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Congress on Thrombosis

The perspective of a clinical-laboratory approach

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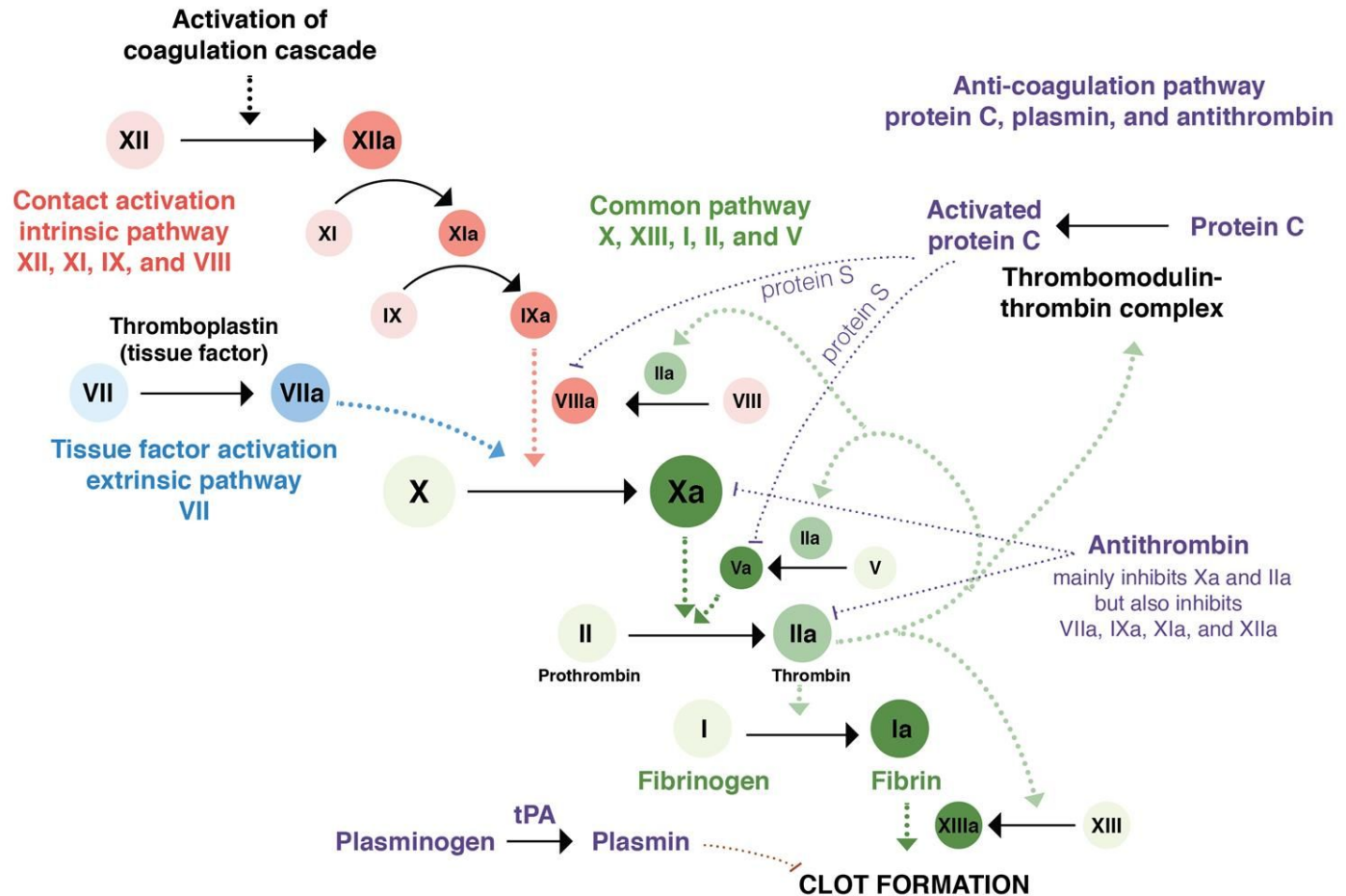
Declaration of Conflict Of Interest

I have no potential conflict of interest to report



How to study alterations in haemostasis?

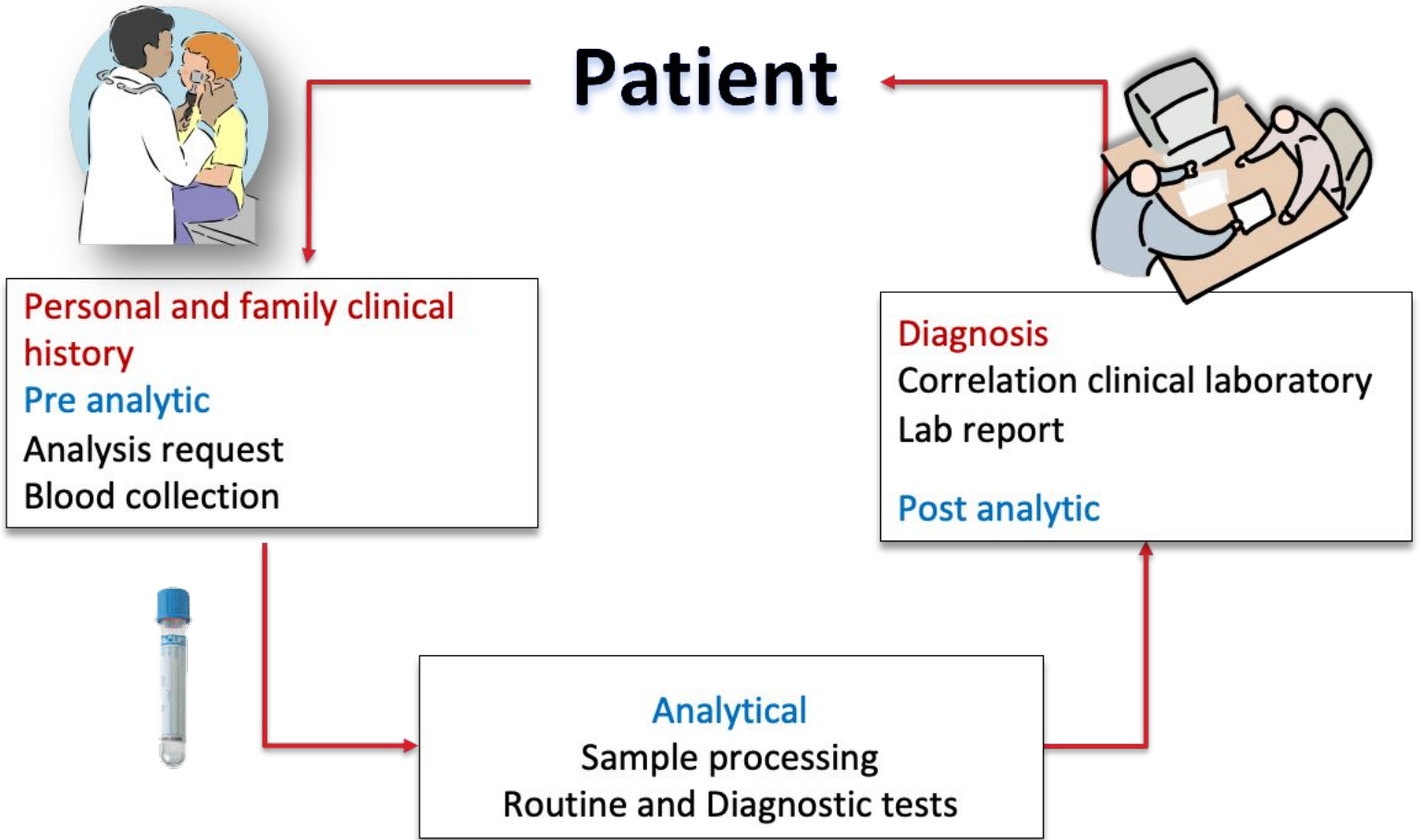
The perspective of a clinical-laboratory approach



Mannucci, P. M., & Franchini, M. (2015). Classic thrombophilic gene variants. *Thrombosis and Haemostasis*, 114(5), 885–889.

Diagnosis strategy

Clinical-Laboratory Concept
Clinic multidisciplinary team



THE IMPORTANCE OF PRE-ANALYTICAL

Category	Variables
Sample collection	Collection tube Patient identification on specimen labeling Phlebotomy Technique Sample volume (see haematocrit)
Sample treatment	Storage Mixing & centrifuging Transport conditions and transport delays

Haemostasis diagnosis is not possible with samples taken under poor conditions!

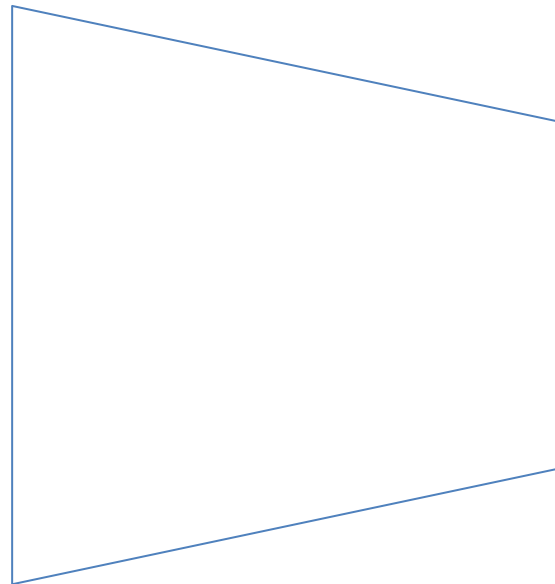
Lippi G and Favaloro EJ. Preanalytical Issues in Hemostasis and Thrombosis Testing. Methods Mol Biol. 2017; 1646: 29-42.

THE IMPORTANCE OF PRE-ANALYTICAL

From sample to quality control

- Sample Collection
- Main Pre-Analytical Variables
- Laboratory Equipment and Laboratory Safety
- Preparation of Normal Plasma Pool (PNP)
- Establishment of Normal Reference Ranges & Reference Times
- Adjust normal values to patient age
- Internal Quality Control and External Quality Control

DIAGNOSTIC STRATEGY



Routine testing

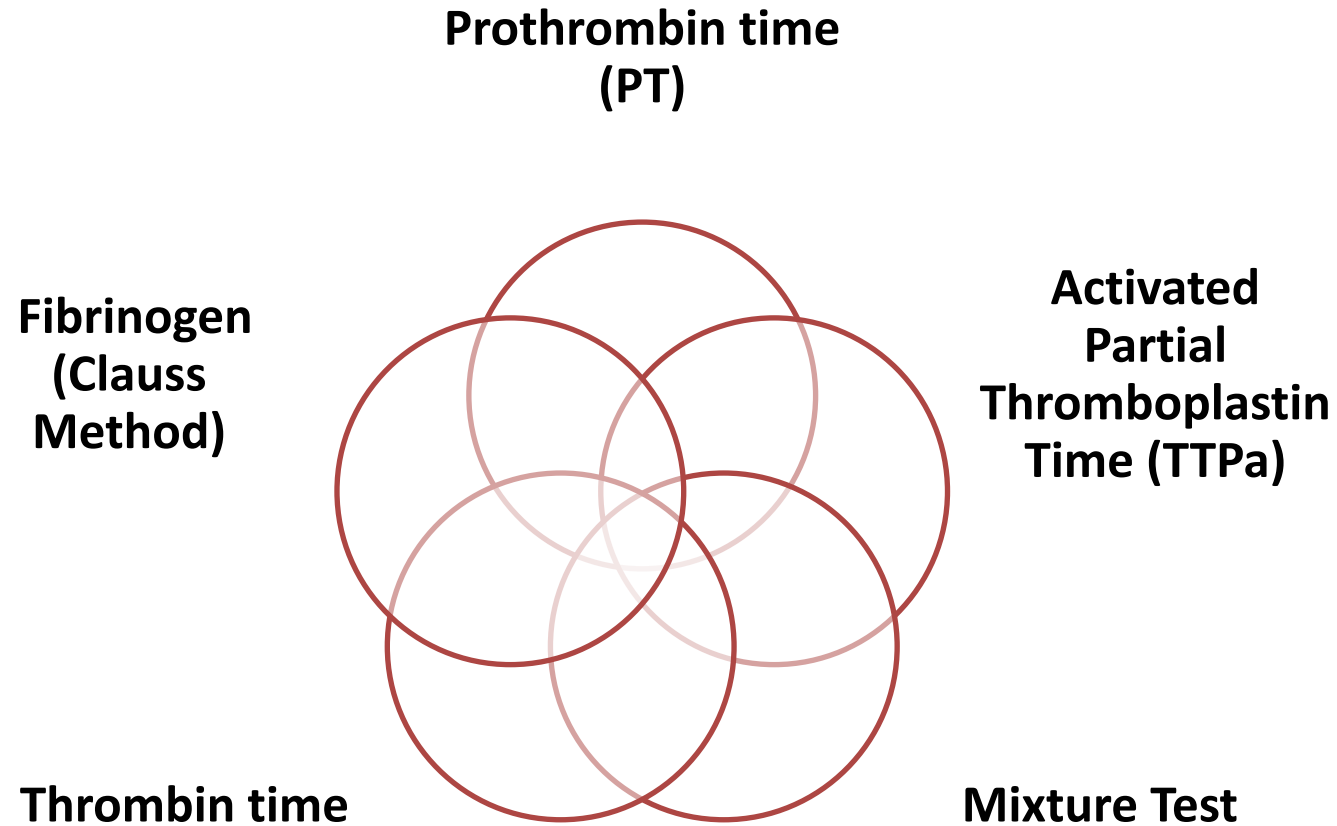
- Study guide
- *when tests are not normal*
- Directing to specific tests

Specific tests

- Identify possible deficiencies or abnormalities
- Detect less common causes for which screening tests are insensitive

Information on Routine Testing

Study orientation



Haemorrhagic Investigation

Specific Tests

Coagulant method

(based on screening test evaluating the respective factor)

- TP (FVII, FX, FV, FII)
- TTP (FVIII, FIX, FXI, FXII)

Chromogenic substrates

(based on enzymatic reactions using chromogenic substrates)

- FVIII
- FXIII
- Alpha-2 antiplasmin
- PAI-1 (Plasminogen activator inhibitor type 1)

Elisa techniques

- FVW

Using these tests in well-defined study algorithms we arrive at a suspected diagnosis

About routine tests



There is no single routine test that allows an assessment of haemostasis



Analytical determinations should be preceded by clinical information or evaluation.



Correlate the evaluation of haemostasis with other tests (e.g. assessment of liver and/or renal function).



Not all haemorrhagic or thrombotic processes result in changes in coagulation tests



Some coagulation abnormalities have no clinical significance



About Specific Tests



If in doubt about the results, **repeat the tests on a 2nd sample** (collected for coagulation only)

Integrated results with the clinical data → save **response time**

- Important in pre-surgery, etc.

**These rules lead to a
Faster and more accurate
diagnosis**

MILD THROMBOPHILIA

Antithrombin, Protein C and Protein S deficiency
Heterozygotes FV Leiden or PRT20210GA

HIGH-RISK THROMBOPHILIA

Homozygotes or multiple defects

Conditions to be investigated

- Antithrombin deficiency
- Protein C deficiency
- Protein S deficiency
- Factor V Leiden
- Variant PT20210G/A
- Homocysteine
- Lupus Anticoagulant

Recommended methodology

- Determination Activity (chromogenic)
- Determination Activity (chromogenic)
- Free antigen determination
- Variant screening
- Variant screening
- Determination by immunoassay
- There is no test of preference

Laboratory Diagnostic of Thrombophilia

How important is it?

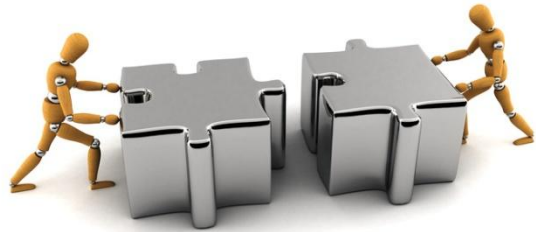
- Determine the pathological basis of the thrombotic episode
- Determine the duration and intensity of AO therapy
- Alert patients to the presence of hereditary risk factors
 - Patient information
 - Genetic counselling
- Prophylaxis in high-risk individuals in situations of increased stimulus
- More specific therapy, when available



HAEMOSTASIS STUDY

Functional Studies

diagnostic orientation



Molecular Studies

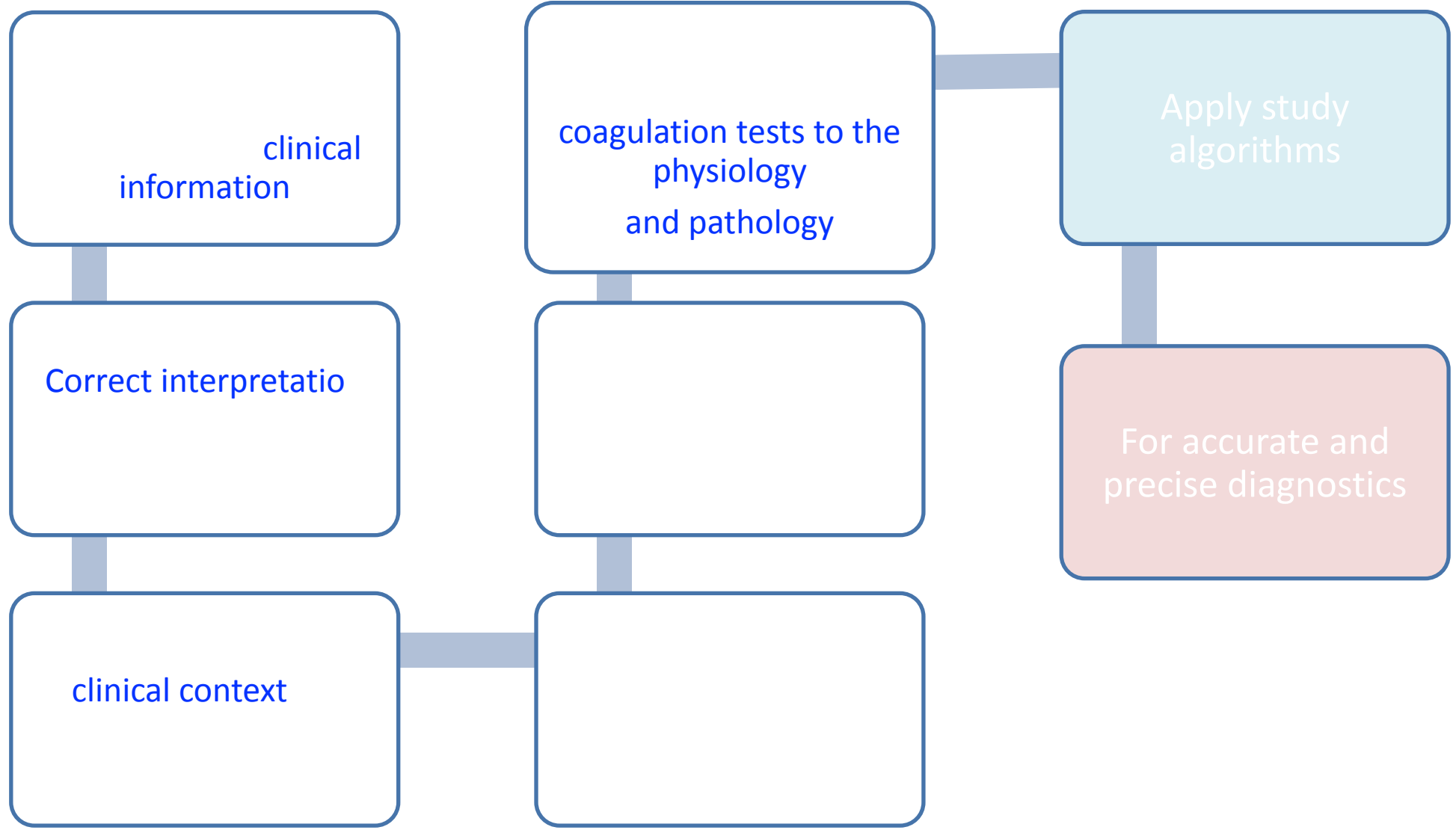
Confirmation of deficiency or deficiencies

NGS (*great resource economy, faster and more effective response*)

that allows the observation of:

- phenotypic variability in individuals with the same genotype
- the low penetrance
- analysis of variants against affected protein domains

The perspective of a clinical-laboratory approach



Clinical data:

- ♀ , 3 months
- Episode of severe neonatal thrombosis (*arterial cerebral thrombosis*)
- Ischaemia with necrosis of three fingers of the right hand

This clinical case because it explains the importance of reliable analytical results and the applicability of the clinical-laboratory concept.

Functional studies (*Acute phase*)

- Antithrombin N, Protein S N, Protein C slightly decreased

What molecular studies should be done?

bjh guideline

Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis

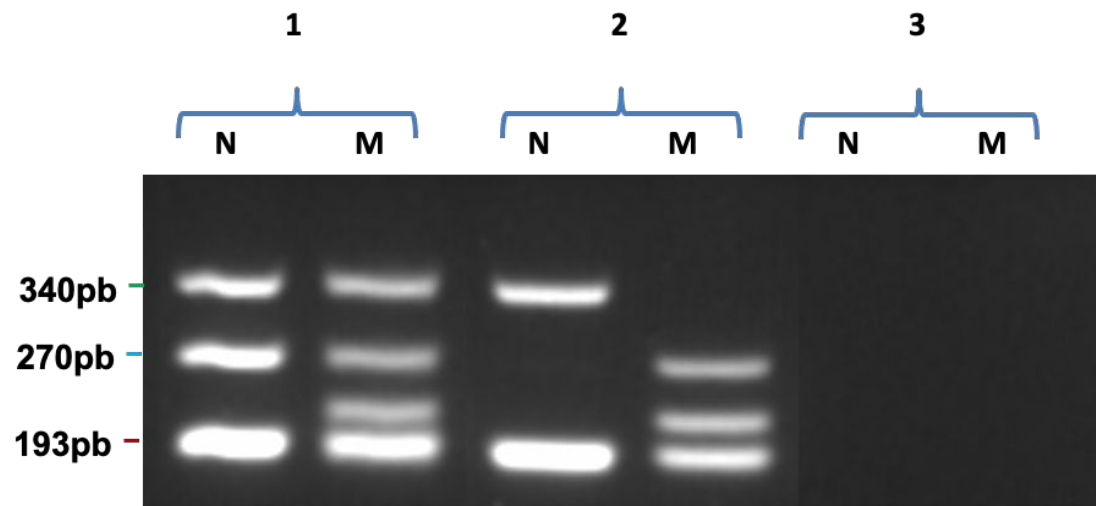
Investigation of a thrombotic tendency

The choice of tests for inherited thrombophilia is described in a previous BSH guideline and may include assays of protein C, protein S and antithrombin, plus tests for the *F2* G20210A (c.*97G>A, prothrombin) and *F5* G1691A [c.1601G>A, factor V Leiden (FVL)] variants.¹⁰³ As when

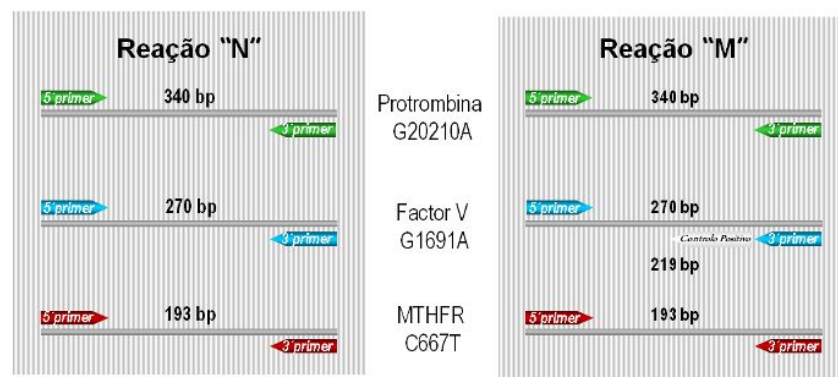
1

Thrombophilia screening - *Factor V Leiden and prothrombin G20210A*

Factor V Leiden and prothrombin G20210A Multiplex Allele-Specific PCR



1. Positive Control
2. **Sample under study**
FV LEIDEN HOMOZYGOUS
3. Negative Control



(Adaptado de Hessner, J. M. *et al.*, 1999)

Clinical case

Clinical data:

- ♀, 3 months
- Episode of severe neonatal thrombosis (*arterial cerebral thrombosis*)
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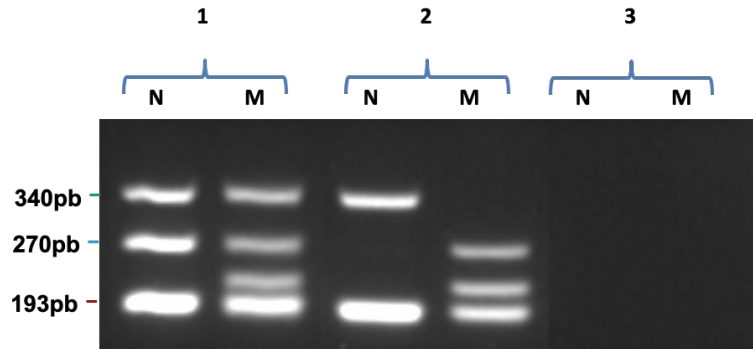
Functional studies (*Acute phase*)

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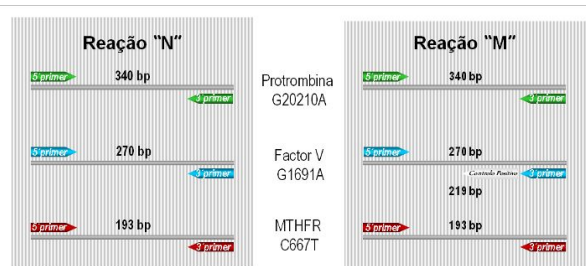
What molecular studies to do next?

**2****Molecular study - PROC gene**

Factor V Leiden and prothrombin G20210A Multiplex Allele-Specific PCR



1. Positive Control
2. **Sample under study**
FV LEIDEN HOMOZYGOUS
3. Negative Control



(Adaptado de Hessner, J. M. et al., 1999)

DNA Sanger sequencing - PROC gene



Exon 9, c.1332G>C (p.Trp444Cys), **heterozygous**

Co-inheritance: homozygous FV Leiden + heterozygous *PROC* pathogenic variant p.Trp444Cys

Clinical case

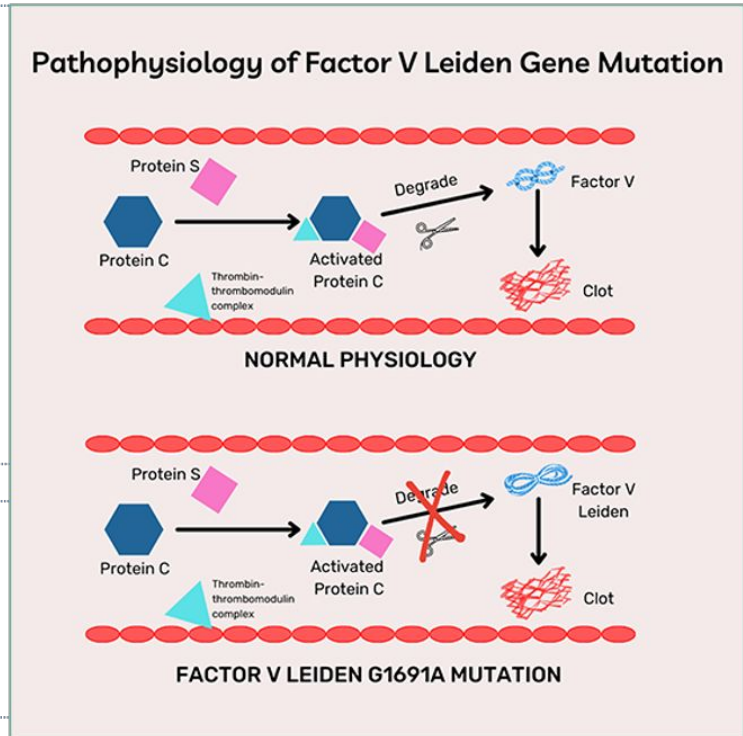
Phenotype

Clinical data:

- ♀, 3 months
- Episode of severe neonatal thrombosis (*arterial cerebral thrombosis*)
- **Ischaemia with necrosis of three fingers of the right hand**

Functional studies (*Acute phase*)

- Antithrombin N, Protein S N, **Protein C decreased**



Genotype

Co-inheritance: homozygous FV Leiden + heterozygous *PROC* patogenic variant (p.Trp444Cys)

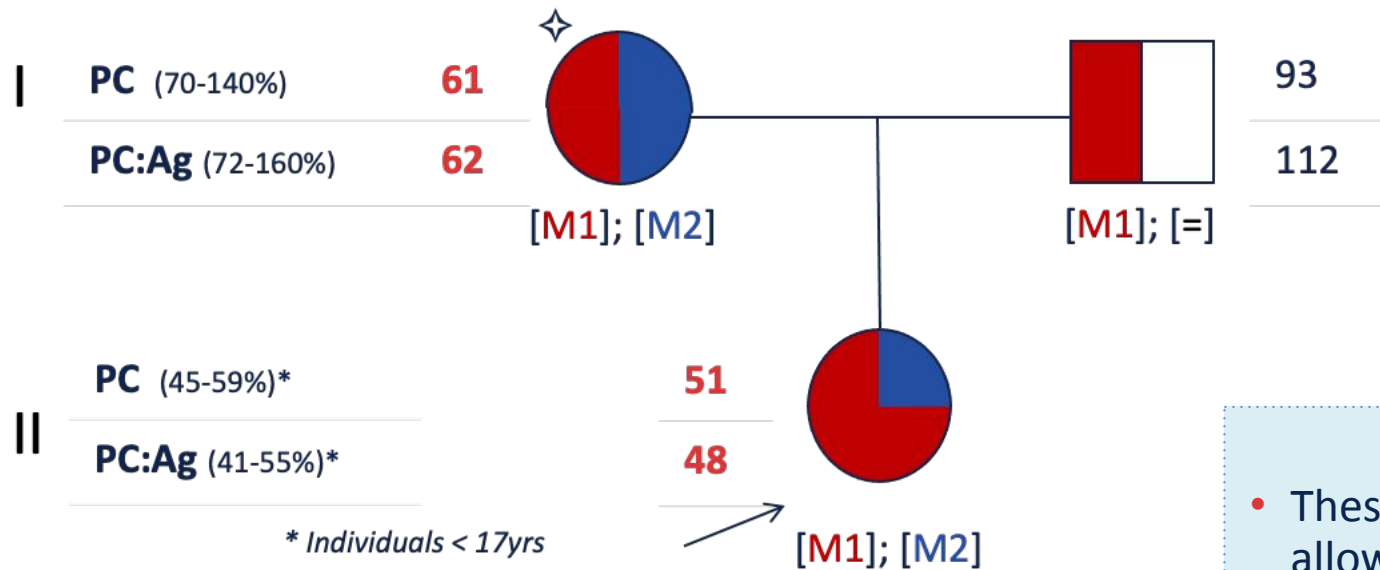
Synergistic effect on the protein C pathway

3

Family study

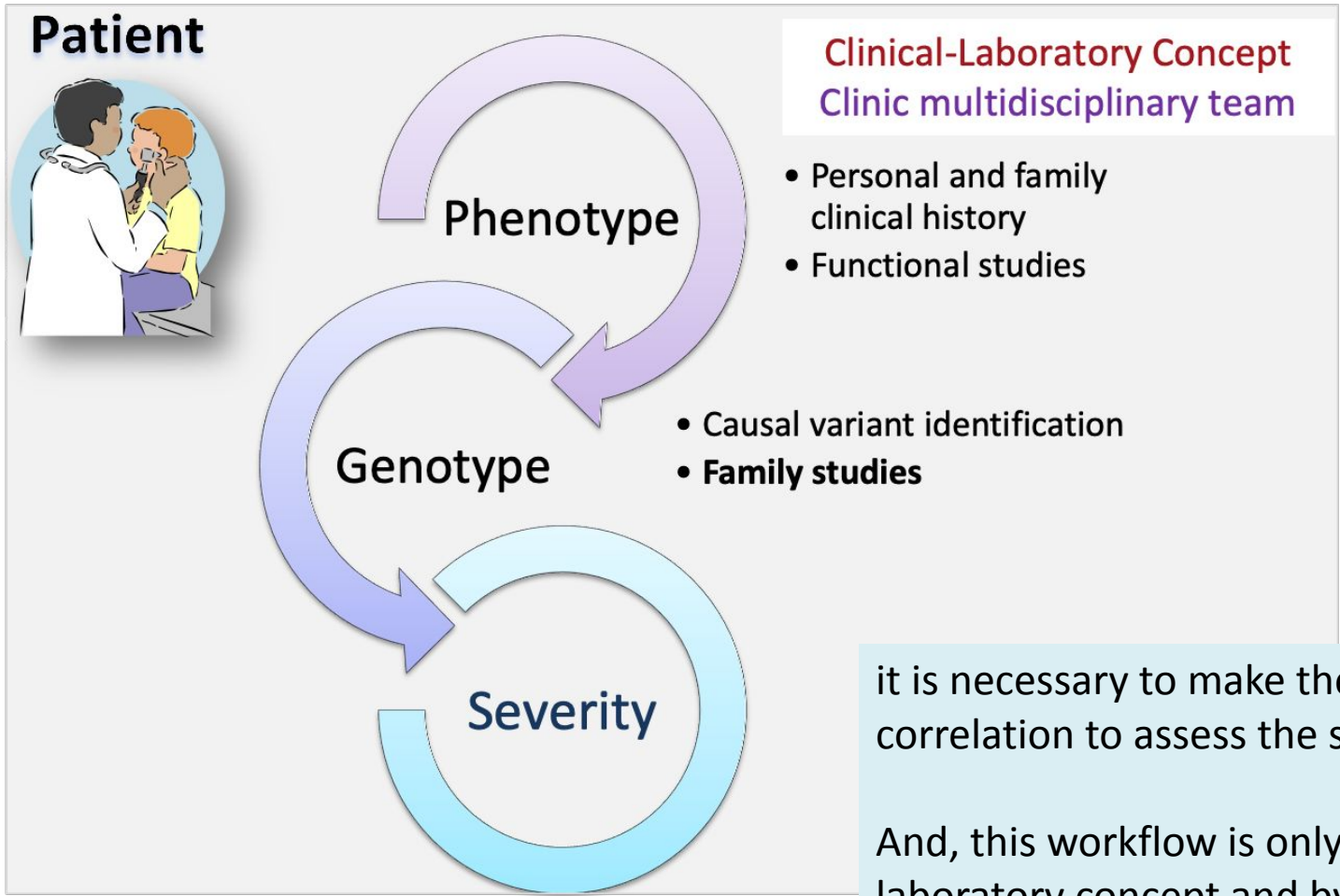
3 Family study

M1: *F5*, NM_000130.5: c.1601A>G, p.Arg534Gln (FV Leiden)
 M2: *PROC*, NM_000312.4: c.1332G>C, p.Trp444Cys



- These genetic family studies allowed the **identification of the unknown carriers and individuals at a higher thrombotic risk within each family**
- permitting the evaluation of the need for prophylactic measures,

The perspective of a clinical-laboratory approach



it is necessary to make the genotype-phenotype correlation to assess the severity in each patient.

And, this workflow is only possible in a clinical laboratory concept and by a multidisciplinary team

Thank you for your attention!



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