Laboratory Methodologic Approach in Prolonged Activated Partial Thromboplastin Time Test

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ABSTRACT
The pathological activated partial thromboplastin time test is a data that we can find in all clinical laboratories routinely. The hemato-coagulative clinical case here reported aims to point out how the laboratory can provide a correct diagnosis by methodological rational setting and, consequently, carry out an appropriate therapy, or reassure the patient that the pathological data will not cause bleeding, even in case of surgery.

Introduction
The time necessary for the clot fibrin formation when the citrate plasma in presence of an activator, (e.g. kaolin, celite, ellagic acid, micronized silica) and phospholipids (e.g. cephalin), is recalcified and characterizes activated partial thromboplastin time (aPTT) test expression (1).

The test is affected by the activity of coagulation factors I (fibrinogen), II (prothrombin), V, VIII, IX, X, XI and XII. When one of the coagulation factors is deficient, such as in A and B hemophilia or in the consumption coagulopathy, the aPTT is prolonged (2).

aPTT test is sensitive in defects of the intrinsic and common pathway coagulation system, particularly in FVIII deficiency (3). Moreover, it is sensitive to the deficiency of some factors involved in the intrinsic system contact phase, such as FXII, prekallikrein (PK) and high molecular weight kininogen (HMWK) (4).

The test is also sensitive to the action of circulating anticoagulants, "lupus-like", which interferes with phospholipid dependent laboratory test of coagulation (5). This inhibitory action on the intrinsic coagulation system triggers a thrombophilic condition.

Before undertaking a more detailed investigation, it’s important to rule out artifactual causes of an abnormal aPTT such as a high hematocrit value, lipemic, hemolyzed (6), or icteric plasma, delays, extreme temperatures and any other causes that underly an incorrect pre-analytic approach that the laboratory must carefully identify (7).

aPTT test is the reference laboratory test for monitoring unfractionated heparin therapy.

Case Report
A 71-year-old woman contacts the attending physician for the occurrence of recurrent episodes of bruising and hematomas located in the upper and lower limbs.

Outpatient laboratory tests highlighted a normochromic and normocytic anemia, white blood cells (WBC) count, differential white blood cell (DWBC) count and platelets (PLTs) count were normal by number and morphology.

The coagulative tests showed an aPTT test prolonged (aPTT = 55.7s - laboratory r. v. = 25 - 36s), while the Prothrombin Time (PT) test was normal (PT = 9.0s - laboratory r. v. = 10 - 13s).
The patient reported a clinical history of arterial hypertension. She does not have a history of past cutaneous and/or mucous bleeding. She does not take drugs that could cause diathesis bleeding. A family history for hemorrhagic diseases was not reported.

After hematology consultation, hospitalization was planned. During hospitalization, the hemorrhagic diathesis persisted, so much that the patient was also subjected to blood transfusions for the presence of severe anemia (Hemoglobin (Hb) < 70 g/L). In Table 1 the main laboratory routine tests are reported. Other routine tests were normal.

**Table 1. Patient’s blood tests values during hospitalization**

| Test       | Values found | Laboratory reference value |
|------------|--------------|----------------------------|
| PT         | 9.0s         | 10-13s                     |
| aPTT       | 59.2s        | 25-36s                     |
| Fibrinogen | 680 mg/dL    | 130-330 mg/dL              |
| Hb         | 65 g/L       | 120-150 g/L               |
| Ht         | 23.8%        | 36-44%                     |
| WBC        | 12x10^9/L    | 4.0-10.0x10^9/L            |
| PLT        | 476x10^9/L   | 150-450x10^9/L             |

**What should the laboratory do in case of pathological aPTT?**

In isolated prolonged aPTT, one of the first evaluations is to consider the fibrinogen test. If its value is < 100 mg/dL, the data would address for quantitative alterations, resulting in a hypofibrinogenemia and consequently prolonged aPTT (Table 2). Fibrinogen test, although simple and not expensive, can be inaccurate in case of disseminated intravascular coagulation, liver disease, kidney disease, dysfibrinogenemia due to thrombolytic therapy, or increased fibrinogen concentration. The fibrinogen dosage must be performed according to the Clauss method (8).

**Table 2. Main causes of hypofibrinogenemia**

| Hereditary afibrinogenemia | Dysfibrinogenemia | Embolism |
|----------------------------|-------------------|----------|
| Hemophilia                 | Disseminated intravascular coagulation | Fibrinolysis |
| Eclampsia                  | Liver disease     | Tumors   |

Thrombin Time (TT) is another test that must be considered if aPTT is prolonged. The test measures the conversion time of fibrinogen into polymerized fibrin (8). The test is pathological in hypo and dysfibrinogenemia, in presence of fibrin and/or fibrinogen degradation products that influence the fibrin polymerization (e.g. paraproteinemia in myeloma), as well as in patients on heparin therapy or heparin sample contamination (e.g. flushing in central venous catheter (CVC) and/or accidental sample collection from the polluted line) (7). The abnormal TT test is corrected in patients with CVC and suspected sample heparin contamination, by the addition of heparin neutralizing agents (e.g. hepzyme or protamine sulfate). In these cases, also the normal reptilase time test indicates the polluted sample.

**Plasma “Mix Test” study**

The test consists of mixing in equal parts (50:50) the pathological specimen plasma with normal plasma; the latter must be obtained from a pool of normal plasmas that have no deficiency of coagulation factors (9). On the obtained mixture, the aPTT test is performed again.

Table 3 shows the procedure, the patient’s baseline aPTT value, the patient’s aPTT values on mix plasma and, finally, the values obtained after comparison vs control plasma.

**Table 3. Main causes of hypofibrinogenemia**

| aPTT | Plasma Mix 50/50 Incubation 30min at room temperature | aPTT | Plasma Mix 50/50 Incubation 2h at 37°C Comparison with control plasma |
|------|------------------------------------------------------|------|------------------------------------------------------------------|
| 59.2s | Normal Pool Plasma = 28.0s (Ratio = 2.13)             | 58.4s | Control Plasma = 27.5s (Ratio = 2.12)                              |

The suspicion of phospholipid inhibitors that can induce eventual thrombosis is justified if, on mixed plasma, the aPTT test is still pathological and a concomitant thrombocytopenia is eventually present (10, 11). If the anti-phospholipid dependent antibodies are negative, the dosage on both coagulation factors and specific inhibitors against coagulation factors is justified (3). Specific anti-coagulation factor antibodies are mainly directed against FVIII causing profuse bleeding.

**What diagnosis was made?**

Summing up, the laboratory excluded the non-appropriateness of the sample and any hypofibrinogenemia.

The laboratory excluded the PK or HMWK deficiencies, also based on the clinical symptomatology characterized by profuse bleeding.
Lupus anticoagulant antibodies presence were excluded by employing LAC, IgG and IgM anti-cardiolipin antibodies as well as IgG and IgM anti-β2-glycoprotein1 antibodies, and procoagulant factor deficiencies tests.

Regarding the mix test, the laboratory analyzed the comparison between seconds and ratio, as well as the Rosner index and the percentage correction, whose results turned out to be

- < 5s, > 1.2, > 15%, < 58%, respectively (12-14).

Based mainly on mix test analysis, the next diagnostic step was both the dosage of coagulation factors and the specific inhibitors detection against coagulation factors, principally FVIII. The most important diagnostic data was the marked FVIII reduction, if not almost absent, due to the presence of marked inhibitor activity (Table 4).

**Table 3. Main causes of hypofibrinogenemia**

| FVIII < 1% | Anti-FVIII inhibitor |
|------------|----------------------|
| Bethesda Unit (BU) = 250* |
| FIX = 57% |
| FXI = 41% |
| FXII = 48% |

*One Bethesda Unit (BU) is the amount of inhibitor that inactivates 50% FVIII of a normal plasma after incubation at 37 °C for two hours

From the analysis results, the laboratory made the diagnosis of "acquired hemophilia A" due to the presence of anti-FVIII antibodies (15). Figure 1 shows the diagnostic pathway carried out by the laboratory after excluding a hypo or dysfibrinogenemia.

**Fig. 1. Diagnostic proceedings in abnormal aPTT**
**Table 5.** Conditions with prolonged aPTT without hemorrhagic diathesis

| Deficiency of:                      |
|------------------------------------|
| FXII                               |
| High molecular weight kininogen (HMWK) |
| Prekallikrein (PK)                 |
| Lupus Anticoagulant (LAC)          |
| Sample anticoagulant excess (citrate) |

**Table 6.** Main causes of acquired Hemophilia A and relational percentages

| Causes                                      | Relational Percentage |
|---------------------------------------------|-----------------------|
| Autoimmune diseases                         |                        |
| SLE, Rheumatoid Arthritis, Sjogren Syndrome, Multiple Sclerosis, Myasthenia Gravis, AHA, Graves-Basedow Disease |                        |
| Solid Tumors                                |                        |
| Prostate, Lung, Colon, Pancreas, Stomach, Head-Neck, Cervix, Breast | 40%                   |
| Oncohematological diseases                 |                        |
| CLL, NHL, MM, MDS, IM                       |                        |
| Drugs                                       |                        |
| Penicillin, sulfamides, phenytoin, methyldopa, chloramphenicol |                        |
| Pregnancy and postpartum                   | 10%                   |
| Idiopathic                                  | 50%                   |

AHA = Autoimmune Hemolytic Anemia; SLE = Systemic Lupus Erythematosus; CLL = Chronic Lymphocytic Leukemia; NHL = Non-Hodgkin Lymphoma; MM = Multiple Myeloma; MDS = Myelodysplastic Syndrome; IM = Idiopathic Myelofibrosis

**Discussion**

In patients with bleeding diathesis, an accurate clinical history for previous bleeding, as well as family history, is mandatory, even in case of previous surgeries or changes in hemostatic status (16). The resulting reports are very important to identify patients who need further and more detailed investigation, since a prolonged aPTT, although pathological in some cases, may not cause hemorrhagic diathesis (Table 5) (17).

In acquired hemophilia A, FVIII inhibitory antibodies are immunoglobulins consisting mainly of heavy chain G4 class and κ light chain; their action is directed against FVIII procoagulant activity (18). Regarding the functional activity, the kinetic inactivation by anti-FVIII antibodies can be type 1 or 2. According to a linear kinetics, type 1 inhibitory antibodies inhibit the FVIII procoagulant activity completely. Type 2 inhibitory antibodies follow an exponential complex kinetics and are not able to inhibit FVIII procoagulant activity completely. Therefore, the Mix test must be performed and interpreted correctly. A short incubation may not show both the presence of type 2 inhibitors, so called "slow inhibitors” which present a complex kinetics (time-dependent kinetics), and low-title inhibitors that show a sensitivity lower than the method used.

In our case, the very significant FVIII deficiency justified the pathological laboratory data (prolonged aPTT), as well as the profuse clinical symptomatology characterized by bleeding with severe anemia.

Acquired hemophilia A has an annual incidence of 1-2 cases per million of inhabitants. In some carriers, bleeding can also be fatal. Major bleeding occurs in 80-90% of cases and mortality is significant, the range is between 10% and 22%.

The causes that can determine the pathology are different; 50% of cases have an idiopathic etiology (Table 6).

The therapeutic approach is mainly applied to treat the hemorrhagic episode in case of emergency, as well as to prevent significant bleeding in surgery. A possible therapeutic association with immunomodulant drugs to eliminate the cellular clone responsible for self-antibody synthesis is also considered.

To monitor anti-hemorrhagic treatment, Factor VIII levels and/or inhibitor titers do not have a therapeutic indication. It is important to emphasize that in about 30% of cases the autoantibody can disappear spontaneously.
The case reported underlines how, through a correct methodological approach, the laboratory can and must provide a correct diagnosis. A pathological aPTT may underline not only hemorrhagic problems but also a prothrombotic condition, two possibilities that impose different therapeutic approaches. Furthermore, although the aPTT test is abnormal, the rational methodological approach can rule out the possibility of bleeding even in case of surgery.

Conflicts of interest
The authors state no conflicts of interest in this study.

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