Pitfalls in Coagulation Testing

Ulrich J. Sachs[1,2]

[1] Department of Thrombosis and Haemostasis, Giessen University Hospital, Giessen, Germany
[2] Institute for Clinical Immunology, Transfusion Medicine, and Haemostasis, Justus Liebig University, Giessen, Germany

Address for correspondence Ulrich J. Sachs, MD, Department of Thrombosis and Haemostasis, Giessen University Hospital, Langhansstr. 2, 35392 Giessen, Germany (e-mail: ulrich.sachs@med.uni-giessen.de).

Hamostaseologie 2023;43:60–66.

Abstract

Assays in the coagulation laboratory are affected by numerous variables. Variables which have impact on the test result can lead to incorrect results, and potentially to consequences for further diagnostic and therapeutic decisions made by the clinician. The interferences can be separated into three main groups: *biological* interferences, with an actual impairment of the patient's coagulation system (congenital or acquired); *physical* interferences, which usually occur in the pre-analytical phase; and *chemical* interferences, because of the presence of drugs (mainly anticoagulants) in the blood to be tested. This article discusses some of these interferences in seven instructive cases of (near) miss events as an approach to generate more attention to these issues.

Keywords

- coagulation testing
- interferences
- case-based learning

Zusammenfassung

Gerinnungsanalysen werden durch zahlreiche Variablen beeinflusst. Variablen, die sich auf das Testergebnis auswirken, können zu falschen Ergebnissen und damit möglicherweise zu Konsequenzen für das weitere diagnostische und therapeutische Vorgehen des Klinikers führen. Diese Interferenzen lassen sich in drei Hauptgruppen einteilen: *biologische* Interferenzen mit einer tatsächlichen Beeinträchtigung des Gerinnungssystems des Patienten (angeboren oder erworben); *physikalische* Interferenzen, die in der Regel in der prä-analytischen Phase auftreten; und *chemische* Interferenzen aufgrund des Vorhandenseins von Arzneimitteln und Chemikalien (hauptsächlich Antikoagulanzien) im zu testenden Blut. In diesem Artikel werden einige dieser Interferenzen anhand von sieben aufschlussreichen Fällen von (Beinahe-) Fehlern erörtert, mit dem Ziel, die Aufmerksamkeit für dieses Thema zu erhöhen.

Schlüsselwörter

- Gerinnungstests
- Interferenzen
- fallbasiertes Lernen

Introduction

Assays in the coagulation laboratory are affected by numerous variables. When they have impact on the test result, incorrect results may be reported to the physician, leading (or, not leading) to consequences for further diagnostic and therapeutic decisions. In this review, I would like to propose a classification of these interferences into three main groups: (1) *biological* interferences—interferences due to an actual impairment of the patient's coagulation system (congenital or acquired); (2) *physical* interferences—interferences due to pre-analytical errors; and (3) *chemical* interferences—interferences due to the presence of drugs (mainly anticoagulants) in the test tube.

Interferences are often more easily recognized by reviewing coagulation tests in concert with the clinical information. On the one hand, however, the laboratory often does not have the clinical information required (or, even worse, no clinical information at all). On the other hand, the patient's physicians may be unaware of the relationship between a confounding variable and its interference with a specific laboratory test. It is therefore recommended that

received
October 28, 2022
accepted after revision
November 17, 2022

Georg Thieme Verlag KG,
Rüdigerstraße 14,
70469 Stuttgart, Germany

DOI https://doi.org/10.1055/a-1981-7939.
ISSN 0720-9355.

appropriate quality systems covering the whole process of a diagnostic test in coagulation—from taking the patient's blood to reporting the results—should be established to minimize the effect of such influences. Educating medical staff involved in this process, in the end, should be a cornerstone in any quality system. Case-based discussions of (near) miss events are one approach to generate more attention to these issues. Seven cases of (near) miss events are reported in this review, and the reader is encouraged to share some or all of them with the medical staff in his institution.

Biological Interferences

A specific coagulation assay or a group of assays that have been requested from the laboratory may be affected by an actual impairment of the patient's coagulation system. This includes congenital impairments (such as a mutation in a relevant gene encoding a clotting factor) and acquired impairments (such as those mediated by inhibitors directed against clotting factors). Often, it is difficult to identify a specific interaction that has impact on a test result. The fewer tests requested, the more difficult it is to detect individual incorrect results. The more different the requested analyses are methodologically (e.g., based on prothrombin time [PT] and activated partial thromboplastin time [APTT], antigen and clotting assays from the same sample), the more likely it is to find inconclusive outliers before a report leaves the laboratory as medically validated.

Interference of the FV R506Q (Leiden) Mutation

Observation

An external laboratory performed a thrombophilia screen and the patient was referred to us with two distinct diagnoses: a homozygous factor V R506Q (Leiden) mutation and protein S deficiency. We confirmed activated protein C (APC) resistance using a functional assay but found free protein S antigen (and later total protein S antigen) within the normal range.

Pitfall

Does this patient have protein S deficiency?[1,2] Protein S is a cofactor of APC and together, they are responsible for inactivating the cofactors FVa and FVIIIa. A protein S activity assay measures the ability of protein S (in the patient's sample) to prolong a one-stage clotting time (PT or APTT) in the presence of a standardized amount of APC. The more protein S available in the sample, the longer the APTT. If the patient's FV is mutated and carries the Leiden variant, it acquires APC resistance. APC resistance shortens the APTT. Accordingly, the protein S activity will be underestimated because in the presence of the same amount of protein S, the APTT is shorter with APC resistance than with no APC resistance.

Remember that the same biological principle is used to identify APC resistance[3]: in an APTT assay, the total clotting time is measured in the patient's plasma in the absence and in the presence of (externally added, high amounts of) APC, and a ratio "APTT with APC added/APTT without APC added" is reported. The ratio is usually <2 if the patient has APC resistance: his APTT is not adequately prolonged because his FV is not sufficiently inhibited. Free protein S antigen is the assay recommended by the ISTH when screening for protein S deficiency.[2]

Interference of Antiphospholipid Antibodies

Observation

A preoperative screen in a 6-year-old boy prior to tonsillectomy revealed normal test results for PT and fibrinogen, but prolonged APTT (92 seconds). Subsequent analysis of single factors revealed normal FXII (82%) and FXI (72%), but severely low FVIII ($<1\%$) and FIX ($<1\%$) activity. The pathologist contacted the clinical team. The child was apparently healthy, did not have any signs or symptoms of bleeding, and his family history was unremarkable. The procedure was postponed for additional diagnostic workup.

Pitfall

A combined diagnosis of severe hemophilia A and severe hemophilia B in an apparently healthy 6-year-old boy seemed unlikely. A plasma mixing study was performed the same day and failed to normalize the APTT, indicating the presence of an inhibitor. Further analysis in a Nijmegen-Bethesda assay gave 3 BU anti-FVIII/mL and 5 BU anti--FIX/mL. Again, development of acquired hemophilia A and B in an apparently healthy child seemed unlikely. The presence of a lupus anticoagulant (LA) was suspected to have caused the alterations. An integrated screen and confirm assay employing normal and elevated levels of phospholipids in a diluted Russell Viper venom time (DRVVT) revealed a ratio of 1.6, confirming the presence of a (weak) LA in the sample. Immunological assays for antibodies of the IgG and IgM type against beta-2-glycoprotein I and cardiolipin, as recommended by the ISTH, were negative. With these results in mind, the first question to address is whether this excludes the presence of inhibitors. In rare case, LA and a specific inhibitor against a clotting factor may be present together.[4] Chromogenic factor assays appear to not suffer from LA interference to the same extent as clotting assays, most likely because of the high dilution of the patient's plasma.[5] Indeed, chromogenic FVIII and F IX were near-normal in this boy (66 and 70%, respectively). Another way to exclude the presence of inhibitors in the presence of LA would be antigenic enzyme-linked immunosorbent assays (unavailable in our laboratory). A preliminary diagnosis of "transient LA in childhood" was made and the boy was cleared for surgery, which was uneventful without any unexpected bleeding. Recovery was quick and complete.

The second question to address is whether the detection of LA is of any relevance to the child. In a recent study in adults, isolated LA was highly associated with thrombosis.[6] In children, a positive LA can be detected in approximately 2% of apparently healthy children, usually discovered during preoperative assessment of coagulation.[7] These LA are usually transient and clinically insignificant, and are most likely induced by infections or vaccinations. Of course, most of

these LA do not show such dramatic impact on clotting factor assays as in they did in this case. Our patient was reevaluated in the coagulation clinic 3 months after surgery, and normal values were obtained for APTT, and APTT-based FVIII and FIX activity. LA was no longer detectable by DRVVT.

Interference of Fibrinogen Variants

Observation

A 22-year-old female patient in gestational week 41 + 3 was transferred from a birth center to the hospital because of obstetrical arrest. After initial assessment, the decision was made to deliver the child by caesarean section. An emergency coagulation panel gave disturbed measurements for PT, APTT, and Clauss fibrinogen, and no values were reported. Reflex testing of fibrinogen in an antigen assay showed 0.81 g/L (reference interval: 1.9–4.3 g/L). Platelet function analysis was normal and von Willebrand antigen and activity were normal with normal activity/antigen ratio. The laboratory and the obstetric team called the pathologist to seek advice.

Pitfall

Most routine coagulation assays use the patient's own fibrinogen to produce either a change in light transmission (photo-optical method) or a mechanically detectable change in the sample (mechanical method). This holds true for the "group tests" (PT, APTT, and TT) and all clotting-based factor assays, such as Clauss fibrinogen, a diluted variant of the TT. Antigen assays, such as the von Willebrand assays or the fibrinogen antigen assay, are not based on clotting but on ligand binding to specific targets, immobilized on latex particles or similar. These assays, and also the chromogenic assays which use an artificial target antigen that is sensitive to FXa- or FIIa-mediated cleavage and will give rise to color after being cleaved, are therefore independent from the patient's own fibrinogen. Interpretation of the aforementioned case was fast and easy because of the reflex testing of fibrinogen, which, for a pregnant woman at term, was unusually low. She initially received 3 g of fibrinogen concentrate and delivery was uneventful. Congenital fibrinogen disorders are classified according to the fibrinogen levels.[8] Congenital dysfibrinogenemia should be suspected in the case of a decreased Clauss fibrinogen and normal antigenic levels, a ratio of $\leq$0.7 is usually applied.[9,10] The term "hypodysfibrinogenemia" was coined to indicate that a low Clauss/antigen ratio is observed together with reduced antigenic levels of fibrinogen. Hypofibrinogenemia, in contrast, is defined by similarly decreased levels of both analytes. Our patient apparently suffers from hypodysfibrinogenemia. A ratio could not be calculated, but her results indicated nonfunctional fibrinogen (Clauss) plus reduced amounts of fibrinogen (antigenic assay). A recent study indicated that a Clauss/antigen ratio of 0.7 would have a sensitivity of 86%.[11] Additional tests are often necessary to distinguish between hypo- and hypodysfibrinogenemia, and genetic testing may help confirm the diagnosis.

(Hypo-)dysfibrinogenemia is a rare disorder. What is seen more often in daily routine are patients with acquired hypofibrinogenemia due to hepatic impairment, massive transfusion, systemic thrombolytic therapy, and acute leukemias. Physicians should be aware that the lower fibrinogen levels are, the more they affect clot-based assays. Severe hypofibrinogenemia (<0.7–0.5 g/L) will result in failure of the assay to clot. In any no-clot sample, the laboratory should also make sure that citrate plasma and not serum was investigated. This mistake is unlikely to occur as long as the primary tube is used for analysis, but may be observed when secondary tubes (aliquots) are received in the laboratory.[12] Be aware that analyzing serum may also give false high FVII and FIX activities. This might be overlooked if these analytes are requested as single tests.

Physical Interferences

A specific coagulation assay or a group of assays that have been requested from the laboratory may be affected by physical impact before it reaches the laboratory. This includes, for example, length of tourniquet application to the patient's arm, increased shear stress due to thin needles, inappropriate mixing of the sample, refrigeration, and long or inappropriate transport to the laboratory. Inside the laboratory, adequate centrifugation, timely separation and analysis, and appropriate freezing and defreezing procedures are required. All steps prior to the analysis are usually covered under the term "preexamination" or "preanalytical" phase, and this is where most errors occur (►Table 1). These errors may lead to platelet activation, consumption of coagulation factors, or in vitro degradation of proteins, finally resulting in incorrect test results and, in the worst case,

Table 1 Preanalytical errors

Error	Impact	Incorrect result
Refrigeration of citrated whole blood prior to centrifugation	Platelet activation Loss of Willebrand factor Loss of FVIII	Low von Willebrand Low FVIII
Long transportation time of citrated whole blood	Protein degradation	Low FVIII, low FV, low protein S; prolonged PT, APTT
Shear forces, e.g., in pneumatic tube systems, heavy breaking in centrifuges	Thrombin generation Platelet/microparticle contamination	Low APTT Negative LA

Abbreviations: APTT, activated partial thromboplastin time; LA, lupus anticoagulant; PT, prothrombin time.

inappropriate patient treatment. Therefore, the preanalytical phase requires special attention, even though some or even all steps may occur outside the control of the laboratory performing the coagulation tests. For a comprehensive overview, see the article by Magnette et al.[13]

Sample Handling in the Laboratory

Observation

A 30-year-old female patient attended the coagulation clinic after two episodes of peripheral vein thrombosis and a transient ischemic attack during the last 2 years. FVIII was ordered from the laboratory as part of the thrombophilia panel.[14] The FVIII clotting assay result was reported as 42%. All other coagulation parameters including PT, APTT, TT, D-dimer, chromogenic protein C, free protein S antigen, LA, antithrombin activity (both Xa-based and IIa-based), and APC resistance were found to be normal.

Pitfall

Two important differential diagnoses in a female patient with reduced FVIII activity are von Willebrand's disease and carrier status of hemophilia A. Further laboratory testing was required and the patient returned to the coagulation clinic for additional blood sampling. Her family history was taken again with a focus on bleeding disorders, but no bleeders were identified. Using the bleeding assessment tool (BAT) from the ISTH,[15] her ISTH-BAT score was calculated with 3 points (cut-off for women of 6 points). Assessing women with carrier status, more than 90% have a positive family history; and more than 60% have an abnormal bleeding score.[16] Assessing women with von Willebrand's disease, a positive ISTH-BAT has a sensitivity of 70 to 100%, but low specificity.[15,17] Overall, additional clinical data seemed not very conclusive for either of the two diagnoses. Repeat FVIII clotting test and chromogenic assay as well as von Willebrand antigen and activity were ordered from the laboratory, together with the group tests and von Willebrand multimers. Same-day results were as follows: PT of 100% (Quick), APTT of 27 seconds, FVIII (clotting) of 88%, FVIII (chromogenic) of 92%, von Willebrand antigen of 92%, von Willebrand activity of 88%, and activity/antigen ratio of 0.9. Multimers were reported as normal with complete triplet structure some weeks later. The discrepancy in FVIII prompted the pathologist to check all FVIII measurements from the specific day when this patient was reported to have 42%. He revealed that all requested assays were run the day the patient had attended the clinic except for FVIII, for which the sample was frozen. The frozen sample was thawed and analyzed 2 days later, together with 20 other samples. Out of the 21 samples analyzed for FVIII clotting that day, 14 (66%) were below the reference range (70–150). Internal quality controls were within their range and did not show any trends. After discussing the issue with the laboratory, it was finally realized that, against the standard operating procedure, frozen plasma samples were thawed on the benchtop for a period of time that could no longer be reconstructed. Frozen samples should always be thawed rapidly (within 5–10 min) in an appropriate device at 37 °C until completely thawed,[18] and close monitoring during thawing is recommended. FVIII, but also FV and protein S, is very sensitive to protein degradation and loss of activity is marked. This also holds true if a sample is refrigerated prior to centrifugation or if the transport time between phlebotomy and the laboratory is too long.

Chemical Interferences

A specific coagulation assay or a group of assays that have been requested from the laboratory may be affected by chemical impact before it reaches the laboratory. This includes the presence of an anticoagulant or a relevant active substance in the patient's blood (such as vitamin K antagonists or direct oral anticoagulants [DOACs]), contaminating anticoagulants present in lines (such as heparins in central venous catheters), or inappropriate amounts of anticoagulants in the test tube, e.g., if the tube is underfilled.

Wrong Anticoagulant in the Collection Tube

Observation

FV activity test was requested as the only test in an outpatient and was very low (<5%). Because homozygous FV deficiency is a very rare disorder,[19] the pathologist contacted the clinical team.

Pitfall

It was revealed that the patient had a family history and a personal history of thromboembolism and that FV activity was ordered erroneously instead of a genetic test for FV R506Q (Leiden). The initial analysis was performed from a secondary tube that was labelled with the patient's details by the external laboratory which referred the sample to us. Analyzing the plasma in this tube revealed gross hyperkalemia, in accordance with the presence of K-EDTA and not Na-citrate as anticoagulant.[20] Potassium has strong effects especially on FV and FVIII activities[20] and should always be suspected when one (or both) of these two analytes are unexpectedly low. Of note, K-EDTA may also affect the Bethesda assay,[21] and if both FV and FVIII inhibitors are detected, which would be very uncommon, the presence of K-EDTA should also be suspected before reports are released by the laboratory. Furthermore, EDTA is known to have high calcium-chelating capacity, which interferes with routine recalcification, which has effects on all clotting assays. Apparently, wrong anticoagulants may affect the test result.

Correct, but Too Strong Concentration of Anticoagulant in the Collection Tube

Observation

We received a preoperative inpatient sample for routine coagulation testing which revealed a PT of 30% (Quick) and an APTT of greater than 120 seconds. Because both results are above the alarm limits for coagulation assays in first-time patients, the technician phoned the ward. The nurses

reported that the patient was clinically fine, was not taking any drugs, and was waiting for surgical shoulder repair after a sport trauma. Under-filling of the tube was suspected, and a new sample was requested. Unexpectedly, results did match the previous findings, and the laboratory contacted the pathologist. A quick review of the additional laboratory results revealed unremarkable results for biochemistry but a hematocrit of 65 L/L (reference range, 37–45 L/L).

Pitfall

If the concentration of the anticoagulant in the test tube is too strong, all clotting times are affected. One frequently seen problem in the coagulation laboratory is under-filling of the primary tube. Blood tubes for coagulation testing should be filled not less than 90% of their nominal filling volume.[22] Filling usually requires an optical check at sample reception, since automated analyzers do not perform this check properly. If the sample is under-filled (meaning that not enough whole blood was added during the blood draw), a higher amount of Na-citrate than required will be present in the patient's blood. Following centrifugation, the patient's plasma will contain more citrate than necessary. When the effect of Na-citrate is then reversed in the analyzer by adding a standard amount of calcium, higher amounts of citrate will bind more calcium than calculated. Accordingly, the sample is not fully recalcified and clotting times are prolonged. Incorrect filling may account for up to 60% of sample rejections in the coagulation laboratory.[23] Of note, although coagulation tubes filling accuracy was recently reported to be within ± 10% for the manufacturers tested, the overall bias was found to be highly variable among manufacturers and lots[24] and lot-specific under-filling cannot be excluded. Also do not forget that vacuum-based blood collection tubes lose their capability of complete filling over time and should not be used after their expiry date.[25] All these potential interferences were excluded in our patient. One important additional reason for the constellation seen in this patient would be high amounts of heparin in the sample. This sometimes occurs if the coagulation sample is taken from existing lines which either had been flushed with heparin or which are used actively for IV heparin therapy.

Notably, the patient had a very high hematocrit and was later diagnosed with polycythemia vera. When the cells are separated from her plasma, a relatively small amount of plasma will be anticoagulated in a relatively high amount of Na-citrate. Comparable to what happens in an under-filled sample, too much citrate remains after recalcification and clotting times will be prolonged, starting somewhere at a patient's hematocrit of 56 to 60 L/L.[26] The amount of citrate in the tube should therefore be adjusted according to the individual hematocrit using the following formula: volume of citrate solution (3.2%) = volume * (100-hematocrit)/(640-hematocrit).

Anticoagulant in the Patient's Plasma

Observation

A patient with recurrent thromboembolic events taking 20 mg rivaroxaban OD after segmental pulmonary embolism 4 months before presented to the coagulation clinic. A partial thrombophilia screening had been performed from a colleague in private practice, indicating absence of protein C, protein S, and antithrombin deficiency, and absence of FV R506Q (Leiden) and prothrombin G20210A mutations. Despite the fact that functional coagulation tests were already performed, a regular thrombophilia panel adopted for patients under direct Xa inhibitors was ordered. Laboratory results were normal for chromogenic protein C activity, and free protein S antigen. The antithrombin results were as follows: antithrombin FXa-based, 126%; antithrombin FIIa-based, 105%; and antithrombin FXa-based after DOAC adsorption, 58%.

Pitfall

In this patient on rivaroxaban for pulmonary embolism, the effect of the DOAC on functional coagulation assays was overlooked. DOACs include both direct thrombin inhibitors (dabigatran) and direct factor Xa inhibitors (apixaban, edoxaban, rivaroxaban). These medications may cause assay interference by falsely increasing or decreasing measured values, depending on the analyte, the drug, and the drug's concentration (which largely depends on the interval between the last drug intake and the time of blood sampling). For an overview, see ►Table 2. Dabigatran and rivaroxaban significantly prolong the PT and APTT in a concentration- and reagent-dependent manner; rivaroxaban has a higher impact on PT and dabigatran on APTT. The presence of dabigatran can lead to falsely reduced Clauss fibrinogen when measured with low thrombin concentration reagents. Antithrombin is overestimated in FXa-based assays in the presence of rivaroxaban, and in FIIa-based assays in the presence of dabigatran. Instrument-related differences can be found for all parameters.[27] Apixaban, in contrast, has only mild effects on coagulation tests.[28] PT and APTT are barely influenced. At 225 ng apixaban/mL, PT and APTT are approximately 1.15 times prolonged, with some reagents being more sensitive than others.[29] However, antithrombin activity measured in FXa-based assays is also overestimated in the presence of apixaban. In comparison with the other two oral Xa inhibitors, the in vitro effects of edoxaban are more similar to rivaroxaban than apixaban.[30] Apparently, our patient has an antithrombin deficiency that was not detected because an anti-Xa-based antithrombin assay was performed despite the fact that she was on rivaroxaban.

Physicians can choose from different strategies to minimize DOAC interference in coagulation testing. The simplest way is avoiding sensitive coagulation tests in patients taking DOACs (►Table 2). If a sensitive test is required under DOAC therapy, the drug may be paused for 2 (or 3) days prior to collecting the blood sample. This interruption may put the patient at increased risk of (re-)thrombosis, and transitioning to low-molecular-weight heparin (LMWH) could be an option. A much more simple and effective way is the use of adsorbing agents that can neutralize DOACs in the patient sample. Several studies have demonstrated that DOAC removal by adsorbing agents is efficient in almost all patient samples.[31] Some centers which receive large amounts of

Table 2 Impact of DOAC on coagulation assays

Impact	Assay	Clinical risk
False increase	Antithrombin activity[a] Clot-based protein C activity Clot-based protein S activity	Deficiency of protein C, protein S, or antithrombin may be overlooked
	APC resistance[b]	FV Leiden mutation may be overlooked erroneously
	LA screen/LA ratio	The presence of LA may be suspected erroneously because of DOAC effect
False decrease	Single factors in clotting assays (PT or APTT based)	Misdiagnosis of single factor deficiency; mixing studies may show (unusual) inhibitor effects that shall not be mistaken as indicative for the presence of an inhibitor
No change	Clauss fibrinogen[c] Fibrinogen antigen D-dimer Chromogenic protein C activity Free protein S antigen Total protein S antigen von Willebrand antigen and activity	–

Abbreviations: APC, activated protein C; APTT, activated partial thromboplastin time; DOAC, direct oral anticoagulant; LA, lupus anticoagulant; PT, prothrombin time.
[a]Apixaban, edoxaban, and rivaroxaban have impact on FXa-based antithrombin assays, and dabigatran on FIIa-based antithrombin assays.
[b]Assay-dependent observation.
[c]In high concentration, dabigatran may lower Clauss fibrinogen in some assays.

plasma samples potentially containing DOAC, such as from regional heart centers, have adopted strategies to double test sensitive parameters in the absence and presence of an adsorber. As seen in our patient, the "true" FXa-based antithrombin activity is 58%, but was increased to 126% in the presence of rivaroxaban. One might argue that the alternative option in this patient would have been to rely on a FIIa-based assay which is unaffected by rivaroxaban. Unfortunately, this assay gave normal antithrombin activity. Later, genetic testing of this patient revealed the presence of a *SERPINC1* c.[236G > A];[=] p.(Arg79His) mutation, also known as antithrombin Rouen I, which is undetectable in FIIa-based assays. The lesson to be learned here is to exclude false normal measurement of the important coagulation inhibitors: clot-based protein C and protein S activity, and antithrombin, because of DOAC. The best strategy is using adsorbers. We also believe that young patients with unexplained thrombosis should also be assessed by at least two functionally different antithrombin assays.

This case can also be used to address another aspect—the question how to identify DOACs in a sample. All DOACs can be measured "specifically" in anti-Xa or anti-IIa assays calibrated with the specific drug. This might be helpful in critical clinical situations, such as deciding the right treatment strategy in a patient with fresh stroke taking DOAC for atrial fibrillation: lysis or no lysis?[32] Of course, "specific" anti-Xa assays are only inasmuch specific as they are calibrated against the drug (e.g., rivaroxaban). Analyzing the rivaroxaban concentration in a patient's plasma in the presence of additional drugs that interfere with the assay system, such as LMWH, does not reflect the true rivaroxaban concentration.[33] Finally, it should be kept in mind that PT and APTT normal values cannot exclude DOAC anticoagulant activity in a patient sample, and PT or APTT prolongation is not always associated with DOAC anticoagulant effect when compared to specific tests.[34]

Conclusion

There are multiple interfering variables that can affect results in the coagulation laboratory. Training of medical staff is relevant to reach an appropriate level of awareness that biological, physical, and chemical interferences may disturb an assay or a group of assays. Whenever a coagulation result is unexpected or seems highly unlikely, close contact between the pathologist and the clinical team is the best way for identifying the root cause.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgement

I would like to thank Anette Kirsch-Altena and Yvonne Schulze, Department of Thrombosis and Haemostasis, Giessen University Hospital, Giessen, Germany, for support in collecting the clinical cases and critically revising the final manuscript.

References

1 Marlar RA, Gausman JN. Protein S abnormalities: a diagnostic nightmare. Am J Hematol 2011;86(05):418–421
2 Marlar RA, Gausman JN, Tsuda H, Rollins-Raval MA, Brinkman HJM. Recommendations for clinical laboratory testing for protein S deficiency: communication from the SSC committee plasma

coagulation inhibitors of the ISTH. J Thromb Haemost 2021;19 (01):68–74
3 Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. Proc Natl Acad Sci U S A 1993;90 (03):1004–1008
4 Jacobs JW, Gisriel SD, Iyer K, Rinder HM. Concomitant factor VIII inhibitor and lupus anticoagulant in an asymptomatic patient. J Thromb Thrombolysis 2022;53(04):945–949
5 de Maistre E, Wahl D, Perret-Guillaume C, et al. A chromogenic assay allows reliable measurement of factor VIII levels in the presence of strong lupus anticoagulants. Thromb Haemost 1998; 79(01):237–238
6 Yin D, de Groot PG, Ninivaggi M, Devreese KMJ, de Laat B. Clinical relevance of isolated lupus anticoagulant positivity in patients with thrombotic antiphospholipid syndrome. Thromb Haemost 2021;121(09):1220–1227
7 Soybilgic A, Avcin T. Pediatric APS: state of the art. Curr Rheumatol Rep 2020;22(03):9
8 Casini A, Moerloose P, Neerman-Arbez M. One hundred years of congenital fibrinogen disorders. Semin Thromb Hemost 2022;48 (08):880–888
9 Prisco D, Zarone N, Liotta AA, et al. Clottable to immunological fibrinogen ratio in plasma from control subjects and hyperfibrinogenemic patients. Haemostasis 1995;25(06):257–263
10 Casini A, Blondon M, Lebreton A, et al. Natural history of patients with congenital dysfibrinogenemia. Blood 2015;125(03):553–561
11 Casini A, Brungs T, Lavenu-Bombled C, Vilar R, Neerman-Arbez M, de Moerloose P. Genetics, diagnosis and clinical features of congenital hypodysfibrinogenemia: a systematic literature review and report of a novel mutation. J Thromb Haemost 2017;15 (05):876–888
12 Favaloro EJ, Adcock Funk DM, Lippi G. Pre-analytical variables in coagulation testing associated with diagnostic errors in hemostasis. Lab Med 2012;43(02):1–10
13 Magnette A, Chatelain M, Chatelain B, Ten Cate H, Mullier F. Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories. Thromb J 2016;14:49
14 Kyrle PA, Minar E, Hirschl M, et al. High plasma levels of factor VIII and the risk of recurrent venous thromboembolism. N Engl J Med 2000;343(07):457–462
15 Rodeghiero F, Castaman G, Tosetto A, et al. The discriminant power of bleeding history for the diagnosis of type 1 von Willebrand disease: an international, multicenter study. J Thromb Haemost 2005;3(12):2619–2626 [Erratum in: J Thromb Haemost. 2006;4(4):925]
16 Carter-Febres ME, Lim MY. Evaluation of ISTH-BAT as a predictor for factor deficiency in haemophilia carriers: a single-centre experience. Blood Coagul Fibrinolysis 2021;32(08):611–613
17 Deforest M, Grabell J, Albert S, et al. Generation and optimization of the self-administered bleeding assessment tool and its validation as a screening test for von Willebrand disease. Haemophilia 2015;21(05):e384–e388
18 Gosselin RC, Honeychurch K, Kang HJ, Dwyre DM. Effects of storage and thawing conditions on coagulation testing. Int J Lab Hematol 2015;37(04):551–559
19 Asselta R, Peyvandi F. Factor V deficiency. Semin Thromb Hemost 2009;35(04):382–389
20 Crist RA, Gibbs K, Rodgers GM, Smock KJ. Effects of EDTA on routine and specialized coagulation testing and an easy method to distinguish EDTA-treated from citrated plasma samples. Lab Hematol 2009;15(04):45–48
21 Moser KA, Adcock Funk DM. Pitfalls in special coagulation testing: three illustrative case studies. Int J Lab Hematol 2013;35(03): 334–338
22 CLSI. H21–A5 Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline. 5th ed. CLSI; 2008
23 Goswami B, Tayal D, Chawla R, Mallika V. Pre-analytical factors that influence the interpretation of prothrombin time in the clinical laboratory: one year experience in a super specialty hospital in India. Clin Chim Acta 2009;410(1-2):93–94
24 Lippi G, Salvagno GL, Radišić Biljak V, et al. Filling accuracy and imprecision of commercial evacuated sodium citrate coagulation tubes. Scand J Clin Lab Invest 2019;79(04):276–279
25 Bostic G, Thompson R, Atanasoski S, et al. Quality improvement in the coagulation laboratory: reducing the number of insufficient blood draw specimens for coagulation testing. Lab Med 2015;46 (04):347–355
26 Marlar RA, Potts RM, Marlar AA. Effect on routine and special coagulation testing values of citrate anticoagulant adjustment in patients with high hematocrit values. Am J Clin Pathol 2006;126 (03):400–405
27 Van Blerk M, Bailleul E, Chatelain B, et al. Influence of dabigatran and rivaroxaban on routine coagulation assays. A nationwide Belgian survey. Thromb Haemost 2015;113(01):154–164
28 Hillarp A, Gustafsson KM, Faxälv L, et al. Effects of the oral, direct factor Xa inhibitor apixaban on routine coagulation assays and anti-FXa assays. J Thromb Haemost 2014;12(09):1545–1553
29 Van Blerk M, Bailleul E, Chatelain B, et al. Influence of apixaban on commonly used coagulation assays: results from the Belgian national External Quality Assessment Scheme. Int J Lab Hematol 2017;39(04):402–408
30 Hillarp A, Strandberg K, Baghaei F, Fagerberg Blixter I, Gustafsson KM, Lindahl TL. Effects of the oral, direct factor Xa inhibitor edoxaban on routine coagulation assays, lupus anticoagulant and anti-Xa assays. Scand J Clin Lab Invest 2018;78(7-8):575–583
31 Slavik L, Jacova J, Friedecky D, et al. Evaluation of the DOAC-stop procedure by LC-MS/MS assays for determining the residual activity of dabigatran, rivaroxaban, and apixaban. Clin Appl Thromb Hemost 2019;25:1076029619872556
32 Berge E, Whiteley W, Audebert H, et al. European Stroke Organisation (ESO) guidelines on intravenous thrombolysis for acute ischaemic stroke. Eur Stroke J 2021;6(01):I–LXII
33 Eller T, Flieder T, Fox V, et al. Direct oral anticoagulants and heparins: laboratory values and pitfalls in 'bridging therapy'. Eur J Cardiothorac Surg 2017;51(04):624–632
34 Testa S, Legnani C, Tripodi A, et al. Poor comparability of coagulation screening test with specific measurement in patients receiving direct oral anticoagulants: results from a multicenter/multiplatform study. J Thromb Haemost 2016;14 (11):2194–2201