PAI-1 4G-4G, MTHFR 677TT, V Leiden 506Q, and Prothrombin 20210A in Splanchnic Vein Thrombosis: Analysis of Individual Patient Data From Three Prospective Studies

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Background: **There are no univocal opinions on the role of genetic thrombophilia on splanchnic vein thrombosis (SVT). We defined genetic thrombophilia the presence of one of these thrombophilic genetic factors (THRGFs): PAI-1 4G-4G, MTHFR 677TT, V Leiden 506Q, and prothrombin 20210A.** ***Objectives:*** **To evaluate the frequencies of these THRGFs in SVT patients, we analyzed individual data of 482 Caucasian patients, recruited from 2000 to 2014 in three prospective studies. SVT was defined as the presence of thrombosis of portal (PVT), mesenteric (MVT), splenic (SPVT), cava (CT), and hepatic vein (Budd Chiari syndrome, BCS). Pre-hepatic SVT (pre-HSVT) was defined as PVT with or without MVT/SPVT, without BCS. Post-hepatic SVT (post-HSVT) was BCS with or without PVT/MVT/SPVT.** ***Methods:*** **We compared 350 patients with liver cirrhosis (LC), 47 hepatocellular carcinoma (HCC), 37 myeloproliferative neoplasm (MPN), 38 associated disease (AD), 10 without any associated disease (WAD), vs 150 healthy controls (HC); 437 patients showed pre-HSVT and 45 post-HSVT.** ***Results:*** **Thrombophilia was present in 294/482 (60.9%) patients: 189/350 LC (54.0%), 31/47 (66.0%) HCC, 29/39 (74.4%) MPN, 35/38 AD (92.1%), and 10/10 (100%) WAD, and 54/150 (36.0%) in HC. In the total group, we found 175 PAI-1 4G-4G, 130 MTHFR 677TT, 42V Leiden 506Q, and 27 prothrombin 20210A; 75 patients showed presence of >1 TRHGF; the more frequent association was PAI-1 4G-4G/MTHFR 677TT, in 36 patients. PAI-1 4G-4G and MTHFR 677TT were significantly more frequent in patients with SVT (*P* values <0.005), whereas V Leiden Q506 and prothrombin G20210A were not. PAI-1 4G-4G and MTHFR 677TT distributions deviated significantly from that expected from a population in Hardy–Weinberg equilibrium. Thrombophilia was significantly less frequent in patients with pre-HSVT (250/437, 57.2%) than in patients with post-HSVT (44/45, 97.8%).** ***Conclusions:*** **Our study shows the significant prevalence of PAI-1 4G-4G and MTHFR 677TT in SVT, mainly in post-HSVT. (J Clin Exp Hepatol 2016;6:10–14)**

There are no univocal opinions on the role of thrombophilic genetic factors (THRGFs) in splanchnic vein thrombosis (SVT); this depends in part on geographical and genetic differences among patients, in part because many studies did not analyze the same THRGFs. We published three articles on the prevalence of PAI-1, MTHFR C677, V Leiden 506Q, and prothrombin 20210A: the first study on liver cirrhosis (LC) and hepatocellular carcinoma (HCC),[1] the second, on abdominal thrombosis in patients without LC or HCC,[2] and the third on a large series of patients with LC.[3] In these studies, PAI-1 4G-4G and MTHFR 677TT were significant risk factors of portal vein thrombosis (PVT) and Budd Chiari syndrome (BCS), in all groups of patients, whereas the role of V Leiden 506Q and prothrombin 20210A was significant, only in subgroups of patients: specifically V Leiden 506Q in BCS, and prothrombin 20210A in HCC patients. To compare the prevalence of these four THRGFs in patients with SVT, studied by our group, from 2000 to 2014, we analyzed individual patient data, from the three studies above described. We show the results below.

Keywords: abdominal thrombosis, hepatocellular carcinoma, cryptogenic cirrhosis, virus related cirrhosis, myeloproliferative neoplasms
Received: 8.09.2015; *Accepted:* 26.11.2015; *Available online:* 6 December 2015
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Abbreviations: AD: associated disease; BCS: Budd Chiarisyndrome; CT: cava thrombosis; HC: healthy controls; HCC: hepatocellular carcinoma; LC: liver cirrhosis; MPN: myeloproliferative neoplasm; MVT: mesenteric vein thrombosis; Pre-HSVT: pre-hepatic SVT (presence of PVT with or without MVT/SPVT, without BCS); Post-HSVT: post-hepatic SVT (BCS with or without other thrombosis sites); PVT: portal vein thrombosis; SPVT: splenic vein thrombosis; SVT: splanchnic vein thrombosis; THRGF: thrombophilic genetic factor; WAD: without any associated disease
http://dx.doi.org/10.1016/j.jceh.2015.11.002

MATERIALS AND METHODS

Patients

We analyzed 482 Caucasian patients with SVT, included into three studies from 2000 to 2014. The first study included 107 patients with LC and 47 patients with

HCC, recruited from January 2000 to December 2007,[1] the second, 85 patients with non-cirrhotic SVT, recruited from January 2005 to June 2011,[2] and the third, 243 patients with LC recruited from June 2008 to January 2014.[3] We built a file with demographic, clinical and genetic data from the individual patients, belonging to the three studies.

Table 1 shows the clinical characteristics of patients, declared in the previous original studies.[1–3] The diagnosis of LC was biopsy proven in patients without ascites and/or esophageal varices (75 in the first and 95 in the third study), and was based on compatible physical signs, laboratory and ultrasound findings in the other patients. For the diagnosis of HCC, we used the criteria of the Italian Association for the Study of the Liver.[4]

In the second study,[2] 85 patients with SVT without LC or HCC, entered into the study. Seventy-five patients showed the presence of some disease or particular clinical status: 37 myeloproliferative neoplasm (MPN) and 38 other associated disease (AD): 12 abdominal surgery, 10 oral contraception or pregnancy, 7 abdominal acute disease, and 9 chronic disease (specifically 3 Crohn's disease, 2 Bechet's syndrome, 1 Gaucher's syndrome, 1 paroxysmal nocturnal hemoglobinuria, 1 hemophagocytic syndrome, 1 nephrotic syndrome). We analyzed these 38 patients all together, as AD patients. Ten patients without associated disease (WAD) constituted the last group.

In the third study, we recruited 243 patients with SVT, of whom 14 with BCS.

All patients underwent to gastroscopy and we registered esophageal variceal size as large-medium/small/absent. Eventual previous bleeding episodes were also registered.

Local human research committee approved the study protocol of the three studies. All patients signed an informed consent, and the study was conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Splanchnic Vein Thrombosis: Diagnosis Criteria

SVT was defined as the presence of one of these localizations: PVT, mesenteric vein thrombosis (MVT), splenic vein thrombosis (SPVT), BCS, and cava thrombosis (CT); moreover we defined pre-hepatic SVT (pre-HSVT), presence of PVT with or without MVT/SPVT and post-hepatic SVT (post-HSVT), presence of BCS, with or without other sites of thrombosis. PVT diagnosis was made, when unambiguous diagnostic evidence (endo luminal material and the absence of flow or presence of cavernous transformation), for extra hepatic PVT was detected by proper imaging techniques (Doppler ultrasound, computerized tomography, or magnetic resonance imaging). BCS was present, when we detected unambiguous evidence for hepatic venous outflow obstruction at any point between the level of the small hepatic veins and the entrance of the inferior vena cava into the right atrium, by proper imaging techniques, as defined above. Presence of MVT, SPVT, and CT was registered.

Thrombophilic Genetic Factors and Definition of Thrombophilia

We performed the genotyping of polymorphisms by polymerase chain reaction-restriction fragment length polymorphism. We analyzed the presence of each TGF mutation in heterozygous and homozygous and their association in patients with SVT and HC.

We defined genetic thrombophilia, when at least one of the following genetic factors was present: PAI-1 4G-4G, MTHFR C677T homozygous, V Leiden Q506 homozygous or heterozygous, prothrombin G20210A homozygous or

Table 1 Main Demographic and Clinical Characteristics in 482 Patients With Splanchnic Vein Thrombosis and Associated Liver Cirrhosis (LC), Hepatocellular Carcinoma (HCC), Myeloproliferative Neoplasm (MPN), Patients With (AD) and Without Associated Disease (WAD).

	LC	HCC	MPN	AD	WAD	Total
No. of patients	350	47	37	38	10	482
Age: median (range)	58 (19–83)	63 (36–83)	53 (21–83)	51 (27–79)	45 (25–72)	57 (19–83)
Male sex	232	35	14	21	6	308
HCV	220	32	3	0	0	255
HBV	53	13	0	0	0	66
Alcohol	45	5	1	0	0	51
>1 etiologic factor	–	5	–	–	–	5
Cryptogenic	83	2	0	0	0	85
Mesenteric and/or splenic vein thrombosis	53	14	15	4	1	87
Esophageal varices: large-medium/small/absent	241/69/40	17/9/21	13/10/14	11/8/19	2/3/5	267/90/125
Patients with previous bleeding episodes	194	11	7	13	2	216