Scholars Journal of Medical Case Reports
Abbreviated Key Title: Sch J Med Case Rep
ISSN 2347-9507 (Print) | ISSN 2347-6559 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Hematology

Interference of Acquired Anti-FVIII and Anti-FXI Inhibitors with One-Stage Clotting Assays of Intrinsic Pathway Factors: A Longitudinal Report of Two Cases

Zineb Jerroundi[1,2]*, Abdelhak Elkhazraji[1,2], Zahid Hafid[1,2]

[1]Department of Hematology and Immunohematology, Mohammed V Military Teaching Hospital, Rabat, Morocco
[2]Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco

DOI: https://doi.org/10.36347/sjmcr.2026.v14i08.039 | **Received:** 07.06.2026 | **Accepted:** 19.08.2026 | **Published:** 29.08.2026

***Corresponding author:** Zineb Jerroundi
Department of Hematology and Immunohematology, Mohammed V Military Teaching Hospital, Rabat, Morocco

Abstract | **Original Research Article**

Introduction: Specific acquired coagulation factor inhibitors may interfere with the interpretation of hemostasis laboratory investigations. Although widely used in routine practice, one-stage clotting assays for intrinsic pathway factors may generate misleading results suggestive of multiple factor deficiencies in the presence of circulating inhibitors. ***Objective:*** To investigate the impact of acquired anti-factor VIII (anti-FVIII) and anti-factor XI (anti-FXI) inhibitors on one-stage clotting assays of intrinsic pathway factors and to analyze the associated analytical biases. ***Materials and Methods:*** We retrospectively analyzed two longitudinal observations: one patient with an acquired anti-FVIII inhibitor and one patient with an acquired anti-FXI inhibitor. Each patient underwent two biological evaluations performed at different time points. Laboratory investigations included activated partial thromboplastin time (aPTT), mixing studies using pooled normal plasma, one-stage clotting assays for intrinsic pathway factors, and inhibitor detection and quantification using the Nijmegen-modified Bethesda assay. ***Results:*** In both cases, the presence of a specific inhibitor was associated with an apparent reduction in non-targeted coagulation factors, mimicking a multiple intrinsic pathway factor deficiency. In the anti-FVIII inhibitor case, the decrease in inhibitor titer during follow-up was associated with normalization of FIX, FXI, and FXII activities. In the anti-FXI inhibitor case, inhibitor disappearance was accompanied by improvement of abnormalities observed in other factor assays. These findings support an analytical interference rather than a true multiple factor deficiency. ***Conclusion:*** Acquired coagulation inhibitors may interfere with one-stage clotting assays and lead to diagnostic errors. An integrated interpretation combining clinical data, functional assays, inhibitor quantification, and alternative analytical methods is essential to avoid misdiagnosis.

Keywords: Acquired inhibitor; factor VIII; factor XI; one-stage clotting assay; chromogenic assay; activated partial thromboplastin time; pseudo-multiple factor deficiency.

INTRODUCTION

Laboratory investigation of coagulation relies on the assessment of the intrinsic, extrinsic, and common pathways to identify abnormalities responsible for bleeding disorders. The intrinsic pathway, mainly involving factors XII, XI, IX, and VIII, plays a critical role in thrombin generation amplification. Its exploration is primarily based on activated partial thromboplastin time (aPTT), followed by functional coagulation factor assays when abnormalities are detected [1–3].

An isolated prolongation of the aPTT may result from a coagulation factor deficiency or from the presence of a circulating inhibitor. Mixing studies are essential to differentiate between a factor deficiency and a plasma inhibitor before performing specific factor assays and inhibitor quantification [1,2].

Coagulation factor activity is most commonly assessed using one-stage clotting assays. These methods rely on the ability of the patient's plasma to correct the clotting time of factor-deficient plasma. However, they evaluate the overall efficiency of the coagulation cascade rather than the isolated activity of a specific factor, which explains their susceptibility to biological interferences [4, 5].

Specific acquired coagulation factor inhibitors represent a challenging situation that may complicate the interpretation of laboratory results. Acquired factor VIII

inhibitors (anti-FVIII inhibitors), responsible for acquired hemophilia A, are the most frequently encountered specific inhibitors and cause a marked reduction in FVIII activity through functional neutralization. Acquired factor XI inhibitors (anti-FXI inhibitors) are much rarer but may also interfere with intrinsic pathway investigations [1–3].

An important aspect of these inhibitors is that their analytical impact may extend beyond the directly targeted factor. Due to the principle of one-stage clotting assays, a circulating inhibitor may result in an apparent decrease in other intrinsic pathway factors, leading to a laboratory profile suggestive of a pseudo-multiple factor deficiency. This situation represents a diagnostic pitfall that may result in incorrect interpretation of coagulation results [5–7].

Distinguishing a true multiple factor deficiency from analytical interference requires an integrated approach combining clinical context, aPTT results, mixing studies, inhibitor detection and quantification, and, when appropriate, alternative analytical methods. Chromogenic assays, which are less dependent on the integrity of the entire coagulation cascade, may provide complementary information in cases of analytical discrepancy [5,6].

Although interference related to anti-FVIII inhibitors has been described, data regarding the longitudinal evolution of abnormalities affecting non-targeted factors, particularly in the presence of anti-FXI inhibitors, remain limited.

The aim of this study was to investigate the impact of acquired anti-FVIII and anti-FXI inhibitors on one-stage clotting assays of intrinsic pathway factors, to analyze the evolution of laboratory abnormalities during follow-up, and to identify analytical biases that may lead to pseudo-multiple factor deficiency.

Materials and Methods

Study design

This was a retrospective descriptive study based on two longitudinal biological observations of patients with specific acquired coagulation factor inhibitors. The study was conducted in the Hemostasis Laboratory of the Department of Hematology and Immuno-Hematology at Mohammed V Military Teaching Hospital, Rabat, Morocco.

The two cases included:

- One patient with an acquired inhibitor directed against factor VIII (FVIII);
- One patient with an acquired inhibitor directed against factor XI (FXI).

Each patient underwent two biological evaluations performed at different time points: an initial assessment at the time of inhibitor diagnosis and a follow-up assessment after a defined interval (14 months for the anti-FVIII inhibitor and 4 months for the anti-FXI inhibitor).

This longitudinal approach allowed evaluation of changes in coagulation factor activities according to the evolution of inhibitor titers.

Blood samples

Blood samples were collected by venipuncture into tubes containing 3.2% sodium citrate (0.109 mol/L), according to international recommendations for hemostasis testing.

After centrifugation, platelet-poor plasma samples were obtained and analyzed according to the laboratory's standard operating procedures. All analyses were performed under standardized conditions to minimize pre-analytical variations that could affect results.

Initial hemostasis assessment

The initial laboratory evaluation included activated partial thromboplastin time (aPTT) and prothrombin time (PT) measurement.

Analyses were performed using the STA Compact Max® analyzer (Diagnostica Stago, Asnières-sur-Seine, France).

The aPTT was measured using the STA®-PTT A5 reagent, and PT was assessed using STA®-Neoplastine 10 thromboplastin.

In cases of isolated aPTT prolongation, additional investigations were performed to differentiate between coagulation factor deficiency and the presence of a circulating inhibitor.

Detection of circulating inhibitors by mixing study

Mixing studies were performed using a standardized 1:1 mixture of patient plasma and pooled normal plasma (Pool NORM).

The aPTT of the mixture was measured immediately after preparation. When indicated, a second measurement was performed after incubation of the mixture at 37°C to investigate time-dependent inhibitory activity.

Correction of the prolonged aPTT was assessed by comparison with normal pooled plasma. Lack of correction or insufficient correction was considered suggestive of a circulating inhibitor.

Results were interpreted together with the Rosner index when available.

Coagulation factor assays

Intrinsic pathway factor activities were measured using one-stage clotting assays.
FVIII activity was assessed using STA® ImmunoDef VIII factor-deficient plasma.
FXI activity was assessed using STA® Deficient XI factor-deficient plasma.

The principle of the assay is based on the ability of patient plasma to correct the clotting time of plasma deficient in the factor being tested. Factor activity was calculated from a calibration curve generated using STA® Unicalibrator and expressed as percentage activity relative to normal reference plasma.

Analytical quality control

Analytical validation of each measurement series was performed using STA® System Control N+P, including normal and pathological control plasmas, according to the laboratory's internal quality procedures.

Detection and quantification of specific inhibitors

Detection and quantification of anti-FVIII and anti-FXI inhibitors were performed using the Nijmegen-modified Bethesda assay.

This method evaluates the ability of patient plasma to inhibit the activity of the target coagulation factor after incubation with normal reference plasma. Inhibitor titers were expressed as Bethesda units per milliliter (BU/mL).

Data analysis

Data analysis was descriptive and based on comparison of laboratory parameters obtained during the two successive evaluations for each patient.

Changes in aPTT, mixing study results, FVIII and FXI activities, and other intrinsic pathway factor activities measured by one-stage clotting assays were analyzed in parallel with variations in inhibitor titers.

Particular attention was paid to apparent reductions in coagulation factors not targeted by the inhibitor in order to identify profiles compatible with analytical interference leading to pseudo-multiple intrinsic pathway factor deficiency.

RESULTS

Characteristics of the cases

Two cases of patients with specific acquired coagulation factor inhibitors were analyzed.

The first case involved a patient with an acquired inhibitor directed against factor VIII (FVIII). Two biological assessments were performed: an initial evaluation at the time of inhibitor diagnosis and a second evaluation after 14 months of follow-up.

The second case involved a patient with an acquired inhibitor directed against factor XI (FXI). Two biological assessments were also performed: an initial evaluation followed by a control assessment after 4 months of follow-up.

Case 1: Acquired anti-factor VIII inhibitor

At the initial evaluation, coagulation testing revealed a marked prolongation of activated partial thromboplastin time (aPTT), with an aPTT of 101 seconds and a ratio of 2.8. The mixing study performed with pooled normal plasma showed no significant correction, suggesting the presence of a circulating inhibitor.

Specific inhibitor testing was positive, with an inhibition rate of 99%. Quantification of the anti-FVIII inhibitor using the Nijmegen-modified Bethesda assay showed a high inhibitor titer of 748 BU/ml

Initial one-stage clotting assay showed severely reduced FVIII activity (1%). In addition, marked reductions in other intrinsic pathway factor activities were observed, including FIX (1%), FXI (1%), and FXII (12%).

After 14 months of follow-up, aPTT remained prolonged (98 seconds; ratio 2.72). Inhibitor testing remained positive, with an inhibition rate of 98%; however, the anti-FVIII inhibitor titer markedly decreased to 12.8 BU/ml

FVIII activity remained severely decreased (1%), whereas previously reduced non-targeted factor activities improved significantly: FIX (113%), FXI (74%), and FXII (71%).
These results are summarized in Table 1.

Table 1: Impact of an acquired anti-FVIII inhibitor on one-stage clotting assays of intrinsic pathway factors: evolution during follow-up

Parameter	Initial evaluation	After 14 months
aPTT (s)	101	98
aPTT ratio	2.8	2.72
Inhibitor detection (inhibition rate)	99%	98%
Anti-FVIII inhibitor titer (BU/mL)	748	12.8
FVIII activity (%)	1	1
FIX activity (%)	1	113
FXI activity (%)	1	74
FXII activity (%)	12	71

Case 2: Acquired anti-factor XI inhibitor

At the initial evaluation, a markedly prolonged aPTT was observed, with a value of 109 seconds and a ratio of 3.02. The mixing study demonstrated absence of correction, consistent with the presence of a circulating inhibitor.

Specific anti-FXI inhibitor testing was positive, with an inhibition rate of 42%. Quantification using the Nijmegen-modified Bethesda assay showed an anti-FXI inhibitor titer of 32 BU/ml.

Initial one-stage clotting assay revealed decreased FXI activity (17%). A concomitant reduction in other factors assessed by one-stage clotting assays was also observed, including FVIII activity (39%) and FIX activity (40%).

After 4 months of follow-up, biological improvement was observed. The aPTT decreased to 61 seconds (ratio 1.7). Inhibitor testing became negative, with an inhibition rate below 25%, and Bethesda assay showed no detectable anti-FXI inhibitor (0 BU/mL).

FXI activity increased to 48%. The activities of the other investigated factors also improved: FVIII (290%) and FIX (61%)

These results are summarized in Table 2.

Table 2: Impact of an acquired anti-FXI inhibitor on one-stage clotting assays of intrinsic pathway factors: evolution during follow-up

Parameter	Initial evaluation	After 4 months
aPTT (s)	109	61
aPTT ratio	3.02	1.70
Rosner index	45%	16%
Inhibitor detection (inhibition rate)	42%	<25%
Anti-FXI inhibitor titer (BU/mL)	32	0
FXI activity (%)	17	48
FVIII activity (%)	39	290
FIX activity (%)	40	61

Summary of findings

In both cases, the presence of an acquired inhibitor was confirmed by positive inhibitor testing and specific quantification using the Nijmegen-modified Bethesda assay.

At the initial evaluation, one-stage clotting assays demonstrated a marked reduction in the factor specifically targeted by the inhibitor, associated with an apparent decrease in additional intrinsic pathway factors.

During follow-up, the decrease or disappearance of inhibitor activity was accompanied by correction of non-targeted factor abnormalities, suggesting that the initial abnormalities were mainly related to analytical interference affecting one-stage clotting assays rather than a true multiple coagulation factor deficiency.

DISCUSSION

Specific acquired coagulation factor inhibitors represent an important cause of discrepancy between laboratory findings and the underlying clinical situation. In this study, the longitudinal analysis of two cases, one involving an acquired anti-factor VIII (FVIII) inhibitor and the other an acquired anti-factor XI (FXI) inhibitor, demonstrated a common phenomenon: the presence of a specific inhibitor may induce an apparent decrease in several non-targeted intrinsic pathway factors when measured by one-stage clotting assays.

One-stage clotting assays are widely used for functional coagulation factor measurement. These assays are based on the ability of patient plasma to correct the clotting time of factor-deficient plasma. However, they do not directly measure the isolated activity of the investigated factor but rather assess the overall capacity of the coagulation system to generate a clot under specific experimental conditions. This explains their susceptibility to biological interferences, particularly in the presence of circulating inhibitors [4,5].

In our first case, the anti-FVIII inhibitor was associated with severely reduced FVIII activity (1%) and a very high inhibitor titer (748 BU/mL). One-stage clotting assays also demonstrated marked reductions in FIX, FXI, and FXII activities, initially suggesting a multiple intrinsic pathway factor deficiency. However, during follow-up at 14 months, the decrease in inhibitor titer to 12.8 BU/mL was associated with normalization of non-targeted factor activities (FIX: 113%, FXI: 74%, FXII: 71%), whereas FVIII activity remained severely decreased. This evolution strongly supports analytical interference induced by the inhibitor rather than a true multiple factor deficiency.

These findings are consistent with previous reports in acquired hemophilia A. Kruse-Jarres *et al.,* described the challenges associated with laboratory interpretation in patients with acquired anti-FVIII inhibitors and emphasized that one-stage assays may generate complex biological profiles extending beyond

the isolated FVIII deficiency [1]. Similarly, Franchini and Lippi reported that laboratory abnormalities observed in acquired hemophilia A may be disproportionate to the isolated functional FVIII defect due to the inhibitory effect on intrinsic pathway coagulation mechanisms [3].

The underlying mechanism is mainly related to inhibition of the intrinsic tenase complex (FVIIIa–FIXa), which plays a central role in thrombin generation amplification. In one-stage clotting assays, this reduction in overall coagulation activity may impair expected correction of factor-deficient plasma and result in artificial underestimation of other factor activities. Marlar *et al.,* highlighted that results obtained from one-stage assays are influenced by several analytical parameters, including reagent composition, assay sensitivity, and the nature of the interfering substance present in plasma [4].

The second case involved an acquired anti-FXI inhibitor, a much rarer condition but one that illustrates a similar analytical challenge. Factor XI contributes to coagulation amplification through thrombin-mediated activation. Therefore, its inhibition may alter global thrombin generation and affect intrinsic pathway-dependent assays. In our patient, initial FXI activity was reduced to 17% with an inhibitor titer of 32 BU/mL, accompanied by apparent decreases in FVIII (39%) and FIX (40%) measured by one-stage assays. After 4 months of follow-up, disappearance of the anti-FXI inhibitor was associated with improvement of FXI activity (48%) and correction of abnormalities observed in the other tested factors.

Because acquired anti-FXI inhibitors are considerably less frequent than anti-FVIII inhibitors, available data remain limited. However, Zanon recently emphasized that rare acquired coagulation inhibitors may generate complex laboratory patterns requiring an integrated diagnostic approach combining clinical information, functional assays, and specific inhibitor testing [2].

Mixing studies represent a key step in investigating isolated aPTT prolongation. In both cases, lack of correction after mixing with normal plasma supported the presence of a circulating inhibitor. However, this test has limitations, particularly in the presence of low-titer inhibitors or inhibitors with slow time-dependent kinetics. Kershaw and Favaloro emphasized that mixing studies should always be interpreted together with clinical findings and specific inhibitor investigations [11]. Miller and Boylan also highlighted that mixing studies alone cannot resolve all complex situations encountered in coagulation laboratories [10].

The Nijmegen-modified Bethesda assay remains the reference method for quantifying neutralizing inhibitors. In our study, monitoring inhibitor titers was essential for interpreting changes observed in one-stage clotting assays. The marked decrease in anti-FVIII inhibitor level and disappearance of the anti-FXI inhibitor were associated with correction of non-targeted factor abnormalities, confirming the reversible nature of these analytical abnormalities.

Chromogenic assays represent a valuable alternative when one-stage clotting assay results are inconsistent with the clinical context or when pseudo-multiple factor deficiency is suspected. These methods rely on a more specific enzymatic measurement and are less dependent on the integrity of the entire coagulation cascade. Bowyer *et al.,* demonstrated their usefulness in situations where inhibitors interfere with conventional functional assays [5]. Novembrino *et al.,* also highlighted their role in resolving analytical discrepancies encountered in complex coagulation disorders [6].

Limitations and Future Perspectives

This study has several limitations, mainly related to the small number of observations. The monocentric and descriptive design does not allow extrapolation of these findings to all patients with acquired coagulation inhibitors.

Moreover, chromogenic assays were not systematically performed in our cases. Their inclusion would have provided direct confirmation of the absence of true deficiencies in non-targeted coagulation factors and would have allowed a more detailed assessment of alternative analytical strategies.

The impact of different analytical platforms and aPTT reagents was not evaluated, although reagent sensitivity to inhibitors may vary significantly.

Despite these limitations, this study highlights a clinically relevant analytical phenomenon that may lead to misdiagnosis of multiple coagulation factor deficiency. Multicenter studies including larger patient cohorts and comparing one-stage and chromogenic assays are required to better characterize these discrepancies and establish practical recommendations for hemostasis laboratories.

CONCLUSION

Specific acquired coagulation factor inhibitors may cause significant discrepancies during laboratory investigation of the intrinsic coagulation pathway. In our study, anti-FVIII and anti-FXI inhibitors were associated with apparent reductions in several non-targeted factors measured by one-stage clotting assays, mimicking a pseudo-multiple factor deficiency.

The improvement of factor activities in parallel with inhibitor reduction or disappearance confirms the

reversible nature of these abnormalities and supports an analytical rather than structural origin.

A comprehensive interpretation combining clinical findings, aPTT results, mixing studies, inhibitor quantification, and alternative methods such as chromogenic assays is essential to improve diagnostic accuracy and prevent misinterpretation.

Acknowledgments

The authors would like to thank all members of the Hemostasis Laboratory of the Department of Hematology and Immuno-Hematology for their technical contribution, availability, and involvement in performing the laboratory investigations required for this study.

Conflict of Interest

The authors declare that they have no conflict of interest related to this work.

REFERENCES

1. Kruse-Jarres R, Kempton CL, Baudo F, Collins PW, Knoebl P, Leissinger CA, *et al.,* Acquired hemophilia A: Updated review and treatment recommendations. *Am J Hematol.* 2017;92(7):695-705. doi:10.1002/ajh.24777.
2. Zanon E. Acquired inhibitors of coagulation factors: Diagnosis and management. *Diagnostics (Basel).* 2023;13(3):420. doi:10.3390/diagnostics13030420.
3. Franchini M, Lippi G. Acquired factor VIII inhibitors. *Blood.* 2008;112(2):250-255. doi:10.1182/blood-2008-03-143586.
4. Marlar RA, Strandberg K, Shima M, Adcock DM. Clinical utility and limitations of one-stage clotting assays for factor activity measurement. *Eur J Haematol.* 2020;104(1):3-14. doi:10.1111/ejh.13339.
5. Bowyer AE, Duncan EM, Antovic JP. The effect of inhibitors on laboratory measurement of coagulation factor activity. *Haemophilia.* 2018;24(4):578-583. doi:10.1111/hae.13520.
6. Novembrino C, Boscolo Anzoletti M, Mancuso ME, *et al.,* Chromogenic assays in the diagnosis and monitoring of coagulation disorders. *Haemophilia.* 2019;25(3):521-526. doi:10.1111/hae.13746.
7. Schütte LM, Hodes LS, van Moort I, *et al.,* Laboratory monitoring of acquired hemophilia A: Challenges and perspectives. *Haemophilia.* 2020;26(5):916-922. doi:10.1111/hae.14106.
8. Kihlberg K, Strandberg K, Rosén S, Ljung R, Astermark J. Discrepancies between one-stage and chromogenic factor VIII activity assays in patients with inhibitors. *Haemophilia.* 2017;23(4):620-627. doi:10.1111/hae.13219.
9. Losos M, Chen J. Laboratory diagnosis of acquired coagulation inhibitors: Current approaches and challenges. *J Clin Transl Pathol.* 2022;2(1):8-11. doi:10.14218/JCTP.2022.00002.
10. Miller CH, Boylan B. Mixing studies in the laboratory diagnosis of coagulation inhibitors. *J Thromb Haemost.* 2021;19(7):1835-1837. doi:10.1111/jth.15360.
11. Kershaw G, Favaloro EJ. Laboratory identification of inhibitors and lupus anticoagulants. *Pathology.* 2012;44(4):293-302. doi:10.1097/PAT.0b013e328353254d.