Results of the ECAT workshop on inhibitor testing

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Participating countries

Australia
Austria
Belgium
Canada
Czech Republic
Denmark
Finland
France
Germany
Greece
Ireland
Latvia
Netherlands
Norway
Slovenia
South Africa
Sudan
Sweden
United Kingdom
USA

Aim of the workshop

To investigate in a group of experts working in the field of haemostasis:

- **the potential to detect coagulation inhibitors in plasma samples without having clinical data of the samples**
- **To find out whether there is a general analytical approach to detect inhibitors on analytical basis**

Limitations

- **No clinical data available**
- **Limited time to analyse 8 samples.**
- **Limited sample volume**
- **Most of the persons have no experience with the available reagents, analyser and testkits**
- **Some reagents were not available and some had “unexpected” specifications.**

~~Limitations~~ Challenge

- **No clinical data available**
- **Limited time to analyse 8 samples.**
- **Limited sample volume**
- **Most of the persons have no experience with the available reagents, analyser and testkits**
- **Some reagents were not available and some had “unexpected” specifications (APTT, dRVVT, anti Xa)**

Design of the workshop (1)

- **8 samples (max. 3 ml) with prolonged APTT and/or PT were presented for analysis of the presence of inhibitor to 10 groups of max. 5 persons (5 groups on Wednesday, 5 on Thursday)**

- **Pt and APTT were pre-analysed**

- **All other assays were analysed centrally on full automatic analysers by expert operators on request of the groups.**

Design of the workshop (2)

- **No clinical data of the samples were available for the groups**

- **On Thursday we made a change in the design: participants could ask us the expert panel for two factor results (F.II, V, VII, VIII, IX, XI, XII) per sample if they had good arguments for it, in order to save time.**

Question to the participants

- **Which samples contain an inhibitor and what is the nature of the inhibitor?**
- **If a sample does not contain an inhibitor, what is the reason for the prolonged APTT and/ or PT.**

Testing procedure

Evaluate provided PT and APTT results.

- **Make a selection of tests you would like to perform on a sample.**
  (make a logical test selection on the basis of the available information)

- **Evaluate obtained results.**

- **When necessary order additional tests.**

- **Give for each of the samples the most likely diagnosis and/or conclusion.**

Tests and equipments

Equipment: AMAX Destiny

Thrombin Time (ref. value: 10.6 – 12.2 sec.)
Fibrinogen (ref. value: 1.75 – 4.00 g/L)
APTT (including mixing study)
PT (including mixing study)
Factor II
Factor V
Factor VIII
Factor IX
Factor XI
Factor XII
Inhibitors against all coagulation factors
APTT lupus sensitive (including mixing study)
dRVVT screen
dRVVT confirm
Heparin anti-Xa

<u>Further available</u>:
Heparin neutralising agent (1 µL per 1 mL plasma)
4 M Imidazole buffer
Normal pooled plasma

Sample 1

Sample that is strongly positive for lupus anticoagulant.

Sample was used in ECAT surveys before.

Sample 2

Sample derived from a patiënt with an acquired FVIII deficiency because of an inhibitor.

Inhibitor was type II inhibitor that is characterised by incomplete inactivation of FVIII activity in the plasma. In this plasma FVIII activity was about 0.10 U/mL.

Sample 3

Sample derived from a patiënt with an acquired FV deficiency because of an inhibitor against FV activity, contaminated with Unfractionated Heparin (~0.3 U/mL).

Sample 4

Sample from a patiënt with afibrinogenemia who is treated with fibrinogen (content 0,3 g Fibrinogen/l).

No inhibitor present.

Sample 5

Artificial sample of a FVIII inhibitor type 1 in lupus positive plasma.

Sample 6

Samples derived from a patiënt that is anticoagulated with coumarin.

No inhibitor present.

Sample 8

Sample with factor XI inhibitor (~ 6BU/mL).

Sample 9

Sample with factor II inhibitor (~ 6BU/mL).

Results

Results

- **We took over the conclusions written on the sheets**
- **We omitted all question marks, whether this was positive or negative**
- **When a group reported “F.VIII inhibitor test pending” for a sample with a F.VIII inhibitor, we considered this as positve result.**
- **When a conclusion was not clear we went back to the results of the analysis whether the conclusion was in line with the assay results**

Sample 1: strongly positive for lupus anticoagulant.

Group	Conclusion	Remark
1	+ lupus	
2	Non specific inhibitor, no lupus	Aberant results for lupus (machine ?)
3	F.V inh. or deficiency	No lupus analysis
4	Moderate + lupus and multiple ↓ factors	
5	↓F.II and + lupus and F.VIII and F.IX inh.	
6 (1)	+ lupus and liver disease or DIC	
7 (2)	↓F.V	??
8 (3)	↓F.V and F.VIII (congen. or acquired)	No lupus analysis
9 (4)	No conclusion	Pos. Confirm but did not report
10(5)	Vit K deficiency and + Lupus	

Lesson 1

Always use two different (types) of reagents for detection of lupus.

Sample 2: acquired FVIII inhibitor type II

Group	Conclusion	Remark
1	F.VIII inh. and liver disease	Low factor V
2	Low F.VIII inh.	
3	F.VIII inh. or deficiency	
4	F.VIII inh. and posssible + lupus	Not sure on type of inhibitor
5	F.VIII deficiency and F.VIII inhibitor	
6 (1)	Mild hemophilia	
7 (2)	No conclusion	Found F.8 10% Inh. Assay pending
8 (3)	F.VIII deficiency	Inh. Assay pending
9 (4)	No conclusion	Found F.8 10%
10(5)	F.VIII deficiency	

Sample 3: FV inhibitor with UFH (0,3 u/ml)

Group	Conclusion	Remark
1	LMWH	Prolonged TT Neutralisation?
2	No conclusion	
3	Contamination of Heparin	TT or Xa assay?
4	↓F.V	
5	↓F.V and heparin	Anti Xa assay
6 (1)	HNF/ UFH	Prolonged TT Neutralisation?
7 (2)	F.V deficiency and + lupus	
8 (3)	F.V deficiency	Inhibitor pending
9 (4)	F.V deficiency and heparin	TT and anti Xa
10(5)	F.V deficency and heparin	Prolonged TT Neutralisation?

Lesson 2

Sometimes you think you have good news but after all it is not !!

Always be aware of the limitations, specificity and sensitivity of the tests that you use.

Sample 4: Afibrinogenemia, no inhibitor

Group	Conclusion	Remark
1	Afibrinogenemia dysfibrinogenemia	
2	↓ Fibrinogen and F.V deficiency	Aberrant F.V result (machine problem?)
3	Possible F.II/V/VII def. (warfarin) or inh.	No data
4	↓ Fibrinogen	
5	LMWH and warfarin	Anti Xa analysis: 0.23, no FG assay
6 (1)	↓ Fibrinogen	
7 (2)	Heparin	TT prolonged, anti Xa 0,11
8 (3)	No conclusion	not completed for Lupus, no FG assay
9 (4)	↓ Fibrinogen	
10(5)	Fibrinogen deficiency	

Lesson 3

Incidence of dys- and a-fibrinogenemia is low but significant (in our institute about 5), so don’t forget to perform the Fibrinogen assay.

Sample 5: lupus sample with FVIII inhibitor type I (2 U/ml)

Group	Conclusion	Remark
1	No conclusion	
2	+ lupus and F.VIII inhibitor	
3	+ lupus	No F.VIII assay
4	F.VIII inhibitor	
5	+ lupus and inhib. effect on F.VIII or IX	
6 (1)	Haemophilia inhibitor and ???	Did not test lupus
7 (2)	No conclusion	No data, FVIII inh test pending
8 (3)	+ lupus and R/O associat. F.VIII inhibitor	
9 (4)	+ lupus and F.VIII inhibitor	
10(5)	+ lupus	F.VIII assay 1%

Lesson 4

Always perform factor analysis (and evt. inhibitors) in lupus positive patients.

Factor assays for inhibitor analysis in presence of lupus are best being analysed with chromogenic substrates to prevent influence of lupus

Sample 6: sample from patient anticoagulated with coumarin

Group	Conclusion	Remark
1	Vit K def	F.V 90%
2	No conclusion	low F.V result (machine problem?)
3	Cross reacting inhibitor	idem
4	F.II and F.V deficiency	idem
5	Liver disease, bleed, ↓ F.II, borderline F.IX	F.V 90%
6 (1)	VKA	F.V 100%
7 (2)	F.II deficiency	F.V 80%
8 (3)	↓F.II and IX, liver disease, vit.K def, warfarin	F.V 80%
9 (4)	F.II def., anticoagulated, ↓K	F.V 80%
10(5)	F.II def	F.V >100%

Sample 8: Sample with factor XI inhibitor (~ 6BU/mL).

Group	Conclusion	Remark
1	+ lupus	Lupus not tested
2	Inhibitor in APTT, def. In PT	F.XI not tested
3	Cross reacting inhibitor	F.XI not tested
4	F.XI inhibitor	F.XI 6 %
5	+ lupus	Pos confirm, F.XI not tested
6 (1)	+ lupus	No confirm and F.XI not tested
7 (2)	F.XI deficiency , inhibitor	F.XI : no clot Inhibitor not tested
8 (3)	Possible + lupus with neg dRVVT	F.XI not tested
9 (4)	No conclusion	F.XI not tested
10(5)	Inhibitor	F.XI not tested

Sample 9: Sample with factor II inhibitor (~ 6BU/mL).

Group	Conclusion	Remark
1	+ lupus and/or F.VIII or IX inhibitor	TT 31
2	Drug? or anti F.II inhibitor	TT 30
3	Strong inhibitor	TT 30
4	Heparin	Anti Xa : no clot
5	Heparin with low F.IX	Anti Xa: 1.4
6 (1)	F.II deficiency	TT 30
7 (2)	↓ F. II, V, X or inhibitor	TT 30, high anti Xa
8 (3)	Likely heparin	TT 30
9 (4)	F. II inhibitor / deficiency	TT 30
10(5)	Hirudin, HIT	TT 30

Lesson 5

You need to perform a heparin neutralising test for TT when it is prolonged.

In the case it does not correct, think about other causes than heparin.

Conclusions for ourselves (organisation) (1)

Next time:

- **More instruments**
- **More time (?)**
- **Better knowledge of the specifications of the tests that are used.**

Conclusions for ourselves (organisation) (2)

The only difference between an expert and a participant is that the expert knows the final results before the analysis.

Conclusions for ourselves (organisation) (3)

The only difference between an expert and a participant is that the expert knows the final results before the analysis.

We were glad not to be a participant because we should be no longer an expert.

Conclusions for ourselves (organisation) (4)

The only difference between an expert and a participant is that the expert knows the final results before the analysis.

We were glad not to be a participant because we should be no longer an expert.

We should not have done it better than you.

Preliminary, short suggestion for a guideline in samples with prolonged PT and/or APTT, as result of this workshop (1)

1. **Perform mixing tests in pH stabilised mixtures with normal plasma at different incubation times.**

2. **If prolonged, perform TT and if prolonged perform heparin neutalisation test to exclude or confirm heparin.**

3. **If heparin is present: is this the only reason for prolongation (e.g. sample 5). Perform neutralisation on Pt/APTT mix?**

Preliminary, short suggestion for a guideline in samples with prolonged PT and/or APTT as result of this workshop (2)

4. **If no heparin is present(no neutralisation in TT):**
   -Perform mix assay of TT
   -Lupus test and clotting assays with lupus insensitive reagents to find out whether there is an isolated factor deficiency (congenital or acquired)

5. **If there is one isolated deficiency perform assay in more dilutions and analyse inhibitor against that factor preferentially with chromogenic substrates.**

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