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An international study of how laboratories handle and evaluate patient samples after detecting an unexpected APTT prolongation

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Abstract

Background: An unexpectedly detected prolonged activated partial thromboplastin time (APTT) can be a harmless laboratory finding, but can also reflect a thrombotic tendency or a bleeding disorder. The assistance of laboratory professionals in the interpretation of an unexpectedly detected prolonged APTT (uAPTT) is often required. The way in which uAPTTs are evaluated in laboratories was assessed in this international study with the aim of determining whether laboratory professionals are able to fulfill this need.

Methods: Postanalytical practices after uAPTT were investigated and the mixing study methodology (if used) was studied by circulating a case report with a questionnaire to staff in the invited laboratories. In addition, the interpretations of those staff regarding the presence or absence of inhibitors in three APTT mixing study scenarios were examined.

Results: Large within- and between-country variations were detected in both postanalytical practices and mixing study methodologies among the 990 responding laboratories, 90% of which were in 13 countries. Shortcomings regarding the investigation of uAPTTs leading to potentially incorrect or delayed clinical diagnoses were found in 88% of the laboratories. Of the laboratories to which the interpretative questions were sent, 49% interpreted all mixing study scenarios correctly. uAPTTs were investigated appropriately and all mixing study scenarios interpreted correctly in parallel in only 9.6% of the participating laboratories.

Conclusions: The clinical requirement for the assistance of laboratory professionals in the interpretation of uAPTTs cannot be met at most of the participating laboratories. Laboratory professionals should be trained in the evaluation of ordinary laboratory tests, such as that for uAPTTs.

Keywords: activated partial thromboplastin time (APTT); clinical interpretation; mixing studies; postanalytical phase.

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Introduction

Activated partial thromboplastin time (APTT) is a frequently requested coagulation parameter in clinical laboratories that is used to screen for intrinsic coagulopathies (hemorrhages) and thrombotic tendencies [e.g., lupus anticoagulant (LA)], and for monitoring unfractionated heparin therapy [1, 2]. An unexpectedly detected APTT prolongation (uAPTT) can be a harmless laboratory finding with a spurious etiology (e.g., preanalytical errors

or a reflection of unknown anticoagulant therapy), or can result from clinically insignificant coagulation factor deficiencies (e.g., factor XII or prekallikrein or high molecular weight kininogen deficiencies), or reflect a severe bleeding disorder (e.g., inherited or acquired hemophilia A) or thrombotic tendency (LA) [1]. The interpretation of a prolonged APTT result requires both comprehensive clinical information regarding the patient and laboratory expertise with respect to coagulation testing and interpretation [3].

After excluding spurious etiologies for the uAPTT, it is essential to determine whether the abnormal APTT is due to an inhibitor effect or a factor deficiency [4–6]. This information can be successfully obtained if the laboratory performs a "mixing study" [7, 8], in which the APTT test is repeated on a mixture of the patient's plasma and normal plasma, and then a decision is made as to the correction or normalization of APTT in the mixture. A correction (normalization) suggests a clotting factor deficiency, while failure of normalization suggests the presence of an inhibitor [7, 8].

Algorithms designed to evaluate a uAPTT [9] and recommendations around how to perform and interpret mixing studies [7, 8, 10] are available. However, the guidelines do not regulate the responsibilities of clinicians and laboratory staff in the differential diagnosis of a uAPTT, and fatal medical errors due to an uninterpreted uAPTT do occur [11]. An increasing number of studies has been published in which it has been reported that some physicians have either found laboratory assistance useful, or required such laboratory assistance in the interpretation of common laboratory tests results, including uAPTTs [12–15]. In addition, prompt laboratory investigation is expected upon the detection of even mild uAPTTs in patients without a prior history of bleeding, due to the potentiality of acquired hemophilias [16].

The aim of the present study was to determine whether the invited laboratories are able to provide the laboratory assistance required for the clinical interpretation of uAPTTs. To this end, the postanalytical practices (i.e., actions performed after the detection of an uAPTT) and mixing study methodologies applied in the evaluation of uAPTTs in these laboratories were explored to determine whether they are appropriate to exclude spurious APTT prolongations and to discriminate between uAPTTs caused by the presence or absence of inhibitors. The skill of the professionals responsible for the coagulation tests in these laboratories to successfully interpret different scenarios of APTT mixing studies results and to decide upon the presence or absence of inhibitors was also investigated.

Materials and methods

This survey was organized by the joint Working Group (WG) on the Postanalytical Phase of the European Federation of Clinical Chemistry and Laboratory Medicine and the European Organisation for External Quality Assurance Providers in Laboratory Medicine (EQALM). The WG developed a case-history-based questionnaire and submitted it to pilot testing in five countries (Croatia, Hungary, the Netherlands, Norway, and Turkey), and adapted the final questionnaire (Supplemental Data, Table 1 that accompanies the article http://www.degruyter.com/view/j/cclm.2015.53.issue-10/cclm-2014-1183/cclm-2014-1183.xml?format=INT) on the web using the SurveyMonkey online survey tool [17]. Laboratories participating in coagulation schemes of different external quality assurance programs in Europe and beyond were invited to participate in the electronic survey between July and October 2012 by member organizations of EQALM. Senior staff at the invited laboratories were asked to forward the questionnaire to the person(s) responsible for routine coagulation tests, so as to obtain the answers that most accurately reflected the everyday situation in their laboratories. Survey completion was expected within approximately 2 weeks, after which a reminder was sent.

Survey content

A schematic summary of the survey content is shown in Figure 1. The survey participants were provided with a case history of an asymptomatic 7-year-old girl and asked to answer a series of single- and multiple-choice questions about it. The questions targeted key practices of a typical laboratory investigation protocol for patients with a prolonged APTT and normal prothrombin time, and the personal and laboratory particulars of the respondents. In addition, three different patterns of APTT mixing study results (mixing study scenarios) were presented to staff at laboratories in which mixing studies were used routinely (Figure 2A). The questions that were asked of staff at laboratories are shown in Figure 1 and Supplemental Data, Table 1.

Laboratory evaluations used to explore uAPTT

The questionnaire addressed two main areas: 1) uAPTT investigations: postanalytical practices after uAPTT detection and the mixing study methodology; and 2) interpretation of mixing study scenarios.

uAPTT investigations

Postanalytical practices after uAPTT detection: Four key postanalytical practices were studied (Figure 1): 1) exclusion of unfractionated heparin presence; 2) performance of mixing studies; 3) performance of or referring samples for further special testing; and 4) providing written interpretation of the results to the requesting physician. Laboratories were classified according to their activities in each single postanalytical action, as follows (Supplemental Data, Table 2): 1) never do; 2) act exclusively "clinically driven" (i.e., performed at the laboratory using exclusively clinical information and no reflex or reflective testing); and 3) act "laboratory driven" (i.e., using reflex or reflective testing).

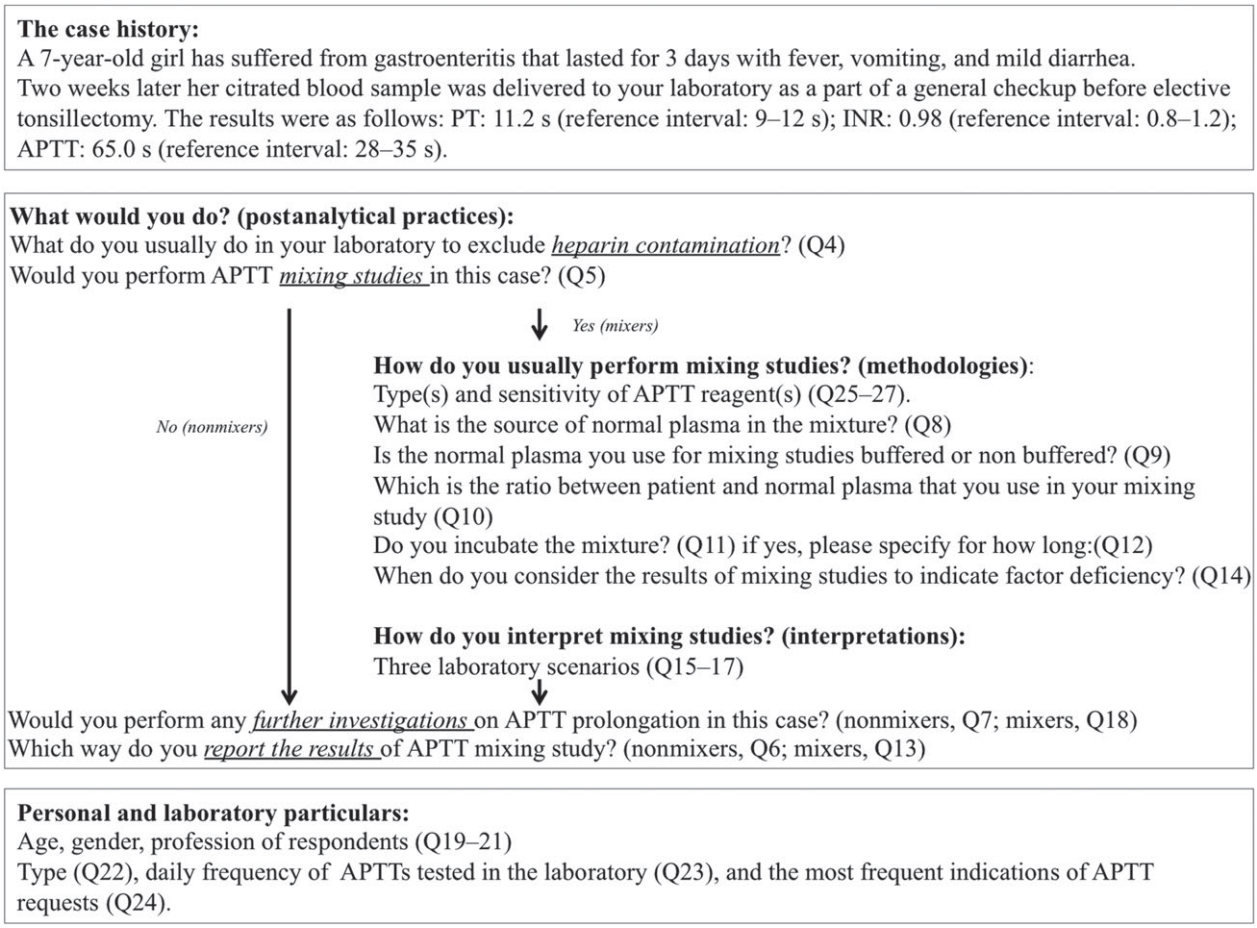


Figure 1 Schematic of survey content.
Q1–Q27: numbers of questions in the electronic questionnaire (Supplemental Data, Table 1). APTT, activated partial thromboplastin time; INR, international normalized ratio; PT, prothrombin time.

Mixing study methodologies: The use of one or more APTT reagents at the laboratories and the staff awareness of the sensitivity of the reagent(s) in use were investigated. In addition, some of the details of the mixing studies, such as the ratio of patient plasma and normal plasma, the source and buffering status of the normal plasma, the conditions of incubation, and the principle used in the interpretation of the results of those mixing studies.

Interpretation of the mixing study scenarios

Interpretation of the mixing study scenarios (Figure 2A) was analyzed according to the individual scenarios and by classifying the performance of staff at individual laboratories in the interpretation of all three case scenarios, as follows: 1) "fully correct" (i.e., good interpretation of the absence or presence of an inhibitor in all three case scenarios); 2) "clinically misleading" (i.e., wrong diagnosis of the absence or presence of an inhibitor in one or more of the three case scenarios); 3) "unresponsive" (i.e., either the option "I do not know" was selected or no answer was given for all three case scenarios); and 4) "other" (i.e., responses on the case scenarios not included in the above three categories; Supplemental Data, Table 2).

Analysis of the responses to the questionnaire

Responses were initially analyzed on basis of the whole study, then according to the following subgroups of respondents: 1) for countries with more than 20 responders, the lowest and the highest frequencies detected in any of these countries are presented in the text as the range of observed frequencies ($range_f$); 2) laboratories in which <50 APTT tests/day (n=392) and ≥50 APTT tests/day (n=356) are usually performed; 3) specialized coagulation laboratories (n=71) and primary care laboratories (n=60); and 4) laboratories for which laboratory specialists (n=373), physicians (n=102), or technologists (n=133) responded.

Statistical analysis of the responses was performed by cross tabulation. The cut-off for statistical significance, which was determined using the t-test and Fisher's exact test, was set at $p<0.05$.

Results

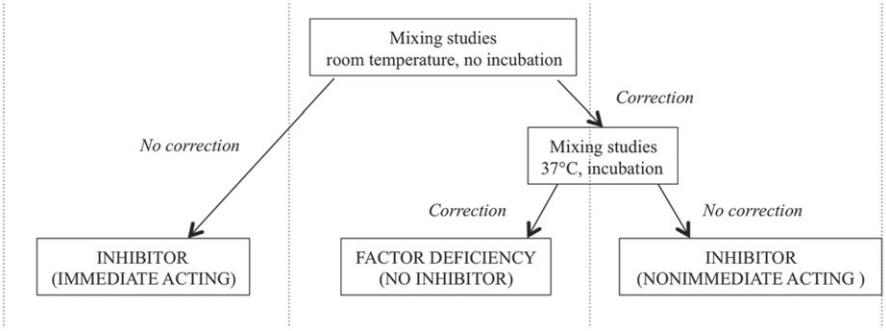
Responses from staff at 990 laboratories were analyzed, 90% of which were located in 13 countries, each providing

A. Instructions and scenarios

You performed APTT mixing studies on your patient sample. What is your most likely laboratory diagnosis for the case history in Fig. 1 (initial APTT=65.0 s) if the results of the mixing study were as follows (APTT reference interval 28.0 – 35.0 s):

	Scenario A (Q15)	Scenario B (Q16)	Scenario C (Q17)
APTT 1:1 mixture of patient and pooled plasma	56 s	33 s	33 s
APTT measured on pooled plasma	32 s		
APTT 1:1 mixture of patient and pooled plasma after incubation at 37°C	59 s	38 s	59 s
APTT measured on pooled plasma at 37°C	35 s		

B. Correct diagnostic approach



C. Results of interpretation by scenarios

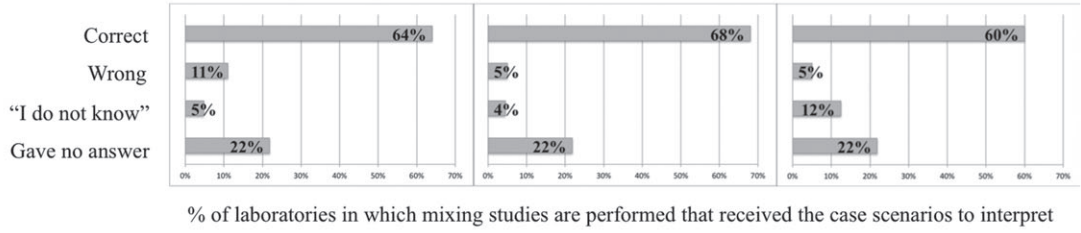


Figure 2 Mixing study results presented to the study participants (A), correct diagnostic approach for evaluation of the mixing studies (B), and interpretations of the respondents (C).

more than 20 responses, with an overall response rate of 19% (range$_f$: 6%–77%). The response rate to individual questions was 78%–100% (Supplemental Data, Table 1). The main characteristics of the laboratories from which responses were received are summarized in Table 1.

Investigations of uAPTT

Postanalytical practices after detection of a uAPTT

Large within- and between-country variations were observed concerning the postanalytical practices performed upon detection of a uAPTT, and the clinically or laboratory driven characteristic of those practices (Table 2). None of the investigated postanalytical practices were performed in only seven laboratories (0.7%), and all of them were performed in 46%. In the remaining laboratories (53%), the applied practice combinations varied between those in which mixing studies were performed (26%) and those in which they were not (27%). The predominant laboratory practice in most countries was to perform mixing tests; however, in some countries 50%–74% of laboratories never performed mixing studies. The percentages of shortcomings in postanalytical practices with the potential consequence of delayed or misdiagnosis in the various investigated laboratory groups are presented in Table 3.

Methodologies used in mixing studies

Only a single APTT reagent was used at 63% of all the responding laboratories (range$_f$: 35%–91%). APTT reagents sensitive to factor deficiencies were most frequently used as the first or the only APTT reagent (34% of all laboratories), while APTT reagents sensitive to LA were used in 19%. In the remaining laboratories, staff either did

Table 1 Main characteristics of the responding laboratories.

Country	No. of invited laboratories	No. of responding laboratories	Response rate to the survey, %	Laboratory type			No. of APTT tests processed daily			Qualification of respondents		
				Special coagulation laboratories	Non-special coagulation laboratories		Median	10th	90th	Medical doctors (physicians) and laboratory scientists		Laboratory technologist
					On secondary or tertiary care	In primary care				With specialization in clinical chemistry or laboratory medicine	Without specialization	
Austria	270	46	17	6%	83%	11%	45	10	280	31%	26%	43%
Croatia	199	36	18	3%	84%	13%	30	3	180	60%	40%	0%
Denmark	155	31	20	19%	81%	0%	18	5	85	44%	11%	44%
France	1000	233	23	5%	90%	6%	50	5	186	56%	39%	5%
Germany	760	49	6	6%	90%	3%	100	10	410	39%	39%	23%
Hungary	160	54	34	3%	90%	8%	35	9	100	70%	28%	3%
Ireland	54	37	69	3%	91%	6%	50	10	150	0%	88%	12%
Italy	280	136	49	10%	83%	6%	90	5	300	65%	26%	9%
Norway	73	35	48	3%	94%	3%	3	1	16	15%	21%	64%
Portugal	88	68	77	6%	83%	10%	15	4	138	48%	31%	21%
Switzerland	211	46	22	0%	75%	25%	10	1	90	19%	47%	34%
The Czech Republic	458	45	10	11%	74%	16%	37	10	115	55%	29%	16%
The Netherlands	129	71	55	15%	77%	8%	20	9	100	62%	18%	20%
Other countries[a]	1452	103	7	32%	68%	0%	100	10	350	32%	52%	16%
Total	**5289**	**990**	**19**	**9%**	83%	**8%**	**40**	**4**	**200**	**48%**	**35%**	**17%**

[a]Albania, Australia, Belarus, Belgium, Canada, Finland, Kosovo, Latvia, Macedonia, Monaco, Poland, Romania, Slovenia, South Africa, Spain, Ukraine, UK. Parameters of the overall survey are shown in bold.

Table 2 The activity of responding laboratories in the investigated postanalytical practices after detection of a uAPTT.

Activity after the finding of a uAPTT	Percentage of key postanalytical practices, mean (range)			
	Heparin exclusion	Mixing studies	Further special testing	Reporting with written interpretation
Never do	3 (0–17)	28 (11–74)	11 (3–27)	26 (10–67)
Practice exclusively clinically driven: Performed by the laboratory using exclusively clinical information and no reflex or reflective testing	58 (47–86)	12 (4–20)	42 (28–81)	NA
Practice laboratory driven: by using reflex or reflective testing	39 (8–51)	60 (6–85)	47 (0–68)	74 (33–90)

Data are percentages of all responding laboratories. The ranges of observed frequencies detected in any of the 13 countries, each providing more than 20 responses, are indicated in parentheses. NA, not applicable.

not know the sensitivity of their APTT reagents (22%), or else they used APTT reagents with unspecified sensitivity (25%). LA-sensitive reagents were most often chosen as the second APTT reagent (in 54% of laboratories in which two reagents were used).

In 90% of the laboratories in which mixing studies were performed (range$_f$: 63%–98%), a 1:1 ratio of patient plasma to normal plasma was used in the testing. The source of the normal plasma in the mixing studies was commercial or home-made pooled plasma for 83% of laboratories (range$_f$: 50%–100%). An incubation step was applied in the mixing studies in 56% of the responding laboratories (range$_f$: 33%–91%). A large variation in the duration of incubation was found, with 2 h of incubation as the most frequent condition (44%; range$_f$: 27%–67%). Plasma buffering was applied in mixing studies by only 17% of the responding laboratories (range$_f$: 9%–50%).

In most countries, all decision rules listed in the questionnaire (Supplemental Data, Table 1, Q14) were applied to some extent for interpretation of the mixing studies, but the Rosner index [20] was the most frequently used (34% of the respondents, range$_f$: 5%–83%).

The frequencies of shortcomings in mixing methodologies with the potential consequence of delayed or misdiagnosis in the investigated laboratory groups are presented in Table 3.

Interpretation of the mixing study scenarios

Only staff at those laboratories in which mixing studies were implemented in everyday practice were asked to interpret the mixing study scenarios. Mixing study scenarios A, B, C, were interpreted correctly by staff at 64%, 68%, and 60% of the laboratories, respectively (Figure 2B and C), with staff at 5%, 4%, and 12% of the laboratories responding "I do not know" for these scenarios (Figure 2C). However, all three mixing study scenarios were interpreted correctly by staff at only 49% (range$_f$: 30%–68%) of the laboratories who assessed them. All of the scenarios were interpreted incorrectly by staff at only one laboratory. An incorrect diagnosis of absence or presence of an inhibitor in one or more of the three scenarios was made by staff at 16% (range$_f$: 9%–19%) of the laboratories; responses were not received for any of the scenarios from staff at 25% (range$_f$: 13%–52%) of these laboratories. Staff at only 9.6% of all participating laboratories reported investigations of uAPTTs appropriately (Table 3) and yet simultaneously interpreted all mixing study scenarios correctly. The ways in which the three mixing study scenarios were interpreted in the different laboratory groups are presented in Figure 3.

Discussion

The findings of this study revealed considerable diversity in both the investigations of uAPTTs in the responding laboratories and in their interpretations of the APTT mixing study scenarios. A significant percentage of the laboratories exhibited shortcomings with respect to investigations of uAPTTs with the potential consequence of delayed or misdiagnosis of the patients. All three mixing study scenarios were correctly interpreted by staff at only 49% of the laboratories in which mixing studies are implemented in everyday practice. It is striking that staff at only 9.6% of all of the participating laboratories performed uAPTT investigations correctly and provided correct clinical interpretations for all three APTT mixing study scenarios. Staff at approximately 25% of all participating laboratories interpreted all three mixing study scenarios correctly

Table 3 Frequency of shortcomings with the potential consequence of delayed or misdiagnosis in investigations of uAPTT.

Practice	Percentage of the practices in the survey	Percentage in laboratories		p-Value	Percentage in		p-Value	Percentage in laboratories with		p-Value
		<50 APTT tests/day	≥50 APTT tests/day		Primary care laboratories	Special coagulation laboratories		Technologists	Specialists MDs	
Shortcomings in postanalytical practices										
Failure to exclude unfractionated heparin presence [7, 8, 18]	61%	76%	42%	<0.01	70%	14%	<0.01	63%	59% 50%	0.54 0.11
Failure in performing mixing studies [7–9, 18]	28%	43%	18%	<0.01	47%	14%	<0.01	51%	26% 21%	<0.01 <0.01
Shortcomings in mixing methodologies										
Sensitivity of the first or only APTT reagent is unknown [19]	17%	32%	10%	<0.01	27%	7%	<0.01[a]	37%	16% 13%	<0.01 <0.01
Sensitivity of the second APTT reagent is unknown [19]	4%	17%	2%	<0.01[a]	30%	0%	<0.01[a]	10%	6% 0%	0.43 0.05[a]
Single donor's plasma used in mixing studies [7–9]	17%	23%	14%	<0.01	41%	2%	<0.01[a]	13%	21% 13%	0.13 1
No incubation in mixing studies [7, 8, 18]	44%	46%	40%	0.17	50%	37%	0.23	43%	38% 41%	0.45 0.80
Any of the above shortcomings	88%	NC	NC	NC	NC	NC	NC	NC	NC	NC
No. of laboratories:	990	392	356	NA	60	71	NA	133	373 102	NA
Median daily no. of APTT tests:	40	13	100	NA	20	150	NA	20	50 60	NA

Detected frequencies are shown as percentages of all responding laboratories in the group. References of the recommended practice are given in parentheses. [a]Calculated using Fisher's exact test. NA, not applicable; NC, not calculated.

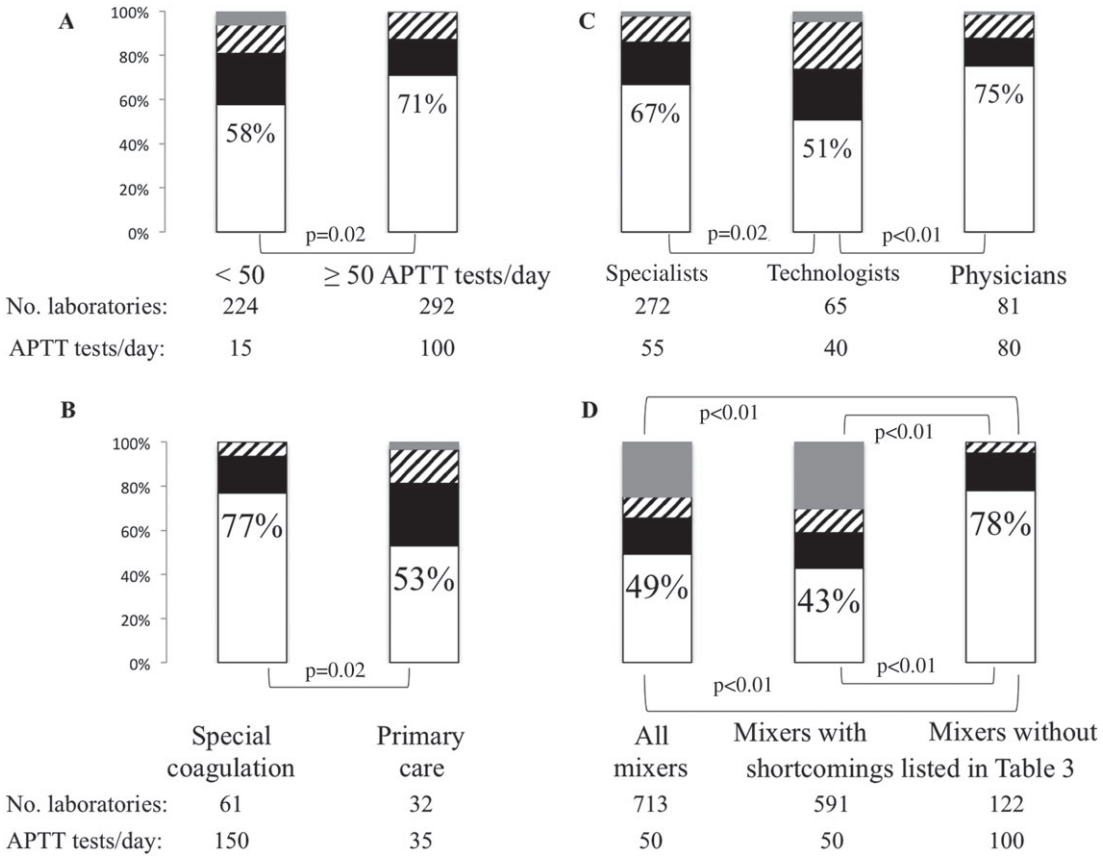


Figure 3 Interpretation of mixing study scenarios in laboratories with different daily APTT workloads (A), in different laboratory types (B), relative to the qualification of the respondents (C), and in laboratories using different postanalytical practices (D).
Stacked columns show the percentage of responding laboratories with the presented interpretative performance: gray, "unresponsive"; stripes, "other response combinations"; black, "clinically misleading"; white, "fully correct".

but some kind of shortfall was found with respect to postanalytical investigations of uAPTTs. These laboratories are at risk of providing a misdiagnosis due to inappropriate laboratory characterization of the abnormality underlying certain uAPTTs.

Investigations of uAPTTs

The timely and appropriate evaluation of a uAPTT is most effective if the responsibilities of both the clinicians and laboratory staff are clear and their practices are correct and concerted. The observed great variations both within and between countries concerning which postanalytical practices were performed in laboratories that participated in this study, and concerning whether the practices were clinically or laboratory driven indicate that either the guidelines [8, 10] were not followed or else they did not deliver harmonized instructions as to how to organize the postanalytical evaluation of uAPTTs.

However, regarding the methodology of mixing studies, the most clearly stated recommendations in existing guidelines were followed by staff at most of the participating laboratories; APTT reagents with known sensitivity, a 1:1 ratio of patient plasma:normal plasma, and pooled normal plasma were applied at 80%–90% of the laboratories [7, 8, 10, 19]. At the same time plasma buffering was rarely (17%) applied and there were large variations between laboratories with respect to the duration of incubation and the applied decision rules regarding the evaluation of mixing studies, likely reflecting the heterogeneity of the existing recommendations [7–10, 18, 21, 22]. Despite the clearly stated recommendations regarding its inclusion in mixing studies [7–9, 18, 21], an incubation step was included in only 56% of laboratories.

In a surprisingly large percentage of the participating laboratories (88%), the practices implemented to investigate a uAPTT had the potential to result in a misdiagnosis or delay in the diagnosis of the patient (Table 3). In general, shortcomings in the diagnostic workup of

a uAPTT were observed less frequently in laboratories in which ≥50 APTT tests/day are performed, in special coagulation laboratories, and sometimes in laboratories where physicians/specialists responded, compared to laboratories in which <50 APTT tests/day are performed, primary care laboratories, or where technicians responded. These findings suggest that more practice and an advanced educational background in coagulation leads to a better methodological understanding, and in turn to fewer shortcomings in uAPTT investigations. The exception was the lack of an incubation step in the mixing studies, which were equally distributed between the analyzed laboratory groups (Table 3), probably due to the rare occurrence of coagulation inhibitors [6, 23]. In agreement with the findings of this study, failure to detect coagulation inhibitors in samples due to the lack of an incubation step has even been observed in specialized coagulation laboratories [18, 24].

Interpretations of uAPTT in mixing study scenarios

It is generally advisable that only professionals with clear expertise in the particular laboratory field should be charged with interpreting laboratory results [25, 26]. It is alarming that although in the present study laboratory professionals responsible for coagulation were asked to interpret the mixing studies, only staff at 49% of the laboratories in which mixing studies were implemented could adequately discriminate between inhibitory and non-inhibitory forms of uAPTT in all three scenarios. These results are similar to those of the postanalytical Quality Assurance Program in Australia, wherein participants were asked to add interpretative comments to a set of non-esoteric laboratory test results, and in which ≥50% of the interpretations were inappropriate and/or misleading [25].

The laboratory groups with fewer shortcomings in the diagnostic workup of uAPTTs (i.e., laboratories in which ≥50 APTT tests/day are performed, special coagulation laboratories, and those in which physicians/specialists responded) provided more frequently correct interpretations of the mixing case scenarios compared with other participants (Figure 3A–C). The laboratories with correct postanalytical investigation performed best regarding the interpretations of case scenarios (Figure 3D). However, it is worrying that even staff at 20% of laboratories that had correct postanalytical practices misinterpreted the mixing study scenarios, suggesting that the implementation of good postanalytical practices for investigations of uAPTTs in itself will not perfect the analyst's understanding of the clinical meaning of the applied laboratory tests. The acquisition and training regarding interpretative knowledge requires a special focus on the education and training of laboratory professionals in this specific field [27].

Limitations and strengths

A limitation of the present survey was that the method of selecting the laboratories to which invitations were to be sent was not fully standardized, resulting in a response rate that varied according to the country and it is not known whether those that responded are representative of the laboratories in their country. Therefore, it was difficult to compare findings from different countries.

The main strengths of this study are that almost 1000 laboratories were included, with response rates to individual questions of 78%–100%, and that the postanalytical laboratory practices regarding investigations of uAPTT and the interpretation skills of the staff at those laboratories were studied in parallel. While the fact that the questionnaire was circulated in English could have been a barrier in some countries, it could also have been considered an advantage since translation errors were avoided. Staff at a large number of laboratories at secondary and tertiary healthcare institutions responded to the survey, suggesting that the targeted population (i.e., laboratories at which uAPTT are detected on a daily basis) was successfully reached.

Conclusions

The clinical requirement for prompt further investigation of even mild uAPTTs in patients without a prior history of bleeding [16] cannot be fulfilled at most of the laboratories included in this study. The present findings reveal a need to harmonize postanalytical uAPTT investigations [28] and suggest that staff at laboratories should be trained in the clinical interpretation of ordinary laboratory tests, such as that for uAPTT. Therefore, training programs designed to educate laboratory professionals in postanalytical investigations and test interpretation, as well as external quality assurance programs focusing on interpretative commenting for ordinary laboratory test results should be organized.

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References

1. Johnsen JM, Konkle BA. Differential diagnosis of the bleeding patient. In: Marder VJ, Aird WC, Bennett JS, Schulman S, White GC, II, editors. Haemostasis and thrombosis: basic principles and clinical practice, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2014:648–58.
2. Tripodi A, Chantarangkul V, Martinelli I, Bucciarelli P, Mannucci PM. A shortened activated partial thromboplastin time is associated with the risk of venous thromboembolism. Blood 2004;104:3631–4.
3. Tcherniantchouk O, Laposata M, Marques MB. The isolated prolonged PTT. Am J Hematol 2013;88:82–5.
4. Kamal AH, Tefferi A, Pruthi RK. How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults. Mayo Clin Proc 2007;82:864–73.
5. Schindhelm RK, Wondergem MJ, Admiraal J, Nap G, Boekel ET, Hani L. A patient with a prolonged activated partial thromboplastin time and a deep intracerebral haemorrhage. Case Rep Neurol 2012;4:131–6.
6. Cugno M, Gualtierotti R, Tedeschi A, Meroni PL. Autoantibodies to coagulation factors: from pathophysiology to diagnosis and therapy. Autoimmun Rev 2014;13:40–8.
7. Kershaw G, Orellana D. Mixing tests: diagnostic aides in the investigation of prolonged prothrombin times and activated partial thromboplastin times. Semin Thromb Hemost 2013;39:283–90.
8. National Committee for Clinical Laboratory Standards. One-stage prothrombin time (PT) and activated partial thromboplastin time (APTT) test; approved guideline. NCCLS document H47-A1996;vol. 28, no. 20.
9. Laposata M, Connor AM, Hicks DG, Phillips DK. Laboratory assays. In: Laposata M, Connor AM, Hicks DG, Phillips DK, editors. The clinical hemostasis handbook, 1st ed. Chicago (IL): Year Book Medical Publisher, 1989:193–279.
10. Pengo V, Tripodi A, Reber G, Rand JH, Ortel TL, Galli M, et al. Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. J Thromb Haemost 2009;7:1737–40.
11. Zeitler H, Ulrich-Merzenich G, Goldmann G, Vidovic N, Brackmann HH, Oldenburg J. The relevance of the bleeding severity in the treatment of acquired haemophilia – an update of a single-centre experience with 67 patients. Haemophilia 2010;16:95–101.
12. Laposata ME, Laposata M, Van Cott EM, Buchner DS, Kashalo MS, Dighe AS. Physician survey of a laboratory medicine interpretive service and evaluation of the influence of interpretations on laboratory test ordering. Arch Pathol Lab Med 2004;128:1424–7.
13. Verboeket-van de Venne WP, Aakre KM, Watine J, Oosterhuis WP. Reflective testing: adding value to laboratory testing. Clin Chem Lab Med 2012;50:1249–52.
14. Laposata M, Dighe A. "Pre-pre" and "post-post" analytical error: high-incidence patient safety hazards involving the clinical laboratory. Clin Chem Lab Med 2007;45:712–9.
15. Reding MT, Cooper DL. Barriers to effective diagnosis and management of a bleeding patient with undiagnosed bleeding disorder across multiple specialties: result of a quantitative case-based survey. J Multidiscip Healthc 2012;5:277–87.
16. Huth-Kühne A, Baudo F, Collins P, Ingerslev J, Kessler CM, Lévesque H, et al. International recommendations on the diagnosis and treatment of patients with acquired hemophilia A. Haematologica 2009;94:566–75.
17. SurveyMonkey Enterprise. Enterprise survey software and research tools. Available from: http://www.surveymonkey.com. Accessed August, 2012.
18. Dardikh M, Meijer P, van der Meer F, Favaloro EJ, Verbruggen B. Acquired functional coagulation inhibitors: review on epidemiology, results of a wet-workshop on laboratory detection, and

implications for quality of inhibitor diagnosis. Semin Thromb Hemost 2012;38:613–21.
19. Fritsma GA, Dembitzer FR, Randhawa A, Marques MB, Van Cott EM, Adcock-Funk D, et al. Recommendations for appropriate activated partial thromboplastin time reagent selection and utilization. Am J Clin Pathol 2012;137:904–8.
20. Rosner E, Pauzner R, Lusky A, Modan M, Many A. Detection and quantitative evaluation of lupus circulating anticoagulant activity. Thromb Haemost 1987;57:144–7.
21. Verbruggen B, Novakova I, Wessels H, Boezeman J, van den Berg M, Mauser-Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. Thromb Haemost 1995;73:247–51.
22. Exner T. Conceptions and misconceptions in testing for lupus anticoagulants. J Autoimmun 2000;15:179–83.
23. Franchini M, Gandini G, Di Paolantonio T, Mariani G. Acquired hemophilia A: concise review. Am J Hematol 2005;80:55–63.
24. Favaloro EJ, Bonar R, Duncan E, Earl G, Low J, Aboud M, et al. Mis-identification of factor inhibitors by diagnostic haemostasis laboratories: recognition of pitfalls and elucidation of strategies. A follow up to a large multicentre evaluation. Pathology 2007;39:504–11.
25. Lim EM, Sikaris KA, Gill J, Calleja J, Hickman PE, Beilby J, et al. Quality assessment of interpretative commenting in clinical chemistry. Clin Chem 2004;50:632–7.
26. Laposata M. Patient-specific narrative interpretations of complex clinical laboratory evaluations: who is competent to provide them? [editorial] Clin Chem 2004;50:472–2.
27. Favaloro EJ, Meijer P, Jennings I, Sioufi J, Bonar RA, Kitchen DP, et al. Problems and solutions in laboratory testing for hemophilia. Semin Thromb Hemost 2013;39:816–33.
28. Aarsand AK, Sandberg S. How to achieve harmonisation of laboratory testing – the complete picture. Clin Chim Acta 2013;432:8–14.

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