Thrombophilia testing: when is it appropriate and when is it not?

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INTRODUCTION

Thrombophilia testing is a laboratory procedure that is often requested inappropriately to identify risk factors for thrombosis in individuals who have previously experienced venous or arterial thrombosis[1]. It is essential to recognize that this testing may yield negative results for a substantial portion of patients, approximately 50%, who have experienced a thromboembolic event. This occurs because the causes of these events are influenced by many factors that are not detectable through laboratory assessments[2]. Furthermore, thrombophilia testing should not interfere with clinical treatment decisions. It is also essential to emphasize that thrombophilia testing should not be used as a diagnostic tool for thrombosis when the event is merely suspected or not sufficiently documented.

THROMBOPHILIA RISK BIOMARKERS, EITHER ACCEPTED OR NOT

Several biomarkers are recognized as risk factors for thrombosis, either venous or arterial. The majority of them are dedicated to the venous side, but are different in terms of frequency in the general population and severity. Factor V Leiden and the prothrombin mutation G20210A are mild risk factors for venous thromboembolism (VTE); however, together they occur in approximately 7% of the healthy population. In contrast, deficiency of natural anticoagulants, such as antithrombin, protein C, and protein S, is a more substantial risk factor for VTE and venous thrombosis. Still, it occurs in 0.02-0.15% in the general population[3]. In addition, anti-phospholipid antibodies are considered strong risk factors for both venous and arterial thrombosis, particularly when they are detected together[4]. Homocysteine is considered a risk factor for both venous and arterial thrombosis, especially when its plasma levels are significantly elevated[5]. On the other hand, for several genotypes there is a lack of scientific evidence to support their classification as risk factors for thrombosis[6]. Nevertheless, they continue to be requested, so creating unjustified anxiety and inappropriate anticoagulant prophylaxis or therapy. The most abused request is that referred to methylenetetrahydrofolate reductase (MTHFR) polymorphism, which has recently been the subject of a call for action to avoid it being prescribed anymore[7] (**Table I**). Moreover, most of them are not reimbursed by the National Health System, at least in Italy, thus forcing people to spend their own money unnecessarily. In other words, a business network has been built in favor of private laboratories. For several years, we have attempted to counteract this undesirable behavior, but with limited success.

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Table I - *Thrombophilia markers and indications for thrombophilia testing*

Thrombophilia markers	
Accepted	**Not accepted**
• Factor V Leiden • Prothrombin mutation G20210A • Antithrombin • Protein C • Protein S • Homocysteine (high values) • Anti-phospholipid antibodies • Factor VIII:C (acute phase reactant protein) • Non-O blood type group (ancillary risk factor)	• Plasminogen deficiency • High PAI-1 levels • Lipoprotein (a) • FXIII Leu34Val polymorphism • MTHFR C677T and A1298C polymorphisms • High coagulation factor levels (FV, FVII, FX) • Thrombomodulin polymorphisms • ACE polymorphisms • PZ/ZPI polymorphisms • High TAFI plasma levels

Indications for thrombophilia testing	
Conditions	**Thrombophilia testing**
Provoked deep vein thrombosis (surgery, fractures, estrogen-progestin use, immobilization, long travel)	No
Patients with unprovoked deep vein thrombosis	Yes (under 45 years)
Family members with VTE or hereditary thrombophilia	Yes
Before estrogen-progestins	Yes
Multiple pregnancy loss	Yes
Arterial thrombosis	Yes (only anti-phospholipid antibodies and homocysteine)
Retinal venous occlusion	No
Splanchnic vein thrombosis	Yes, and *JAK2*^{V617F}

PAI-1: plasminogen activator inhibitor-1; FXIII: factor XIII; FV: factor V; FVII: factor VII; FX: factor X; ACE: angiotensin-converting enzyme; PI/ZPI: protein Z/protein Z-dependent protease inhibitor; TAFI: thrombin activatable fibrinolysis inhibitor; VTE: venous thromboembolism.

WHO ARE THE CANDIDATES FOR THROMBOPHILIA TESTING, AND WHO ARE NOT?

Table I also presents various indications for whether thrombophilia testing should be offered or not. It is essential to briefly note some key points. Thrombophilia testing is not indicated for provoked VTE[8]. Still, it should be provided to patients under 45 years old who have experienced an unprovoked VTE because it may help to decide the duration of the therapy if a strong risk factor is present[9]. Before assuming oral contraceptives, we believe that searching for factor V Leiden and prothrombin mutation G20210A could help to choose the association with the least thrombotic risk[10]. This is in contrast with all the guidelines, which are against thrombophilia testing before starting oral contraceptives[11]. Still, it should be noted that the thrombotic risk is multiplicative, not additive, especially in women over 35 years old[12]. After multiple pregnancy losses, thrombophilia testing is indicated because searching for thrombophilia may be a tool for increasing the probability of having a live birth. This assessment is based on the Ottilia study[13], a prospective, multicenter cohort study conducted in 12 hospitals across three countries between 2012 and 2019. The odds ratio for pregnancy losses in women with inherited or acquired thrombophilia (mild and severe) without any treatment was 2.9 (95% confidence interval: 1.4-6.1) while a prophylaxis with lower molecular weight heparin showed higher odds of live births (odds ratio=10.6; 95% confidence interval: 5.0-22.3). Splanchnic vein thrombosis is another indication for thrombophilia testing, along with a search for *JAK2*^{V617F} mutation, which is associated with a very high odds ratio for splanchic vein thrombosis[14]. In particular, myeloproliferative diseases, such as polycythemia vera, essential thrombocythemia, and primary myelofibrosis, accounted for 37%, 37%, and 26% of cases, respectively, in a study involving a total of 181 patients with myeloproliferative diseases from 23 European centers[15]. A much more limited thrombophilia testing for arterial thrombosis has been identified, with only anti-phospholipid antibodies and high homocysteine levels found to play a role in the complex pathogenesis

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of the disease[16]. Finally, no thrombophilia testing is to be performed in cases of retinal venous occlusion because the prevalence of the thrombosis risk factors was found to be similar to that in healthy subjects, as reported by Romiti *et al.* in a meta-analysis of 94 studies[17]. To add something new to the field of thrombophilia testing, a study by Crocchiolo *et al.* is published in this issue of *Blood Transfusion*[18]. These authors performed thrombophilia testing on 99 healthy donors of hematopoietic stem cells to be collected for allogenic bone marrow transplantation. Thrombophilia testing included the following biomarkers: plasma levels of functional protein C, free protein S and antithrombin, prothrombin time, partial thromboplastin time, and lupus anticoagulant, but, surprisingly, not anti-cardiolipin anti β_2 glycoprotein IgG antibodies, and homocysteinemia for all potential donors; testing for factor V Leiden and the G20210A mutation of prothrombin was performed only in donors with a first-degree relative with a history of VTE while potential donors with a personal history of prior venous or arterial thromboembolism were excluded. Results showed that no arterial or venous thromboembolic events occurred during mobilization, donation, or follow-up, which lasted a median of 47 months. This report is noteworthy as it emphasizes the concept that no thrombophilia testing is required for hematopoietic cell donors, while an accurate medical history is crucial for these individuals. The authors effectively achieved their objectives. Additionally, these findings corroborate previous research that demonstrated transient neutrophil activation and hemostatic changes in healthy donors, indicating a hypercoagulable state[19]. Importantly, no adverse events related to arterial or venous thrombosis were observed, indicating a temporary condition with no clinical impact.

The Italian Bone Marrow Donor Registry foresees such a screening for all unrelated donors and, since November 2022 (CSR n.231 30 Nov 2022)[20], according to Italian legislation, the same tests must be conducted on all related donors to exclude those with high-risk thrombophilia even in the absence of any prior thrombotic event. On the one hand, all healthy donors should be protected against possible adverse events. However, we have difficulties in accepting that this methodological approach comes without any approved international statement. The Italian Bone Marrow Donor Registry does not represent any global agreement on this topic. In fact, international guidelines do not indicate the performance of thrombophilic screening for all donors, at least to our knowledge. This approach seems to represent defensive medicine which, in general, is a behavior that should be avoided since it does not add anything of value to patients' care, while resulting in a loss of time, anxiety, and waste of resources for the community.

We therefore request that the registry be modified to adopt a more realistic approach for donors of hematopoietic stem cells.

The Authors declare no conflicts of interest.

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