Management of an elderly male patient with subconjunctival bleeding associated with pemphigus in the emergency department

Claudia Bellini[1], Luca Puccetti[2], Eleonora Franceschini [3], Lucrezia Galasso[3], Daniela Fineschi[3], Alessandra Terrosi[1], Maria Ida Bonetti[1], Marco Mancini[1], Gian Piero Caldarelli[1], Agostino Ognibene[4], Paola Calzoni[3]
[1]Clinical Chemistry Laboratory Analysis Unit, Misericordia Hospital of Grosseto, South East Tuscany AUSL
[2]Hemathology Unit/Coagulative Disorders, Santa Maria alle Scotte University Teaching Hospital of Siena, University of Siena
[3]Coagulative Disorders Laboratory Unit, Santa Maria alle Scotte University Teaching Hospital of Siena
[4]Department of Transfusion and Laboratory Medicine, South East Tuscany AUSL

Questo lavoro è stato in parte presentato al 54° Congresso Nazionale SIBioC - Genova, 5-7 ottobre 2022, essendo stato selezionato come Speaking Award

ABSTRACT

A 82-year-old man suffering from pemphigus and Parkinson's disease presented at the emergency department of the Misericordia Hospital in Grosseto (Tuscany, Italy) with subconjunctival bleeding. Laboratory blood tests showed a prolonged activated thromboplastin time (aPTT), with normal prothrombin time (PT) and slightly reduced haemoglobin. The negative family and personal history of haemorrhagic disease rose the suspicion of the presence of an acquired inhibitor. The patient was referred for further diagnostics to the University Hospital of Siena where second level tests were performed at the Coagulation Unit. The aPTT mixture test revealed a non-correction both at room temperature and, more markedly, after incubation at 37°C for 2h, confirming the presence of an intrinsic pathway inhibitor. Among the measured factors, only the activity level of factor VIII was extremely low. The titration of FVIII inhibitor confirmed the diagnosis of Acquired Haemophilia A (AHA).

Key words: *haemophilia, bleeding, abnormal aPTT*

CASE REPORT

This case report involves an 81-year-old man who accessed the Emergency Department (ED) of the Misericordia Hospital in Grosseto (Tuscany, Italy) for subconjunctival bleeding accompanied by soft tissue small hematomas. Laboratory blood tests showed a prolonged activated thromboplastin time (aPTT) (ratio 2.46, r.i. 0.8-1.2), with normal prothrombin time (PT) (ratio 1.12, r.i. 0.8-1.2) and slightly reduced haemoglobin (131 g/L, r.i. 140-180 g/L). About the medical history, his family members reported Parkinson's disease and pemphigus on corticosteroid treatment. Neither the patient nor any member of his family reported a history of bleeding-type syndromes.

Therefore, following communication between ED and the clinical laboratory, given the negative medical history, the hemorrhagic symptoms, the physical examination, and on the absence of any anticoagulant therapy, the suspicion of an acquired inhibitor emerged. It was therefore deemed necessary to proceed to second level tests.

The patient was then referred to the Coagulation Unit of the University Hospital of Siena. The PT mixture test was not performed as the PT was in the normal range. The aPTT mixture test, which involves mixing the plasma of the patient with a normal pool plasma in equal volumes (1:1) and performing an aPTT on the three samples (patient, pool and mixture) both at room temperature (RT) and after incubation at 37°C for 2 hours, revealed a non-correction both at RT and, more markedly, after incubation, indicating the presence of an intrinsic pathway inhibitor. The Lupus Anticoagulant assays were negative and the Coagulation Unit decided to proceed to the determination

Corrispondenza a: Claudia Bellini, U.O.C. Analisi Chimico-Cliniche, Azienda USL Toscana Sud Est, Sede Operativa Grosseto, via Senese, 169, Email: cbellini.md@gmail.com

Ricevuto: 14.02.2023 Revisionato: 04.03.23 Accettato: 07.03.23

Pubblicato on-line: 13.03.2023 DOI: 10.19186/BC_2023.012

of the coagulation factors. The factors were measured on the analyzer ACL TOP 750 (Werfen, Bedford, MA USA) with plasmas deficient in FVIII, FXI, FXII, FIX, FX, FV, FII, FXIII from the same producer. Only the activity level of factor VIII was extremely low (FVIII:C=0.1%, r.i. 55-150%). Von Willebrand Antigen was 224% (r.i. zero blood group: 50-150%; non-zero blood group: 60-200%) and Von Willebrand Ristocetin Cofactor (Ri:Cof) >200%(r.i. zero blood group: 45-150%; non-zero blood group: 50-200%). The titer of FVIII inhibitor (Bethesda modified Nijmegen) resulted in 6 Bethesda Unit (BU)/mL (r.v. <0.50 BU/mL), confirming the diagnosis.

Since the Computerized Tomography examination excluded internal bleeding and there were no significant anaemia or large muscle hematomas, a bypass agent was not employed. The patient was treated with corticosteroids for the eradication of the inhibitor. After 21 days of therapy, aPTT returned to normal ranges (ratio=0.96) with an increase in FVIII activity (FVIII:C=54%) and an increase in haemoglobin (142 g/L). The close collaboration between the laboratories of the two institutions made it possible to effectively support clinicians in the early detection of the clinical condition and the correct management of the patient.

DISCUSSION

Abnormalities in haemostasis tests, whether or not accompanied by haemorrhagic manifestations in individuals without a personal or family history of coagulative disease, may be due to the presence of specific anti-factor inhibitory autoantibodies, most frequently directed against FVIII (Acquired Haemophilia A, AHA).

AHA can be suspected either on the basis of the clinical picture, especially in the elderly and in young women in the peripartum or postpartum period presenting with abnormal bleeding that has recently occurred, with prolonged aPTT and normal PT, or on the basis of laboratory tests, when it occurs asymptomatically. In this latter case, the warning sign is an isolated prolongation of aPTT, with normal PT, in a patient not receiving anticoagulant therapy (1).

In the majority of cases, the disease begins with spontaneous bleeding events or induced by minor trauma or invasive procedures such as the placement of venous catheters, endoscopic investigations, intramuscular injections or arterial sampling, in individuals with no personal or family history of bleeding.

The most frequent haemorrhages (80% of cases) are muscle and soft tissue haematomas, which can be very extensive and have the potential to cause severe anaemia and/or compression of nerves and vessels, resulting in compartment syndrome (2). In contrast, the typical joint bleedings of the congenital form is rare in AHA. Other bleeding may involve mucosae with epistaxis, gingival bleeding, metrorrhagia and urinary tract haemorrhages, but serious life-threatening bleedings may also occur (gastrointestinal, retroperitoneal, or intracranial bleedings, or psoas muscle haematomas).

Furthermore, some more unusual sites of bleeding seem to emerge as a possible expression of AHA, especially in its initial form, such as the subconjunctival haemorrhage as in the present case. This aspect must therefore be taken into consideration because it could lead to an early diagnosis and rapid identification and an adequate management of the case without risking most serious situations (3).

In a proportion of patients, suspicion of AHA occurs in the absence of bleedings, following abnormalities in coagulation tests (mainly prolonged aPTT) during diagnostic work-up or in preliminary examinations prior to invasive procedures or surgery. Sometimes, suspicion arises in haemorrhagic patients with prolonged aPTT, who receive blood products without correction of the laboratory abnormality and/or haemostatic efficacy (2).

These disorders are not always detected and treated promptly and still have a high mortality rate due to haemorrhagic complications (3-15% in the most recent registers). The development of anti-factor inhibitors is associated with autoimmunity (9-17%, depending on the number of cases in the various registres) and solid cancer or haematological malignancies (6-22%), pregnancy and the postpartum period (2-15%), following surgery or drug administration, and less frequently with dermatological diseases (1-4%), such as pemfigo affecting the patient (4). However more than 50% of cases are idiopathic although some of these can be considered post-infectious (5).

From the laboratory's perspective, the diagnostic pathway starts with the detection of a prolonged aPTT, which must lead to further investigations. It is always necessary to discriminate a pre-analytical error, causing a non-conformity of the sample, from a true prolongation of the aPTT, which may be due to various causes: anticoagulant therapies, deficiencies of coagulation factors including fibrinogen, antiphospholipid antibodies and specific anti-factor antibodies (6).

Therefore, communication with the clinic to confirm the suitability of the sample collection and to clarify the cause of the prolongation, is essential. It may be appropriate to consider a number of issues: whether only the aPTT is prolonged or the PT is also involved, whether it is appropriate to test for fibrinogen deficiency. It is also appropriate to ask the clinician if the patient shows signs of bleeding, if the patient has taken anticoagulant drugs, and if the patient has a personal or family history of coagulation disorders.

Once pre-analytical problems or interference by anticoagulant drugs have been ruled out, if the isolated prolonged aPTT is accompanied (or not) by haemorrhage, in a patient with no personal or family history of bleeding disorder, a suspicion of acquired inhibitor haemorrhage syndrome is imposed and the next diagnostic step is to perform a mixing test, which involves mixing patient's plasma with a normal pool plasma (NPP) usually in a 1:1 ratio (or 50:50; i.e., equal volumes) (7), as previously indicated.

In general, PT and APTT are in range when the factors involved, of the extrinsic and intrinsic pathway respectively, have activity of at least 50%.

The principle underlying the mixing test is that a 50% level of a coagulation factor is sufficient to bring the altered test back to normal, leading to a "correction" of the test. For example, by mixing NPP, which by definition contains 100% activity of the various factors, with the plasma of a patient with 0% of a certain factor, a level of 50% of the deficient factor would be reached in the mixture, which is sufficient to bring the altered test back to normal (8).

It therefore follows that the result of the test can be either a correction of the aPTT, which directs the diagnostic process towards a factor deficiency, or a non-correction, which shows the presence of an inhibitor that, by blocking the activity of the factor(s) concerned, not only in the patient's plasma but also in the NPP, inhibits coagulation and does not allow a reduction of the coagulation time and thus a correction of the test. The inhibitor can be a non-specific inhibitor such as Lupus Anticoagulant (LAC) or an antibody against a specific factor, as is the case of AHA.

In the first stage, aPTT is performed on the 3 samples (plasma patient, NPP and mixture) at RT. Some inhibitors may exhibit their presence at RT with a failure to correct the so-called "direct" or "immediate" mixture. If, otherwise, the mixture does correct, to assess the presence of a time- and/or temperature-dependent inhibitor, it is necessary to incubate the three samples for 2h at 37°C and then repeat the tests. Only then would a correction rule out the presence of an inhibitor, revealing a factor deficiency. The purpose of incubation is to allow any time- and/or temperature-dependent 'slow' antibodies to manifest their activity.

Nonspecific inhibitors (such as LAC) are usually not time-dependent and are already evident in the direct mixture, leading to non-correction of the mixture at RT. Conversely, many factor-specific inhibitors (especially anti FVIII) are time-dependent and may not prolong the direct mixture time (false correction), but only become manifest after incubation (non-correction). Therefore, in the case of correction of the mixture at RT, it is always necessary to incubate for a longer time in order to avoid missing cases.

To identify the correction, various methods can be used, among which the most commonly used are:
- correction within the normal reference interval (NRI)
- index of circulation anticoagulant (ICA)
- percent correction method.

All of them provide effective discrimination for immediate follow-up in the vast majority (>95%) of cases where the method has been validated by the laboratory (7).

Once the failure to correct aPTT is revealed, the diagnostic pathway continues with the determination of the factors involved in the intrinsic pathway, which in the case of AHA will show low levels of FVIII. If more than one factor appears to be inhibited, the plasma must be tested for LAC. Confirmation of the diagnosis is given by titration of anti-FVIII antibody using the modified Bethesda method (9). The diagnostic flowchart of AHA is summarised in Figure 1.

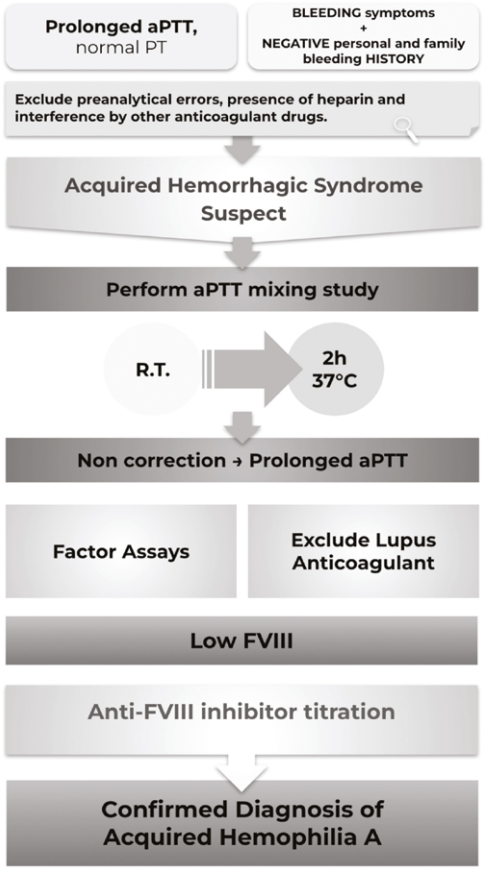

Figure 1

Diagnostic flowchart of Acquired Haemophilia A. aPTT,activated partial thromboplastin time; PT,prothrombin time; RT,room temperature; FVIII, coagulation factor eight.

CONFLICT OF INTEREST

None.

REFERENCES

1. Kruse-Jarres R, Kempton CL, Baudo F, W Collins P, Knoebl P, Leissinger CA, et al. Acquired hemophilia A: Updated review of evidence and treatment guidance. Am J Hematol 2017;92:695-705.
2. Coppola A, Franchini M, Tripodi A, Santoro RC, Castman G, Marino R et al. Acquired haemophilia A: Italian Consensus Recommendations on diagnosis, general management and treatment of bleeding. Blood Transfus 2022;20:245-62.
3. Puccetti L, Ciofini S, Fruschelli M, Calzoni P, Fineschi D, Guazzi G, et al. Subconjunctival bleeding as uncommon first hemorrhagic sign in patient with acquired A Haemophilia. ACMCR 2019;1:1-9.
4. Knoebl P, Marco P, Baudo F, Collins P, Huth-Kühne A, Nemes L et al. Demographic and clinical data in acquired hemophilia A: results from the European Acquired Haemophilia Registry (EACH2). J Thromb Haemost 2012;10:622-31.
5. Puccetti L, Bacchiarri F, Calzoni P, Santoni A, Bocchia A. A fatal unsuspected case of acquired A hemophilia. Misleading role of therapy with acetylsalicylic acid? Intern Emerg Med 2021;16:2339-40.

6. Rasmussen KL, Philips M, Tripodi A, Goetze JP . Unexpected, isolated activated partial thromboplastin time prolongation: A practical mini-review. Eur J Haematol 2020;104:519-25.
7. Favaloro EJ. Coagulation mixing studies: utility, algorithmic strategies and limitations for lupus anticoagulant testing or follow up of abnormal coagulation tests. Am J Hematol 2020;95:117-28.
8. Associazione Italiana Centri Emofilia (AICE). Documento di Consenso. Procedure per il Laboratorio di Emostasi: Test di miscela e misura del fattore VIII e IX. A cura del gruppo di lavoro qualità dei laboratori dell'AICE. Edizione Agosto 2022. https://aiceonline.org/wp-content/uploads/2022/09/Procedure-per-il-laboratorioFVIII-FIX.pdf (last access: February 2023).
9. Verbruggen B, van Heerde WL, Laros-van Gorkom BA. Improvements in factor VIII inhibitor detection: from Bethesda to Nijmegen. Semin Thromb Hemost 2009;35:752-9.