The future of laboratory diagnosis of thrombophilia

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ECAT Participants' Meeting 2008

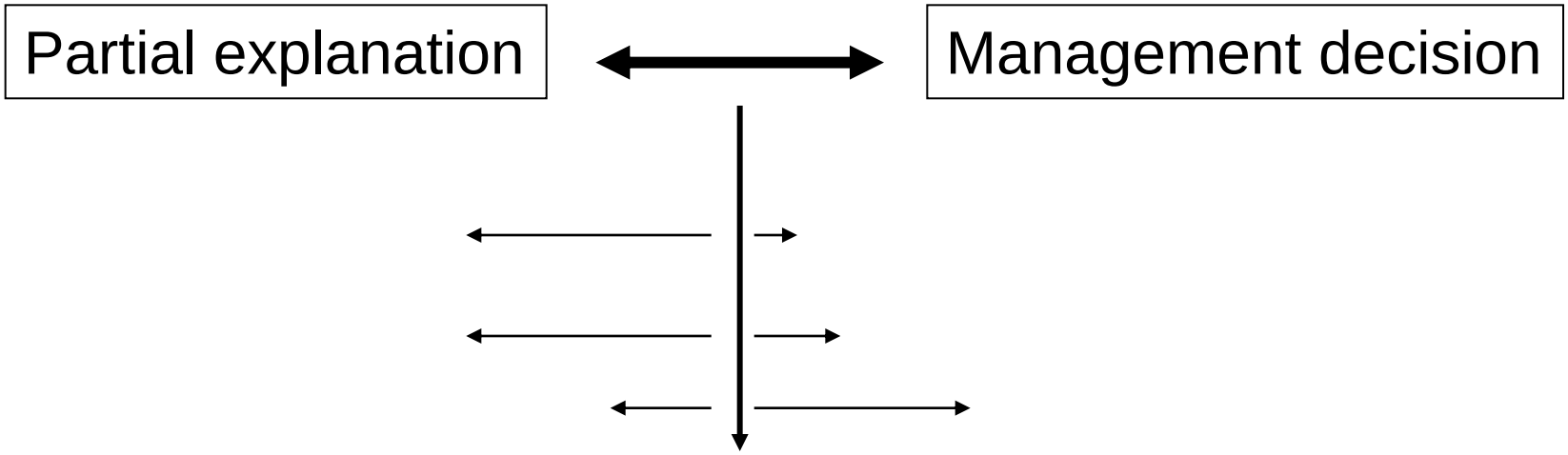
Thrombophilia

- Tendency to develop (venous) thrombosis
- Genetic and non-genetic factors (and their interactions) contribute to this tendency
- Some of these factors can be diagnosed in the laboratory (**thrombophilia screening**)

Thrombophilia screening

- Why do we test?
- Who should be tested?
- What to test for?
- Which tests need to be done?
- When to perform the tests?

Thrombophilia screening: why?



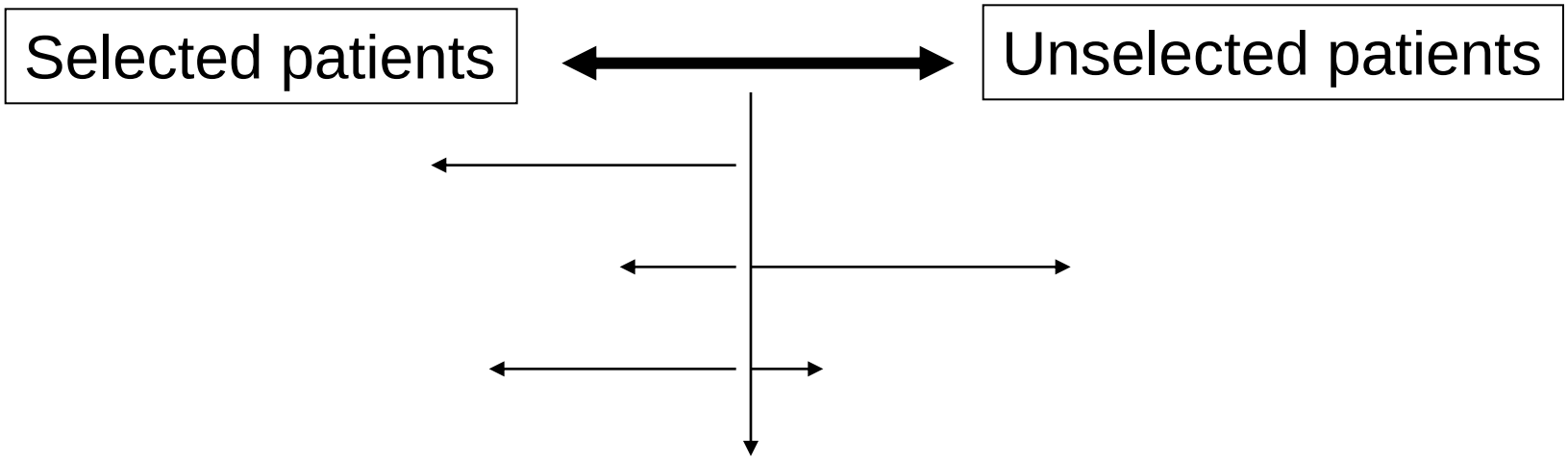
Why do we test?

- To improve treatment
  - prevention of recurrent events
  - prevention of posttrombotic syndrome (PTS)

- To prevent thrombotic events by adequate counseling and prophylaxis in risk situations

- To inform the patient and the doctor.

Who should be tested?



Who should be tested?

<1994

- Selected patients with thrombophilia
  - Young patients
  - Positive family history
  - Recurrent (idiopathic) events
  - Thrombosis at unusual site
  - Coumarin induced skin necrosis
  - Neonatal purpura fulminans

- Family members of patients with a thrombophilic defect (deficiencies of antithrombin, protein C and protein S)

Screenings program (<1994)

- Antithrombin defciency
- Protein C deficiency
- Protein S deficiency

- Dysfibrinogenemia

- Lupus anticoagulant
- Anticardiolipin antibodies

Who should be tested?

>1994

Criteria have become less restrictive due to the finding of novel genetic risk factors for venous thrombosis that are common in the population

Screenings program (>1994)

- Antithrombin defciency
- Protein C deficiency
- Protein S deficiency

- Dysfibrinogenemia

- Lupus anticoagulant
- Anticardiolipin antibodies

- APC resistance
- Factor V Leiden
- Prothrombin 20210A

Thrombophilia screening (>1994)

- Initially less restrictive patient selection

Hypercoagulability: Too Many Tests, Too Much Conflicting Data

Kenneth A. Bauer, Frits R. Rosendaal, and John A. Heit

It is now possible to identify hereditary and acquired risk factors in a substantial percentage of patients presenting with a venous thrombotic event. The clinician is faced with an ever-growing number of laboratory tests that can be ordered in such patients, and there is considerable uncertainty as to how this information should be utilized in patient management. Some have argued that widespread testing of thrombosis patients for prothrombotic abnormalities such as the factor V Leiden and prothrombin G20210A mutations has been prematurely adopted into clinical practice as there are few data that their identification leads to improved clinical outcomes.

Dr. Rosendaal provides an overview of the epidemiology of venous thrombosis with an emphasis on hereditary and acquired risk factors. The presentation will include information obtained from properly designed case-control studies as well as family studies.

While some have suggested treatment strategies for managing patients with hereditary thrombophilia with prior thrombotic events or for managing patients undergoing procedures associated with increased thrombotic risk, clinical decision making is complicated by the need to assess the risk of recurrence and the likely benefit of prolonged anticoagulation versus the associated bleeding risk. Drs. Bauer, Heit, and Rosendaal discuss their approaches to patient management. Case presentations are used to illustrate the impact of laboratory test results on decisions.

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Thrombophilia screening (>1994)

- Initially less restrictive patient selection

- Development of guidelines

Who should be tested?

Only those individuals/patients where it is expected that the outcome of the test will influence the management decision of the physician using evidence based guidelines (balance of risks)

Thrombophilia screening (>1994)

- Initially less restrictive patient selection
- Development of guidelines
- Many novel (genetic) risk factors for venous thrombosis were identified and not included in the thrombophilia screen

Novel risk factors for first VTE

Common plasma phenotypes

	OR	(95% CI)
FVIII>P90	2.8	(2.0-4.2)
FIX >P90	2.3	(1.6-3.5)
FXI >P90	2.2	(1.5-3.2)
Fbg >P90	2.1	(1.4-3.1)
FII >P90	1.9	(1.2-2.8)

Leiden Thrombophilia Study

Novel risk factors for first VTE

Other plasma phenotypes

Anti beta 2 glycoprotein antibodies

Elevated plasma homocysteine

APC-resistance (non-factor V Leiden)

Novel risk factors for first VTE

Novel genetic risk factors?

	P(%)	RR
Blood group A1/B	55	2
MTHFR-677TT	10	1.2
F5-HR2	7	1.2
PROC-CCGG	19	1.3

Novel risk factors for first VTE

And many more risk alleles….

	P(%)	OR
FGG-10034TT	5	2.0
IL1RN-H5H5	1.5	3.9
……	…..	…..
……	…..	…..
……	…..	…..
CYP4V2 (rs 134146272)	42	1.5
SERPINC1 (rs 2227589)	18	1.34

Novel risk factors for VTE

Different risk alleles in non-Caucasians…

Factor V R485K: mild APC-resistance
China: 485K allele: 42%

G-33A in Thrombomodulin gene: ↓TM?
Asia: -33A allele: 7.5%

Protein S K155E: (mild) PS deficiency
Japan: 155E allele 0.75%

Protein C R147W: PC deficiency
Taiwan: 147W allele 0.75%

Thrombophilia screening (>1994)

- Patient selection less restrictive
- Development of guidelines
- Many novel (genetic) risk factors for venous thrombosis were identified and not included in the thrombophilia screen
- External quality assessment schemes

Questions for the future

- Who should be tested and why?

- What tests need to be done?

- Do we have sufficient guidelines?
- Do we use these guidelines?

Who should be tested and why?

- Prevention of a first thrombotic event
- Prevention of a recurrent event
- Explanation of the thrombophilia

Aim: prevention of first event

- Screening of selected populations for FV Leiden or Prothrombin 20210A seems not to be cost effective
  - Before surgery
  - Before use of oral contraceptives
  - Before use of hormone replacement therapy ?
  - Before pregnancy
- Screening of asymptomatic individuals for private mutations in thrombophilia families?
- Identify the remaining risk factors for a first event

Aim: prevention of a recurrency

Most of the known (genetic) risk factors for a 1st VTE seem not to influence the risk for a second event in adults

(*De Stefano et al, NEJM 1999*; *Christiansen et al, JAMA 2005*)

More data are needed to assess the effect of

- Deficiencies of Protein C, Protein S and Antithrombin
- Combined defects (including homozygous FVL and PT20210A)

Identify risk factors for a recurrent event

Risk factors for recurrent events

Elevated FVIII?

Hyperhomocysteinemia?

Clinical risk factors associated with the 1st episode (e.g., idiopathic/ provoked)

Post-treatment residual thrombus

Gender

Post treatment D-dimer or APTT?

Aim: explanation thrombophilia

- Young patients
- Positive family history
- Recurrent (idiopathic) events
- Thrombosis at unusual site
- Coumarin induced skin necrosis
- Neonatal purpura fulminans

Probandi of thrombophilia families (n=211)

- FVL 36.5%
- PT20210A 6.6%
- PC deficient 5.1%
- PS deficient (I) 7.6%
- PS deficient (III) 10.5%
- AT deficient 4.0%
- No defect 41.3%

Non O blood group 83%

- No defect 41.3%
- One defect 51.2%
- Two defects 6.4%
- Three defects 1.2%

Questions for the future

- Who should be tested and why?
- What tests need to be done?
- Do we have sufficient guidelines?
- Do we use these guidelines?

Which tests?

- Prevention of a first event

  Private mutations or complete screen in asymtomatic relatives of thrombophiliacs

- Prevention of a recurrency
  - PC, PS, AT…….?
  - D-dimer, APTT, thrombin generation?

- Explanation (familial) thrombophilia
  - Complete screen

Which tests?

- PT, APTT and thrombin time

- Protein C activity (> antigen)
- Protein S activity or free antigen (> total antigen)
- Antithrombin activity (> antigen)

- Modified APC resistance (> FVL genotype)

- Prothrombin 20210 A genotype

- Lupus anticoagulant

Additional tests?

- APC resistance (non Factor V Leiden)
  - APTT-based
  - ETP- based
- FVIII, FIX, FXI, fibrinogen
- Homocysteine

- Blood group (phenotype or genotype)

- Other polymorphisms reported to be associated with venous thrombosis risk

Questions for the future

- Who should be tested and why?
- What tests need to be done?
- Do we have sufficient guidelines?
- Do we use these guidelines?

Problems

Diagnosis Protein C deficiency

- Overlap between heterozygotes and normals
- Treatment with oral anticoagulants
- FVL and FVIII might interfere with some PC anticoagulant assays

→ false positive and negative results

- Routine sequencing PC gene not yet feasible

Problems

Diagnosis Protein S deficiency

- Selection of test (activity, free and/or total antigen)
- Use of seperate normal ranges (men, women +/- OC)
- Overlap between heterozygotes and normals
- Treatment with oral anticoagulants
- Influence acute phase and pregnacy
- FVL and FVIII might interfere with PS anticoagulant assay

→ false positives and false negatives

- Routine sequencing PS gene not yet feasible

To think about

- Blood group and thrombophilia screening (possible interaction blood group and FVL)
- Development of post treatment tests that predict recurrence risk

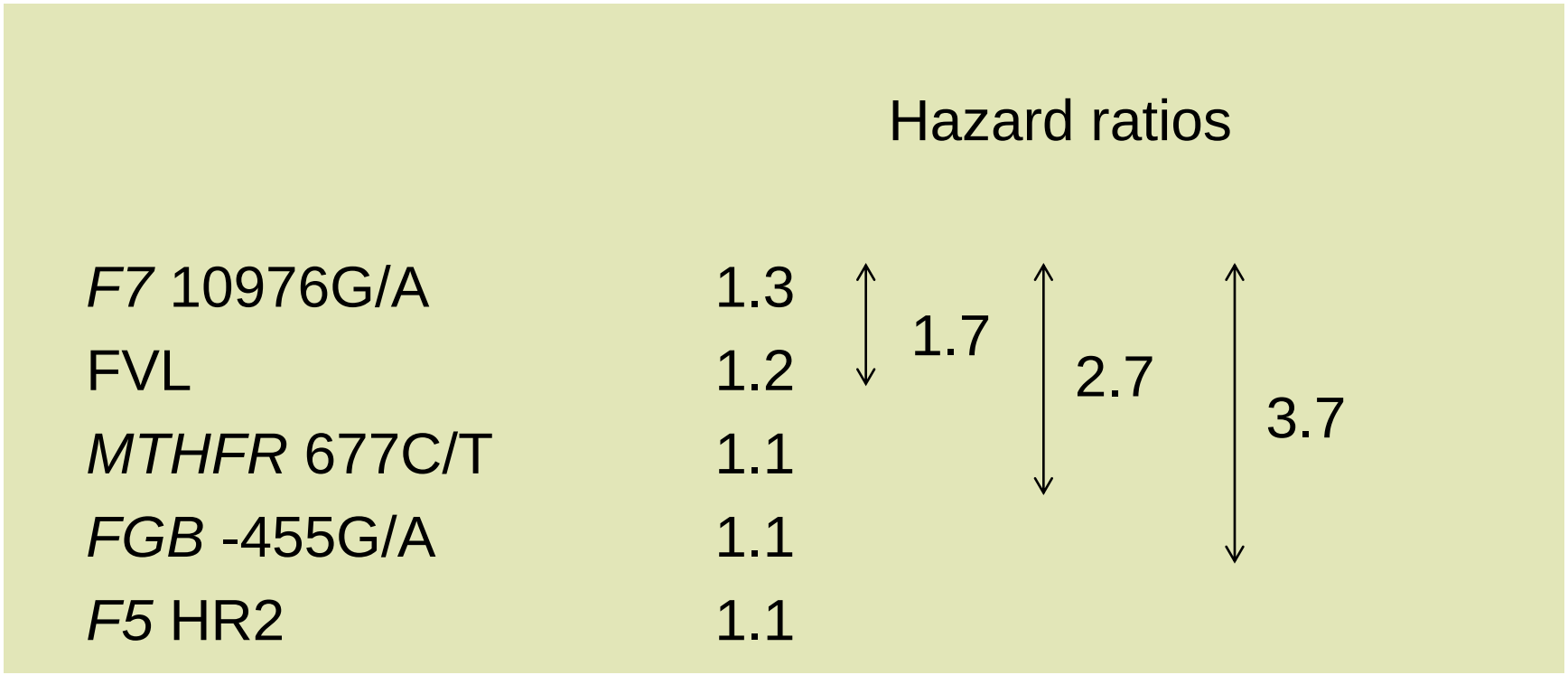
To think about

- Further development of APC resistance tests

  (many of the known risk factors result in dysregulation of prothrombinase activity)

- Development of platforms for multiplex genotyping of thrombosis alleles

Multiple SNP analysis and the risk of recurrent venous thrombosis



Hylckama Vlieg et al, JTH 2008;6:751