



Assessment of Prevalence and Risk Factors for Subclinical Splanchnic Thrombosis in Patients with Myelofibrosis

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Background

Splanchnic thrombosis is characteristic feature of MPN-associated prothrombotic state. Unlike ET and PV current prognostic scoring systems for MF do not incorporate parameters for thrombotic risk assessment. Such clinical dissociation may partly be attributed to the inherently worse clinical course of MF than concentrates the focus of disease management on disease-modifying rather than on anti-thrombotic strategies. On the other hand, prevalence of thrombotic episodes in MF may also be underestimated because their occurrence may be masked by worsened disease status. We hypothesized that prevalence of subclinical splanchnic thrombosis is increased in patients with MF and that its occurrence is directly related to Jak2 allele burden, WBC and IPSS.

Objective

To determine the prevalence of subclinical ST in patients with myelofibrosis and assess disease-related and hereditary factors as potential risk predictors.

Materials & Methods

Patients were considered eligible if diagnosed with myelofibrosis according to WHO 2016 criteria (incl. post-PV and post-ET), >18 years, no symptoms of overt ST, and no previous history of thrombosis. 21 subjects were recruited. Patients underwent CT scan angiography to detect the presence of vascular occlusion in the splanchnic circulation. Digital droplet PCR was performed to quantitate JAK2 mutant and wild-type allele burden in copies per nanogram DNA. Hereditary thrombophilia was analyzed for a panel of four genes: f.V(R506Q), f.II(20210G>A), PAI(4G/5G), and MTHFR(677C>T) by RT-PCR. Statistical analysis was performed in R v.4.0.4. All patients provided written informed consent.

Results

Table 1. Clinical characteristics of patients

Characteristics	Value
Age, years	
Mean ± SD	67 ± 10.51
Range	43 -83
Gender, % (n)	
Female	53 (11)
IPSS, % (n)	
Low/int-1	52 (11)
Int-2/high	48 (10)
Ruxolitinib, % (n)	
Yes	57 (12)
HGB g/l	
Mean ± SD	113.14 ± 36.22
Range	58 – 190
WBC G/l	
Mean ± SD	8.94 ± 5.66
Range	1.4 – 23.34
PLT G/l	
Mean ± SD	271.23 ± 162.49
Range	68 - 657
Jak2mut copies/ng DNA	
Mean ± SD	63.69 ± 65.34
Range	0 – 191.62
SVT presence, % (n)	
Yes	33 (7)
No	67 (14)

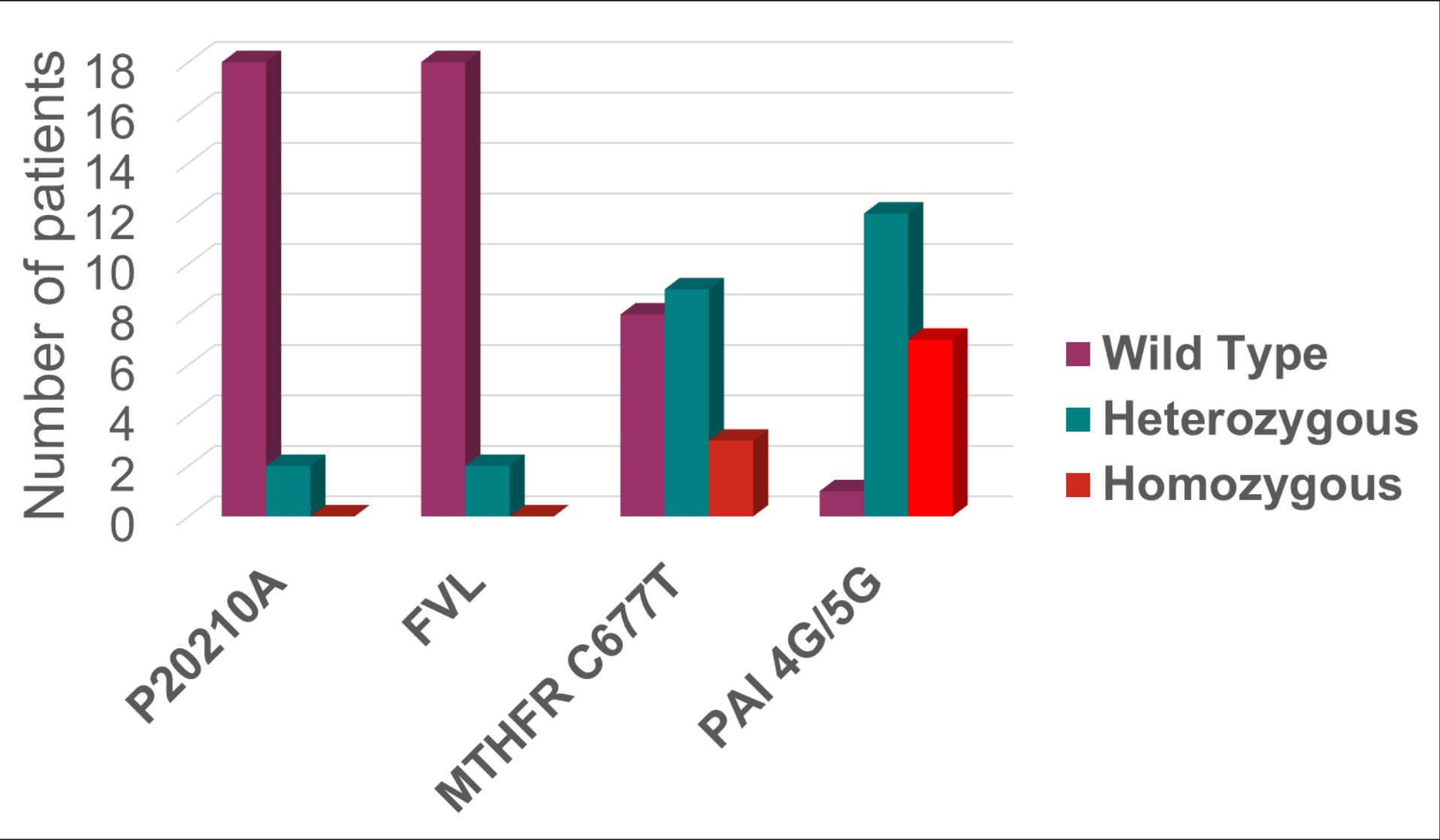


Fig. 1. Distribution of prothrombotic genes carrier state

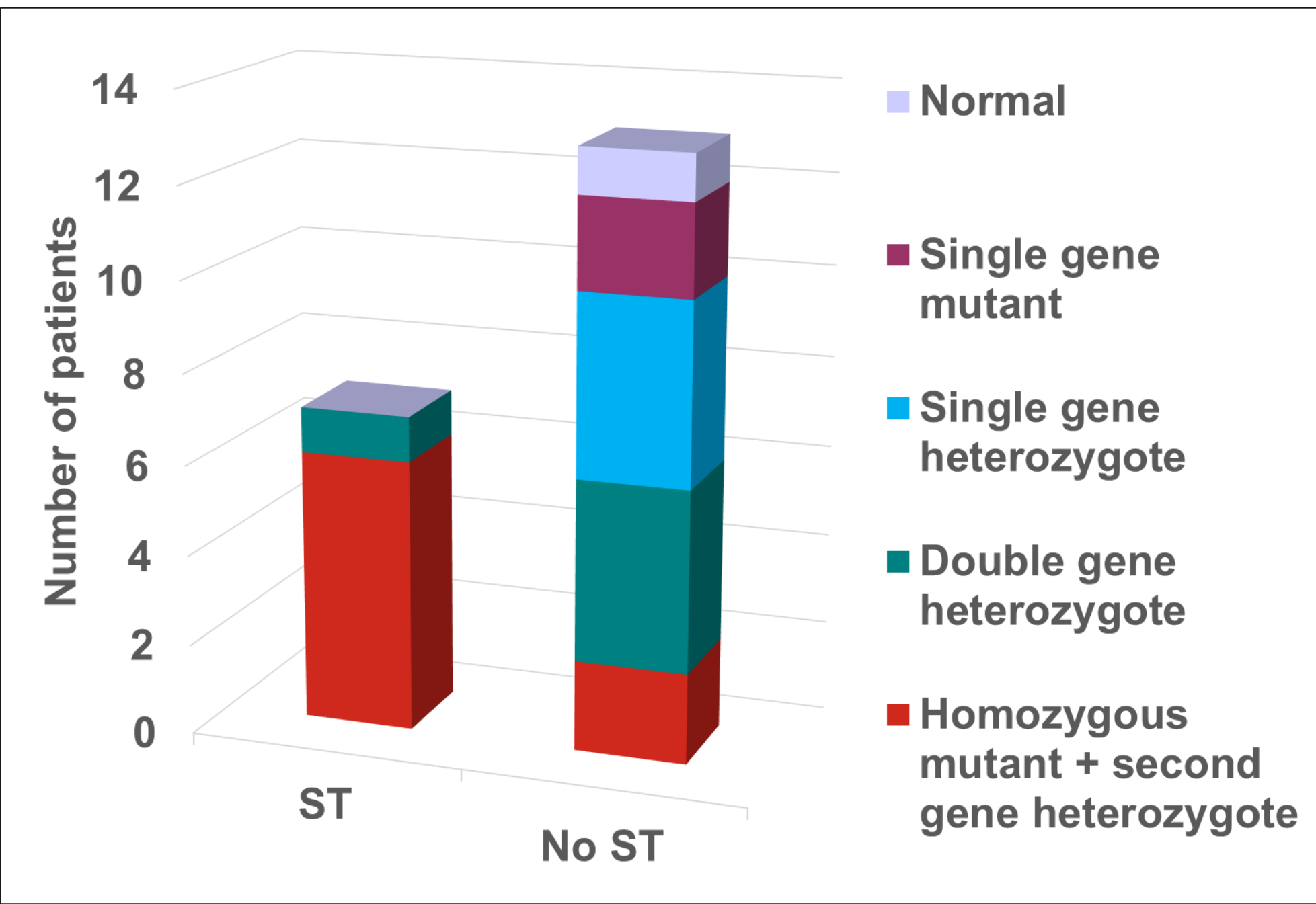


Fig. 2. Prothrombotic carrier state in patients with and without splanchnic thrombosis

Table 2. Univariate Logistic regression for the presence of splanchnic thrombosis

Predictor	Coefficient CI 2.5% ÷ 97.5%	Odds Ratio CI 2.5% ÷ 97.5%
Age, years	-0.01 -0.102 ÷ 0.08	0.98 0.90 ÷ 1.09
Gender Male	1.50 -0.37 ÷ 3.70	4.5 0.68 ÷ 40.69
HGB, g/l	0.006 -0.019 ÷ 0.034	1.007 0.981 ÷ 1.034
WBC, G/l	0.20 0.0169 ÷ 0.463	1.222 1.017 ÷ 1.589
PLT, G/l	-0.0005 -0.007 ÷ 0.005	0.999 0.993 ÷ 1.005
IPSS Low/Int-1	-0.575 -2.50 ÷ 1.256	0.563 0.082 ÷ 3.512
Jak2mut copies/ng DNA	-0.0006 -0.016 ÷ 0.0139	0.999 0.984 ÷ 1.014
Ruxolitinib Yes	0.916 -0.952 ÷ 3.0868	2.5 0.386 ÷ 21.907
Spleen (cm below costal margin)	0.010 -0.159 ÷ 0.182	1.011 0.852 ÷ 1.2
Homozygous mutant + 2 nd gene heterozygote**	3.497 1.219 ÷ 6.755	33 3.387 ÷ 858.62

Reference category for gender is “Female”, for IPSS is “High”, for Ruxolitinib is “No”, for mutant+heterozygote is “No”.

**p-value <0.01

Summary

Primary outcome was presence of thrombosis (arterial and/or venous) in the splanchnic circulation. ST was detected in 7 patients (33%), Table 1.

FV(R506Q) and f.II(20210G>A) were detected in two patients each (9%), PAI(4G/4G) was detected in 10 patients, only one patient was wild-type homozygous, 3 patients were MTHFR(677TT), and 9 were heterozygous, Fig. 1.

6 out of 7 patients with thrombosis were homozygous mutant [either PAI(4G/4G): 5 patients or MTHFR(677TT): 1 patient] and heterozygous for at least one of the other three genes, Fig. 2.

Disease related factors: Jak2mut/ng DNA, WBC, HGB, PLT, spleen size and ruxolitinib as well as age and gender were not found to be significant predictors by univariate logistic regression, Table 2.

Concurrent mutant homozygosity and heterozygous carrier state was significantly predictive of ST and was associated with 33 times greater odds for the presence of ST than single gene polymorphism or double gene heterozygous carrier state, Table 2.

Discussion

Prevalence of subclinical ST in patients with MF is relatively high (33%). We found significant association of splanchnic thrombosis and prothrombotic genes (PAI 4G/5G and MTHFR C677T). We did not find association of ST with any of the studied disease related factors.

Although prevalence of Jak2 mutation among patients with idiopathic ST has been considered risk factor for thrombosis, in this study absolute quantity of Jak2 mutant allele copies were not found to be predictive of ST presence.

Testing for prothrombotic polymorphisms is not recommended in patients with clinically evident ST, because it wouldn’t change therapeutic management . However, prothrombotic genetic factors emerge as potential risk factors for subclinical ST in patients with MF. Further investigation of PAI(4G/5G) and MTHFR(677C>T) screening utility is warranted