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Editorial

In this eighth issue of The ECAT Newsletter we would like to start with a Focus article about the diagnosis of hereditary antithrombin (AT) heparin-binding site deficiencies. It is interesting to see how different commercial AT activity assays have different sensitivities for detecting AT heparin-binding site mutations.

The 9th ECAT Participants' Meeting, to which we are pleased to be able to invite you, will be held in November. In this issue you will find the programme for this meeting under 'ECAT information'. We would also like to pass on information to you about the introduction of two different method codes for the Siemens Berichrom FXIII method in the ECAT external quality assessment (EQA) surveys and the reason why the two method codes are introduced. The last item in ECAT Information is that the ECAT has recognized the benefit of developing an algorithm for the laboratory diagnosis of a prolonged APTT. This algorithm is now available in poster format.

Some clinical diagnoses are rare and therefore not always easy to detect. This time the diagnosis of a Factor XIII deficiency in a 13-year-old girl is described in the Case Report.

Finally in this issue we have chosen a different approach for the Literature Review. Review articles of state-of-the-art lectures of the recent ISLH congress in The Hague are featured; these have all recently been published in the International Journal of Laboratory Hematology.

I hope you will enjoy reading this issue.

Petra ter Hark

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News

Nineth ECAT Participants' Meeting

Course: Laboratory Testing for Lupus Anticoagulant

Course: The use of a flowchart for the interpretation of deviating EQA results

12, 13 and 14 November 2014
Holiday Inn Hotel
Leiden
The Netherlands
(for further information see page 5)

Discount on CLSI Lupus Anticoagulant guideline

All participants of the 9th ECAT symposium as well as participants in the course on Laboratory Testing for Lupus Anticoagulant will receive a **50% discount** on the recently published CLSI guideline "Laboratory Testing for Lupus Anticoagulant" (H60-A). The regular non-member price is $ 130,=. More information can be found under meeting information at the ECAT website.

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Focus Article:
Diagnosis of hereditary antithrombin heparin binding site deficiencies

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Inherited AT deficiency

Antithrombin (AT) is the major physiological inhibitor of thrombin and other coagulation proteases. It is a serine protease consisting of 432 amino acids and is synthesized in the liver. Antithrombin exerts its inhibitory activity by recognizing the target coagulation protease as a substrate and inactivates it by proteolytic cleavage. This action is potentiated at least a thousand times in the presence of heparin [1].

Inherited AT deficiency was the first defect of the physiological coagulation inhibitors to be described in 1965 by Egeberg [2]. It is a rare autosomal dominant disorder causing a predisposition to venous thrombosis. Homozygous cases are incompatible with life except for certain subtypes. The prevalence rates vary from 1 in 500 to 1 in 5000 in the overall population [3,4]. Despite this low prevalence, inherited AT deficiency is one of the most severe risk factors for venous thromboembolism (VTE), with the first event often presenting before the third decade.

VTE risk in AT-deficient individuals is about 5- to 50-fold higher compared to the general population. Thrombotic phenotypes seem to vary according to the (sub)type. Classification of inherited AT deficiency is based on the results of AT activity and antigen. Type I deficiency is characterized by a parallel reduction of AT activity and antigen in the plasma, most often caused by the reduced secretion of functionally normal antithrombin. Type II is characterized by reduced functional AT and (nearly) normal antigen levels. Type II deficiencies are subclassified depending on whether the mutation affects binding to heparin (Heparin Binding Site, HBS), the reactive site (Reactive Site, RS) or both (Pleiotropic Effect, PE) [5]. Type II HBS variants are thought to be less thrombogenic than type I or other type II deficiencies. However, if present in a homozygous state, HBS variants are associated with arterial thrombosis in childhood [6].

Diagnosis of inherited AT deficiency

The diagnosis of hereditary antithrombin deficiency should be established only after the exclusion of acquired causes of AT deficiency, such as liver disease, consumptive coagulopathy, heparin therapy, nephrotic syndrome and sepsis.

The test of choice for identifying patients with AT deficiency is the activity measurement. Current methods quantify AT activity by chromogenic tests measuring the inhibition of thrombin or factor (F) Xa by AT in the presence of heparin. The first step in these assays is the addition of heparin and excess thrombin/FXa to the diluted patient's plasma. The AT rapidly neutralizes the thrombin/FXa in the presence of heparin. Residual thrombin/FXa is determined by the rate of hydrolysis of the chromogenic substrate. The para-nitroaniline release measured at 405nm is inversely proportional to the antithrombin level in the plasma.

Antigen determination by immunological methods (immuno-electrophoresis, ELISA,…) allows differentiation between type I and type II deficiency. AT variants with an altered heparin affinity can be identified by a progressive AT activity assay (i.e. AT activity in the absence of heparin). Finally, genetic analysis of the AT gene, SERPINC1, can confirm the diagnosis at the molecular level. However, these additional tests are only performed in specialized laboratories.

Pitfalls in the diagnosis of AT deficiency

Today, most commercially available methods to measure AT activity have a good sensitivity for detecting type I AT deficiency. However, there is some concern about the sensitivity of these assays for detecting type II HBS deficiencies. Several reports have already addressed this issue [7,8,9]. Patients with HBS mutations can show variable activity levels depending on the assay conditions used and may therefore erroneously be classified as normal. Major determinants of assay sensitivity are heparin concentration and incubation time [10].

We evaluated the suitability of several commercial AT activity assays for the identification of HBS variants and aimed to improve detection by adjusting assay conditions. We used plasma samples from 34 patients with AT heparin binding site deficiency, confirmed by molecular analysis. These patients shared a total of five different mutations: p.Pro73Leu, p.Arg79His, p.Arg79Cys, p.Leu131Phe, p.Gln150Pro (amino acid numbering according to HGVS recommendations: the +1 amino acid is Met, encoded by codon ATG).

Antithrombin activities were measured using three different commercial anti-Xa based tests: HemosIL Liquid AT® (Instrumentation Laboratory), Coamatic® AT (Chromogenix)

Table 1. Characteristics of the different commercial assays used in this study

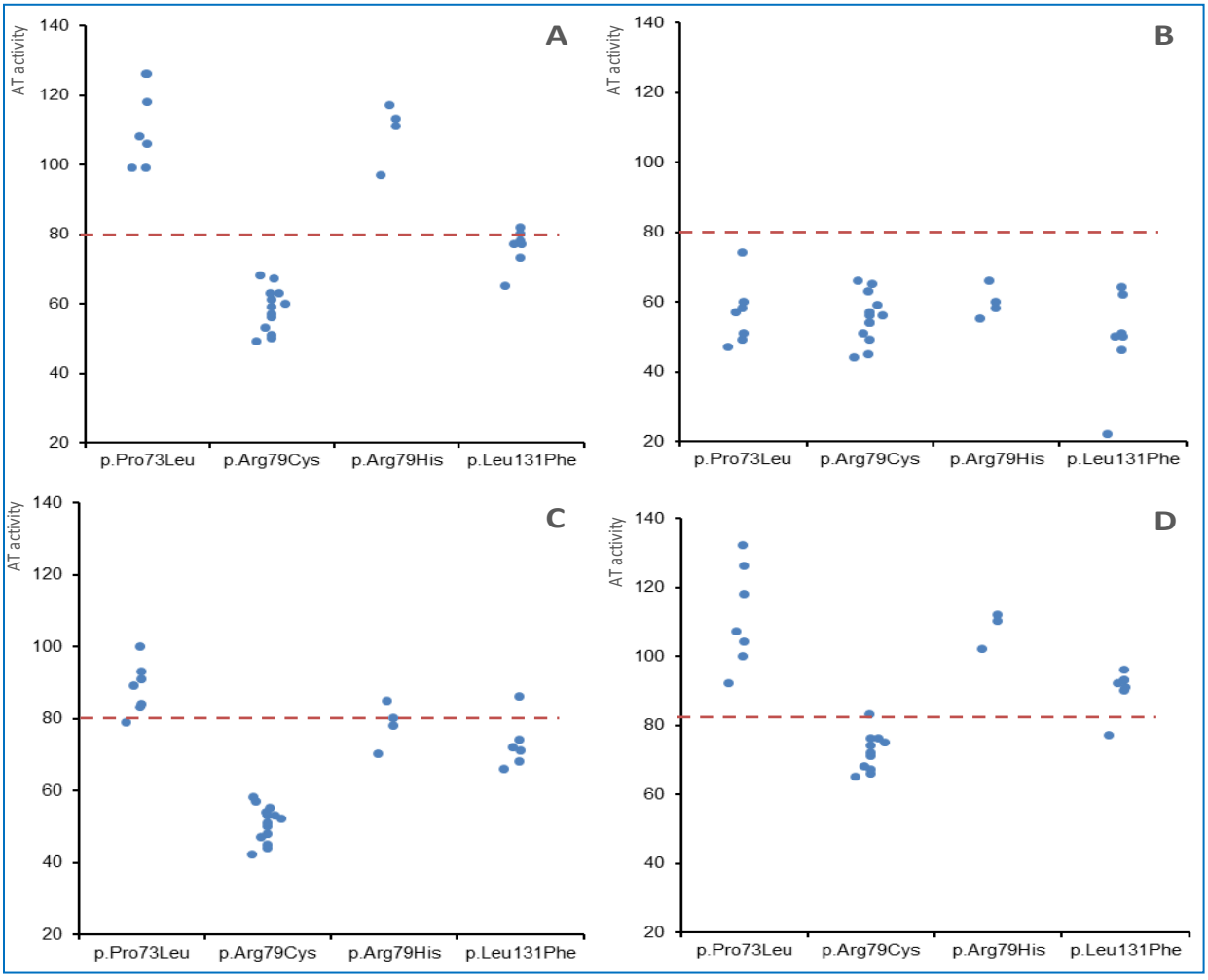
	Liquid AT	Coamatic AT	Innovance AT	Biophen anti-IIa
Substrate source	Bovine FXa	Bovine FXa	Human FXa	Bovine FIIa
Incubation time	100-140s	100-140s	180-190s	60s
Heparin concentration	3000U/mL	3000U/mL	1500U/mL	5000U/mL
Sensitivity (%)	60	74	100	39
Specificity (%)	100	100	100	100

and Innovance® Antithrombin (Siemens). One anti-IIa activity test was evaluated, Biophen® AT anti-IIa (Hyphen Biomed). All assays were performed on ACL TOP500 (Instrumentation Laboratory). Relevant characteristics of these commercial tests are represented in table 1. The reference interval was the same for all assays: 80-120%.

The sensitivity of the anti-Xa assays for HBS deficiencies was 60%, 74% and 100% for HemosIL® Liquid AT, Coamatic® AT and Innovance®, respectively. Two specific mutations are responsible for this high variability between assays, namely the p.Pro73Leu and p.Arg79His mutations (figure 1). The anti-IIa assay showed a sensitivity of 39% and was only able to detect some the p.Arg79Cys variants. The anti-IIa assay yielded higher activity levels when compared to the Coamatic® anti-Xa assay with an average increase of 23%.

A first attempt to improve sensitivity was the adaptation of the incubation time. For the Coamatic® AT test, we changed the incubation range that varies between 100-140 seconds to a fixed 110 seconds. The incubation time of the anti-IIa test was shortened to 30 seconds, based on the publication by Harper *et al* [10]. This resulted in improvement of the sensitivity, especially for the Coamatic® AT test where it improved from 74% to

Figure 1. antithrombin activity per mutation measured with HemosIL® Liquid AT (A), Innovance® AT (B), Coamatic® AT (C) and Biophen® anti-IIa (D). - - - - - Lower limit of normal range



86%. Adaptation of incubation time had no impact on the reference interval.

Further efforts to improve sensitivity included changes in sample dilution and variations in heparin concentration (where possible). However, these adjustments did not significantly alter the diagnostic capacity of the tests and induced a loss of specificity in some settings (data not shown).

Discussion

Our study confirms the wide variation in the ability of commercial AT activity assays to detect AT HBS mutations, as previously observed [11,12]. Our data show that this variation is due to different sensitivity for specific HBS mutations, especially for the p.Pro73Leu and p.Arg79His mutations. Of the critical variables known to influence sensitivity, only reduction in incubation time improved the detection of HBS mutations.

We observed higher AT activity levels when measured with the anti-IIa method compared to the anti-Xa methods. Thrombin-based assays have the potential problem that the added thrombin may be inhibited by heparin cofactor II (HC-II), a second heparin-catalyzed thrombin inhibitor present in plasma. This effect can lead to an overestimation of the measured AT concentration by 5-10%. It has been shown that bovine thrombin is not, or only minimally, inhibited by HC-II.

Today, most commercial anti-IIa based tests use bovine thrombin, as does the Biophen® anti-IIa AT assay, and results should therefore be minimally affected by HC-II. However, we found a substantial difference in our experiments.

Nowadays, the majority of clinical laboratories use anti-IIa-based methods for the measurement of antithrombin activity. This is reflected in the distribution of methods among the ECAT participants in the year 2013: approximately 55% of all participants used a thrombin-based assay. Moreover, the laboratories using an anti-Xa based method frequently use the Liquid Antithrombin assay which, in our hands, seems to be less adequate in diagnosing particular HBS deficiencies.

Taking our data into account, one could raise the question about the real prevalence of HBS deficiencies. In the light of the present findings, AT deficiency of the HBS subtype could be underdiagnosed due to the use of insensitive reagents. Although known as a mild thrombophilic risk factor, HBS defects contribute to a markedly increased risk for venous thromboembolism if associated with other thrombophilia factors such as Factor V Leiden or the prothrombin G20210A mutation. Laboratories are encouraged to choose their assay for functional antithrombin activity carefully.

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ECAT information:

9th ECAT PARTICIPANTS' MEETING

On 13 and 14 November 2014 the 9th ECAT Participants' Meeting will be held in the Holiday Inn Hotel, Leiden, The Netherlands. Below you will find the outline of the programme.

Thursday 13 November (9.45 – 17.15 hr.)

How to improve quality in the pre-analytical phase	S. Church
The future of anticoagulation	H. ten Cate
Guideline for the laboratory testing of direct anticoagulants	A. Tripodi
ECAT survey results on direct anticoagulant testing	P. Meijer
The new CLSI guideline on Lupus Anticoagulant testing	G. Moore
How to measure low levels of Factor VIII and IX	S. Rosen
ECAT survey results for low levels of Factor VIII and IX	H.W. Verbruggen
Parallelism of dilution curves: the real story	C. Kluft
A practical approach to establish reference values	J. Ruinemans
Case studies	K. Devreese
EQA results for POCT INR testing	P. Meijer
Application of thromboelastography in clinical practice and how to control quality	Y. Henskens

Haverkate Lecture:

Pathophysiology of Lupus Anticoagulant and the consequences for the laboratory diagnosis	Ph. de Groot

Friday 14 November (9.00 – 15.15 hr.)

ADAMTS13 and the pathogenesis of thrombotic thrombocytopenic purpura	B. Lämmle
ECAT survey results on ADAMTS13 testing	R. Niessen
Case studies	K. Devreese
Introduction on Inhibitor Testing	H.W. Verbruggen
Procedures and barriers for the introduction of a new or improved method	t.b.a.
Do we need a new mind in the laboratory?	t.b.a.
Forum discussion on: Pros and cons for the introduction of a new or improved method	
Quality assurance of the entire diagnostic process	M.P.M. de Maat
The pathophysiology of HIT	K. Krauel
The laboratory diagnosis of HIT	L. Porcelijn
ECAT survey results for HIT	F. Haas
The role of molecular biology in the diagnosis of impaired haemostasis	P. Reitsma

More information about the meeting, including registration, can be found on the ECAT website (www.ecat.nl)

Courses

In conjunction with the 9th ECAT Participants' Meeting two courses will be organised on **Wednesday 12 November 2014**. These course will also be held in the Holiday Inn hotel in Leiden, The Netherlands. Both are full day courses and start at 10 am.

Course 1: **The laboratory diagnosis of Lupus Anticoagulant.**

This course will discuss in detail the diagnostic strategy for LA, including the currently available guidelines. The full day workshop includes besides education on LA testing also interactive discussions on patient cases.

Course leader : Dr. G.W. Moore

Course 2: **The use of a flowchart for the interpretation of deviating EQA results**

This course will discuss a systematic approach for the interpretation of deviating results in EQA surveys. What can be the cause? How to initiate corrective actions? Etc. The use of a flowchart for such an systematic approach will be introduced. Practical examples will be discussed.

Course leader : Dr. P. Meijer

More information about these courses, including registration, can be found on the ECAT website (www.ecat.nl)

The measurement of low Factor XIII activity with the Siemens Berichrom method

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Introduction

Factor XIII (FXIII) is responsible for cross-linking fibrin, resulting in a stable fibrin network. Severe deficiency (FXIII < 1%) is a rare but important bleeding disorder. But even mild deficiencies (FXII between 5 and 40%) may be associated with severe bleeding complications [1]. It is therefore important that FXIII can be measured accurately with sufficiently sensitive methods. For the quantitative measurement of FXIII activity chromogenic methods are used [2]. In this report we evaluate the quantitative Factor XIII activity results obtained in the ECAT external quality assessment (EQA) surveys.

EQA surveys

Samples used in the ECAT surveys are produced by Technoclone (Vienna, Austria). The FXIII activity in these samples varied between < 2% and normal levels. The levels in these samples were confirmed by antigen testing. As usual in the ECAT surveys, the samples were distributed to the participants as lyophilised plasma. In each survey clear instructions for the reconstitution of the samples were given. Results are mainly reported to the ECAT using the web application for returning results. Information on the method and equipment used is given by the participant. Survey results are evaluated according to the standard survey procedure using robust statistics. Currently approximately 65 laboratories are participating in the quantitative FXIII activity surveys.

Results

Samples with a FXIII level > 50% are classified as normal by the vast majority (> 90%) of the participants, while samples with a FXIII level < 50% are classified as abnormal by the vast majority of participants (> 95%). Table 1 shows the between-laboratory variation (CVbetween) at different activity levels. It is clear that there is an inverse relationship between FXIII activity levels and the CVbetween.

Interestingly a remarkable difference is observed when the method-specific consensus values of the Siemens Berichrom are compared with those of FXIII methods of other companies (mainly Stago and Technoclone) for samples with an activity of < 20% (see fig. 1). For FXIII activities > 20 % no systematic differences between the methods used can be observed.

Table 1. The between-laboratory variation (%) at different FXIII activity levels

FXIII level (%)	CV between (%)
> 50	10 - 15
11 - 49	10 - 26
2 - 10	37 - 43
< 2	66 - 78

Fig 1. The relationship between the method-specific consensus values and the overall consensus values for FXIII activity

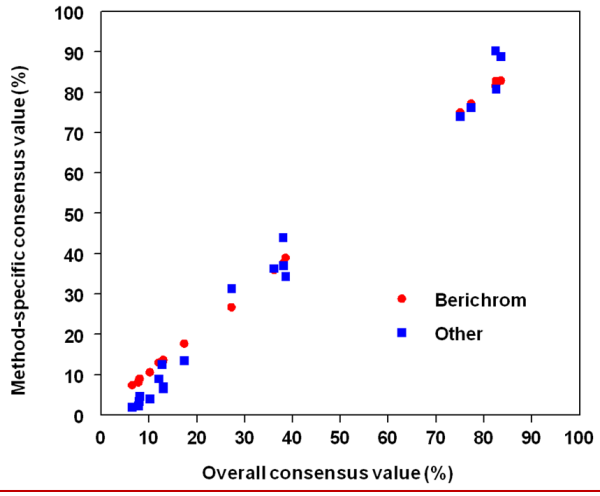

Table 2. The consensus of FXIII activity levels (%) for the Siemens method and other methods in different samples used in the EQA surveys in 2013

Survey	Method	Siemens	Other methods
	No. of participants	± 55	± 10
2013 - 1	Sample 1	37	39
	Sample 2	12	4
2013 - 2	Sample 1	79	76
	Sample 2	17	13
2013 - 3	Sample 1	38	38
	Sample 2	10	3
2013 - 4	Sample 1	14	9
	Sample 2	78	86

Table 3. The consensus of FXIII activity levels and the range of results (%) for the Siemens method in samples with a low FXIII level used in the EQA surveys in 2013

Survey		Consensus value	Range
2013 - 1	Sample 2	12	1-25
2013 - 2	Sample 2	17	5-25
2013 - 3	Sample 2	10	1-26
2013 - 4	Sample 1	14	0-23

This was further explored for the samples used during the surveys in 2013 (see table 2). From table 2 it can be appreciated that for samples with a low FXIII activity level (survey 2013-1: sample 2 ; survey 2013-2: sample 2 ; survey 2013-3: sample 2 and survey 2013-4: sample 1) there is a significant difference in the consensus value between the Siemens method and the other methods used.

However, when the results of the Siemens method are further explored it can be observed that there is a wide range of results reported (table 3). In addition, a substantial number of participants (up to 30%) using the Siemens method also reported results below their lower limit of detection (LLD). Frequently an LLD of 15% is used. This means that those participants are not able to diagnose a severe FXIII deficiency.

It is known that for the subtraction of a plasma blank it is very important to have a sensitive FXIII activity method for measuring samples in the low range [3]. From the wide range of reported results within the Siemens Method group we have the impression that this is a heterogeneous group of laboratories using the method with and without blank correction. We therefore decided to introduce two different method codes for the Siemens Berichrom method, one with and one without blank correction. If you are participating in the FXIII quantitative activity surveys and you are using the Siemens method, please select the appropriate method.

New method codes Siemens Berichrom FXIII method

4010 = Siemens Berichrom FXIII (without blank correction)

4011 = Siemens Berichrom FXIII (with blank correction)

Note: when you are using the web application for result submission you will only see the names of the method and not the method code.

References

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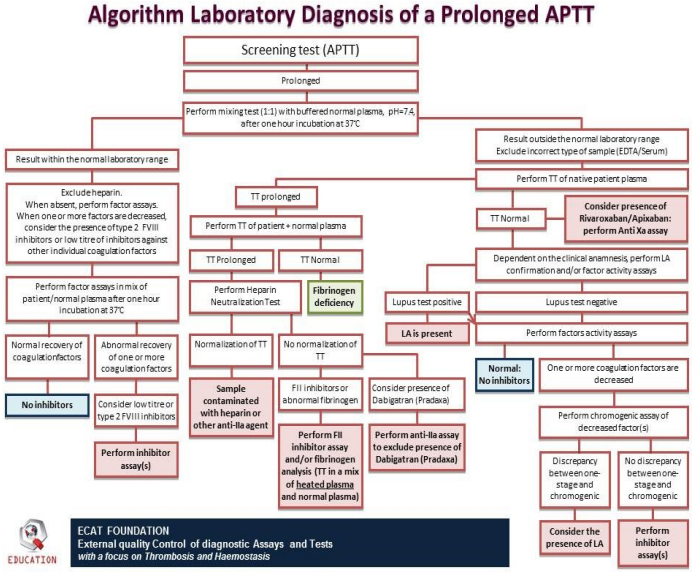
ECAT Education, The diagnostics of a prolonged APTT

On a regular basis each laboratory has to deal with samples from patients with a prolonged APTT. This can have very diverse causes. For example, it can be caused by a reduced quantity of a coagulation factor, the presence of a whether or not specific inhibitor, or the treatment of a patient with oral anticoagulants.

On the basis of experiences during the workshop at the 8th ECAT meeting in 2012 and the presentation of M. Dardikh during this meeting, the ECAT has come to recognize the benefit of developing an algorithm for the laboratory diagnosis of a prolonged APTT. This algorithm makes it possible to determine the cause of a prolonged APTT by using a systematic approach to laboratory testing. The use of a clear step-by-step procedure promotes the recognition of all possible abnormalities and saves time, as well as being cost-effective.

If you are interested, a poster format of the algorithm can be ordered by sending a request email to info@ecat.nl. The price of the poster is € 15,= (Europe) or € 25,= (outside Europe) including delivery costs.



Case report:

Diagnosis of a previously unnoticed factor XIII deficiency after ovarian haemorrhage in a 13-year-old girl with extradural haematoma and defective wound healing in her medical history

K. Devreese, MD, PhD

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Case report

We report on a girl newly diagnosed with congenital factor XIII deficiency at the age of thirteen. She was born after 39 weeks of pregnancy. She is the first child of non-related Caucasian parents. There were no reports of umbilical cord bleeding, the first and most characteristic symptom of FXIII deficiency.

At the age of 3 she was admitted to the hospital with an extradural haematoma after minor trauma capitis. Trepanation was performed. Laboratory analysis showed no abnormalities in screening coagulation test results (prothrombin time (PT), activated partial thromboplastin time (aPTT) and platelet count). Levels of von Willebrand (VW) factor antigen and activity were normal as well as platelet function tests. Later on there were several reports of extensive, but superficial bruising over the whole body, which even lead to the suspicion of child abuse.

When she was 12 years old a botriomycoma was removed from her right flank with seriously impaired wound healing. Since no abnormalities in routine coagulation were detected, self-mutilation was considered as a possible cause.

Presently, while still in wound care follow-up she presented at the emergency room with nausea and abdominal pain. Ultrasound and MRI showed an abdominal mass near the left ovary. An explorative laparotomy was performed and revealed a massive bleeding (most probably due to rupture of the follicle during first ovulation) and 1150 mL of blood was removed.

Table 1. Results of FXIII antigen and activity in plasma and platelets

	FXIII Activity %	FXIII-A2B2 Antigen %	FXIII-A Antigen %	FXIII-B Antigen %
Reference Interval	69– 143	67– 133	67– 133	64– 136
Patient's plasma	<1	<0,05	<0,03	37,8
Patient's platelets	<1		<0,03	

FXIII Activity analysed with TECHNOCHROM® FXIII kit (Technoclone, Vienna, Austria); FXIII-A2B2 complex antigen, FXIII-A antigen, and FXIII-B antigen were measured with TECHNOZYM® FXIII Ag, FXIII-A SUB and FXIII-B SUB ELISAs (Technoclone).

Eventually a clot solubility test (CST) was performed because routine coagulation, platelet function tests, screening for VW disease and factor VIII and IX were normal. The qualitative CST turned out positive, indicating severe factor XIII deficiency. The FXIII level (subunit A) was measured quantitatively in plasma with Hexamate™ (MBL), a latex immuno-assay (LIA), and by Zymutest (Hyphen BioMed), an ELISA, and was below 4%. The FXIII activity (Berichrom, Dade Behring) was 2.5%. Then plasma and platelet samples were sent to Debrecen for exploring the full laboratory phenotype and for molecular genetic analysis. In the laboratory of L. Muszbek additional tests were performed to confirm the diagnosis of a type I FXIII-A deficiency. These parameters confirmed severe FXIII-A deficiency. FXIII-B antigen was above 30% in the plasma. See table1.

Later on, molecular genetic analysis revealed a homozygous single nucleotide deletion in exon 3 (c.215 delA) that led to an early stop codon (in codon 74), which explains the severe deficiency and the lack of FXIII-A in plasma and in platelets. Her mother was heterozygous for the same mutation; the father was not available for investigation.

After administration of fresh frozen plasma postoperatively, factor XIII was 17%. To stop bleeding 30 U/kg FXIII-concentrate (FIBROGAMMIN P, CLS Behring, Marburg, Germany) was administrated IV. 14 days after treatment her FXIII level was 12%. Today, she receives prophylactic administration of 10 U/kg F XIII every two to four weeks. With this dose her FXIII levels remains above 5%. Her wound is finally healing and she no longer presents with bleeding or easy bruising. See figure 1.

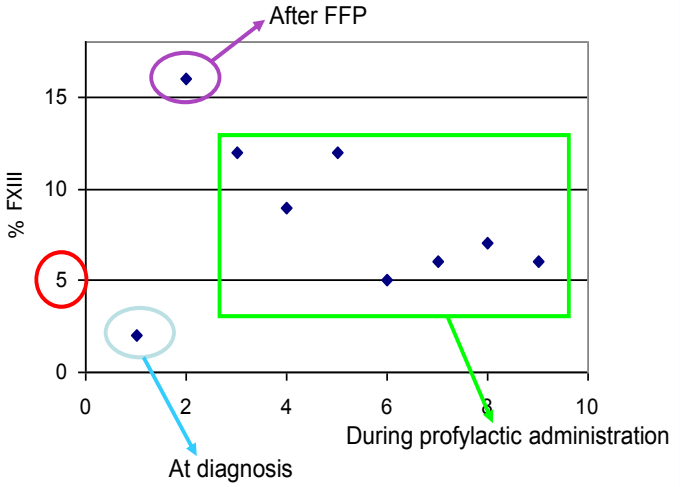
Discussion

Coagulation factor XIII (FXIII) exists both in plasmatic and cellular forms. Plasma FXIII is a heterotetramer consisting of two catalytic A subunits (FXIII-A) and two inhibitory/carrier B subunits (FXIII-B). The gene coding FXIII-A (F13A1) is located on chromosome 6 at t6p25.3–p24.3 position and contains 15 exons, whereas the FXIII-B gene (F13B) is located on chromosome 1 at 1q31–32.1 position and contains 12 exons. The cellular form of FXIII, a dimer of FXIII-A, is exempt of FXIII-B. A huge amount of cellular FXIII is present in platelets (3% of the total platelet proteins) and it is also expressed in monocytes, macrophages, osteoblasts, osteocytes, chondrocytes.

Activated FXIII is a transglutaminase, which cross-links glutamine and lysine side-chains by peptide bonds. Its main task in haemostasis is to cross-link fibrin á-, and ã-chains and á2-plasmin inhibitor to fibrin. The cross-linking of fibrin chains mechanically stabilizes fibrin and protects it from shear stress [1].

Inherited FXIII deficiency is an autosomal recessive trait; patients with severe disease are homozygotes or compound heterozygotes. FXIII deficiency is a rare bleeding disorder with an incidence of approximately 1: 2000 000. More than 70 causative mutations in

Figure 1. FXIII level (%) in function of time (weeks).



Follow-up FXIII subunit A (LIA)

the FXIII-A gene have been published, but only five mutations in the FXIII-B gene. Inherited FXIII deficiencies are classified as FXIII-A and FXIII-B deficiencies; subtypes I and II of FXIII-A deficiency represent quantitative and qualitative defects, respectively. Acquired FXIII deficiency is described caused by autoantibodies against a FXIII subunit, as well as a reduction of FXIII A subunit levels caused by decreased synthesis or consumption. and other moderate FXIII deficiencies caused by decreased synthesis of a FXIII subunit (impaired bone marrow function, liver disease) or consumption of FXIII or by dilution coagulopathy [1, 2, 4].

FXIII activity below 1% causes severe bleeding diathesis. Such low FXIII activity occurs in inherited FXIII-A deficiency, while in patients with inherited FXIII-B deficiency, FXIII activity is higher (usually 5-10%) and the bleeding diathesis is mild to moderate. FXIII deficiency is considered the most under-diagnosed bleeding diathesis. Unawareness at the clinical side and incorrect diagnostic practices in laboratories contribute to this problem. A recommendation on diagnosis and classification of FXIII deficiencies was published as an official communication of the Scientific and Standardization Committee, International Society on Thrombosis and Haemostasis [1, 3].

Although clinical symptoms may be suggestive of FXIII deficiency, the diagnosis relies on laboratory tests. The routine screening tests for coagulopathies – prothrombin time, activated partial thromboplastin time, and thrombin time – do not show prolongation in cases of FXIII deficiency. Therefore, if clinical symptoms indicate a bleeding diathesis, full evaluation of the clotting system should include a test that detects FXIII deficiency [4]. FXIII deficiency is considered the most underdiagnosed bleeding diathesis. The recommended algorithm for its diagnosis and classification could improve the diagnostic efficiency.

The SSC recommends the following algorithm [3]:

1. A quantitative functional FXIII activity assay that detects all forms of FXIII deficiency should be used as a 'first-line' screening test.
2. If plasma FXIII activity is decreased, the subtype of FXIII deficiency is to be established by using the following tests.

a. Measurement of the FXIII-A2B2 antigen concentration in the plasma. If the FXIII-A2B2 antigen concentration is decreased, FXIII-A and FXIII-B antigens should also be measured. Alternatively, measurement of both isolated subunits is sufficient for the classification.

b. Measurement of FXIII activity and FXIII-A antigen in platelet lysate.

3. Detection of autoantibodies against FXIII subunits:

a. Mixing study for the detection of neutralizing antibodies against FXIII-A.

b. Binding assays for the detection of non-neutralizing antibodies against FXIII-A and FXIII-B.

4. Additional test for the evaluation of fibrin crosslinking by SDS-PAGE.
5. Detection of molecular genetic defect.

Traditionally, the solubility of fibrin clot in concentrated urea, acetic acid or monochloroacetic acid solution was used to screen for FXIII deficiency. This is a qualitative method that detects only very severe FXIII deficiency. The drawbacks of this test are the poor standardization and its sensitivity depending on the fibrinogen level, the clotting reagent (thrombin and/or Ca2+), and the solubilizing agent and its concentration. Depending on these variables, the detection limit of the clot solubility assay varies between <0.5% and 5% FXIII activity. Although useful as first screening test when interpreted with care, the use of this method as the screening test for FXIII deficiency is not recommended by the SSC [2, 3, 4].

The preferred choice for substitution therapy is FXIII concentrate. Primary prophylaxis (10/20 U/kg FXIII every 4–6 weeks) is recommended for patients with severe FXIII deficiency in order to prevent spontaneous severe bleedings, abnormal wound healing and recurrent miscarriages in women. Although fresh frozen plasma (FFP) and cryoprecipitate contain FXIII, highly purified and heat-treated FXIII concentrate is preferred for long-term prophylaxis. The half-life of FXIII is the longest among coagulation factors (11–14 days). It has been suggested that a level of 5% is sufficient to prevent spontaneous bleeding. Now, fibrogammin P, a purified pasteurized concentrate, is available for prophylaxis in a recommended dosage of 10–20 U/kg once every 4–6 weeks [4].

Conclusion

In this case the clinical history clearly shows a severe bleeding diathesis. Delayed umbilical stump bleeding has been reported in approximately 80 % of new-borns with FXIII-A deficiency. However, its lack does not exclude the diagnosis and one always has to be suspicious when intracranial bleeding occurs at an early age. Intracranial bleeding occurs in 30% of FXIII-A deficient patients not on prophylactic substitution therapy. In this case the clinical history clearly shows a severe bleeding diathesis [1, 2]. Characteristic umbilical bleeding did not occur or it was not prominent enough to be recognized. She was also free from intra-cerebral bleeding. The case emphasizes that when severe bleeding diathesis is recognized in the absence of positive haemostasis screening tests a FXIII assay should be performed. Positive clot solubility test, 2.5% FXIII activity by the BERICHROM assay and <4% FXIII-A antigen by the latex immunoassay ensured the diagnosis of FXIII deficiency, but these tests did not indicate the severity of the disease. FXIII activity between 1-5% usually causes moderate bleeding diathesis [1]. It is known that FXIII activity assays may overestimate FXIII activity in the low activity range [5]; this explains the discrepancy between the result obtained by both assays used (BERICHROM assay and TECHNOCHROM FXIII assay).

A complete evaluation of the laboratory phenotype with the establishment of the causative mutation is presented here. The severe clinical symptoms, the non-detectable FXIII activity and FXIII-A antigen in plasma and platelets harmonize with the molecular defect. This case report demonstrates that early diagnosis is essential to avoid severe clinical consequences; awareness of the disease and adequate laboratory support are important in achieving this goal [1]. The girl is currently being treated prophylactically with Fibrogammin P that keeps her free of bleeding.

References

This case report is based on the article

Katona É, Muszbek L , Devreese K, Kovács K, Bereczky Z, Jonker M, Shemirani A, Mondelaers V, Ermens A. Factor XIII deficiency: complete phenotypic characterization of two cases with novel causative mutations, Haemophilia. 2014 Jan;20(1):114-120.

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Literature review:
ISLH congress state-of-the-art lectures

During the ISLH congress, which was held from 15 – 17 May 2014 in The Hague, The Netherlands, a number of outstanding state-of-the-art lectures were given in the field of thrombosis and haemostasis.

From a number of these lectures a review article was published in the International Journal of Laboratory Hematology (Volume 36, number 3, June 2014).

We recommend reading at least the following articles:

Diagnosis and treatment of disseminated intravascular coagulation
(M. Levi, IJLH 2014; 36: 228-236)

A short introduction is given on the clinical aspects of disseminated intravascular coagulation (DIC). The greater part of this review deals with laboratory findings in DIC. The role of the measurement of platelets, coagulation factors and global clotting times is examined. Also the measurement of biomarkers involved in clot dissolution, like plasminogen activators and plasminogen activator inhibitors, and molecular biomarkers, like prothrombin activation fragment F1+2 and fibrinopeptide A are discussed. Another aspect which is described is the role of the use of thromboelastography in the diagnosis of DIC. There is commentary on the scoring system for the diagnosis of DIC devised by the International Society of Thrombosis and Haemostasis. Lastly, the state of the art with respect to the treatment of DIC patients described.

Interpretation of coagulation test results under direct oral anticoalgulants
(H. Mani, IJLH 2014; 36: 261-268)

It is well known that the direct oral anticoagulant, which is increasingly used in clinical practice, may effect both global and specific haemostasis assays.

This article briefly reviews the laboratory influence of dabigatran, rivaroxaban and apixaban on these assays. It discusses both how the test can be influenced by the drug as well as which test is suitable for monitoring.

Laboratory testing issues for protein C, protein S and antithrombin
(R. Marlar, J.N. Gausman, IJLH 2014; 36: 289-295)

Protein C (PC), protein S (PS) and antithrombin (AT) are the major parameters in the laboratory diagnosis of thrombophilia. This review discusses the major analytical issues relevant for measuring PC, PS and AT. Besides analytical issues also pre- and post-analytical issues are examined. This review concludes with some recommendations relevant for testing PC, PS and AT.

Current insights into the laboratory diagnosis of HIT.
(T. Bakchoul, H. Zöllner, A. Greinacher, IJLH 2014; 36: 296-305)

The laboratory diagnosis of heparin-induced thrombocytopenia (HIT) is challenging because of the risk to overdiagnosis due to the potential measurement of clinically non-relevant antibodies by the currently available immunoassays for the detection of anti-platelet 4/heparin antibodies. Therefore it is important to use a combination of a clinical likelihood estimation for HIT and laboratory testing. This review describes the currently available diagnostic procedures as well as their limitations.

Technological advances in diagnostic testing for von Willebrand disease: a new approach and challenges.
(C.P.M. Hayward, K.A. Moffat, L. Graf, IJLH 2014; 36: 334-340)

Von Willebrand disease (VWD) is the most common bleeding disorder. Therefore a proper diagnosis is very important. In recent years there have been applied several technological innovations for von Willebrand Factor (VWF) assays to further improve the laboratory diagnosis of VWD, such as improved VWF ristocetin cofactor activity assays and VWF activity assays. The pros and cons of these assays are discussed in this review. The authors conclude that implementation of these improved assays may have a positive impact on the correct diagnosis of patients as well as monitoring therapy.

Antiphospholipid antibody testing and standardization.
(K.M.J. Devreese, IJLH 2014; 36: 352-363)

The laboratory criteria for the diagnosis of the antiphospholipid syndrome (APS) includes besides the measurement of Lupus Anticoagulant also the measurement of anticardiolipin antibodies (ACL) and anti-ß2 glycoprotein I (aß2GPI). Over the past decades much effort has been invested in testing improvement. However, standardization of the methods available has not yet been attained. This review describes the efforts and achievements in standardization as well as the weaknesses and strengths of the currently available methods.

Commonalities and contrasts in recent guidelines for lupus anticoagulant detection.
(G.W. Moore, IJLH 2014; 36: 364-373)

Currently there are three different guidelines for the detection of lupus anticoagulant: the guideline of the International Society on Thrombosis and Haemostasis (ISTH), the guideline of the British Committee for Standards in Haematology (BCSH) and the Clinical and Laboratory Standards Institute (CLSI). The last guideline is the most recently published one (2014). This review describes the cross-guideline agreements as well as their differences. One remarkable difference is the role of the mixing test in the testing panels. CLSI reprioritise the test order to screen-confirm-mix to reduce false-negative results because of the the dilution of the antibody titre below the detection limit of the assay.