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ADAMTS13 Testing Methodologies and Thrombotic Thrombocytopenic Purpura (TTP): Conflicting Results Can Pose a Clinical Dilemma

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Abstract. ADAMTS13 testing plays a critical role in confirming the clinical diagnosis of acquired idiopathic thrombotic thrombocytopenic purpura (TTP) and distinguishing it from other forms of thrombotic microangiopathies (TMA). Serial measurements of ADAMTS13 activity and inhibitor levels are also helpful in determining response to treatment and/or subsequent relapses. Numerous ADAMTS13 assays have been developed recently, including some with rapid turnaround times. Despite the good inter-assay correlation of different ADAMTS13 methodologies in published case studies, discrepancies have been shown to occur. Here we present a case where discrepant results were obtained using two different assays, posing a clinical treatment dilemma.

Introduction

Acquired idiopathic TTP is a rare disease caused by deficiency of the von Willebrand factor cleaving protease ADAMTS13 (A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) [1,2]. Clinical diagnosis of TTP is often challenging due to overlapping features with other thrombotic microangiopathies (TMA) [3]. Mild to moderately reduced ADAMTS13 activity levels (20-60%) may be seen in other conditions, including atypical hemolytic uremic syndrome (aHUS), hematopoietic stem cell [4] and solid organ transplantation, liver disease [5], disseminated intravascular coagulation (DIC), sepsis [6], pregnancy [7], with certain medications (e.g. ticlopidine, clopidogrel, cyclosporine, mitomycin, quinine) and in TTP patients who have received plasma infusions.

Given the high mortality rate of TTP, emergent therapeutic plasma exchange (TPE) is generally initiated in the presence of microangiopathic hemolytic anemia (MAHA) and unexplained thrombocytopenia. Despite a low threshold to initiate TPE promptly in such cases, a thoughtful approach is still required since TPE has its own significant patient risks [8,9]. Severely reduced ADAMTS13 activity (<10%) and the presence of an inhibitor support the clinical diagnosis of TTP. In the past decade, ADAMTS13 testing has evolved significantly and multiple assays are now available, each with their own strengths and shortcomings, and most requiring use of a reference laboratory. Case studies have shown overall good correlation between the different testing methodologies at low ADAMTS13 activity levels (<10%) [10-16], although discrepant results have been reported and clinical judgement is recommended [17] in such cases. We present a case where we received widely discrepant results using two different testing methodologies, one result supportive of TTP and the other not supportive of TTP, in a patient where clinical judgment supported the negative results.

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Case Report

A 52 year old African American female presented with acute cholecystitis and thrombocytopenia. She reported a past history of ITP (treated elsewhere with steroids) and chronic anemia. She denied fever, bruising, epistaxis, melena, hematuria, or any other bleeding, as well as any recent blood transfusions.

Significant laboratory results on admission included: anemia (HgB 7.7 g/dL), thrombocytopenia (platelet count 14K/μL), elevated LDH (647 IU/mL), elevated total serum bilirubin (1.6 mg/dL), low haptoglobin (5

mg/dL), and presence of schistocytes (2/HPF) on peripheral blood smear. Additional results included a normal DIC panel, normal plasma free hemoglobin and negative direct Coomb's test. As clinical judgement by both hematology physicians and transfusion medicine physicians did not suggest TTP, ADAMTS13 testing was performed in-house as part of a validation study using comparison to a reference laboratory with rapid turnaround time. Additional testing performed included complement levels (C3, C4; within normal limits), antiphospholipid antibody panel (normal results), platelet-specific and HLA antibody analysis. Platelet-specific antibody analysis (GTI-Pak 12®, Immucor GTI diagnostics, Norcross, GA) showed the presence of antibodies against multiple human platelet antigens (HPA) as well as HLA antigens; however, HLA antibody profile (LABScreen® Single Antigen determination, One Lambda®, Canoga Park, CA) showed no significant antibodies against Class I HLA antigens, suggesting the possibility of interference by another antibody or unknown substance with the GTI-Pak 12® test.

The ADAMTS13 testing performed in-house (using the Fluorescence resonance energy transfer assay/FRET methodology; Lifecodes ATS-13® Assay, Immucor® GTI Diagnostics, Waukesha, WI) supported the clinical impression that this was not TTP, with ADAMTS13 activity 33% (normal range >70%) and a negative inhibitor screen. However, ADAMTS13 testing from the same specimen performed at a reference laboratory using the chromogenic immunoassay (TECHNOZYM® ADAMTS-13 activity, Technoclone, Vienna, Austria) was supportive of TTP, with ADAMTS13 activity <1% (normal range 40-130%) and presence of an inhibitor, as well as an anti-ADAMTS13 IgG antibody (64 units; normal <18 units) (TECHNOZYM® ADAMTS-13 INH, Technoclone, Vienna, Austria). As part of our own testing validation process, another specimen was drawn the following day without intervening receipt of plasma or plasma exchange, and sent for ADAMTS13 testing to another reference laboratory using the FRET methodology we are validating (FRET assay; Lifecodes ATS-13® Assay, Immucor® GTI Diagnostics, Waukesha, WI). The specimens were collected in sodium citrate anticoagulant for all three ADAMTS13 assays. The patient's laboratory parameters remained stable during this repeated testing period (platelet count 14-22 K/μL, LDH 647-651 IU/L, hemoglobin 7.7-7.3 g/dL, total serum bilirubin 1.6-1.2 mg/dL, no increase in free hemoglobin or lipemia). While waiting for the results from the second reference laboratory, TPE was initiated using cryo-poor plasma. The results from the second reference laboratory were comparable with our own and did not support a diagnosis of TTP, with ADAMTS13 activity 23% (normal range >67%) and a negative inhibitor screen. However, after institution of TPE the patient showed clinical improvement, with increased platelet count and decreased LDH. After 7 TPE the platelet count (174 K/μL; >150 on 2 consecutive days) and LDH (246 IU/L) were within normal limits and TPE was stopped. Repeat ADAMTS13 testing 3 days after the last TPE by the reference laboratory using FRET methodology showed mildly decreased ADAMTS13 activity (65%; normal range >70%) and no inhibitor. Chromogenic immunoassay was not repeated due to reimbursement issues, nor was it possible to send a sample to a reference laboratory using a different FRET assay.

Discussion

ADAMTS13 activity assays are useful in confirming a clinical diagnosis of TTP and distinguishing it from other forms of TMA, although some argue strongly that clinical judgment should outweigh the test results [16]. Several assays for determining ADAMTS13 activity are available, based on the cleavage of plasma-derived or recombinant VWF multimers by test plasma and the direct or indirect detection of cleaved VWF by ADAMTS13. Direct assays detect VWF cleavage products. The fluorescence resonance energy transfer assay (FRET-VWF73 assay) is one such direct assay that utilizes a truncated synthetic 73-amino-acid VWF peptide as a substrate for the determination of ADAMTS13 activity. Cleavage of substrate by the ADAMTS13 in the test plasma results in fluorescent emission that is directly proportional to the protease activity. Indirect assays measure either the residual substrate (i.e. VWF) or its disappearance. The chromogenic VWF73 enzyme-linked immunosorbent assay (ELISA) is one indirect assay that measures the residual, cleaved VWF fragment by using a HRP conjugated monoclonal antibody that only recognizes the cleaved VWF fragment. The FRET-VWF73 assay and the chromogenic VWF73 ELISA assays described above are the most commonly used assays.

Prior studies have shown overall agreement between the different ADAMTS13 activity assays [10-16]. Mackie et al. showed good correlation between assays (Collagen Binding Assay/CBA, an in-house

FRET-VWF73 and a chromogenic VWF73 immunoassay) for samples with <11% ADAMTS13 activity [15]. Joly et al. also observed good correlation between the assays (chromogenic VWF73 immunoassay and an in-house FRET-VWF73 assay) for ADAMTS13 activity <10% [16], although ADAMTS13 activity was overestimated in 12% of samples using the chromogenic assay, resulting in a false negative diagnosis. However, a discrepancy leading to a false positive result was extremely rare (only 1 of the 52 TTP patients in remission tested as false positive with the chromogenic VWF73 immunoassay).

A number of substances are known to cause interference with testing and to result in an apparent reduction of activity, particularly with the FRET methodology, including significantly elevated bilirubin, elevated plasma free hemoglobin, and sample collection in EDTA [18,19]. However, other variables causing discordant results between different assays are not completely understood. Within the discordant samples, Mackie et al. [15] found no relation among disease status, level of ADAMTS13 activity, antigen or antibodies, or the different time points when the samples were collected from the same individual. Likewise, Joly et al. found no relation among the type of sample (from healthy volunteers, acute TTP, TTP in remission or other forms of TMA), anti-ADAMTS13 IgG titer, or technical issues [16]. However, both Mancini et al. [20] and Palla et al. [21] in their study comparing FRET-VWF73 and CBA showed that the samples with discordant results had a higher frequency of anti-ADAMTS13 antibodies with low titer and inhibitory activity. They believed that dissociation of anti-ADAMTS13 antibodies (from immune complexes with ADAMST13) by the denaturing agent (urea) used in the CBA could result in the overestimation of ADAMTS13 activity by CBA (FRET-VWF73 was unaffected). However, these results could not be confirmed in all the discordant samples. Additionally, there are no known reasons for the observed discrepancies between the chromogenic VWF73 immunoassay and FRET-VWF73 assays in the study by Joly et al. [16]. Thus incompletely understood variables exist that affect activity assays.

Since most clinical labs use a single assay and few patients are treated per year at a given facility, such discrepancies may go unnoticed unless the clinical presentation and results are widely inconsistent, as was the case here.

Severely reduced ADAMTS13 activity (<10%) and the presence of an inhibitor are the hallmarks of acquired idiopathic TTP. However, a case report has described normal ADAMTS13 activity (by immunoblotting and FRET) and absence of inhibitor at first presentation, with detection of reduced ADAMTS13 activity and presence of an inhibitor on subsequent relapses [22]. This has been attributed to the pathophysiology of the disease, suggesting the presence of low-titers of anti-ADAMTS13 antibodies at initial episode resulting in severely reduced ADAMTS13 activity on subsequent episodes. As a result, treatment decisions may not be based solely on ADAMTS13 results, but may be made in conjunction with clinical findings and other laboratory results given the discordant results between the various assays.

In the present case, our patient showed presence of an inhibitor and anti-ADAMTS13 IgG antibodies only by the chromogenic assay. Although the clinical suspicion of TTP was low in this case and was not corroborated by testing results using the FRET methodology, we elected to continue daily TPE since the platelet count rose in apparent response to the treatment.

Conclusions. This case highlights the importance of using alternate testing methodology when the laboratory results conflict with the clinical picture. ADAMTS13 testing is still evolving, and International Standardized ADAMTS13 plasma has been established by the World Health Organization (WHO) [23], with proficiency tests offered by some organizations to reduce inter-laboratory assay variability. Availability of ADAMTS13 testing has greatly facilitated clinical decision making; however, it is still unclear which test has the most clinical utility, nor whether clinical judgment alone is sufficient to choosing between discordant laboratory results.

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