CLOT-ED

The Clotting times

December 2012
Issue 6

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Editorial

With this sixth issue of The Clotting Times we have reached the end of 2012. In this issue we start with a Focus Article describing the results of a survey, performed in the Netherlands, showing the diversity in testing for platelet function disorders by light transmission aggregometry. This survey was initiated by the WHD (Working Group on Haemostasis Laboratory Diagnosis) subgroup of the NVTH (Dutch Society for Thrombosis and Haemostasis).

In November the 8th ECAT Participants' Meeting was held. The day before the meeting a wet workshop on inhibitor testing and an interactive course on the role of external quality control in laboratory management took place. In this issue you will find a report about this meeting, workshop and course.

We are also glad to inform you about the new initiatives of the ECAT Foundation. It is our pleasure to introduce to you in this issue The Clotting Times Advisory Committee.

In a case report, the phenotypical change of the activated protein C resistance ratio after liver transplantation is described by Dr Katrien Devreeze from the University Hospital of Gent, Belgium. In the rubric entitled "Literature Reviews" we would like to highlight a recent article on the use of an APTT reagent with ellagic acid in the diagnosis of lupus anticoagulant.

The editorial board wishes you a healthy 2013.

Yours sincerely, Petra ter Hark

Content

News

New logo

Since the ECAT Foundation was established in 1994 there have been many changes in the activities of the ECAT Foundation. Though we started as an external quality assessment (EQA) provider in the field of thrombophilia, nowadays our programme covers the whole field of thrombosis and haemostasis. Another important change is the shift from being a European-oriented EQA provider to being a global EQA provider. Therefore, after almost 20 years we decided to look for a new modern logo. We are proud to present to you here our new logo, which will be introduced in 2013.

The large Q indicates our major focus: Quality. This is borne out not only by the fact that we are a provider of an EQA programme but also by our scope for assisting laboratories in quality improvement in laboratory diagnosis in the field of thrombosis and haemostasis. The Q also means that ECAT would like to work according to high-quality standards. By the Q you may appreciate also the reference to the six-ring structure from our previous logo. This represents the basic structure of the fibrin network, which can be recognized in the continents on the globe in the middle of the logo. The globe represents the fact that we are nowadays a global organization. The colour red in the logo represents the colour of blood, while the blue represents the fact that quality in laboratory diagnosis is related to the safety of patients.

Although our logo has been changed one important issue still remains the same. The ECAT aims to be an EQA provider using high-quality standards.

News

The Clotting Times Advisory Committee

In 2000 Marlies Ledford-Kramer started with the publication of The Clotting Times, which was followed in 2003 by the establishment of the educational website CLOT-ED. In 2010 the ECAT Foundation became the owner of CLOT-ED. We continued with the publication of The Clotting Times. From that time onwards Marlies served as an advisor for the Clotting Times. Her very helpful advice and comments were very much appreciated. Unfortunately by mid 2012 she decided to completely step down as an advisor for The Clotting Times. Although we respect her decision, we regret this very much and will miss her good advice. On this occasion we would like to thank Marlies for her co-operation.

This was a good point at which to start with a new international advisory committee for The Clotting Times.

We are pleased to introduce to you the following members of the advisory committee:

Betsy Van Cott, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States;

Katrien Devreese, University Hospital, Gent, Belgium;

An Stroobants, Academic Medical Center, Amsterdam, The Netherlands

Dirk Peetz, Institute for Laboratory Medicine, Helios Clinic, Berlin, Germany.

We welcome Betsy, Katrien, An and Dirk into our advisory committee and are looking forward to working together on The Clotting Times to maintain it as an interesting and important resource for relevant information on laboratory diagnosis in the field of thrombosis and haemostasis.

Focus Article:

Diversity in testing for platelet function disorders by light transmission aggregometry in the Netherlands

P. Verhezen BSc

Working group on Haemostasis laboratory Diagnostics (NVTH -WHD)

Background

Diagnosis of platelet-function disorders is very important for resolving the cause of a bleeding tendency in patients. Different types of platelet function disorders are known, which can be caused by both hereditary and acquired factors [1, 2]. Examples are abnormalities of platelet receptors, abnormalities in signal-transduction mechanisms, and deficiency of granular content [2].

The most important and common test to diagnose platelet function disorders is Light Transmission Aggregometry (LTA). This method was discovered almost 50 years ago by Born and O'Brien [3, 4]. LTA is based on the measurement of increasing light transmission of platelet-rich plasma by aggregation of the platelets after the addition of an agonist. By using different kinds of agonists it is possible to test for several types of common and rare platelet function disorders.

Although this method is lacking standardization, is time-consuming, and quality control is difficult to perform, it is still the 'gold standard' method for the testing of platelet function disorders [1]. Proposals for standardization have been recommended in the past, but a universal guideline was lacking. Recently the following guidelines were published: Clinical and Laboratories Standardisation Institute (CLSI 2008) [5], British Society for Haematology British Committee for Standards in Haematology (BSH BCSH 2011) [6], and the North American Consensus Guidelines (QMP-LS and NASCOLA 2010) [7]. The Platelet Physiology Subcommittee of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis (SSC-ISTH) is currently working on a guideline.

Methods

The working group on haemostasis laboratory diagnostics (WHD) of the Dutch Society on Thrombosis and Haemostasis (NVTH) has performed a survey to make an inventory of the ways in which platelet function testing is performed among its members. One of the main topics in this survey was the performance of LTA. This paper will focus on these LTA results, and will give a summary of the current degree of standardization of LTA performance in the Netherlands. The results are also compared with the recent available guidelines. A total of 19 laboratories completed the questionnaire, and 15 of them performed LTA. Because LTA is a challenging technique, which depends on different variables, the discussion of the results is divided into the following topics: blood collection; centrifugation; time between blood collection and LTA; type and concentration of agonists.

Results

Blood collection

According to the CLSI [5] and the BSH BCSH [6] guidelines, blood for LTA should be collected using evacuated tubes or

syringes, and the blood collection has to be performed with a minimum of tourniquet pressure. In the past, concerns have been raised about the use of evacuated tubes for LTA, because it was suggested that the use of these tubes may lead to platelet activation. Because comparison studies showed minimal differences, the guidelines approve both methods.

Results: For the blood collection 50% of the laboratories use evacuated tube systems and 50% use syringes. Both methods are in line with the guidelines as discussed above. Two participants use a so-called open-collection system in which the blood flows from an open needle into an open tube. Although this method is historically the best method to prevent platelet activation, concerns have to be raised for both the operator safety and the impact of the blood collection on the patient. Because there are commercially available syringe systems in which the blood is collected without using evacuated tubes, this open-collection technique is not suitable for common practice.

Eighty-one per cent of the participants use a tourniquet, but it is directly released when the blood flow starts, resulting in a minimum of tourniquet pressure as recommended in the guidelines. Nineteen per cent of the participants do not use a tourniquet at all.

Centrifugation

For LTA it is essential to prepare platelet-rich plasma (PRP) and platelet-poor plasma (PPP). PRP and PPP can be obtained by centrifugation of whole blood. It is very important that a good separation occurs, so that the PRP only consists of platelets and that the red- and white blood cells are removed from the PRP [5].

If red blood cell contamination occurs, it disturbs the LTA measurement. Because the red blood cells are large cells, they will absorb the transmitted light, which will result in a falsely decreased aggregation response [5]. This red cell contamination can occur due to improper centrifugation, use of the (centrifuges) brake and by disturbing the cellular component of the centrifuged sample when separating the PRP from the other cells. According to the CLSI guidelines [5], PRP should be prepared by centrifugation for 15 minutes at 170 G. The BSH BCSH guideline [6] advises the preparation of the PRP by centrifugation for 10 minutes at 170-200 G. Both guidelines advise not using the brake.

Results: According to the responses of the questionnaire, PRP is prepared by centrifugation at between 120 and 240 G for 8 – 20 minutes. It is very remarkable that all laboratories use their own method for PRP preparation and that that there are no exact similar methods used by the 15 laboratories that perform LTA. It is also very disturbing to notice that forty per cent of the participants use a brake during PRP preparation. For the preparation of PRP we can conclude that there is a major lack of standardization. The laboratories that centrifuge by less then 170 G and use the brake could have problems with the quality of the prepared PRP (red cell contamination).

For the preparation of PPP all guidelines advise preparing PPP by centrifugation at 1500 G for 15 minutes. According to the results of our questionnaire, PPP is prepared by centrifugation at 1200 – 16000 G during 5 - 20 minutes. The laboratories that use a very high centrifugation speed, prepare the PPP in two different centrifugation cycles. The first cycle is used for the separation of plasma from the cells. The second step (after the plasma has been transferred into another tube) is used for the complete preparation of PPP. Although there are some differences in the guidelines for the preparation of PPP, this is less problematic than the differences observed for the PRP preparation.

Time between blood collection and LTA

According to both the CLSI [5] and BSH BCSH [6] guidelines LTA has to be performed within 4 hours after blood collection. The British guidelines [6] also state that the PRP sample has to rest 30 minutes before analysis. The CLSI guideline [5] is not clear whether it is mandatory to rest the platelets before analysis.

Results: All participants perform LTA within 4 hours after blood collection. Forty-three per cent of the participants perform LTA within 3 hours after blood collection and

thirty-three perform LTA in even less than 3 hours after blood collection.

About the resting of the PRP before analysis, a major range (0 – 65 minutes) was observed. Only 2 participants do not rest the platelets, or rest them for less than 30 minutes before testing. Both guidelines are respected by the participants for the maximum age of the sample after blood collection and most participants follow the minimum requirements of resting before LTA according the BSH BCSH guidelines.

Type and concentration of agonist used

There are different types of agonist that can be used in LTA. The CLSI [5] recommends the use of the following agonists: Adenosine diphosphate (ADP), collagen, epinephrine, ristocetin and arachidonic acid. The CLSI guideline is not clear about which exact concentrations should be used for LTA, but instead they advise common ranges of agonist concentrations. The BSH BCSH guideline [6] advises using the same agonists as the CLSI as a baseline panel. Instead of the CLSI guideline, the BSC BCSH guideline advises using the following starting concentrations: ADP 2.5 μM, collagen 1.25 μg/ml, epinephrine 5 μM, ristocetin 1.2 mg/ml and arachidonic acid 1.0 mM. Whenever there is an impaired response to an agonist, a higher concentration of that agonist has to be tested.

Results: Of the common agonists ADP, ristocetin, collagen

and arachidonic acid are used by most participants. Epinephrine is only used by 36% of the participants. Except for epinephrine, the guidelines are followed for the type of agonists. The results of the different concentrations of agonist used show a very broad range. For most agonists, multiple concentrations are used.

Conclusion

On the basis of the results of this questionnaire, we conclude that standardization of LTA in the Netherlands is very poor and that improvement is necessary. Especially preparation of PRP and the concentrations of agonists used should be standardised in the Netherlands. The need for standardization (worldwide) in LTA is also observed in other questionnaires (e.g. SSC-ISTH 2009 and 2011 [8], and NASCOLA [9]). The use of an available guideline will improve the current lack of standardization of LTA in the Netherlands. This is only possible when consensus is reached about which guideline is going to be used. The best solution would be one internationally approved consensus guideline for all laboratories that perform LTA. A possible option could be the guideline that is now being developed by the Platelet Physiology Subcommittee of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis (SSC-ISTH).

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ECAT Information:

ECAT programme 2013

In 2013 we will start with a new module on Factor IX Inhibitor testing. This module will run twice a year (survey 2013-1 and 2013-3). The module includes two samples per survey. The design of the survey is similar to those for Factor VIII Inhibitor.

The new initiative will also start with case-based surveys of bleeding disorders. These surveys will be organized in cooperation with INSTAND in Germany. In these surveys a short patient description, together with plasma and probably also DNA will be provided. The participants are asked for a proper diagnosis. They are free to measure any parameter they find to be relevant to reach a diagnosis. When DNA is provided we also ask for the detection of the gene defect. This new survey will run twice a year.

On the basis of the questionnaire for new parameters in the ECAT programme, which we performed in 2012, two pilot studies are scheduled for 2013. The first study is on testing for ADAMTS13. The measurement of this parameter is important in the diagnosis of Thrombotic thrombocytopenic purpura (TTP). The pilot study includes the measurement of ADAMTS13 activity and antigen as well as ADAMTS13 inhibitor and is scheduled for the spring. The second pilot study is on the measurement of abnormal fibrinogen. Both hyper- and dysfibrinogenaemia may have significant clinical impact and because of this, proper diagnosis is needed. We will therefore perform a pilot study on the measurement of abnormal fibrinogen. This pilot study is scheduled for Autumn 2013.

If you are interested in one of the above-mentioned new initiatives please contact the ECAT office (info@ecat.nl).

ECAT Information:

8th ECAT Participants' Meeting

On 7 – 9 November 2012 the 8th ECAT Participants' Meeting was held in Leiden, The Netherlands.

On Wednesday we started with a wet workshop on Inhibitor testing. Twenty participants took part in this workshop. In the morning the algorithm for the laboratory follow-up after the detection of a prolonged APTT as well as the proper testing for clotting factor inhibitor was discussed. In the afternoon several clinical samples related to this topic were tested. Siemens kindly supported the workshop and measured all the requested tests of the participants on the CA7000 analyser. We wish to thank Dr. Verbruggen for giving this course and for his contribution which gave the participants new insights regarding inhibitor testing. At the same time also an interactive course about the role of external quality control in laboratory management took place for 15 participants presented by Dr. Meijer, the director of the ECAT Foundation. Here the participants had the chance to learn by performing exercises, and to discuss the outcomes.

On Thursday and Friday the 8th ECAT symposium was held. In total 190 participants took part in this symposium. A variety of different topics were discussed, such as Pre-analytical Variables, Gene therapy in Haemophilia treatment, Performance of FVIII and FIX measurement, Interpretation and management of INR results, New Oral Anti-Coagulation drugs, D-Dimer age-dependent cut-off values, Lupus anticoagulant, POCT, and 'What do with a prolonged APTT?'. Also this year we included case studies in the programme, which were clearly presented by Dr. Devreese from Gent, Belgium. During these case studies an audiovisual response system made it possible to take part actively.

A highlight of the meeting was the Haverkate Lecture, this time given by Professor J. Rosing, entitled: "Global haemostasis assays: what will be the future?".

The ECAT Foundation is proud to receive officially the accreditation certificate . ECAT is accredited by the Dutch Accreditation Council (RvA), which is by law appointed as the national accreditation body for The Netherlands. The accreditation was based on an assessment carried out according to the requirements of ISO/IEC Guide 17043:2010 standard. This ISO Guide, entitled: Conformity assessment – general requirements for proficiency testing, is the international standard for accreditation of EQA and proficiency-testing (PT) programmes.

This 8th ECAT symposium was a great success. If you are interested in the presentations given at this symposium, most of the lectures and abstracts are available in the educational part of the CLOT-ED section on the ECAT website, www.ecat.nl/meeting .

ECAT Information:

CLOT-ED: ECAT Education

Since 2010 the ECAT Foundation has been the owner of the former educational website CLOT-ED. We incorporated the contents of the CLOT-ED website into our own website under the menu item, CLOT-ED.

Now, after 3 years, it is time for a change. The board and director of the ECAT Foundation have decided to pay more attention to educational activities.

Besides maintaining and expanding the educational website we will also focus on the organisation of courses and workshops. All these activities will be grouped together under the title, ECAT Education. At our new website, which will be launched as soon as possible in 2013, you will find these changes in place.

Please visit the Education menu on our website on a regular basis to see new items and activities.

Scientific Article:

External quality control for CoaguChek XS INR monitors

Introduction

External quality assessment (EQA) of test results is a way of establishing the quality of laboratory testing. The major focus of EQA is to establish whether a laboratory is able to produce accurate results. In addition, it is also a method for establishing the between-laboratory variation for a particular test. EQA is nowadays a well-known and accepted procedure for the assessment of the quality of regular laboratory testing. However, EQA for point-of-care testing (POCT) has not yet been generally adopted. One of the problems is the inadequate availability of suitable control material. POC monitors are designed for the use of whole blood. Unfortunately it is difficult to distribute stable whole blood samples suitable for measurement using the International Normalised Ratio (INR) to participants. Fortunately with the CoaguChek XS monitor, the most widely used in Europe, it is also possible to use citrated plasma. The ECAT Foundation therefore designed an EQA programme for the CoaguChek XS INR monitors using lyophilised plasma pools of patients who are already receiving anticoagulants. Because of the direct relationship between the INR value measured and the treatment of the patient, it is important that a POCT can monitor the correct INR value. Therefore in our EQA approach for the CoaguChek XS we focus on the level of accuracy of the measurement.

CoaguChek EQA programme

The ECAT CoaguChek XS EQA programme consists of a set of 4 different plasmas covering the whole therapeutic range (INR 2 – 4.5). Because we use assigned values for the 4 samples (see below) the results of users of these QC sets can be immediately evaluated by the ECAT on receipt. This also means that quality control can be performed by the user at any time convenient for them.

It has also been shown that the samples are stable for 6 hours after reconstitution. This makes it also possible to evaluate multiple monitors during the day with the same control set.

The results of the 4 samples are evaluated in an integrated linear regression model. With this model we are able to assess whether it is possible to obtain accurate results over the entire therapeutic range. Acceptance criteria are based on the deviation of the target value, slope, intercept and correlation coefficient.

The assigned values are established by using multiple measurements with different lot numbers of test strips as well as different monitors. The measurements are performed by the Dutch Reference Laboratory for Anticoagulation (RELAC). The assigned values, including their uncertainty, are shown in Table 1.

Table 1. The assigned values and results of quality control performance with the ECAT QC set for the CoaguChek XS

	Sample 1	Sample 2	Sample 3	Sample 4
Assigned value	1.9	2.7	3.9	4.1
Uncertainty of assigned value	1.0%	0.0%	0.6%	0.4%
Mean INR	2.0	2.8	4.1	4.3
Coefficient of variation	5.3%	2.1%	2.3%	2.4%

Results

Since we introduced this programme in 2012 in total 103 monitors were checked. Five sets of results were excluded from the evaluation because of an error in one of the samples. From the 98 monitors left, 94 fulfilled the acceptance criteria. A summary of the results is given in Table 1. Test strips with five different lot numbers were frequently ($n \geq 5$) used by the participants. On average no differences were observed in the INR values of test strips with different lot numbers.

Conclusion

The ECAT EQA programme for CoaguChek XS monitors has been shown to be a valuable tool for quality control of POC INR testing. From the first results obtained in this programme it can also be observed that overall Coaguchek XS monitors show a stable performance.

Information

If you are interested in the ECAT EQA programme for CoaguChek XS monitors please contact the ECAT office (E: info@ecat.nl ; T: +31 88 8669718).

Case Report:

Phenotypical change of the activated protein C resistance ratio after liver transplantation

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Case report

A female patient (°1955) with autosomal dominant polycystic kidney disease (ADPKD) presented with a pulmonary embolism in 1997. The thrombophilia screening in 2002 revealed resistance for activated protein C (APCr) on two consecutive plasma samples (APCr ratio = 1.44 and 1.36; reference range >2) but no other prothrombotic abnormalities. Mutation 1691 G>A in the Factor V gene on chromosome 1q23 (Factor V Leiden) was confirmed using a real-time PCR assay for allelic discrimination, which demonstrated heterozygosity (see Table 1). A few years later, her chronic kidney disease progressed, resulting in renal replacement therapy. She was placed on a waiting list for kidney transplantation in 2008. Because of a second lung embolism in 2009 coumarine therapy was started. After an elective nefrectomy (2011) due to infected renal cysts, she developed a persistent ascites production. In view of the latter problem and the massive cystic enlargement of the liver a combined liver and kidney transplantation was planned and was carried out on August 25th 2011. Pre-transplantation laboratory findings showed a haemoglobin concentration of 10.1g/dl (11.7-15.7 g/dl), a leucocyte count of 5480/µl (4000-10000/µl), a platelet count of 224 000/µl (177 000-393 000/µl), a serum creatinin concentration of 4.01 mg/dl (0.55-0.96 mg/dl), an alanine aminotransferase (ALT) of 34 U/L (7-31 U/L), an aspartate aminotransferase (AST) of 53 U/L (0-31 U/L), a ã-glutamyltransferase (GGT) of 618 U/L (9-36 U/L), and an alkaline phosphatase of 673 U/L (30-120 U/L). Prothrombin time (PT) was 29% (70-120%) and the INR 2.73 (0.9-1.1). Post-transplantation, imaging demonstrated a good perfusion of the kidneys, a good arterial flow of the liver but a reduced portal flow. The elevated liver enzymes and serum creatinin decreased to normal values in the succeeding week. Considering the presence of the FVL mutation heparin therapy was immediately started after surgery. At discharge, the antivitamin K therapy was reintroduced regarding the FVL mutation and history of lung embolism. Six weeks later, the patient had recovered well and liver and kidney imaging was normal. The liver parameters were stabilised and a control sample was sent to the laboratory to check for APCr ratio and FVL mutation. The APCr ratio appeared to be normal (2.59; reference range >2), although the heterozygous FVL mutation was

Table 1. Patient's laboratory haemostatic values pre- and post-transplantation

Laboratory test	Pre-transplantation	Post-transplantation	Reference value
Antithrombin	104.0 %		80.0 - 120.0 %
Protein C activity	102.0 %		70.0 - 130.0 %
Protein S antigen	106.0 %		50.0 - 134.0 %
APCr ratio → control sample	1.44 1.36	2.59	> 2.00
Lupus anticoagulant	Absent		/
Prothrombin mutation	Homozygous normal		/
FVL mutation	Heterozygous mutant	Heterozygous mutant	/
aPTT	37.3	34.1	28.9 - 38.1 s
INR	/	2.97	0.9 - 1.1

APCr: activated protein C resistance; FVL: Factor V Leiden; aPTT: activated partial thromboplastin time; INR: international normalized ratio

reaffirmed (see Table 1). The discrepancy between the APCr ratio and the FVL mutation can be explained by the preceding liver transplantation. The elevated INR is explained by the use of anti-vitamin K therapy. Despite the normalised APCr ratio, life-long anticoagulant therapy was indicated due to the past recurrent pulmonary embolism [1].

Discussion

Although liver failure is uncommon in ADPKD patients, liver transplantation is occasionally indicated for these patients in the case of massive cystic enlargement of the liver [2]. The quality of life, abdominal pain, anorexia or fatigue often improve after transplantation, but these patients seem to be highly susceptible to infections after such high risk intervention [2]. This patient had a high serum creatinin concentration, an important variable of the MELD model (scale from 6 to 40), and the prognostic model for end-stage liver disease [3]. A score of 15 or more indicates hepatic dysfunction. As she also used oral anticoagulants, she had an artificially high MELD score due to the contribution of the INR in its calculation [3]. Furthermore, polycystosis is a standard exception in this model. This means patients can be listed with 20 points independently of the laboratory values or receive additional points each three months [4]. Despite the fact that the elevated creatinin level raises the MELD points, pre-existing renal disease can cause a reduced survival and renal failure requiring dialysis after liver transplantation, except for the hepatorenal syndrome [2]. Thus, a combined liver and renal transplantation can be an excellent option for carefully selected patients since this must be weighed against the risk of depriving renal transplant recipients of donor organs [2]. The successful combined transplantation in this patient cured the polycystosis but developed a discrepancy between APCr assay and the FVL genotype.

APCr, first described by Dahlback *et al*. in 1993, means the patient's plasma is resistant to the anticoagulant effect of activated protein C [5]. Approximately 95% of these patients have a mutation in the Factor V gene, called Factor V Leiden, first characterised in Leiden in the Netherlands [6]. It describes a G1691A nucleotide transition, a missense mutation, resulting in an amino acid substitution of arginine by glutamine (R506Q) which causes resistance to proteolysis by APC. The FVL mutation, with a prevalence of 5 to 8% in the normal population, is associated with an about 5- fold increased risk of venous thrombosis in heterozygous patients and an 80-fold increased risk in homozygosity [7]. Patients with FVL usually present with a deep venous thrombosis of the leg veins [7].

Regarding the laboratory diagnosis, the most commonly used phenotypic screening test for FVL is the modified APCr coagulation assay, requiring predilution with FV-deficient plasma, which excludes other clinical conditions causing a decreased APCr ratio. This functional test is based on a prolongation of the APTT by addition of APC and can be managed in anticoagulated patients. Results are expressed as an APCr ratio, the ratio between the APTT measured in the presence and absence of added APC [8]. The FVL mutation detection on DNA extracted from peripheral blood leucocytes can be performed to confirm abnormal APCr results [7, 9].

Before transplantation our patient's hepatic tissue contained the same DNA with heterozygosity for FVL as in the leucocytes. By transplantation she received a donor liver producing wild-type FV which phenotypically cured the APCr, despite heterozygosity for FVL in the patient-derived haemopoietic cells.

80% of the Factor V circulates in the plasma, while the residual 20% can be found in the platelets, respectively synthesised in the liver and a smaller pool originating from the megakaryocytes [10]. Even though the patient's APCr in plasma is corrected by liver transplantation, the platelet-derived FVL could contribute to the persistence of the

prothrombotic state [11, 12]. Namely at the side of vascular injury, platelet FV concentration is 600 times higher than that of the plasma FV within a platelet aggregate [10]. However, Camire *et al.* showed that most of platelet FV is endocytosed by megakaryocytes from the plasma [13] instead of endogenous synthesis. Consequently, the risk of venous thromboembolism would be decreased due to the production of normal FV and the plasma correction of APCr after liver transplantation [14, 15]. Equally, when a patient receives an hepatic allograft from a heterozygous FVL donor, the mutant FV results in an abnormal APCr ratio and an increased thrombotic risk regardless of the patients' normal genotype [16, 17]. Similarly, normal genotype stem-cell recipients can receive the bone marrow of a heterozygous FVL donor achieving a normal APCr ratio and a mutant FV allele. Despite this FVL mutation, the thromboembolic risk would not be increased because of normal circulating FV [16] (see Table 2).

Table 2. Discrepancy of phenotype (APCr ratio) and genotype (FV Leiden mutation) in several conditions

	aPC-r	FV Leiden
Stem cell recipient		
Pre-transplant	2.7	G/G
Post-transplant	2.7	G/A
Stem-cell donor	**1.7**	**G/A**
Stem-cell recipient		
Pre-transplant	1.7	G/A
Post-transplant	1.7	G/G
Stem-cell donor	**2.7**	**G/G**
Liver recipient		
Pre-transplant	2.7	G/G
Post-transplant	1.7	G/G
Liver donor	**1.7**	**G/A**
Liver recipient		
Pre-transplant	1.7	G/A
Post-transplant	2.5	G/A
Liver donor	**2.5**	**G/G**

Conclusion

We describe a patient with a discrepancy between the FV genotype (FVL) and phenotype (APCr) after liver transplantation. Before transplantation, our patient suffered from two episodes of unprovoked pulmonary embolism, a rather unusual presentation of heterozygosity for FVL. Therefore, we decided to continue the anticoagulant therapy despite the normalization of the APCr ratio [1] and the lack of guidelines in the case of normal phenotype/abnormal genotype discrepancy.

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Literature review:

APTT reagent with ellagic acid as activator shows adequate lupus anticoagulant sensitivity in comparison to silica-based reagent

(G. Kumano et al. J Thromb Haemost 2012; 10: 2338 – 2343)

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The subcommittee on Lupus Anticoagulant and Antiphospholipid Antibodies of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis has recommended the use of a silica-based APTT reagent for the detection of Lupus Anticoagulant (LA) [1]. It has already been shown by Jennings *et al* that there are many laboratories which use ellagic acid-based APTT reagents [2]. In the November 2012 issue of the Journal of Thrombosis and Haemostasis Kumano and colleagues published an interesting article on the comparison of ellagic acid and silica-based APTT reagents on the basis of their sensitivity to LA [3].

In this publication the authors compared two APTT reagents prepared in house, one on the basis of ellagic acid and the other on the basis of silica. To both activators the same phospholipid solution, including a mixture of synthetic phospholipids, was added. This phospholipid mixture is similar to the phospholipid composition of the APTT-SLA reagent from Sysmex. In addition 4 different commercially available APTT reagents were included in the study: two ellagic acid-based reagents (APTT-SLA, Sysmex and Actin FSL, Siemens) and two solica-based reagents (APTT-SP, Instrumentation Laboratory and PTT-LA, Stago). The lupus sensitivity was measured by establishing the cut-off levels for the index for circulating anticoagulant activity (ICA) in lupus-negative samples.

Subsequently a set of 22 lupus-positive samples was measured. A summary of the results is given in the table below.

APTT reagent	In-house	In-house	APTT-SLA	Actin FSL	APTT-SP	APTT-LA
Activator	Silica	Ellagic acid	Ellagic acid	Ellagic acid	Silica	Silica
ICA cut-off	12.9	11.5	13.2	15.6	14.3	14.0
Lupus sensitivity	91%	96%	68%	46%	91%	86%

Both in-house reagents showed good lupus sensitivity. This indicates that even an APTT reagent on the basis of ellagic acid may display good sensitivity to LA. The in-house ellagic acid-based reagent showed better lupus sensitivity than the APTT-SLA reagent. However, in the APTT-SLA reagent the concentration of phospholipids is twice that of the in-house reagents. This indicates that the concentration of phospholipids is probably more important than the type of activator.

The authors therefore conclude, that 'ellagic acid-based reagents showed high sensitivity to LA as compared with silica-based reagents in a low phospholipid condition and had adequate sensitivity to detect LA. The LA sensitivity of an APTT reagent is dependent on the phospholipid concentration and not on the activator employed.'

The study may imply the need for a reconsideration of the current SSC guideline on the detection of LA.

For further details about this publication see reference 3.

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