

THROMBOPHILIA SCREENING

Introduction

The regulation of haemostasis

Normally, when a clot occurs, it exactly occurs where it has to be and does not grow more than necessary due to the action of the haemostasis regulation process. This regulation process mainly involves a containment mechanism to ensure that clot will be limited to the injured parts. This process is largely activated by coagulation itself when coagulation activated enzymes will reach intact endothelial cells (protein C system).

Thrombophilia

Thrombophilia is defined as an increased tendency to thromboembolic event due to one or several conditions (not always pathological), inherited or acquired.

Several mechanisms may induce this increase risk:

1. Defective inhibition
 - a. Natural inhibitor's deficiency: Antithrombin, protein C, protein S
 - b. Activated Protein C Resistance: most are induced by F V Leiden, a F V normal for its procoagulant properties but mutated at its main site of cleavage by protein C.
2. Elevated coagulation factors
 - a. Factor II (prothrombin): several mutations, (mainly 20210 G>A mutation, known also as factor II Leiden) in the factor II gene have been associated with an increased level of factor II and risk of TE event.
 - b. Factor VIII: high levels of factor VIII may be congenital (multifactorial process) or acquired. Levels above 150% are considered as a moderate thrombophilia
3. Antiphospholipid syndrome
 - a. Antiphospholipid syndrome (APS) is an auto-immune disease characterized by recurrent venous or arterial thrombosis and/or foetal losses and/or thrombocytopenia associated with typical biological markers. These include persistent lupus anticoagulant (clotting test) and/or elevated levels of antibodies (Elisa) directed against complexes of proteins (mainly β 2 glycoprotein I and/or prothrombin) and anionic phospholipids like cardiolipin. This latter assay is usually known as "anticardiolipin antibodies" or "antiphospholipid antibodies".
4. Vascular wall alteration with loss of endothelial cells function
 - a. Hyperhomocysteinemia Homocysteine is an amino acid formed by the demethylation of methionine. Several inherited or acquired conditions can cause mild ($>15 \mu\text{mole/L}$) to severe ($>100\mu\text{mole/L}$) hyperhomocysteinemia. It

seems to act through alteration of endothelial cells layer alterations which will lose its anticoagulant properties.

The table points out the main characteristics of the common disorders implied in the venous thromboembolic disease (1-5).

The standard screening

The screening is based on the determination of specific elements and a basic exploration of haematology and chemistry to be able to interpret accurately these specific elements.

Preliminary conditions and basic exploration

Due to the high cost of the screening and the frequency of interfering situations (see table), the thrombophilia screening is supposed to be performed after general clinical and biological examination, in patient without any inflammatory process. A basic coagulation exploration will also be performed (Prothrombin Time, Activated Partial Thromboplastin Time, Fibrinogen, Thrombin Time).

Specific elements

Activated Protein C Resistance
Antithrombin
Protein C
Protein S
Lupus anticoagulant.
Anticardiolipin antibodies
Factor VIII
Homocysteine (fasting)
20210 G>A Factor II

For review of the most appropriated methods and risk of interferences, see references 6 and 7.

Other elements

Some laboratories and protocols may propose or request other tests or methods, mainly for research trials. Tight collaboration with haematology and vascular medicine staff is always recommended.

Who to screen ?

Patient with a documented (duplex ultrasonography or phlebography) venous thromboembolic event occurring before 45 years old and one of the following characteristics:

- Recurrent events, mainly in the case of different venous territories
- Spontaneous event
- Minor triggering factor or triggering factor with adequate thrombo-prophylaxis.
- Unusual site: visceral vein thrombosis, cerebral vein thrombosis, upper extremities vein thrombosis.
- Familial history of thromboembolic event
- Skin necrosis at the initiation of AVK treatment.
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Patient with a history of recurrent foetal loss or major obstetrical complications (severe preeclampsia, HELLP syndrome, ..)

Patient not fulfilling the criteria

- A screening limited to potentially acquired elements in older patients, mainly in patients with spontaneous or recurrent events.
- A screening limited to frequent elements in young patients with TE event related to clear cut triggering factor.
- Patients with unusual site thrombosis (Budd –Chiari,...) are frequently enrolled in clinical trials that provide precise guidelines.
- Patient with arterial thrombosis: patient with a documented thromboembolic event occurring before 45 years old and one of the following characteristics:
 - Absence of arteriosclerosis
 - Recurrent event despite adequate prophylactic treatment
 - History of familial venous thrombosis

When to screen?

There is a general consensus that the most appropriate moment to perform the thrombophilia screening is at the end of the anticoagulant treatment (usually one month later). The acute thrombosis period is not appropriate since activation of coagulation due to the thromboembolic event may induce perturbation of coagulation with false positive or negative results.

However, in some circumstances, some tests will be performed at diagnosis, in tight collaboration with a specialist of thrombophilia and laboratory staff:

- Lupus anticoagulant and factors evaluation in case of abnormal APTT
- Protein C, protein S and APCR in case of skin necrosis
- Antithrombin in case of unusual site of thrombosis or young patient.

As most of the analysis are not influenced by AVK therapy, the screening is sometimes initiated during or near the end of anticoagulation (see table).

Psychological aspects of thrombophilia screening

A severe thromboembolic event will frequently induce some psychological impact. In most of the case the patient wishes a rational explanation of his problem. However, the thrombophilia screening may induce stress and anxiety and so should only be done after an informed consent (8, 9).

The familial screening

A familial screening is to be considered in case of a characterized genetic abnormality inducing a clear cut thrombophilia. The interest of the step is evident in the case of a severe genetic thrombophilia like Antithrombin deficiency. To the opposite, the interest is far less evident in case of a mild thrombophilia like factor II Leiden.

Even more than for patient's screening, risk to induce stress, anxiety, culpability or overprotection reactions (children) are to be taken into account. So the procedure should only

be initiated after full explanations on the pathology, the nature of the transmission, the interest of the knowledge, the appropriate conditions and the exact performance in term of specificity and sensitivity of the test to use. In consequences, this screening is to perform in tight collaboration with an haemostasis specialist.

Common thrombophilic disorders

	Thrombophilia associated with	% in Caucasian general population	% in VTE patients	Relative risk of VTE (case control studies)	Do not perform if	Interpretation's restriction
Antithrombin	Low level *	0.02-0.16	0.5-4.9	20 *		UFH/LMWH treatment (10 days) , Oestrogen (1 month)
Protein C	Low level *	0.14-0.5	1.4-8.6	6.5 *	AVK treatment stopped since less than 2 weeks	APCR, lupus anticoagulant
Protein S	Low level *	0.1	14-7.5	5.0 *	AVK treatment stopped since less than 4 weeks	APCR, lupus anticoagulant, oestrogen (1 month), pregnancy
APC resistance	Low ratio					Lupus anticoagulant, inflammatory syndrome, pregnancy.
F V Leiden	Hetero- or homozygous	4.8	18.8 - 28.8	Heteroz: 5 –10 Homoz: 50-80		
F II Leiden	Hetero- or homozygous	2	7.1	2 - 4		
F VIII	Increase > 150% *		25	4.8 *	Inflammatory syndrome, UFH/LMWH treatment	Oestrogen (1 month)
Lupus anticoagulant	Present and persistent	2	4		UFH/LMWH treatment	AVK treatment, coagulation activation (recent TE, pregnancy), recent infectious syndrome, pregnancy.
Anticardiolipin antibodies	Present and persistent, mainly IgG					recent infectious syndrome.
Homocystein	Increase *	5	11	2.95 *	Not fasting	Unusual diet

UHF: unfractionated heparin LMWH: Low molecular weight heparin

* risk is proportional to the degree of abnormality

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