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Morphologic Diagnosis of Thrombotic Thrombocytopenic Purpura

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The diagnosis of thrombotic thrombocytopenic purpura (TTP) rests on evidence of microangiopathic hemolytic anemia and thrombocytopenia in the absence of disseminated intravascular coagulation and other known causes of thrombotic microangiopathy. Highly specific diagnostic tools such as serum levels of ADAMTS13 are not routinely available for immediate clinical diagnosis. The presence of schistocytes on a blood smear is the morphologic hallmark of the disease, but no guidelines exist as to the number of schistocytes required to differentiate TTP from other thrombotic microangiopathies. We studied 6 patients with TTP and compared their schistocyte counts with those of 40 normal subjects, 28 patients with chronic renal disease, 5 with preeclampsia, and 5 with normal functioning mechanical heart valves. The mean schistocyte count for the TTP patients was 8.35% versus 0.05% for normal subjects, 0.2% for renal patients, 0.25% for preeclamptic patients, and 0.18% for patients with mechanical valves ($P < 0.001$). Schistocytes were found on 100% of blood films of TTP patients and ranged from 1.0% to 18.4% of red cells. Schistocytes are found on the smears of 58% of normal individuals and on 80–100% of the other patient groups studied, but always comprise less than 0.5% of the red cell population. An initial schistocyte count of greater than 1% strongly suggests a diagnosis of TTP in the absence of other known causes of thrombotic microangiopathy. Am. J. Hematol. 75:18–21, 2004. © 2003 Wiley-Liss, Inc.

Key words: schistocytes; thrombocytopenia; purpura; microangiopathy; hemolysis

INTRODUCTION

Thrombotic thrombocytopenic purpura (TTP) was first described by Moschowitz in 1924 [1]. The clinical manifestations of the disease include thrombocytopenia, microangiopathic hemolytic anemia, varying neurologic and renal function abnormalities, and fever. During the early 1970s plasma exchange was introduced as an effective treatment for TTP, making early and rapid diagnosis urgent. Diagnostic criteria were relaxed so that today the diagnosis of TTP is based mainly on the finding of thrombocytopenia and microangiopathic hemolytic anemia in the absence of other identifiable causes, such as DIC, cancer, or preeclampsia [2]. Thrombocytopenia is defined as a platelet count of less than 100,000 per cubic millimeter, and microangiopathic hemolytic anemia is diagnosed by the finding of schistocytes on the peripheral blood smear along with a negative Coombs' test. No minimum number of schistocytes has ever been defined.

Schistocytes, which are fragmented red cells, are not specific to TTP. Cardiac valve disease and malfunctioning prosthetic valves have long been known to cause a mechanical hemolytic anemia leading to schistocytosis [3–8]. Women with preeclampsia and eclampsia also exhibit microangiopathic hemolytic anemia with peripheral schistocytes and thrombocytopenia [9–11]. Red cell fragmentation is also seen

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after solid organ or bone marrow transplantation [12], as well as with diabetic microangiopathy [13].

There have been no studies to determine whether or not there is a numerical threshold of schistocytes above which the diagnosis of TTP can be confidently made. We therefore defined a reference range for schistocytes in normal individuals and among patients with various diseases. We compared the number of schistocytes on the blood smears of TTP patients with those of normal individuals as well as patients who had undergone cardiac valve replacement, who had preeclampsia, and those with severe renal disease in order to better define a simple and meaningful diagnostic tool for TTP.

METHODS

IRB approval was obtained to produce and examine peripheral blood smears from left over blood samples obtained from normal subjects and patients during the course of routine clinical care. Data were gathered from 40 normal blood donors, 28 chronic renal failure patients on hemodialysis, 5 preeclamptic patients, and 5 patients with well-functioning mechanical heart valves. None had clinical evidence of hemolysis. Lack of hemolysis was defined as having normal hemoglobin, reticulocyte count, serum LDH, and bilirubin. The number of schistocytes per 2,000 red blood cells was counted using a Miller optical disk at 1,000-power magnification. Results were then expressed as number of schistocytes per 1,000 red cells.

For the TTP patients, a single stored admission blood smear of each of 6 patients who had undergone plasma exchange at Montefiore Medical Center over a 3-year time period was analyzed.

We also analyzed observer bias in identifying schistocytes on blood smears by asking 5 well-trained hematology fellows and 5 experienced Hematology Attendings to analyze 10 blood smears for the presence of schistocytes. They were each given coded normal smears and asked to evaluate and record any erythrocyte abnormalities on a zero to 4+ basis on a scoring sheet that included among other abnormalities the presence or absence of schistocytes. The same normal slides were then re-coded and given again to the same observers for evaluation after their first, being told that the smears were from patients with microangiopathic hemolytic anemia.

RESULTS

None of the normal subjects or patients outside of the TTP group had clinical or laboratory evidence of hemolysis. Despite this, schistocytes were seen in 58% of the normal individuals with a mean of 0.05% and a range of 0–0.27% of all red cells; 93% of chronic renal failure patients had schistocytes with a mean of 0.21% and a range of 0–0.6% ($P < 0.01$) of all red cells; 80% of the preeclamptic women had schistocytes with a mean of 0.25% of all red cells and a range of 0–0.45% ($P < 0.01$). All of the patients with normally functioning prosthetic cardiac valves had schistocytes with a mean of 0.18% and a range of 0–0.48% of all red cells.

The TTP patients had considerably more schistocytes than all of the other groups, with a mean of 5% and a range of 1.1–9.4% ($P < 0.001$, Table I). In addition, 100% of the TTP patients had schistocytes.

In the observer bias part of the study, each of the 10 observers reported considerably more schistocytes on a smear if they were first told to specifically look for them (average 3+ vs. 0–1+; complete data not shown).

DISCUSSION

There are few published articles that quantitate schistocytes in normal subjects or different disease states and no articles that suggest a reference range for the specific morphologic diagnosis of TTP. In line with other studies of morphologic bias [14], we have demonstrated that the mere sensitivity to the possible presence of schistocytes on a smear creates positive observer bias that detects the presence of these cells, even among expert observers. Several studies have dealt tangentially with the quantitative presence of schistocytes on blood films.

Rao et al. studied erythrocyte survival in patients with porcine xenograft valves and found that in 9 patients with normal valve function the mean schistocyte count was 0.56% of all red cells and in 1

TABLE I. Incidence of Schistocytes on Peripheral Blood Smears

Patient groups	*N*	Prevalence (%)	Mean ± SD (%)	Range (%)
Normals	40	58	0.05 ± 0.03	0–0.27
Chronic renal disease	28	93	0.21 ± 0.18	0–0.6
Preeclampsia	5	80	0.25 ± 0.08	0–0.45
Mechanical valves	5	100	0.18 ± 0.15	0–0.48
TTP	6	100	8.35 ± 2.74	1.0–18.4

patient with double valve prostheses and aortic paravalvular regurgitation, the mean schistocyte count was 4% [3]. In a study of intravascular hemolysis after cardiac valve replacement with different types of mechanical prostheses, Ismeno et al. reported that a few schistocytes were found regardless of model and position and was not clinically significant ($P > 0.05$). The percentage of schistocytes reported for patients with different types of mechanical prostheses in aortic and mitral positions ranged from 0.8 to 1.8% [4].

Ducrou et al. also studied hemolysis after heart valve replacement and reported fragmented cells in only 3 of 33 patients. One patient had less than one fragmented cell per HPF, one patient had less than one fragmented and helmet cell per HPF, and one patient had 1–2 fragmented and helmet cells per HPF [5].

Cox et al. studied hemolysis and anemia associated with acute antepartum pyelonephritis. They report a significant increase in the proportion of abnormal red blood cells in general and schistocytes specifically. They report a schistocyte count of 2.2% (0.6–5.4% range) in 18 women with pyelonephritis versus 0.5% (0–2.8% range) in 25 normal pregnancies ($P < 0.0001$) [10].

Cunningham et al. studied erythrocyte morphology in preeclampsia and eclampsia. Nine eclamptic women had a mean of 12.8% schistocytes compared with 12 normally pregnant controls who had 1.3% schistocytes. Twelve preeclamptic women had 1.0% schistocytes compared with 25 normally pregnant controls who had 0.5% schistocytes [11].

The presence of schistocytes in TTP relates to the pathophysiology of the disease. Von Willebrand factor (vWF) is a large multimeric protein synthesized and released by endothelial cells. It is a key component in facilitating the platelet thrombi that form in this disease. vWF is normally produced and secreted as an extra large polymer known as unusually large von Willebrand factor (ULvWF). ULvWF has the ability to spontaneously bind platelets [15,16]. In normal circulation, when ULvWF encounters abnormal shear stress it is unfolded to reveal a cleavage site and is rapidly depolymerized to smaller sized multimers [17]. This is accomplished by a plasma zinc metalloprotease, now known as ADAMTS13 [17]. An actual or functional deficiency of the vWF-cleaving protease apparently leads to the accumulation of UL vWF in the plasma and predisposes patients with TTP to platelet thrombosis [17]. Tsai et al. point out that the thrombotic lesions implicated in the pathogenesis of TTP contain not only platelets, but also abundant vWF [15].

The mechanism of schistocyte formation in TTP is somewhat unclear. Red cells will undergo fragmentation when exposed to high levels of shear stress in a cone plate viscometer [18]. Recent studies reveal that inhibitory IgG antibodies against vWF-cleaving metalloprotease occur in patients with acute TTP [15]. Some believe that patients with the sporadic form of TTP may have such inhibitory antibodies while those with the autosomal recessive form of TTP suffer from a defect in protease synthesis or function [19,20]. Whatever the cause, a deficiency of this metalloprotease leads to high levels of un-cleaved vWF and life-threatening thrombosis, thrombocytopenia, and anemia [20]. It is possible that schistocyte formation occurs due to high levels of shear stress in association with platelet thrombi which form in the microvasculature secondary to high levels of ULvWF.

It is clear from our results that schistocytes are seen on the peripheral blood smears of most healthy individuals as well as in various conditions affecting the heart and rest of the vascular system. Therefore, the mere presence of some schistocytes on a blood film cannot be taken as specific evidence of the presence of TTP. Indeed, patients with renal disease, preeclampsia, and various cardiac valve abnormalities routinely have large numbers of schistocytes present in their blood. In other words, the finding of schistocytes on a blood smear is a highly sensitive but not at all specific diagnostic finding for TTP. This is especially true due to the observer bias introduced when one is specifically looking for schistocytes on the blood smear of a patient suspected of having TTP. This is in contradistinction to DIC, in which the presence of schistocytes is less common than widely perceived and has a low sensitivity of about 25%. We therefore did not include this condition as a control group [21,22]. However, based on our data, there appears to be an identifiable range for the percentage of schistocytes on peripheral blood smear that is specific for TTP. A schistocyte count greater than 1% appears to be diagnostic of TTP in the appropriate clinical setting.

Classic Bayesian analysis of sensitivity and specificity or ROC analysis of the utility of a specific schistocyte count would not be helpful in clinical practice as there are clear-cut clinical conditions where a schistocyte count of greater than 1% would be expected. Examples include malfunctioning prosthetic heart valves, systemic lupus erythematosis with vasculitis, HELP syndrome, and DIC. We therefore simply recommend that when the diagnosis of TTP is suspected a quantitative schistocyte count be performed. In the absence of known valvular conditions, preeclampsia, lupus, DIC, or pre-existing renal disease, the finding of more than 1% schistocytes on a peripheral blood smear along with thrombocytopenia and hemolysis should be taken as putative evidence of TTP and treatment begun. Given the limited number of TTP patients studied we cannot exclude the

possibility that some patients with TTP may have fewer than 1% schistocytes on presentation. Nevertheless, given the fact that other microangiopathic conditions consistently have fewer than 0.5% schistocytes on smear, we feel confident in our recommendation. As more specific tests for TTP, such as quantitative ADAMTS13 levels, become readily available for immediate diagnosis, this morphologic tool will recede into historical importance.

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