An Overview of the Laboratory's Role in the Diagnosis and Treatment of Thrombotic Thrombocytopenic Purpura

Abstract:

Thrombotic thrombocytopenic purpura (TTP) is a multi-faceted disease for a clinical laboratory with diagnosis data and treatment spread across many different laboratory sections. Encompassing results from hematology, chemistry, molecular, and coagulation sections with treatment from the transfusion medicine/Blood Bank section of the laboratory, clinicians are able to accurately diagnose and treat TTP.

Abbreviations:

ADAMTS- a disintegrin and metalloprotease with thrombospondin type 1 repeats, ADAMTS13 - a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13, aPTT - activated partial thrombosplastin time, CBC – complete blood count, ED – emergency department, HUS - hemolytic uremic syndrome, IgG - immune globulin G, IgM - immune globulin M, LDH - lactic acid dehydrogenase, MAHA – microangiopathic hemolytic anemia, PT - prothrombin time, TPE – therapeutic plasma exchange, TTP – thrombotic thrombocytopenic purpura, VWF-von Willebrand Factor

Index Terms: Thrombotic Thrombocytopenic Purpura, ADAMTS13, von Willebrand Factor

Objectives:

1. Describe the role the laboratory has in diagnosis of thrombotic thrombocytopenic purpura.
2. List the classical diagnostic pentad of TTP and the more modern diagnostic criteria.
3. Describe the treatment method used for congenital and acquired TTP.

Introduction:

Thrombotic thrombocytopenic purpura (TTP) is a hematologic disorder that results in microthrombi forming in the small capillaries of the circulatory system. While sometimes confused with other hematologic disorders, TTP is classically seen with thrombocytopenia, microangiopathic hemolytic anemia (MAHA), fragmented erythrocytes, neurological defect and fever. TTP can be rapidly fatal, requiring quick assessment of symptoms and start of treatment to reduce mortality.

Thrombotic Thrombocytopenic Purpura (TTP)

TTP was first described in 1924 when a 16 year old girl presented to the emergency room with fever, anemia, and weakness. Dr. Eli Moschcowitz was the first physician to describe the disorder after the girl's death. Autopsy findings showed disseminated hyaline thrombi in arterioles and capillaries of the heart, liver, and kidney. No larger vessels were affected by these observed thrombi.[1,4] In 1966, the pentad of TTP symptoms were described and included fever, hemolytic anemia, thrombocytopenia, neurological symptoms and kidney involvement, however over time the expectation of this classical pentad has decreased. In 1982, the ultra large von Willebrand factor multimers were seen in patients presenting with TPP and the role of vonWillebrand Factor (VWF) was determined.[3] In 2001, with new techniques in gene cloning, the 13th member of the a disintegrin and metalloprotease with thrombospondin type 1 repeats (ADAMTS) protein family was discovered and it was shown that a decrease in a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13 (ADAMTS13) activity was consistent with TTP.[3] Currently, TTP should be suspected and excluded by laboratory assays and

the presence of microangiopathic hemolytic anemia (MAHA) with red blood cell fragmentation and thrombocytopenia alone.[2] Additional clinical signs may allow for a faster diagnosis as well. These clinical signs may include petechiae, purpura, epistaxis, cerebral and retinal hemorrhages. Neurologic symptoms that may present include headache, confusion, aphasia, and even coma.[4]

Since the identification of ADAMTS13, TTP can be identified as one of two forms: congenital or acquired deficiency. Clinically there are many other syndromes, including eclampsia and hemolytic uremic syndrome (HUS), that are considered MAHA disorders and that could closely resemble TTP.[5] Most cases of TTP are due to an autoimmune mechanism that interferes with ADAMTS13 acquired TTP.

Laboratory's Role

Laboratory confirmation of TTP is determined by a profound decrease in ADAMTS13 enzyme activity. The follow-up to a decreased ADAMTS13 activity assay is to detect autoantibodies to ADAMTS13, confirming the diagnosis of acquired TTP.[2] Additional laboratory testing includes a complete blood count (CBC), clinical chemistry panel, and urinalysis. Specific testing includes lactic acid dehydrogenase (LDH), haptoglobin, bilirubin, prothrombin time (PT) and activated partial thrombosplastin time (aPTT) and other indicators of hemolysis.[2] The hemolysis in TTP arises from increased shear stress on red blood cells in arterioles and capillaries narrowed by microthrombi.

TPP is still considered a life-threatening disease with a mortality rate of 10-20%. Even though TTP is a serious hematologic emergency that is almost always fatal in untreated cases, an

understanding of its pathophysiology can lead to successful treatment strategies resulting in improved patient management and outcomes.

Aside from routine laboratory findings, TTP is a disease that is classified by abnormal functioning of the ADAMTS13 protease. ADAMTS13 protease impairment can be caused by genetic mutations at the gene level or through autoantibodies that are formed within the circulation. Congenital mutations account for about 5-10% of the TTP population while the acquired version is more common. The acquired version of TTP is due to inhibitory and non-inhibitory autoantibodies that effect the ADAMTS13 protease. Both congenital and acquired TTP are treated through transfusion therapy with therapeutic plasma exchange (TPE). TPE is used to remove the autoantibodies and any mutated ADAMTS13 proteases in the circulation while providing the addition of normal functioning ADAMTS13 to the circulation. TPE is removal and retention of plasma through n apheresis machine that allows all cellular products to be returned to the circulation.[6] TPE was first employed in 1952 and by the 1970s was a multi-use treatment for many different diseases. The efficiency of TPE depends on the plasma volume that is being removed and pathogenic substrate (IgG and IgM antibodies in TTP). One volume exchange is equivalent to 65% of the initial component removed with 75-85% substrate removal within two TPE procedues.[6]

Case Study

This Focus Series is designed to provide a comprehensive review of Thrombotic Thrombocytopenic Purpura (TTP) for the laboratory scientist. The following articles will provide in-depth understanding of the etiology, pathogenesis, immunology, laboratory findings, and

treatment of TTP. This case study is the review of diagnosis and treatment of a 40 year old female who presents to the emergency department (ED). The original laboratory findings can be seen in Table 1. The presented results which are analyzed in the following Focus article demonstrate why prompt and accurate diagnosis and treatment are needed in cases of TTP.

Table 1. Original patient laboratory values for a 40-year-old female who was seen in the emergency department with abdominal pain over 3 days.

Hematology:	**Reference Interval**	**Original patient values**
WBC bil/L	3.3-10.7	4.7
RBC tril/L	3.87-5.08	3.3
Hemoglobin g/dL	12.1-15	9.0
Hematocrit %	34.4-44.2	27.8
Mean Cell Volume (MCV) fL	80-100	84
Mean Cell Hemoglobin (MCH) pg	28-32	27
Mean Cell Hemoglobin Concentration (MCHC) %	32-36	32
Red Cell Distribution Width (RDW) %	11.5-15.5	20
Platelet bil/L	150-400	32
Mean Platelet Volume (MPV) fL	8.5-12.5	14
Immature platelet fraction (IPF)%	1-11	15
Neutrophils bil/L	1.6-7.2	3.2
Lymphocytes bil/L	1.1-4.0	1
Monocytes bil/L	0.0-0.8	0.4
Eosinophils bil/L	0.0-0.5	0.0
Basophils bil/L	0.0-0.1	0.0
Immature Granulocytes bil/L	0.00-0.03	0.01
RBC Morphology	<1/hpf	Schistocytes 3-5; polychromasia 1-3
Coagulation:		
Prothrombin Time (PT) sec	9.5 - 12.3	11.8
aPTT sec	24.3 - 32.2	29.7
Fibrinogen (mg/dL)	180-400	319
ADAMTS13 activity	50-160%	<5%
ADAMTS13 Inhibitor (BU)	<0.4	2
Chemistry:		
LDH U/L	140-280	362
Creatinine	0.5-1.0	1.2
Bilirubin mg/dL	0.2-1.2	3.6

Urinalysis-Completed Day prior to above results:		
Test Strip Results		
Color	Yellow	Dark Yellow
Clarity	Clear	Cloudy
Glucose	Negative	Negative
Bilirubin	Negative	Negative
Ketones	Negative	Negative
Specific Gravity	1.003-1.035	1.017
Blood	Negative	3+
pH	5.0-8.0	7.0
Protein mg/dL	Negative	100
Urobilinigen mg/dL	0.2-1	2
Nitrates	Negative	Negative
Leukocyte Esterase	Negative	Negative
Urinary Sediment		
RBC	0-2 (negative)	0-2 (negative)
WBC	0-2 (negative)	3-10
Epithelial, Squamous	0-5 (negative)	>50
Casts, Hyaline	0-2 (negative)	0-2 negative
Bacteria	0-1+ (negative)	1+

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