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Interference of M-protein on Thrombin Time Test: A Case Report

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ABSTRACT

Objective: A case of interference of monoclonal protein (M-protein) on thrombin time (TT) test in a 39-year-old Caucasian male patient is presented.

Methods: Coagulation screening tests were performed where altered results only for TT result (>150 seconds) and activated partial thromboplastin time (aPTT) result (36 seconds) were measured. Further specific coagulation testing included measurement of individual coagulation factors FII, FV, FVII, FVIII, FIX, FX, FXI, and FXII. Diagnostic steps in detection and identification of monoclonal protein included serum protein electrophoresis and immunofixation (both serum and urine specimen).

Results: Monoclonal protein immunoglobulin G kappa detection and identification in serum and urine clarified the situation.

Conclusion: Unexpectedly altered results of screening coagulation tests without any appropriate clinical signs and symptoms in a patient without any anticoagulant therapy needs to be critically considered in the context of extended next diagnostic steps in order to clarify the cause of pathological test results.

Keywords: M-protein, thrombin time test, interference, coagulation, immunology, hematology.

Clinical History

A 39-year-old Caucasian man, previously diagnosed with testicular cancer, was admitted for orchiectomy of the right testicle at the Department of Urology, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia, in December 2018. During preoperative preparation, screening coagulation tests were ordered. In the first specimen, prolonged thrombin time (TT) higher than the upper measurement range (ie, >150 seconds [reference range (RR), 14–21 seconds]) and slightly prolonged activated partial thromboplastin time (aPTT) of 39 seconds (RR, 23–32 seconds) were measured. The results for prothrombin time (PT) and fibrinogen were within their respective RRs. aPPT and TT tests were repeated from the same specimen. The results obtained for TT greater than 150 seconds and aPTT of 41 seconds were almost the same; however, due to suspected contamination of the specimen, the results were not reported to the clinician (sample 1, **Table 1**). The results of all coagulation tests performed at different time points are presented in **Table 1**.

In communication with the clinician treating the patient, possible heparin contamination of the first specimen was excluded. However, a new blood specimen was still requested, to exclude heparin contamination of the specimen. After a new plasma specimen was analyzed, the results for TT greater than 150 seconds and aPTT of 36 seconds were measured again, and the results were reported (sample 2, **Table 1**).

Abbreviations:

TT, thrombin time; RR, reference range; aPTT, activated partial thromboplastin time; PT, prothrombin time; RT, reptilase time; FII, factor II; Ig, immunoglobulin; M-IgG-κ, monoclonal immunoglobulin G kappa; FLCs, free light chains; β2M, beta-2 microglobulin; SMM, smoldering multiple myeloma; PPP, platelet-poor plasma; FDPs, fibrin(ogen) degradation products; DIC, disseminated intravascular coagulation; MGUS, monoclonal gammopathy of undermined significance; MM, multiple myeloma; INR, international normalized ratio; NP, not performed

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Table 1. The Results of Coagulation Tests Performed at Different Time Points

Date		December 7, 2018		December 12, 2017	December 14, 2018
Sample Number		Sample 1	Sample 2	Sample 3	Sample 4
Analyte	Reference Range				
PT (%)	≥70	86[a]	89	85	77
INR	Therapeutical interval, 2.0–3.5	1.1[a]	1.1	1.1	1.2
aPTT (s)	23–32	39/41[a]	36	33	36
aPTT (ratio)	0.8–1.2	1.4/1.5[a]	1.2	1.1	1.3
Fibrinogen	1.8–3.5	2.1[a]	2.1	2.3	2.0
TT (s)	14–21	>150/>150[a]	>150	>150	>150
Factor II (%)	70–120	NP	201	205	NP
Factor V (%)	70–140	NP	NP	157	NP
Factor VII (%)	70–120	NP	NP	139	NP
Factor VIII (%)	70–150	NP	NP	153	NP
Factor IX (%)	70–120	NP	NP	145	NP
Factor X (%)	70–120	NP	NP	134	NP
Factor XI (%)	70–120	NP	NP	137	NP
Factor XII (%)	70–150	NP	NP	115	NP

PT, prothrombin time; INR, international normalized ratio; aPTT, activated partial thromboplastin time; TT, thrombin time; NP, not performed.
[a]Results were not released because of suspicion of possible heparin contamination. A new blood specimen was requested.

Although reptilase time (RT) is a useful screening assay for the detection of heparin contamination and qualitative fibrinogen abnormalities, this test was not performed because it is not available in our laboratory. For the same reason, plasma specimen material was not treated with heparinase enzyme or protamine sulfate for exclusion of heparin or endogenous heparin-like anticoagulants presence in the specimen. In our laboratory, only the Clauss fibrinogen activity test is available, so we did not use an immunochemical method for fibrinogen concentration determination. However, due to normal functional activity of fibrinogen (2.1 g/L) and the patient history, we assumed that the possibility of dysfibrinogenemia for this patient was low, so our intention was to investigate other possible causes of such a prolonged TT result.

Because of unmeasurably prolonged TT, we measured the activity of coagulation factor II (FII) to exclude the presence of FII inhibitors. FII inhibitors could cause such prolonged TT result, but the obtained results for activity of FII excluded the presence of FII inhibitors and its deficiency (sample 2, **Table 1**). Screening coagulation tests were repeated after 5 days. Values for TT and aPTT did not differ significantly (TT > 150 seconds; aPTT = 33 seconds). To exclude any other factor deficiency, activities of coagulation factors V, VII, VIII, IX, X, XI, and XII were ordered, and none was deficient (sample 3, **Table 1**). In cooperation with the clinician caring for the patient, and due to the lack of any sign of bleeding, the patient underwent an operation. In the morning before the operation, the coagulation tests were repeated again, and the values for TT and aPTT (TT >150 seconds, aPTT = 36 seconds) were almost the same (sample 4, **Table 1**). The surgical procedure was performed successfully, with no bleeding complications related to abnormal results of coagulation tests.

After excluding coagulation disorders related to individual coagulation factors and heparin contamination as possible causes of prolonged TT and aPTT, we assumed that the presence of paraprotein could cause such an interference. Therefore, further laboratory diagnostics was directed toward detection of eventually-present monoclonal protein in serum. Measured concentration of total protein was 87 g per L (RR, 66–81 g/L), immunoglobulin (Ig)G concentration was 23.6 g per L (RR, 5.4–18.2 g/L), IgA concentration was 0.53 g per L (RR, 0.63–4.84 g/L), and immunoglobulin M IgM concentration was 0.26 g per L (RR, 0.22–2.4 g/L). An electropherogram reading (**Figure 1**) showed a peak in beta fraction at a concentration of 16.5 g per L. The presence of cryoglobulins was excluded due to visually-absent precipitate in the plasma specimen material that was left overnight at 4°C. To identify monoclonal protein observed in the electropherogram readings, we performed immunofixation of serum and 24 hours urine specimen after collection. Monoclonal IgG kappa (M-IgG-K) was identified in serum and urine (**Figure 2**). Quantification of serum free light

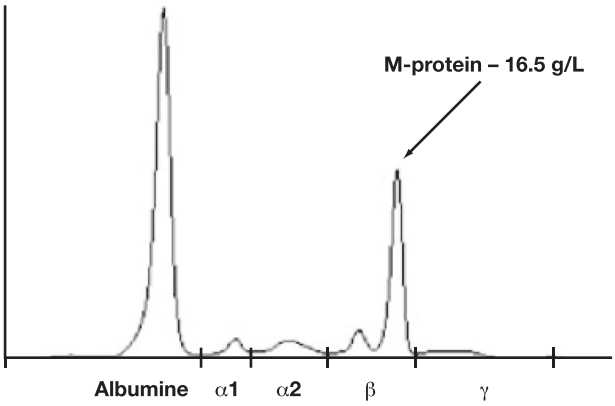

Figure 1

Capillary zone electrophoresis of serum proteins detected M-protein in beta fraction at a concentration of 16.5 g per L.

chains (FLCs) showed an abnormal kappa/lambda ratio of 27.09 (RR, 0.31–1.56). Concentrations of free kappa chains were 279.0 mg per L (range, 6.7–22.4 mg/L) and lambda chains, 10.3 mg per L (RR, 8.3–27.0 mg/L). Concentration of beta-2 microglobulin (β2M) was 2.2 mg per L (range, 0.8–2.4 mg/L).

All laboratory tests reported herein were performed in our laboratory. Coagulation tests were performed on the Behring Coagulation XP analyzer (Siemens AG) using original manufacturer-provided reagents and protocols. PT and APTT were determined with coagulometric methods using Dade Innovin and Actin FS reagents (both by Siemens AG). TT testing was performed via the coagulometric method using BC Thrombin reagent (Siemens AG), and fibrinogen level was determined by Clauss clotting method with Multifibren U reagent (Siemens AG). The activities of coagulation factors II, V, VII, VIII, IX, X, XI, and XII were determined by the coagulometric method, with appropriate deficient plasmas for individual factors and PT and aPTT reagents. Further, capillary zone electrophoresis was performed on a CAPILLARYS 2 instrument (SEBIA), measurement of total serum proteins, IgG, IgA, IgM, and β2M concentrations using original manufacturer-provided reagents on ARCHITECT c8000 clinical chemistry analyzer (Abbott Laboratories, Inc.). Immunofixation of serum and urine specimen 24 hours after collection was performed on agarose gel using he semiautomated method, on a HYDRASYS instrument (SEBIA) and quantification of serum FLCs was performed using original manufacturer-provided reagent and protocol within the BN II System nephelometer (Siemens AG).

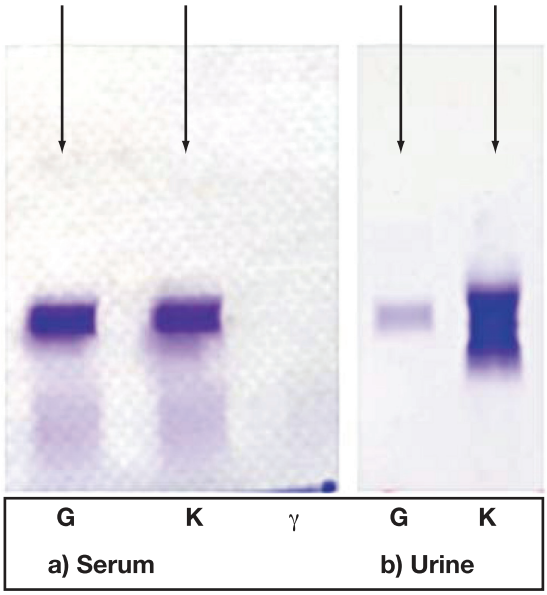

Figure 2

Immunofixation identified monoclonal immunoglobulin G kappa (M-IgG-K) in serum (**A**) and urine (**B**).

After operation and complete laboratory diagnostics, the results of which indicated the presence of monoclonal protein in the circulation, the patient was advised to visit a hematologist. At the Department of Hematology, Sestre Milosrdnice University Hospital Center, the patient was fully examined. After appropriate diagnostic procedures (X-ray, myelogram), he was diagnosed with smoldering multiple myeloma (SMM).

Discussion

The *TT test* is a screening coagulation assay that measures the ability of fibrinogen to be converted into fibrin, after the addition of bovine or human thrombin, in excess to platelet-poor plasma (PPP). Such prolonged TT testing results might be caused by underlying disorders associated with decreased or dysfunctional fibrinogen, increased fibrin(ogen) degradation products (FDPs), disseminated intravascular coagulation (DIC), advanced liver disease, thrombolytic therapy, heparin, and direct thrombin inhibitor therapies (dabigatran).[1]

Analytical interference by paraproteins result from chemical or immunological interference from specific or nonspecific

binding to a particular analyte or reagent component and also interfering with formed precipitate. Preanalytical interference by paraprotein can result from binding to electrolytes, enzymes, hormones, and other proteins.[2,3]

Lymphoproliferative disorders are frequently associated with disturbances of hemostasis and hemorrhagic complications. Because higher risk of bleeding complications or even thrombotic events are present in these patients, constant monitoring of coagulation test results is needed.[4,5] SMM is classified as a stage between monoclonal gammopathy of undermined significance (MGUS) and multiple myeloma (MM); usually, it is asymptomatic, as in our case individual. SMM diagnosis has a higher incidence in the older population (typical age at diagnosis is –70 years), which was not the case with our patient.[6] The main difference between SMM and MGUS is clinical, due to the risk of progression to malignancy.[6] The impact and interference on any particular screening hemostasis assay are different among individual patients with monoclonal gammopathic manifestations.[7]

In this case study, we present the case of a patient with an unmeasurably prolonged TT test result without any clinical signs and symptoms or history of bleeding. As mentioned by Zangari et al,[8] prolonged TT can be frequently observed in patients with MM but is not always accompanied by clinical manifestation of bleeding diathesis.[8] However, paraproteins of all subtypes can show inhibitory activity on fibrin polymerization and abnormal clot formation. In their study, Zangari et al presented a case of a patient with long-standing IgG-kappa monoclonal gammopathy with accompanied severe bleeding diathesis.[8]

In the literature, the interference of M-protein on coagulation test results has already been described[9,10] and mainly includes cases describing interference on PT and aPTT results. We assume that the main reason for this finding is because TT is not ordered in the basic preoperative coagulation screening profile. Rather, the TT test is mainly performed in specialized coagulation laboratories, making this phenomenon less frequently noticeable.

Huang et al[7] described the effect of serum monoclonal protein concentration on hemostasis abnormalities in patients with MM. Prolonged TT was found in most patients (77%) and was positively correlated with light-chain concentration. Prolonged PT was found mostly in patients with MM of the IgA and IgG types. Further, these authors showed that prolonged PT result was strongly related to higher M-protein concentration. Based on their comprehensive study, the authors concluded that abnormal hemostasis test results are not always accompanied by clinically apparent hemostatic complications.[7]

In the case report by Llamas et al,[1] prolonged TT was observed in 3 patients with different hematological malignant neoplasms: chronic lymphocytic leukemia, T-prolymphocytic leukemia, and MM. In all 3 cases, a prolonged TT result was found in a routine coagulation screening. Because the TT result was corrected with the addition of protamine sulfate, this finding suggested the presence of endogenous circulating heparin-like anticoagulant activity originating from neoplastic cells as the cause of the initially prolonged TT result. The severity of bleeding in these 3 patients ranged from severe epistaxis and deep-site hematoma to bleeding of the biopsy site and occasional ecchymosis. One of these 3 patients (ie, one of those who were diagnosed with MM, had TT of 122 seconds (RR, 16–21 seconds) with accompanying normal results for PT and aPTT, and had only occasional ecchymosis as a bleeding manifestation.

We consider that the mechanism of M-protein interference on the TT test is the result of impaired fibrinogen polymerization. M-protein interacts with the gamma chain of fibrinogen and, consequently, inhibits formation of a fibrin clot. Huang et al[7] have described that FLCs that occur due to nonspecific binding to fibrinogen have a stronger effect on TT result.

It is clear to us, from the results of different studies, that although prolonged TT has been shown to be a commonly present abnormal coagulation test result in patients with monoclonal gammopathic manifestations, it is not always associated with clinical bleeding manifestations. In the case of our patient, extremely prolonged TT higher than the upper measurement range (>150 seconds) did not result in any clinically evident bleeding. One possible explanation could be the relatively low concentration of M-protein at the time of diagnosis. This theory is in accordance with results of other studies[1,7] showing that despite relatively frequent prolonged results of TT testing in patients with monoclonal gammopathic manifestations, hemostasis disorders are not always present. This finding can be explained with the assumption that impaired fibrin polymerization correlates with the concentration of M-protein. This assumption suggests that clinically evident bleeding could be expected at high concentrations of M-protein rather than at lower M-protein concentrations.

Laboratory Role in Diagnosis

Following the rules of strong laboratory practice, laboratory experts should always be aware of possible preanalytical and analytical factors that could impact laboratory-test results in terms of interference. Knowing the analytical limits of the method used and excluding specimens with the most common preanalytical interferences, such as hemolysis, icteria, and lipemia, are crucial steps in preventing errors in laboratory practice. Further, special consideration should be given to less-common preanalytical and analytical interfering factors. The presence of paraprotein could be a cause of such interference.

Our case report has clearly shown that reflex testing and collaboration of laboratory professionals and clinicians are necessary for accurate interpretation of laboratory test results, especially when these factors are not associated with expected clinical conditions and/or symptoms. In the case of our patient, a markedly abnormal TT test result was the only indicator that focused further laboratory diagnostics on detection of the presence of monoclonal protein and led to formulating the SMM diagnosis. We emphasize that in this case, the patient is a young man; due to his age and lack of symptoms, SMM probably could not be suspected and/or diagnosed if a normal TT value was measured. The clear TT result in our patient, without any symptoms and/or history of bleeding and without any anticoagulant therapy, confirmed the conclusion described in a case report previously published by Margetić et al.[9] These findings suggest that diagnostic search for the presence of paraprotein should be considered in all patients who have not undergone anticoagulant therapy but have had unexplained and unexpected coagulation test results.

An algorithm suggested and described described by Margetić et al[9] can be followed to exclude possible paraprotein interference. This algorithm includes repeated blood-specimen collection at 37°C; refrigeration of the plasma specimen overnight at 4°C. It also involves measurements of total protein and immunoglobulins G, A, and M concentrations; serum protein electrophoresis; urine and serum immunofixation; and β2M concentration.[9]

The key message from the case of our patient is that diagnostic search for possible paraprotein presence should be considered in patients whose clinical condition cannot explain the pathological results of individual screening coagulation tests, such as PT, aPTT, and/or TT. Altered laboratory test results need to be carefully interpreted, and if there is no pathophysiological explanation for the obtained test result(s), one should consider paraprotein interference as a possible cause.

Patient Follow-Up

According to the current standard practice for SMM, clinicians decided to observe the patient without any treatment at the moment. Since then, as of the publication of this article, there were no request for the coagulation tests in our laboratory. In the future, the patient will be under observation in the Department of Internal Medicine, Sestre Milosrdnice University Hospital Center. **LM**

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