Transfusion and Apheresis Science 49 (2013) 447–452

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Analysis of clinical and laboratory characteristics in 42 patients with thrombotic thrombocytopenic purpura from a single center in China

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ARTICLE INFO

Article history:
Received 30 April 2012
Received in revised form 30 June 2013
Accepted 26 July 2013

Keywords:
TTP
Chinese
Clinical feature
ADAMTS 13
T lymphocytic subset

ABSTRACT

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disease characterized by microvascular platelet deposition and thrombus formation with resulting microangiopathic hemolytic anemia. Deficiency of the von Willebrand factor cleavage protease, also known as ADAMTS 13, has been implicated as an important etiological factor in TTP. Little studies were obtained on Chinese patients with TTP until now. Our aim was to analyze the clinical features, outcome and laboratory characteristics of Chinese TTP patients, and determine whether plasma ADAMTS 13 activity is decreased in TTP and its diagnostic value for TTP. Forty-two TTP patients (29 females; 13 males) admitted to our hospital from 1998 to 2010 were analyzed. There were 34 patients (81%) with the triad of TTP, including hemolytic anemia, thrombocytopenia and neurologic abnormalities; 7 (16.7%) had the classical pentad of TTP. Major etiologic factors were acquired autoimmunological abnormalities (31%); no familial TTP was identified in this series. The schistocytes of peripheral blood smears were present in all cases with a mean frequency of 4.6% (range from 0.3% to 13.4%). Plasma ADAMTS 13 activity was determined in 22 patients with the FRET-vWF86 assay. Only 4 idiopathic TTP patients (18.2%) had severe ADAMTS 13 deficiency (activity < 10%); 9 (40.9%) had moderate decrease of ADAMTS 13 activity (activity: 10–40%); another 9 (40.91%) had normal ADAMTS 13 activity (>40%). T lymphocyte subpopulation was measured in 23 TTP patients with FACS Calibur; 14 of the 23 (60.9%) had significantly decreased CD4 cells count and CD4/CD8 ratio, suggesting cellular immune dysfunction may be involved in the pathogenesis of TTP. In the studies, plasmapheresis is the main therapeutic method. 26 of 31 patients (83.9%) accepting plasmapheresis achieved complete remission; those patients who only underwent plasma infusion had low remission rate (18.2%) and high mortality (9/11; 81.8%). Four patients with packed RBC infusion manifested transient exacerbation of neurologic or psychiatric symptoms. In conclusion, the diagnosis of TTP in China is still based on clinical features including evidence of microangiopathic hemolysis. Severe ADAMTS 13 activity deficiency might be a valuable indicator for idiopathic TTP diagnosis. Further studies are needed to determine the real value of ADAMTS 13 activity for TTP diagnosis and whether T lymphocytes subset dysregulation plays important role in TTP pathogenesis.

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http://dx.doi.org/10.1016/j.transci.2013.07.026

1. Introduction

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disseminated thrombotic microangiopathy characterized by ultra-large molecular weight vWF multimer formation, which leads to microangiopathic hemolytic anemia, consumptive thrombocytopenia and widespread microvascular thrombosis with diffuse ischemic damage [1,2]. Before plasma exchange (PE) was introduced as an effective therapy, TTP was often fatal with a mortality rate exceeding 90%. Plasma exchange has a response rate of approximately 80% and a survival rate greater than 90%, indicating that early and rapid diagnosis is urgent to ameliorate the prognosis of TTP patients [3,4]. Currently, the diagnosis of TTP is mainly based on the clinical manifestations of microangiopathic hemolytic anemia and thrombocytopenia without reliable specific laboratory golden criteria [5]. Although Furlan and Tsai et al. have confirmed the existence of severe vWF-cleaving protease (ADAMTS 13) activity deficiency in some TTP patients,implying that it may be used as a specific laboratory indicator for TTP diagnosis [1,2], the diagnostic value of the ADAMTS 13 activity assay remains controversial [6]. In China, few data on TTP are available regarding the clinical features of the disease or the routine laboratory tests obtained from studies of large size sample. In the present study we analyzed the clinical characteristics of 42 TTP patients admitted to our department from March 1998 to October 2010. In addition, ADAMTS 13 activities using the FRET-vWF86 substrate method were performed in 22 patients to assess its significance in general clinical practice. The value of the frequency of schistocytes in the peripheral blood film for TTP diagnosis was also evaluated in our study. Furthermore, T lymphocytic subpopulation assay was performed in 23 patients to better understand the pathogenesis of TTP based on immune dysfunction.

2. Materials and patients

2.1. Patients

Patients with TTP diagnosed between January 1998 and October 2010 were identified by a retrospective review of records of the Second Xiang-Ya hospital. Forty-two patients were included, 29 females and 13 males, with a median age of 39 years (range 22–71). The diagnostic criteria were based on: (1) thrombocytopenia ($<100 \times 10^9$/L) without other identifiable causes; (2) a negative Coombs' test and hemolytic anemia with schistocytes on the peripheral blood smear; and (3) Only those patients who fulfilled the criteria for TTP, both on presentation and through their clinical course, were included in this study. Other thrombotic microangiopathies were excluded, including DIC, cancer and preeclampsia. Routine laboratory tests such as peripheral blood cell counts, reticulocyte count, bone marrow aspirate, serum LDH, bilirubin, serum creatinine, direct Coombs' tests, coagulant mechanism examination and urinalysis were all performed.

2.2. Methods

2.2.1. Schistocyte count on peripheral blood smears

Peripheral blood smears were routinely prepared from all TTP patients. The frequency of the schistocyte in the blood film was obtained by counting 5000 red cells at 1000-power magnification.

2.2.2. ADAMTS 13 activity assay

ADAMTS 13 activity was determined in 22 TTP patients. Citrated platelet-poor plasma was prepared by centrifugation at 2800g for 15 min and stored at −80 °C until analysis. ADAMTS 13 activity was measured by a FRET (fluorescence resonance energy transfer) assay using FRET-vWF86 substrate (American Diagnostica Inc., Stanford, USA) as described [7] with some modifications. In brief, 100 mL of each diluted standard or patient sample was incubated at 37 °C in a 96-well white plate for 3 min. Then, 100 μL preheated ALEXA488-vWF86 FRET substrate was added into each well and the increase in fluorescence was measured in a varioskan microplate reader (Thermofisher, USA) equipped with a 485 nm excitation filter and 535 nm emission filter. Fluorescence was measured every 20 s interval for 20 min and the rate of change in fluorescence was calculated. A standard curve was constructed by plotting the rate of change in fluorescence for each standard sample versus the corresponding ADAMTS 13 activity. The ADAMTS 13 activity of the test samples was calculated against a standard curve.

2.2.3. T lymphocytic subpopulation assay

A T lymphocytic subpopulation assay, including CD3, CD4, CD8 cells, was performed in 23 TTP patients and 25 healthy controls on a FACS Calibur (BD, USA); the CD3/CD8/CD45/CD4 assay kit was purchased from BD Biosciences. Meanwhile, leukocyte differential counts were also performed; the CD4 and CD8 T cell absolute counts were calculated according to the lymphocyte absolute counts obtained by leukocyte differential count. The results were expressed as CD4 and CD8 T lymphocyte absolute count and CD4/CD8 ratio as per the manufacturer's instruction.

2.3. Statistical analysis

A Student's *t* test was used to determine the statistical difference of ADAMTS 13 activity before and after plasma exchange therapy in 9 patients with TTP and of the CD4 and CD8 T lymphocyte absolute counts and CD4/CD8 ratio between 23 TTP patients and 25 normal controls. All statistical treatments were performed using the SPSS 16.0 for windows. A P value of <0.05 was considered as significant.

3. Results

3.1. Clinical characteristics

In 42 patients with TTP, according to the potential etiology, the patients were allocated to various clinical categories (Table 1). TTP occurred in one patient after a severe fungus infection and in another one following oral