient had a mutation in enhancer of zeste homolog 2 (*EZH2*), which is a histone-lysine N-methyltransferase enzyme.

While the *JAK2* V617F and *DNMT3A* mutations clearly have a pathogenic role in MPN, it is worth noting that other genes are consistently mutated in SVT patients, as reported in Table 1 (lower part), but further investigations are essential to assess the relevance of this observation. Even if found to be cancer-associated mutations, most of these additional aberrations have a VAF of 50% or 100%, suggesting a potential germline involvement. Notably, 2/15 (13%) patients had *ZRSR2* mutations at the same hot spot (between Ser445 and Ser447), which has never been reported in MPN. *ZRSR2* is a minor spliceosome component, which has been associated with the pathogenesis of both myelodysplastic sdynromes and MPN[12,13] and a predisposition to cancer.[14] This observation suggests that *ZRSR2* mutation at the Ser445/Ser447 site could predispose to the development of SVT. Notably, the two patients were male, coherently with the location of the *ZRSR2* gene on the X chromosome. If biologically relevant, it could be speculated that this mutation should be assessed in males with SVT at diagnosis.

Overall, our data suggest that idiopathic SVT is associated with CH, and reveal a potentially new subgroup of patients with *DNMT3A* mutations. Larger cohorts of patients with idiopathic SVT should be assessed to better describe the incidence of CH in this rare condition. The findings should have clinical implications, since the identification of CH in SVT patients should imply closer follow-up over time to check for the potential evolution of the disease into a hematologic cancer, and the need for extended anticoagulation because of the risk of recurrent thrombosis. It would also be informative to model CH in mice to investigate whether CH, including *DNMT3A* mutations, causes SVT as a phenotypic manifestation of a MPN or simply acts as risk factor for thrombosis. Indeed, the existence of "gray zone disease" could be speculated, with a phenotypic presentation resembling that of MPN but still without a clear morphological classification at histology. In this respect, it is worth noting that a distinct MPN, defined as clonal megakaryocyte dysplasia with normal blood values, has been described to be associated with SVT.[15] The integration of genetic analyses, in particular *DNMT3A* mutational status, with clinical and histological assessments could eventually lead to the identification of a novel entity with therapeutic implications.

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Contributions

GC, IR, BM and RP analyzed data; EG performed the next-generation sequencing analyses; SC, GR and EB evaluated patients; DC reviewed the manuscript and data; AM conceived the study and wrote the manuscript.

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Data-sharing statement

Data are available upon request.

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