ECAT Newsletter

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Editorial

It is our pleasure to present to you the ninth issue of the ECAT Newsletter. This issue starts with a Focus Article describing how to establish the mean and standard deviation of the internal quality control samples necessary to construct control charts. For this the Bayesian approach is used with an example of D-dimer. A practical tool for this common problem is included.

We are happy to announce that the abstracts and presentations of the 9th ECAT Participants' Meeting are now available on the ECAT website.

In the section 'ECAT information' we ask for your attention to the correct use of the < and > estimators in the result submission facility. Furthermore, information is given about an external quality assessment (EQA) programme for the CoaguChek XS/XSpro (POCT). A case report describes a patient with Lupus Anticoagulant-Hypoprothrombinemia Syndrome. The rubric entitled "Literature Reviews" highlights a recent study where the minimum citrate tube fill for routine coagulation testing was investigated.

We hope you enjoy reading this informative newsletter.
The editorial board and advisory committee wish you a healthy 2015.

Petra ter Hark

Content

News

Abstracts and presentations for the ECAT symposium

Abstracts and presentations from the last ECAT symposium are now available on the ECAT website.
Select the homepage "ECAT Education" followed by "Education". Log in with your labcode and password and select subsequently "ECAT Meeting". Here you can find the abstracts and presentations of the 9th International symposium.

EQA for the CoaguChek POCT INR

The ECAT have started a special External Quality Assessment programme for the CoaguChek POCT INR monitor.
More information about this programme can be found on page 6 of this Newsletter.

Customer Satisfaction Questionnaire

We are interested in your opinion about the services of the ECAT external quality assessment programme. If you have not yet done so, please complete the customer satisfaction questionnaire which can be found in the member section of the ECAT website. Completion of the questionnaire takes approximately 10 -15 minutes. We highly appreciate your cooperation.

Template for calculating the mean and standard deviation necessary for an Internal Quality Control Chart

In this issue you can read a contribution from Dr. F. Sobas, Lyon, France who has developed a practical tool to established the mean and standard deviation of an internal control sample necessary to conduct a control chart. This Excel tool is now available on the ECAT website. Go to via the log in function to the member section. Select "IQC template". Here you can download the Excel spreadsheet mentioned in the contribution of Dr. Sobas. Also to be found there are a short guideline on how to use the Excel spreadsheet as well as a document with more background about the Bayesian statistical approach for establishing the mean and standard deviation for internal quality control samples in the preliminary phase.

Focus Article:
How to establish the mean and standard deviation of internal quality control samples to construct control charts.
The Bayesian approach with an example of D-dimer

Panagiotis Tsiamyrtzis[1], Frédéric Sobas[2] and Claude Négrier[2]

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2: Hemostasis Laboratory, Edouard Herriot Hospital, Lyon, France

Abbreviations:
FAP: False alarm probability
IC: In control
IID: Independent identically distributed
IQC: Internal quality control
BCC: Bayesian control chart
SPC: Statistical process control
$\hat{\mu}$: Estimated prior target mean value for the control material under monitoring
$\hat{\sigma}$: Estimated maximum acceptable inter-assay standard de viation (SD) according to the manufacturer
$\hat{\tau}$: Estimated inter-assay SD during method validation in each laboratory

Introduction

Internal quality control (IQC) is an important tool for establishing whether an analytical test system produces reliable results. For IQC one or more quality control (QC) samples are run prior to or simultaneously with patient samples. If the results of the control samples are within certain predefined acceptance limits, the results of patients' samples can be released. QC sample test results are frequently displayed in so-called control charts. To evaluate these QC sample results and look into trends statistical process control (SPC) methods are used. One of the well-known QC rules is the 1_{2s} QC rule [1,2] which signals anything that exceeds two SD from the expected value. For each new batch of IQC samples the expected value (mean value) and SD need to be established to construct a control chart. This requires two phases: I (preliminary) & II (testing). The former is done in an off-line mode (i.e. the QC sample is not yet used for making a decision about the status of an analytical system), where first we estimate the parameters used to build the chart and then examine the data retrospectively, i.e. once phase I is completed all the phase I data will be examined for conformance with the established limits. On the other hand phase II runs on-line, i.e. each new reading is plotted on the chart and on-line inference is available. It is important not to forget the statistical aspect of the IQC management regarding the assumption of approximating the normal distribution when enough data are observed. In other words the phase I management is very crucial with respect to the conventional phase II reliability.

In this contribution we focus our attention on the conventional preliminary phase, where the major goals are both to be able to perform efficient QC monitoring even when we have very few data points available and to obtain "reliable" estimates of the mean and the inter-assay SD for the next long-term conventional QC management. It is possible to run an off-line preliminary phase in advance, using new control batches before actually changing batches. But there is considerable technical and economic interest in getting round this conventional preliminary phase management with regard to the high number of laboratory tests especially when the measurement series are not frequent. Unreliable estimates of the mean and the inter assay SD can have from serious to catastrophic results on the performance of the classical control chart in both phases I & II [3,4]. This means that QC results can either be falsely rejected or accepted. This implies the risk of a waste of resources (falsely rejected) or the risk of a wrong interpretation of patient sample results (falsely accepted).

The classical approach during the preliminary phase (off-line method), assumes the process is in the in-control (IC) state (i.e. no abnormal cases should be present), with independent identically distributed (IID) observations. The longer the preliminary phase, the more accurate the estimates, but simultaneously the more likely it is that the process will deviate from its IC state. Typically, the mean and inter-assay SD are estimated from at least 20 and 30 control values respectively [5]. The preliminary phase uses up a great deal of resources, given the large panel of tests a laboratory has to carry out. This is especially true as laboratory examinations are rarely performed in continuous series, resulting in overlaps with currently used QC samples that are very hard to manage.

This work proposes an alternative monitoring mechanism that will not require a preliminary phase, allowing on-line inference from the beginning (i.e. from the second measurement onwards). It is based on the Bayesian logic where we utilize available manufacturer's prior information. The two main manufacturer prior parameters are:

1. Prior target mean of assayed quality control materials (manufacturer control materials with assigned values).
2. Maximum acceptable inter-assay SD value on methods with reagents and device both provided by the manufacturer (technical notices specifying the maximum acceptable inter assay SD)

We provide an Excel spreadsheet (downloadable on the ECAT website) where the proposed monitoring method can be applied to the process readings once we specify three parameter values:

i) $\hat{\mu}$: the prior target mean value for the assayed control-material being monitored (i.e. the target value provided by the manufacturer).

ii) $\hat{\sigma}$: the maximum acceptable SD specified by the manufacturer.
iii) $\hat{\tau}$: each laboratory's inter-assay SD determined during the method-validation phase (with at least 30 data points [5]).

The data can be immediately used for on-line monitoring. The data is put sequentially in the Excel file, as they arrive and have immediate inference for the process, (i.e. "ALARM" if there is a loss of statistical control state). At the end of phase I the Excel tool provides a mean (when we have at least 20 observations in phase I), which can then be implemented in a conventional control chart for the testing phase. With 30 data points, the Excel tool will also calculate the inter-assay SD value that one can compare to the inter-assay SD determined during the validation phase of the method in order to identify an underlying matrix effect [6].

Methods

Estimating the required parameter values for the Bayesian control chart

The construction of the Bayesian control chart (BCC) requires three parameters: $\hat{\mu}$ and $\hat{\sigma}$ which are obtained from manufacturer specifications and $\hat{\tau}$ which reflects the accuracy of measurements in the laboratory and is established during the laboratory validation process.

For estimating $\hat{\mu}$ and $\hat{\sigma}$, we can use the fact that the manufacturer normally provides the acceptance range for the IQC results (i.e. the mean +/- 2 * SD) and a coefficient of maximum acceptable variation CV for a given process. The data have a high probability of being within these limits if the process is under the in-control state. Then an estimate of $\hat{\mu}$ and $\hat{\sigma}$ can be calculated by :

$$\hat{\mu} = \frac{L+U}{2} \quad \text{and} \quad \hat{\sigma} = \hat{\mu} \times CV$$

(L = lower limit of acceptance range; U = upper limit of acceptance range)
Example : L = 0.8 IU/mL ; U = 1.0 IU/mL ; CV = 5 %
$\hat{\mu}$ = (0.8 + 1) / 2 = 0.9 ; $\hat{\sigma}$ = 0.9 * 0.05 = 0.045
The parameters are used in the Excel template.

Regarding $\hat{\tau}$, i.e. the accuracy of the measurement, it depends on various laboratory factors: equipment, experience of the technician, etc. It can be assessed during in-laboratory method validation upstream of implementation, as the inter-assay SD, varying according to the degree of control over these nuisance parameters [7].

As data are obtained sequentially, they are entered into the Bayesian chart and from the second observation it is valid to perform inference. During the preliminary phase the parameters $\hat{\mu}$ and $\hat{\sigma}$ are continuously updated based on the actual IQC results obtained. The initial prior settings of $\hat{\mu}$ and $\hat{\sigma}$, will affect the performance of the chart only for the very first few observations, and their effect will vanish as more data become available. Thus, as long as we avoid extreme choices (such as very small $\hat{\sigma}$), the chart will be quite robust even when poor estimates were used for these prior settings. On the other hand, the chart will be more sensitive to the parameter $\hat{\tau}$, which reflects the accuracy of the laboratory, since it will be fixed and not updated at any stage. A sensitivity analysis in the Results section examines the effect of parameter misspecification in the case study considered.

Technical details of the BCC construction, along with an Excel BCC template that runs the suggested methodology can be found in the member section of the ECAT website and can be tested by interested users.

Results

Case study: Reagent and automated coagulation analyzer

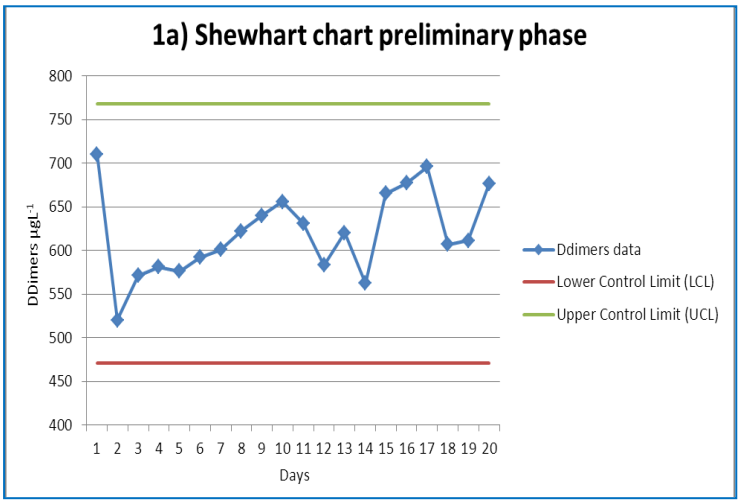
An Instrumentation Laboratory (IL) (Bedford, MA, USA) automate and reagents were used (analyzer: ACLTOP 500 CTS®; reagent: D-Dimer HS 500® for D-Dimer quantification in citrated human plasma). Control material was a low-control sample (D-Dimer HS 500® control level 1), with prior allowable inter-assay SD defined by IL as 65 ($\hat{\sigma}$) and prior mean value 544 μgL^{-1}.

Acceptability of the 20 control values was confirmed as they had been collected during a phase of overlap with another control batch with the same reference, in turn collected respecting the 1_{3s} rule. In the new IQC batch, IL's prior target value was 544 μgL^{-1}, and the maximum acceptable inter-assay SD should not exceed 65 when validated in each laboratory. In the checking phase, inter-assay SD in our laboratory was 49 ($\hat{\tau}$) [7].

Analysis of preliminary phase data

After the preliminary phase, performed on the 20 values, the mean was 620 μgL^{-1} and inter-assay SD 49, while the control limits were set at ±3.016 so that a 5% false alarm probability (FAP) was achieved for the whole sequence of 20 values (Figure 1a). This is called the 1_{3s} method. To be equivalent to

Figure 1. a) Shewhart chart and b) Bayesian control chart (BCC) during preliminary phase



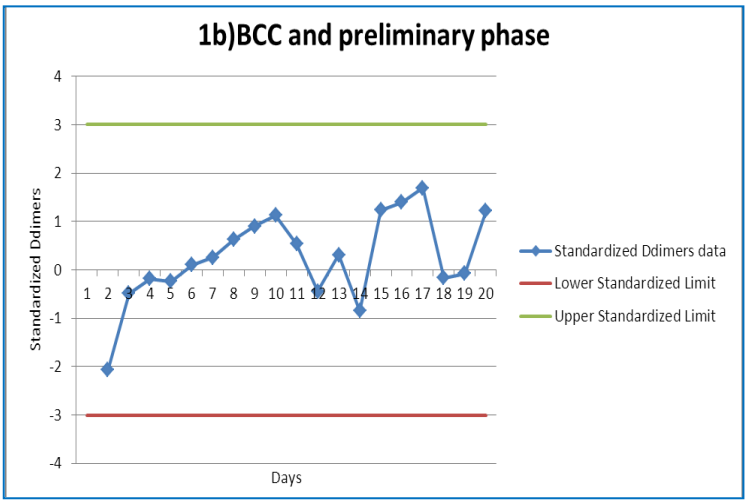
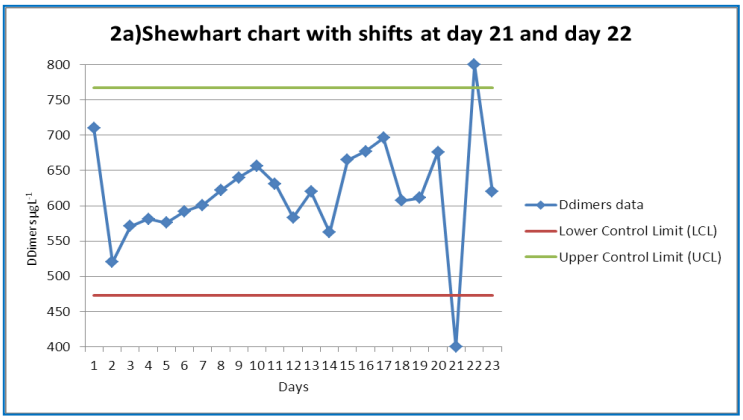


Figure 2. a) Shewhart chart and b) Bayesian control chart (BCC) with shifts at day 21 and day 22



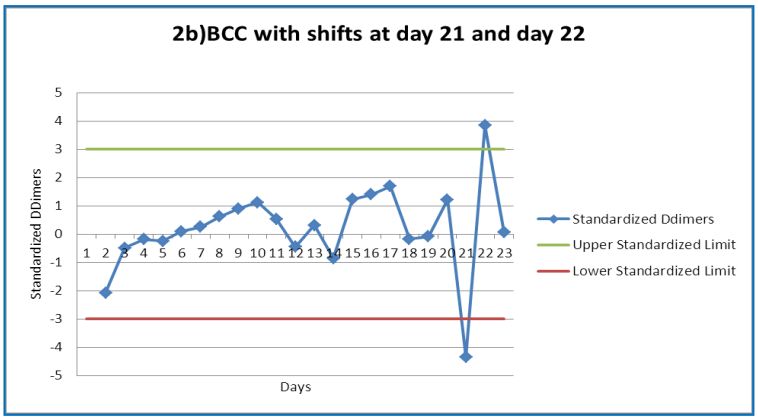
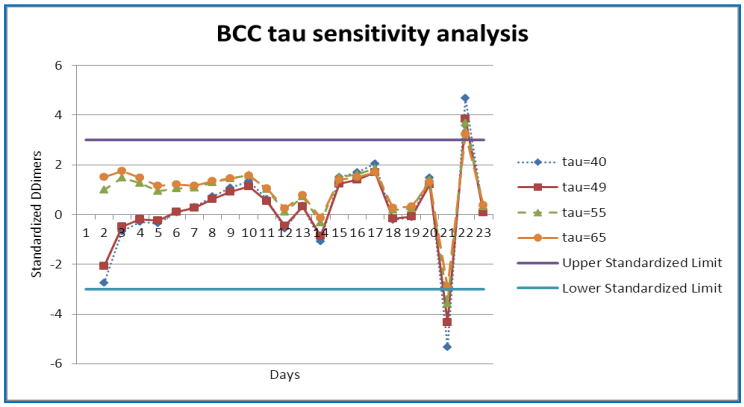


Figure 3. Bayesian control chart (BCC) with shifts at day 21 and day 22 and sensitivity analysis on « own inter assay SD estimated at method validation phase (tau) »



the BCC, the 1_{3s} chart was selected which plots the control limits at 3.01599 SD from the center line, achieving a 5% overall FAP.

The BCC identified no outliers during a preliminary phase well controlled by the overlap phase: i.e., the BCC was not subject to false rejection with $\hat{\tau}$ =49 (Figure 1b).

Analysis of data after the preliminary phase with a shift scenario

The 1_{3s} rule and BCC with $\hat{\tau}$ =49 detected results outside the established limits simulated after the preliminary phase (Figures 2a and 2b).

Examining the sensitivity to the parameter estimates we found that the BCC detected simulated shifts after the preliminary phase with $\hat{\tau} < 65$, while for $\hat{\tau} > 65$, it no longer detected all alarms (Figure 3). This is expected as the large value of $\hat{\tau}$ is associated with rather inaccurate laboratory measurements that can help outlying observations to escape detection. For the remaining two parameters, $\hat{\mu}$ and $\hat{\sigma}$ the sensitivity analysis showed very minor differences as we alter them. Furthermore, these differences were observed only in the very first few data in the process.

Discussion

With the introduction of a new batch of control samples it is necessary to run a preliminary phase. During this phase QC data for accepting or rejecting an analytical was run with the ongoing batch. However, running a preliminary phase can be costly and time-consuming, especially when particular measurements are not frequently performed [4]. Therefore there is considerable technical and economic interest in getting round this problem.

The laboratory may be thought to focus only on manufacturer specifications to define control value acceptability. However it is not unreasonable to use manufacturer specifications (i.e. manufacturers' prior target values and allowable analytic performance are derived from plentiful data from multiple machines and batches), if the analytic system is a good one.

Laboratories with good analytic practice (small inter-assay SD) benefit most from BCCs (Figure 3). They are better able to detect outliers than laboratories with poorer analytic performance. Laboratories must therefore be as careful as possible in estimating prior inter-assay SD in the validation phase.

The Bayesian approach is also useful for both methods which are rarely done and methods using a small batch of IQC samples. It is possible to monitor this kind of method with this short-term Bayesian approach.

Thus, both theoretically and practically, the laboratory is bringing its method under control as soon as it begins implementing its IQC values. At least this Bayesian model can serve as a complement to a conventional approach, which can be reintroduced as soon as there are enough reliable IQC data.

Acknowledgements

The authors would like to thank Dr P. Meijer (ECAT Foundation, Leiden, Netherlands), Dr W.L. Nichols (Mayo Clinic College of Medicine, Rochester, MN, USA) for their kind support and Iain Mc Gill for the translation.

References

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ECAT information:
The correct use of the < and > estimator in the result submission facility

We have a strong indication that not all participants use the < and > estimators in the result submission facility correctly. This is shown in the example of antithrombin activity below.

Method	Equipment	Unit	Result 1	< or >	Result 2	< or >
Stago Stachrom ATIII	STA	%	65	<	95	

It seems that this participant means by their use of the estimator for Result 1 that this result is below the reference range and not that this result is smaller than 65% (< 65%). This is an incorrect use of these estimators. This may lead to the wrong expression of your result in your survey report. Checking the whole survey data file for these potential errors is time-consuming and makes a short turn-around-time for issuing a survey report more difficult.

These estimators should only be used to indicate whether a result is below the lower limit of detection (<[value]) or above the upper limit of the measurement range (>[value]). This is shown in the example below for samples with a very low and high Factor VIII level.

Method	Equipment	Unit	Result 1	< or >	Result 2	< or >
Stago Cephascreen	STA	%	1	<	175	>

Here Result 1 means a Factor VIII activity < 1% and Result 2 means a Factor VIII activity > 175%. The correct way to use the < and > estimators is as follows:

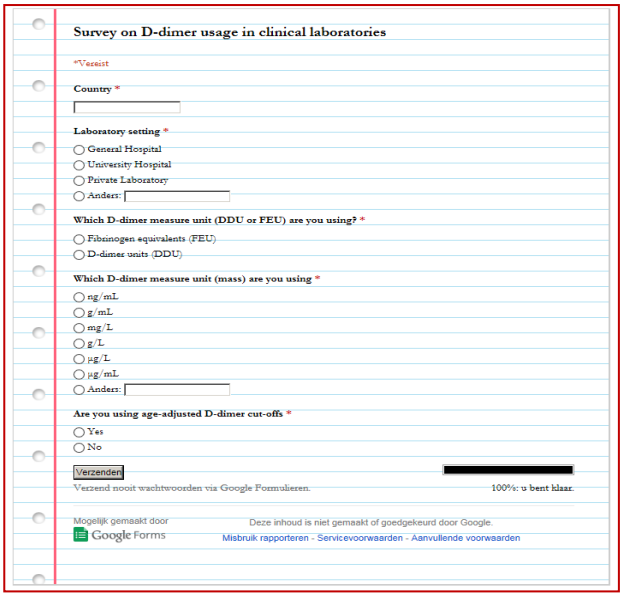
- Report in the result field the lower limit of detection or the upper limit of the measuring range.
- Select in the "< or >" field the corresponding estimator.

Never add an estimator to the result in the result field! The result field only accepts numerical data and no text. When text is added to a numerical field an error occurs during submission.

D-Dimer questionnaire

Our colleagues from the RCPA in Australia have drafted a questionnaire on the use of D-Dimer. D-dimer is now regarded as the biochemical gold standard for assisting the diagnosis of venous thromboembolism (VTE) and predicting the recurrent risk of thrombosis. However, the interpretation of D-dimer values with increasing patient age remains challenging due to many factors. These include: (i) the development of age-related changes in both the microcirculation and blood coagulation, which ultimately contribute to the generation of a hypercoagulable state; (ii) a gradual increase of D-dimer concentration with ageing; and (iii) increasing rates of thrombosis with increasing age, with ageing also recognized to be a risk factor for thrombosis. Thus, questions have been raised regarding the appropriateness of conventional D-dimer cut-off values for older populations. This short survey (<1 minute to complete) aims to identify current practice related to D-dimer testing in this scenario.

We kindly invite you to participate in this short survey. You can find the link to this questionnaire in the member section of our website (www.ecat.nl).


Survey on D-dimer usage in clinical laboratories

*Vereist

Country *

Laboratory setting *
- General Hospital
- University Hospital
- Private Laboratory
- Anders:

Which D-dimer measure unit (DDU or FEU) are you using? *
- Fibrinogen equivalents (FEU)
- D-dimer units (DDU)

Which D-dimer measure unit (mass) are you using *
- ng/mL
- g/mL
- mg/L
- g/L
- µg/L
- µg/mL
- Anders:

Are you using age-adjusted D-dimer cut-offs *
- Yes
- No

Verzenden

Verzend nooit wachtwoorden via Google Formulieren. 100%: u bent klaar.

Mogelijk gemaakt door Google Forms

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Misbruik rapporteren - Servicevoorwaarden - Aanvullende voorwaarden

External quality control for CoaguChek XS INR monitors

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Introduction

Because of the direct relationship between the measured INR and the treatment of a patient with vitamin K antagonists an accurate measurement of the INR is necessary. Today for the measurement of INR the CoaguChek XS/XSpro point-of-care monitor is widely used. The ECAT Foundation has therefore developed an external quality assessment (EQA) programme using control samples with an assigned value for the CoaguChek XS/XSpro. Below this EQA programme is presented.

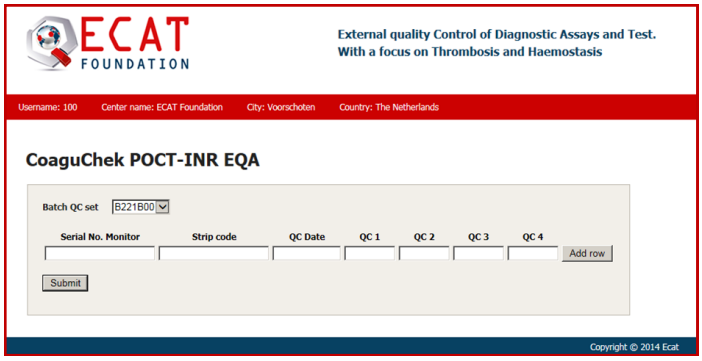
CoaguChek Control Set

For this EQA programme the ECAT uses a control set with 4 lyophilised plasma samples, prepared from pools of plasma of anticoagulated patients with an assigned INR value ranging between 2 and 4.5. The uncertainty of the assigned values is less than 1%. The set includes also vials with water and calcium chloride solution. Because of the use of assigned values the quality control can be performed at any time convenient for the user. It has also been demonstrated 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.

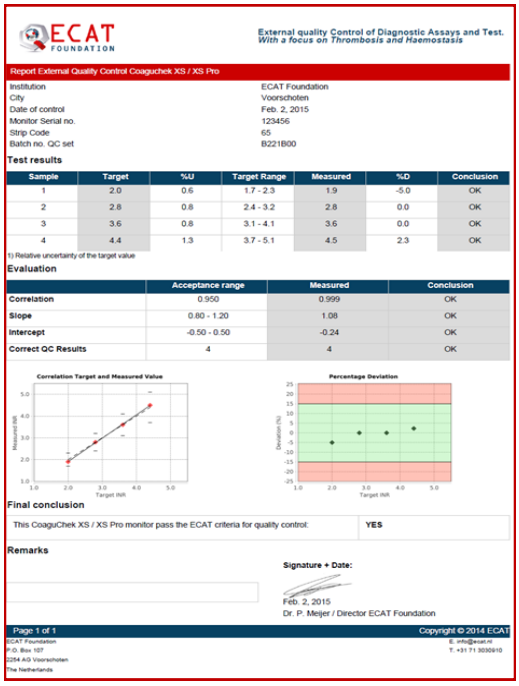
Performance evaluation

The quality performance of a CoaguChek XS/XSpro monitor is assessed by integrated comparison of the INR measured and the value assigned using a linear regression model. Acceptance criteria are based on the deviation of the target value, slope, intercept and correlation coefficient. Because of the use of assigned values the performance evaluation can be done immediately after the INR measurement. An easy-to-use online tool (see picture 1) is now available at the ECAT website for the evaluation of quality performance. With this tool the participant may perform the evaluation themselves. The report is created in PDF format (see picture 2).

Picure 1. Online tool



Picture 2. ECAT report



Results

Since we introduced this programme in The Netherlands approximately 1400 monitors have been evaluated with in total three different lot numbers of the QC sets. Independent of the QC set and the lot number of test strips used, the between monitor variation is on average less than 4%. The failure rate of monitors to pass the performance acceptance criteria is less than 5%.

Participation

There are two possibilities to participate in the ECAT POCT external quality assessment programme.

1) You order the required number of QC sets and perform the quality control of your CoaguChek monitors at any time convenient to you. The price for one QC set is € 60,= (excl. VAT).

2) You subscribe to an annual programme and receive automatically at the beginning of each quarter the required number of QC sets. These sets are used to control your CoaguChek monitors during this quarter Each quarter you receive a different lot number of QC sets. At the end of each quarter an overall report is conducted of all the monitors evaluated in this period which shows the results for all the monitors together as well as at the level of different lot numbers used by the participants. The price for participation in this annual programme is € 295,= (excl. VAT) for one QC set per quarter and € 240,= (excl. VAT) for any additional QC set.

Information

If you are interested in the ECAT EQA programme for CoaguChek XS/XSpro monitors please contact the ECAT office (E: info@ecat.nl ; T: +31 71 3030910) or look at the ECAT website (www.ecat.nl) and select POCT.

Case report:
Lupus Anticoagulant-Hypoprothrombinemia Syndrome

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Case report

A 21-year-old woman was admitted with a recent history of excessive menstrual bleeding, gingival bleeding and epistaxis. Three weeks prior to her presentation she was diagnosed with systemic lupus erythematosus (SLE). She suffered from increased fatigue, arthralgia and synovitis, especially of the proximal interphalangeal joints. Other notable symptoms included alopecia and Raynaud's phenomenon. She had no past history of bleeding disorders or thrombotic events, and her family history was not significant for any coagulation disorder. Her medication included meloxicam (since one month, 15 mg once daily), hydroxychloroquin sulfate (since 2 weeks, 200 mg twice daily), tranexamic acid and iron supplementation.

At presentation, the complete blood count revealed a white blood cell (WBC) count of $5.02*10^9$/L (normal range, $3.65\text{-}9.30*10^9$/L), platelet count of $227*10^9$/L (normal range, $171\text{-}374*10^9$/L), and hemoglobin of 87 g/L (normal range, 118-148 g/L). The initial coagulation screening showed a prolonged activated partial thromboplastin time (aPTT) and prothrombin time (PT), and a normal fibrinogen level of 329 mg/dL. In the diagnostic work-up, we performed a mixing study of patient plasma and normal pooled plasma in a 1:1 proportion. The mixing study resulted in a correction of the PT, while the prolonged aPTT with lupus anticoagulant (LA) -sensitive aPTT reagent (PTT-LA, Diagnostica Stago, Asnières, France) did not improve, suggesting the presence of LA. This was confirmed by using the Staclot-LA® assay (Diagnostica Stago, Asnières, France). The dilute Russell's Viper Venom Test (dRVVT) (LA screen, Life Diagnostics, DSRV, Clarkstons, USA), the mixing test, and the confirmation test (LA confirm, Life Diagnostics) were also positive and confirmed the presence of LA in the dRVVT system. Factor assays revealed a low prothrombin level (Factor II) and low factor (F) VIII-, IX- and XI-levels. Clotting factor activities were measured in one-stage PT (II, V, X) or APTT (VIII, IX, XI) clotting assays using factor-deficient plasmas (Diagnostica Stago) in a standard 1:10 predilution of patient's plasma. The nature of the decreased levels of coagulation factors was studied by repeated testing at increasing plasma dilution (1:40 and 1:100). FVIII-, FIX- and FXI-levels increased towards normal at a 1:40 and 1:100 dilution, suggesting the presence of a non-specific inhibitor (typical pattern of LA). FII-activity remained low even when high dilutions of the patient's plasma were tested. Further evaluation revealed the presence of IgG and IgM anticardiolipin antibodies (aCL), IgG and IgM anti-β_2-glycoprotein I (aβ2GPI) (measured by HemosIL AcuStar, Instrumentation Laboratories, Bedford, MA, USA), and IgG and IgM anti-phosphatidylserine-prothrombin (aPS/PT) antibodies (measured by ELISA Quanta Lite®, INOVA Diagnostics, San Diego, California, USA). The anti-phospholipid 10 Dot (Generic assays GmbH, Dahlewitz, Germany) showed IgG and IgM anti-prothrombin antibodies (aPT). Additionally, laboratory tests showed the presence of anti-dsDNA, anti-Smb, anti-RNP-A, anti-RNP-C and anti-Ribo-P antibodies. Laboratory results are presented in Table1.

Fresh frozen plasma and packed red blood cells were given to control menorrhagia, and high-dose corticosteroid therapy (methylprednisolone 125 mg) was immediately started due to a suspected autoantibody. Prednisolone therapy was slowly tapered and after 1 month of follow-up, both the PT and aPTT and FII were normalized, prothrombin antibodies had disappeared, and no further bleeding episodes were seen. In addition, joint symptoms improved well on corticosteroid therapy. LA, aCL, aβ2GPI and aPS/PT remained positive, even after 3 months of follow-up.

Discussion

We describe here a patient with hemorrhagic symptoms in combination with LA (LA-hypoprothrombinemia syndrome, LA-HPS) associated with SLE. We report what is currently known about the pathogenesis, clinical features, diagnosis, treatment and prognosis of LA-HPS after a literature search. We found 90 cases reported in the literature (between 1960 and February 2014) ; we combined these data with our two new cases reported recently (one of which is decribed here) [1] to summarize the characteristics of LA-HPS.

Pathogenesis

Antiphospholipid antibodies (aPL) are a heterogeneous group of antibodies directed at plasma proteins with an affinity for anionic surfaces (e.g. phospholipids). β2GPI is the principle cofactor protein for aPL; but aPL can also bind with another cofactor, prothrombin [2, 3].

In 1960, the first patient with bleeding symptoms with a LA-associated acquired hypoprothrombinemia was described [4]. The underlying mechanism of LA-hypoprothrombinemia is supposed to be the rapid clearance of prothrombin-antiprothrombin antibody complexes from the circulation, causing a factor II deficiency and hemorrhagic tendencies [5, 6].

Patient characteristics

The incidence of acquired hypoprothrombinemia associated with LA appears to be higher in the pediatric age group, as 55% of cases present in patients under the age of 16, with a median age at disease onset of 13 years (range 1- 86 years). The disease affects females more often than males, with an overall female: male ratio of 1.5:1. The sex difference in disease prevalence in females is even greater in patients ≤ 16 years, with a female: male ratio of 2.5:1 [1].

Underlying diagnosis

The two diseases most frequently associated with LA-HPS are SLE and viral infection. Association with other autoimmune diseases (primary antiphospholipid syndrome (APS), incomplete SLE, discoid lupus, celiac disease, autoimmune hepatitis) has been described [1]. Less frequently, LA-HPS was associated with lymphoma, multiple myeloma, or was drug-

Table 1. Results of coagulation testing of case 1, on admission, after 1, 2, and 3 months' follow-up

Assay	Normal range	On admission	After 1 month	After 2 months	After 3 months
PT (%)	70-120	45	97	106	110
Control (%)		95			
Mix 1:1 (%)		57			
aPTT (PTT-A) (sec)	28.9-38.1	72.3	38.8	38,9	37.1
TT (sec)	<16.8	16.9			
Fibrinogen (mg/dL)	200-400	329			
aPTT (PTT-LA) (sec)	<45.6	106.3	51.7	47	47
Mix 1:1 (sec)	<40.7	181.9	51.9	44.8	43.7
Staclot®					
aPTT no PL (sec)		>180	85.4	77.9	80.0
aPTT PL (sec)		86	59.6	58.1	61.3
Δ = aPTT no PL – aPTT PL (sec)	<8	>94	25.8	19.8	18.7
dRVVT (LA screen) (sec)	<46.1	204.4	79.4	66.2	71.3
LA screen mix 1:1 (sec)	<42.8	216.2	82.1	61.0	56.7
LA confirm (sec)	<41.3	93.9	42.4	37.9	38.2
Ratio LA screen/LA confirm	<1.26	2.18	1.87	1.75	1.87
FII (%)	70-120	16	77	94	87
Dilution 1:40 (%)		18			
Dilution 1:100 (%)		26			
FV (%)	70-120	95			
FX (%)	70-120	88			
FVIII (%)	60-150	17.1			
Dilution 1:40 (%)		30.7			
Dilution 1:100 (%)		86.5			
FIX (%)	60-150	6.3			
Dilution 1:40 (%)		10.4			
Dilution 1:100 (%)		65.6			
FXI (%)	60-140	15			
Dilution 1:40 (%)		29			
Dilution 1:100 (%)		50			
aCL					
IgG (U/mL)	<20	186	66		51
IgM (U/mL)	<20	164	146		126
aβ2GP1					
IgG (U/mL)	<60	765			233
IgM (U/mL)	<20	210			56
Anti-prothrombin antibodies					
aPS/PT IgG (U/mL)	≤30	>150	>150	>150	>150
aPS/PT IgM (U/mL)	≤30	>150	95.8	>150	>150
Anti-phospholipid 10 Dot IgG and IgM					
Prothrombin	Negative	Positive	Negative	Negative	Negative
Phosphatidyl-serine	Negative	Positive	Positive	Positive	Positive

PT, prothrombin time; aPTT, activated partial thromboplastin time; TT, thrombin time; PL, phospholipids; dRVVT, dilute Russell's viper venom time; LA, lupus anticoagulant; F, factor; aCL, anticardiolipin; aβ2GP1, anti-β2-glycoproteïn 1; aPS/PT, antiphosphatidylserine/prothrombin complex; IgG, immunoglobulin G; IgM, immunoglobulin M.

induced. In 10% of cases no underlying diagnosis can be identified.

Clinical features

In the cases reported, bleeding episodes varied in severity from easy bruising to life-threatening postoperative bleeding. The minority of patients (7%) did not show any episodes of bleeding. Clinical bleeding features are summarized in Table 2. Thrombosis (both venous and arterial thrombosis) was reported in 12% of cases with LA-HPS. Three cases with recurrent miscarriages were described. All cases describing patients with thrombosis or recurrent miscarriages had LA-HPS associated with SLE or APS.

Diagnosis

LA-HPS was most often diagnosed after patients presented with bleeding symptoms and a prolonged PT in association with the presence of an LA. Occasionally, patients without prior clinical evidence of bleeding were diagnosed on the basis of coagulation abnormalities during routine blood screening. The laboratory features observed in the reported cases are summarized in Table 3. The most common laboratory finding was the

Table 2. Clinical bleeding features in 72 patients with LA-HPS and bleeding

Bleeding feature	n (%)
Epistaxis	27 (35%)
Ecchymosis	34 (44%)
Petechia	5 (6%)
Gingival bleeding	10 (13%)
Bleeding after tooth extraction	4 (5%)
Gynecologic bleeding	11 (14%)
Hematuria	12 (15%)
Digestive tract hemorrhage[a]	9 (12%)
Intracerebral hematoma/bleeding	5 (6%)
Soft tissue bleeding[b]	7 (9%)
Post-surgery bleeding	5 (6%)
Post-surgery hematoma	2 (3%)
Scrotal hematoma	1 (1%)
Ulcer/cutaneous bleeding	3 (4%)
Retinal or congunctival bleeding	2 (3%)

[a]includes melena, hematemesis, gastrointestinal bleeding, hemorrhagic diarrhea, and rectal bleeding
[b]includes intramuscular hematoma

Table 3. Laboratory features of 92 cases with LA-HPS

Assay	n	Median [range]	Positivity (%)
PT (N of sec)	49	1,7 [1,0-6,0]	
aPTT (N of sec)	67	2,2 [1,1-4,2]	
FII (%)	80	12 [<1-49]	
Lupus anticoagulant (n)	92		91 (99%)
aCL (n)	52		39 (75%)
aCL Ig G (N of titer)	19	7,9 [1,1-373]	
aβ2GPI (n)	19		14 (70%)
aβ2GPI (median [range])	10	11,3 [1,3-150]	
Antiprothrombin antibodies (n)	55		50 (91%)

N, number of times the upper value of the normal range or, if not available, of the normal control; n, number of patients with available data.

combination of prolonged aPTT and PT, normal thrombin time, fibrinogen and normal platelet count. A mixing study should be performed to differentiate between a factor deficiency and the presence of an inhibitor, as mixing of patient plasma with normal pooled plasma in a ratio of 1:1 will normalize aPTT and PT in factor-deficient patients. In contrast, in LA-HPS only PT will normalize after mixing, while aPTT remains prolonged. To further investigate the prolonged aPTT, testing for LA should be performed. LA may occasionally prolong PT and FII deficiency should always be considered if LA is associated with a prolonged PT. In most cases FII levels will be severely decreased. The median FII level reported in case studies was 12% (range <1%-49%). Activity of other coagulation factors may be artificially reduced, but repeat testing in increasing dilutions of plasma should elucidate the true level of these coagulation factors, whilst FII activity will remain low at all dilutions. Similarly, the Bethesda assay for quantifying a factor-specific inhibitor can be false positive due to interference with LA, and an immunoassay for the identification of factor inhibitors could then be used to exclude inhibitors.

When LA-HPS is diagnosed, aPL other than LA should be tested. However, standardization of the assays to do so remains a problem [7]. Therefore, antibodies against prothrombin (aPT) are not included in the current classification criteria to define APS [8]. Nevertheless, antibodies against prothrombin are major antigen targets for aPL and are frequently found in patients with APS. Two types of antibodies are described: antibodies against prothrombin alone (aPT) and those against the phosphatidylserin-prothrombin complex (APS/PT), which are strongly associated with APS [3, 9]. In the cases reported in the literature, data on the presence of aPL other than LAC are limited. aCL and aβ2GPI tested positive in 70-75% of cases. The presence of aPT (evaluated in 51 cases) was positive in 90% of cases [1].

Treatment

Treatment options are summarized in Table 4. Currently, there are no standardized guidelines for the treatment of LA-HPS. The fundamental objectives are to control bleeding (if necessary) and initiate immunosuppression to eradicate the inhibitor. However, prothrombin deficiency associated with transient LAC mostly resolves spontaneously.

Relapse and outcome

Relapse was not reported in cases describing patients with LA-HPS associated with infections. In contrast, relapses of bleeding were described in cases associated with SLE, one with autoimmune hepatitis, one with APS, one with incomplete lupus and two with idiopathic LA-HPS. Fatal bleeding was presented in two cases [1].

Conclusion

We report the case of a girl with LA-HPS associated with SLE, one of the most frequently associated diseases. We presented the results of an extensive literature search on case reports documenting patients with LA-HPS. LA-HPS is a rare disease, with only 92 patients reported in the literature so far. The nature of bleeding episodes presented in these case studies varies greatly in severity. The diagnosis for this acquired hemorrhagic disorder, both clinically and on the basis of laboratory parameters, is difficult, but should be suspected when both aPTT and PT are prolonged, in combination with the presence of LA. There is no consensus on a treatment strategy, but corticosteroids are mostly used as first-line treatment. However, prothrombin deficiency associated with transient LA often resolves spontaneously without therapy. In the case of LA-HPS described in this paper, the patient presented with severe bleeding diathesis.

Table 4. Treatments administrated in 77 patients with LA-HPS

Treatment		n (%)	failure of treatment, n (%)
Supportive treatment			
Fresh frozen plasma		24 (31%)	
Packed red blood cells		16 (21%)	
Platelet concentrate		1 (1%)	
Prothrombin complex concentrate		3 (4%)	
Recombinant factor VIIa		1 (1%)	
Vitamin K		8 (10%)	
Immunosuppresion			
Corticosteroids	alone	24 (31%)	3 (13%)
	in combination with other immuosuppresion	22 (29%)	3 (14%)
Cyclophophamide	in combination with or after CS	9 (12%)	0 (0%)
Azathioprine	in combination with CS	11 (14%)	0 (0%)
IVIg	alone	7 (9%)	0 (0%)
	in combination with CS	1 (1%)	0 (0%)
Rituximab	alone	1 (1%)	1 (100%)
	alone followed by CS	1 (1%)	0 (0%)
	in combination with or after CS	1 (1%)	0 (0%)
Other			
Plasma exchange		2 (3%)	
Hydroxychloroquine		7 (9%)	
Danazol®		1 (1%)	

N, number of cases; CS, corticosteroids

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Literature review:

Minimum citrate tube fill volume for routine coagulation testing

(*L. Pretorius et al. Int. J. Lab. Hem. 2014; 36: 493 – 495*)

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Currently the Clinical and Laboratory Standards Institute (CLSI) recommends a tube filling of 90% for citrate vacutainers or the ratio of blood to citrate in collection tubes should be 1:9 [1]. Under-filled tubes will have a too high citrate concentration which may lead to increased clotting times [1, 2]. Recently Pretorius *et al* published a study where they investigated the minimum citrate tube fill for routine coagulation testing [3]. The study included citrated blood samples from 31 healthy volunteers and 10 patients on long-term oral anticoagulation therapy with vitamin K antagonists. They varied the tube filling for each of the donors between 60% and 100%. Laboratory testing was performed for APTT, PT, INR and fibrinogen. In their publication they show that with 80% tube filling no significant differences with respect to 100% could be observed for all four parameters. The authors also investigated the percentage of test results exceeding their reference limits for APTT, PT and fibrinogen. An overview of the results is given in the table 1.

Table 1. An overview of the percentage of test results

Tube filling (%)	Percentage of test results within reference limits		
	APTT	PT	Fibrinogen
60%	74.2%	100%	93.5%
70%	90.3%	100%	97.0%
80%	100%	100%	96.8%
90%	96.8%	100%	100%
100%	100%	100%	100%

Although there was a small gradual increase (14%) observed for the mean INR values between 100% and 60% tube filling, there was no significant difference from the 100% tube filling mean INR value.

The findings of this study are comparable with those published by Ver Elst *et al* [4]. Lippi *et al* showed that the tube filling for APTT should be at least 89% [5].

The authors concluded that, on the basis of the fact that these 4 parameters are mostly measured from the same specimen, the minimum tube filling should be 80%.

For further details about this publication see reference 3.

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