Received: 6 December 2017 | Accepted: 26 January 2018

DOI: 10.1002/jcla.22415

BRIEF REPORT

WILEY

What should be the laboratory approach against isolated prolongation of a activated partial thromboplastin time?

Mesude Falay[1] | Mehmet Senes[2] | Dogan Yücel[2] | Turan Turhan[3] | Simten Dagdaş[1] | Melike Pekin[1] | Namik K. Nazaroglu[4] | Gülsüm Özet[1]

[1]Department of Hematology Ankara Numune Training and Research Hospital, Ankara, Turkey

[2]Department of Medical Biochemistry Ankara Training and Research Hospital, Ankara, Turkey

[3]Department of Medical Biochemistry Ankara Numune Training and Research Hospital, Ankara, Turkey

[4]Synlab Laboratories, Ankara, Turkey

Correspondence
Mesude Falay, Department of Hematology, Ankara Numune Training and Research Hospital, Ankara, Turkey.
Email: mesudey@gmail.com

Background: This study is a retrospective evaluation of patients who were subject to mixing study in our laboratory due to prolonged APTT. The preliminary diagnoses, clinical manifestations, and results of additional ordered tests were reviewed. The study aims to investigate whether repeating APTT test with a different assay prior to performing mixed study in patients with prolonged APTT would be a better alternative algorithmic approach in order to save both time and costs.

Methods: We retrospectively evaluated 166 patients (65 females and 101 males) who were subject to mixing study due to isolated prolonged APTT. Additional ordered tests to identify the etiology and clinical findings were reviewed. All patients who had prolonged APTT as a result of testing with Hemosil Synthasil APTT reagent in ACL TOP analyzer were repeated with Stago Cephascreen APTT reagent in STA-R coagulation analyzer.

Results: APTT test was requested preoperatively in 72.2% of cases. Only 6.6% of the cases had history of bleeding. Correction with mixing study was achieved in 122 (73.5%) cases, among which 75 (45%) cases were found to have APTT test results within reference range when tested with Cephascreen reagent. In 44 (26.5%) cases, mixing study did not result in correction. Only 4 cases were confirmed to have lupus anticoagulants (LA), while 4 cases were diagnosed with hemophilia with inhibitors.

Conclusion: Prolonged APTT results should always be retested using a different assay prior to mixing study. The clinician and the laboratory specialist should collaborate at the postanalytical phase.

KEYWORDS
APTT, coagulation, mixing test

1 | INTRODUCTION

Activated partial thromboplastin time (APTT) testing is used for monitoring heparin treatment, screening for congenital or acquired deficiencies of FVIII, FIX and FXI or inhibitors, lupus anticoagulants (LA), and as a first-line test for diagnosis of hemophilia.[1-3] Although it is not a screening test for predicting bleeding, many clinicians order this test preoperatively for screening purposes. Patients with no history of bleeding but slightly prolonged APTT are further investigated for possible presence of acquired hemophilia. After ruling out pre-analytical errors, mixing study is the first line of testing in such patients.[2-5] If mixing study corrects the test results, the patient possibly has a coagulation factor deficiency. Failure to correct the results with mixing study raises suspicion for possible presence of an inhibitor (heparin, direct thrombin inhibitors (DOACs), antibodies to specific factors, or nonspecific antibodies such as LA).[1-5] In either case, advanced testing is required. In this context, mixing study is considered as a first-line test.[1,6,7]

APTT is not a standardized test, and many limitations to APTT testing exist. Unexpected APTT elevations do not always indicate a pathology. It is not always easy to identify the cause of elevation in APTT, even when there is some clinical background (bleeding or thrombosis).[7] Although the testing is performed in in vitro conditions and free from the influences of molecules and systems playing role in vivo, the test results are used to interpret patient's condition.[3] For instance, despite a lack of clinical bleeding in FXII, prekallikrein or HMWK deficiencies, APTT can be prolonged. Also, sepsis, malignancy, and atherosclerosis are also associated with disturbance in physiological hemostasis and abnormal coagulation test results.[8-11] These should always be considered when interpreting APTT results. Another challenging factor for standardization of APTT assays is the content of these assays. APTT reagents have two main components: an activator (kaolin, celite, silica, polyphenol, ellagic acid, etc.) and a phospholipid source. Activator's role is to provide a negatively charged surface required for activation of the coagulation factors. Phospholipid content of the reagents is highly variable in terms of both concentration and source.[3,10] The diversity in concentration and composition of the APTT reagents results in remarkable differences in response to heparin, coagulation factor deficiencies, and LAs. Current Clinical and Standards Institute (CLSI) approved guidelines for prothrombin and APTT recommend that an APTT reagent confers a clear prolongation in APTT with plasmas, which contain <30% of factors VIII, IX, or XI because factor levels above this level were not considered to cause an increased risk of bleeding.[12] The measurement technique employed in the automatic analyzers (mechanical and optic) also contributes to the variation in test results.[2,3,10]

The purpose of this study was to retrospectively evaluate patients who were subject to mixing study in our laboratory during the last 1 year due to isolated prolongation APTT, by examining their clinical and laboratory findings, and to investigate whether it would be a better approach to repeat APTT test with a different assay prior to performing mixed study.

2 | MATERIAL AND METHOD

Totally 166 cases (65 females and 101 males) aged between 18 and 84 years who had requisition of mixing study due to isolated prolonged APTT within the last 1 year in our Hemostasis Laboratory were examined retrospectively in terms of their additional tests ordered for the aim of identifying the etiology, as well as their clinical findings. In our institution, the laboratory specialist is not authorized to perform a reflex test, including mixing study for elevated APTT results. Blood samples for coagulation tests were drawn into tubes containing 0.109 M sodium citrate (1:9 by volume) (Greiner Bio-One, Kremsmünster, Austria) following 12 hours fasting. Plasma was obtained by centrifugation at 2200 *g* and +18°C for 15 minutes, within 2 hours after sampling, and was immediately analyzed.

APTT: APTT was measured using Hemosil Synthasil (Instrumentation Laboratory, IL, Bedford, MA, USA) reagent (reference range: 25.5-36.8 seconds) with ACL TOP 700 analyzer (Instrumentation Laboratory, IL, Bedford, MA, USA) device. For cases who had prolonged APTT, a repeat APTT testing was performed on a different day, with freshly drawn blood sample. All cases who had prolonged APTT in the repeat study were subjected to mixing study. Also, a repeat APTT testing using another APTT reagent this time: Stago Cephascreen with STA-R coagulation analyzer (reference range: 24.8-36.5 seconds) (Diagnostica Stago, Asniéres, France).

Mixing test: Mixing tests were performed using a plasma pool (APTT: 30.2 seconds) prepared by mixing plasma from 20 healthy individuals (10 females and 10 males), which were stored at -70°C as 0.250 μL aliquots. For each patient, 2 tubes were prepared. The first tube included 0.250 μL patient plasma and 0.250 μL plasma pool, whereas the second tube included 0.250 μL healthy plasma pool and 0.250 μL imidazole buffer. Both tubes were tested immediately after preparation and after 2 hours of incubation in water bath at 37°C. At the end of the incubation, the tubes were placed on ice to stop the reaction, and APTT was measured using ACL TOP analyser. If the difference in postincubation APTT results between the control tube and the patient tube was >10%, the result was interpreted as inhibitor screening (+), and if the difference was <10%, the result was interpreted as correction achieved.

FVIII, FIX, FXI, and FXII levels were measured using STA-deficient (VIII, IX, XI, and XII) (Diagnostica Stago) reagents with STA-R coagulation analyzer (the reference range was 50% to 150%). LA test was performed with both LA-sensitive PTT-LA screening reagent and also STA-Staclot dRVV Screen and STA-Staclot dRVV Confirm reagents on STA-R coagulation analyzer.[13] For those that were suspected to have an inhibitor, inhibitor testing was performed with modified Bethesda assay. In Bethesda assay, titers >0.6 BU/mL were accepted as inhibitor-positive.[14]

All plasma pools were thawed before use at 37 Co for 2-3 minutes and filtered through a pore size of 0.22 mm. Quality controls were performed using both normal and abnormal quality controls.

2.1 | Statical analysis

Comparison of groups (ACL Top, STA-R and mixing study) for APTT results was performed using ANOVA test, with Tukey HSD as a post hoc test and regression analysis. Statistical analyses were carried out with SPSS statistical software version 17.

3 | RESULTS

Totally, 25.000 APTT tests were performed in our laboratory in 1 year. Mixing study was requested in 166 cases with isolated APTT elevation (65 female [aged 18-44 years], 101 males [18-81 years]). APTT test was requested as part of preoperative work-up in 120 (72.2%) cases and for indications shown in Table 1 in 46 (27.8%) cases. Only 6.6% of cases had history of bleeding. The mixing study resulted in correction in 122 (73.5%) cases, whereas no correction

was achieved with mixing study in 44 (26.5%) cases. The repeat APTT testing using Cephascreen reagent yielded APTT results within normal range in 75 (45%) of 166 patients. None of these cases had history of bleeding or thrombosis, etc. Seventy-two cases were requested for preoperative screening, and 3 were using DOACs.

Figure 1 shows requested tests and the factor deficiencies detected among cases with correction in mixing study. Of the 122 cases who had correction with mixing study, the requested factor tests were FVIII in 80 (65.5%), FIX in 72 (59%), FXI in 66 (54%), and FXII in 22 (18%) cases. Only 6 of these cases had history of bleeding (4 cases with FVIII <5%, 1 case with FIX <5%, and 1 case with vWF deficiency). The numbers of cases with FVIII, IX, FXI, and FXII levels between 30% and 50% were 13, 10, 14, and 3, respectively (data not shown). The other cases had factor levels above 50%. Three cases were previously diagnosed with afibrinogenemia.

Figure 2 shows additional requested tests among 44 (26.5%) cases who did not have correction with mixing study. Four cases had hemophilia with inhibitor, and 4 cases were previously diagnosed with LA. All of these cases had prolonged APTT with Cephascreen reagent.

Upon review of patient medical files, 20 cases were detected to use direct thrombin inhibitor (direct oral anticoagulants [DOACs]). In all cases using DOACs, mixing study was requested. While mixing study resulted in correction in 6 cases (2 cases had mild FVIII deficiency, 2 cases had FIX deficiency, and 2 cases had FXI deficiency), no correction was achieved in the remaining 14 cases. LA test was requested for all these 14 cases, and 2 cases were found to be positive for LA. Inhibitor testing was requested for remaining 11 cases (1 case was detected to have 1 Bethesda unit (BU) inhibitor). Thrombin time was not requested in any of the cases.

APTT results obtained with Synthasil and Cephascreen assays were compared in regression analysis, and accordingly, APTT was 52 ± 21.5 seconds (x ± SD) (37-170 seconds) in measurements with Synthasil on ACL TOP coagulation analyzer and 44.7 ± 18.3 seconds (x ± SD) (31-140 seconds) in measurements with Stago Cephascreen on STA-R coagulation analyzer (Figure 3). APTT was significantly lower when measured with Cephascreen reagent ($P < .001$) (Figure 4).

TABLE 1 Patient characteristics

Age	18-84 y
Sex (M/F)	101 M/65 F
Indication for testing	Preoperative work-up 72.2%
	History of bleeding 6.6%
	Deep vein thrombosis /PE 3.2%
	Vascular disease 1.2%
	Afibrinogenemia 1.8%
	Sepsis 6.6%
	Cancer 8.4%

4 | DISCUSSION

In this study, we examined the records of 166 cases, either inpatient or outpatient, who were subjected to mixing study due to prolonged APTT. None of these cases' preliminary diagnosis or clinical information was conveyed to our laboratory, and additional tests for investigation were requested by the clinician. Majority of the tests were ordered as part of the routine preoperative work-up. Only 6.6% had history of bleeding, while 3.2% had history of pulmonary embolism or deep vein thrombosis. APTT is not very sensitive in predicting the risk of perioperative bleeding.[2-5] If there is no history of bleeding or thrombophilia at the preoperative phase, APTT may lead to confusion and result in many unnecessary testing, or unnecessary treatments such as administration of fresh frozen plasma, causing delays in operations.[3-5,10,15] Prolonged APTT does not necessarily indicate a pathology.[10,15] In our study, no pathology was detected in majority of the cases. Among 44 cases who did not achieve correction with mixing study, LA testing was requested for 40 cases, of which 6 were found to be LA-positive. Inhibitor assay was requested for 11 cases, and 5 of them were detected to have inhibitor (4 cases had hemophilia with inhibitor). A review of medical files of the 2 LA-positive cases showed that they used DOACs due to atrial fibrillation in one case and cerebrovascular event in the other case. One case who was detected to have low-titer inhibitor was also using DOACs due to atrial fibrillation. Most clinicians and laboratory specialists are aware of the errors in coagulation test results due to heparin or

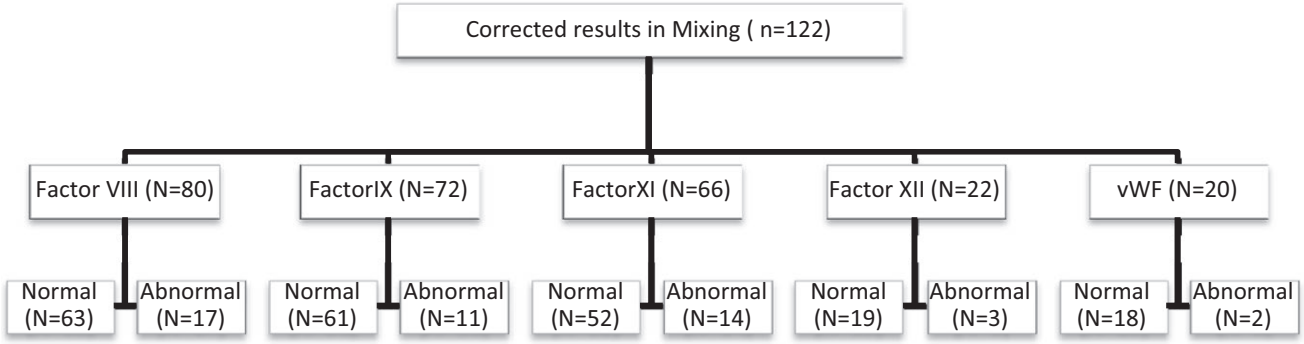

FIGURE 1 Cases with corrected results in mixing study

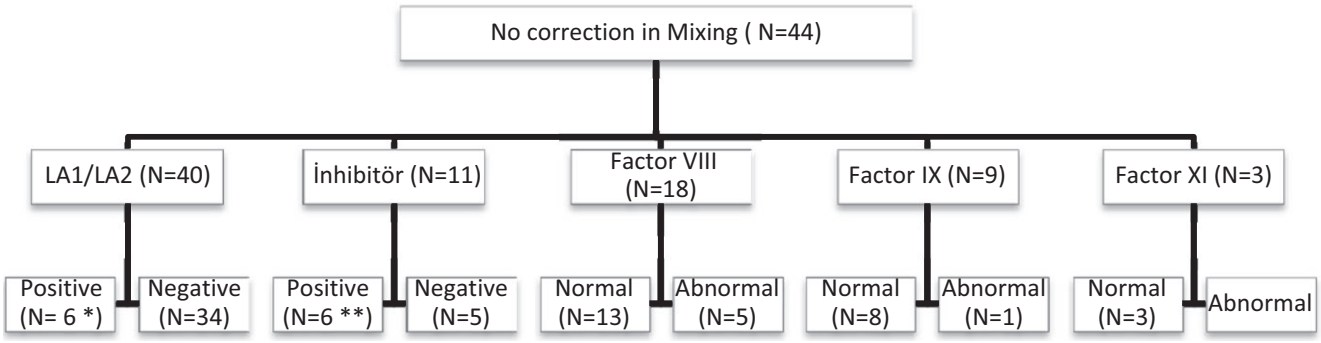

FIGURE 2 Cases with n no correction in mixing study and Cephascreen APTT prolonged. *2 cases use DOAC. **2 cases use DOAC

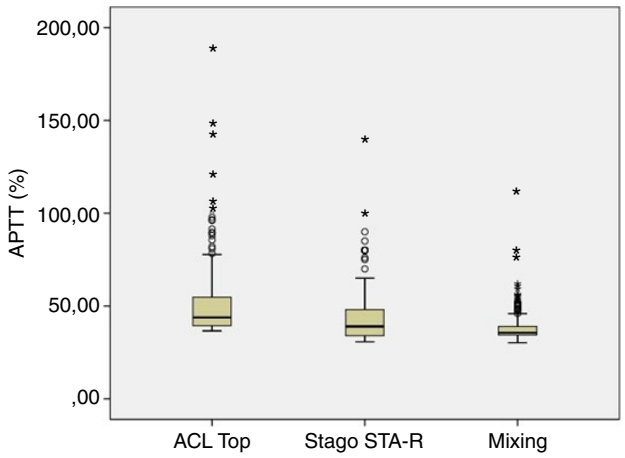

FIGURE 3 APTT test results showed significant difference between ACL Top, Stago STA-R, and mixing study groups ($P < .0001$)

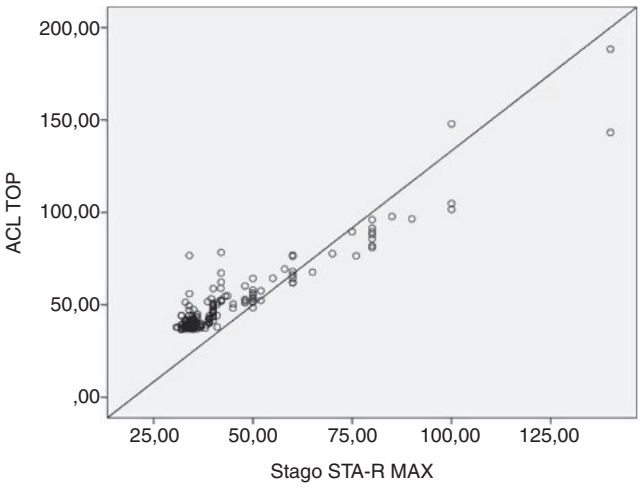

FIGURE 4 ACL TOP and Stago STA-R regression analysis correlation graphic ACL Top = 1.109 Stago STA-R + 2.48 ($r = .943$, $P < .001$)

oral anticoagulant use. However, though it is becoming increasingly common, the impact of DOACs on coagulation tests is not well recognized.[16,17] As it is seen in our results, almost all cases using DOACs had requisition for LA testing, and if LA was found negative, inhibitor testing was requested. However, DOACs have been shown to interfere APTT-based tests, cause false positivity or false negativity in LA, cause false positivity in inhibitor assays, and interfere factor assays.[16,17] The impact of DOACs on APTT test is not fully understood yet. Related studies do not show a clear picture of how DOACs influence APTT reagents from different manufacturers.[17] Further studies should explore how DOACs affect APTT reagents produced by different manufacturers.

APTT test is not standardized as prothrombin time (PT).[18] A normal APTT result does not always indicate normal hemostasis, and not all APTT reagents have the same level of sensitivity for coagulation factors.[6,18-20] Assuming that LA is excluded, prolonged APTT should prompt advanced testing such as FVIII, IX, XI, XII, PKK, or HMWK deficiencies.[6,18] Because FXII, HMWK, and PKK are not associated with bleeding phenotype, sensitivity is not sought for these factors.[6,18] The activator and phospholipid components of the reagent determine the sensitivity of the test. Mahalossan et al showed that the phospholipid composition and concentration of APTT reagents are an important determinant of single-step testing and APTT variation.[19] APTT is prolonged when factor level drops down below 30 U/dL.[12] Although the general conception is that APTT is prolonged when factor levels drop to 30%-50% of normal,[1,4] its sensitivity varies according to assays from different manufacturers or between various reagents produced by the same manufacturer.[19-22] In our laboratory, we use Hemosil Synthasil APTT reagent. In the present study, prolonged APTT results by Hemosil Synthasil reagent were repeated using Stago Cephascreen APTT reagent with STA-R coagulation analyzer, and 75 cases were found to have normal results in the repeat testing. None of these cases had history of bleeding or thrombosis. Although both reagents contain silica as activator, the composition and source of both silica and phospholipid are different. Although both assays have similar reference ranges, there was significant difference regarding mean APTT results ($P < .001$). This difference may have resulted from differences in both reagent sensitivity and the measurement method. Based on our findings, it would be a more effective approach to repeat prolonged APTT test results using a different reagent and measurement method prior to mixing studies, in order to save both time and costs, especially for those cases tested for preoperative screening purposes. As factor

levels were not requested in all cases, we could not determine which factors affected our assays.

FXII deficiency is the most common cause of prolonged APTT (responsible for 30%-50%)[1,4] In our study, FXII deficiency was detected in 2.4% of cases. The reason we had such a low rate may be because not all cases were tested for FXII level.

There are some limitations to our study. First, this was a small-scale study with retrospective analysis and was not designed to clearly identify coagulation factor disorders in all patients. Mixing study was performed only once in majority of the cases. Second, factor levels could not be measured in all patients with coagulation screening anomalies. Similarly, factor level test could not be performed for all patients with risk of bleeding, because the treating physician did not find it necessary due to APTT results within normal range. As the laboratory in our institution is not authorized to perform reflex test, the laboratory specialist could not request advanced tests when deemed as necessary.

This analysis sheds light on the interesting trends regarding current laboratory test methods. First, currently a great number of coagulation screening tests are requested, and many of these test orders are unnecessary, bringing substantial burden of cost and workload to the laboratory. In consideration of the studies showing that APTT has no use in prediction of bleeding risk, there should be a restraint on requesting preoperative coagulation screening without any information about patient's clinical or genetic background. Even so, prolonged APTT results should be confirmed using a different reagent and measurement method with a different sensitivity.

Second, coagulation tests are considered as part of the routine laboratory work-up along with complete blood count and clinical chemistry test. However, unlike these tests, abnormal coagulation test results require a thorough understanding of the coagulation system, and clinicians may not have sufficient knowledge about the significance of abnormal test results and about further tests that should follow in the face of abnormal test results. In our study, many tests were requested for no reason, and most possibly, whether these tests were indicated or not were not considered. Another worrying fact is that some of the cases who were subjected to mixing study were not followed up by clinicians. For such cases, possible coagulation disorders may have gone undiagnosed. In our study, thrombin time was not requested in any of the cases to rule out heparin contamination. The laboratory specialist should be authorized to perform reflex test when necessary and should be able to guide the clinician in this respect. Many patients are either tested unnecessarily when there is no need or else not investigated when there is high risk of bleeding and go undiagnosed. The clinician and the laboratory specialist should always collaborate when interpreting the test results or deciding on the selection of additional tests.

In conclusion, rather than functioning as a routine test laboratory, coagulation laboratories should be arranged as coagulation disorders investigation laboratories, and standardization should be achieved in this respect, with collaboration between the clinician and the laboratory specialist.

ORCID

Mesude Falay http://orcid.org/0000-0001-7846-3476

REFERENCES

1. Kershaw G, Orellana D. Mixing tests: diagnostic aides in the investigation of prolonged prothrombin times and activated partial thromboplastin times. *Semin Thromb Hemost*. 2013;39:283-290.
2. Jennings I, Kitchen DP, Kitchen S, Woods TAL, Walker D. Investigation of prolonged APTT. Different approaches taken by laboratories to achieve the same diagnosis. *Int J Lab Hematol*. 2013;35:177-182.
3. Fowler A, Perry DJ. Laboratory monitoring of haemostasis. *Anaesthesia*. 2015;70:68-72.
4. Kottke-Marchant K. Algorithmic approaches to hemostasis testing. *Semin Thromb Hemost*. 2014;40:195-204.
5. 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;82:864-873.
6. 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;137:904-908.
7. Ajzner É, Rogic D, Meijer P, Kristoffersen AH, Carraro P, Sozmen E, et al. An international study of how laboratories handle and evaluate patient samples after detecting an unexpected APTT prolongation. *Clin Chem Lab Med*. 2015;53:1593-1603.
8. Cugno M, Gualtierotti R, Tedeschi A, Meroni PL. Autoantibodies to coagulation factors: from pathophysiology to diagnosis and therapy. *Autoimmun Rev*. 2014;13:40-48.
9. Thachil J. Coagulopathy of sepsis from nonspecific inhibitors. *Int J Lab Hematol*. 2015;37:e170-e171.
10. Lippi G, Franchini M, Favaloro EJ. Diagnostics of inherited bleeding disorders of secondary hemostasis: an easy guide for routine clinical laboratories. *Semin Thromb Hemost*. 2016;42:471-477.
11. Lippi G, Favaloro EJ. Laboratory hemostasis: milestones in Clinical Chemistry and Laboratory Medicine. *Clin Chem Lab Med*. 2013;51:91-97.
12. Clinical and Laboratory Standards Institute (CLSI) (2008) One-stage prothrombin (PT) test and activated partial thromboplastin time (APTT) test. Approved guideline H47-A2. 28 (20).
13. LA Clinical and Laboratory Standards Institute (CLSI). Laboratory Testing for the Lupus Anticoagulant; Approved Guideline. CLSI document H60-A. Wayne, PA: CLSI, 2014.
14. Gringeri A, Mantovani LG, Scalone L, Mannucci PM. Cost of care and quality of life for patients with hemophilia complicated by inhibitors: the COCIS Study Group. *Blood*. 2003;102:2358-2363.
15. Thorell SE, Nash MJ, Thachil J. Clinical implications of clotting screens. *Int J Lab Hematol*. 2015;37:8-13.
16. Favaloro EJ, Lippi G. Laboratory testing and/or monitoring of the new oral anticoagulants/antithrombotics: for and against ? *Clin Chem Lab Med*. 2011;49:755-757.
17. Lippi G, Favaloro EJ. Recent guidelines and recommendations for laboratory assessment of the direct oral anticoagulants (DOACs): is there consensus? *Clin Chem Lab Med*. 2015;53:185-197.
18. Lippi G, Favaloro EJ. Activated partial thromboplastin time: new tricks for an old dogma. *Semin Thromb Hemost*. 2008;34:604-611.
19. Milos M, Herak D, Zadro R. Discrepancies between APTT results determined with different evaluation modes on automated coagulation analyzers. *Int J Lab Hematol*. 2010;32(1p2):33-39.
20. Bowyer A, Kitchen S, Makris M. The responsiveness of different APTT reagents to mild factor VIII, IX and XI deficiencies. *Int J Lab Hematol*. 2011;33:154-158.

21. Tcherniantchouk O, Laposata M, Marques MB. The isolated prolonged PTT. *Am J Hematol*. 2013;88:82-85.
22. Li R, Swaelens C, Vandermijnsbrugge F, Cantinieaux B. Applying a direct aPTT ratio (PlatelinLS/ActinFS) permits to identify rapidly and reliably a bleeding-related factor deficiency or a lupus anticoagulant sequential to an isolated prolongation of aPTT in paediatric pre-operative screening. *Eur J Haematol*. 2016;96:578-585.

How to cite this article: Falay M, Senes M, Yücel D, et al. What should be the laboratory approach against isolated prolongation of a activated partial thromboplastin time? *J Clin Lab Anal*. 2018;32:e22415.
https://doi.org/10.1002/jcla.22415