Eastern Journal of Medicine 15 (2010) 15-20

Original Article

Assessment of patients with thrombotic thrombocytopenic purpura

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Abstract. Thrombotic thrombocytopenic purpura (TTP) is a severe microvascular occlusive thrombotic microangiopathy characterized by systemic platelet aggregation, organ ischemia, profound thrombocytopenia, and fragmentation of erythrocytes. Unexplained occurrence of thrombocytopenia and anemia should prompt immediate consideration of the diagnosis and evaluation of peripheral blood smear for evidence microangiopathic hemolytic anemia. Excellent remission and survival rates were achieved by therapeutic plasma exchange. We reviewed characteristics and response rates to plasmapheresis of our TTP patients. A total of 25 cases were diagnosed. The parameters of hemoglobin and platelet were analyzed at presentation, as well as the number of plasmapheresis sessions and adjunctive treatment given. We found a response rate of 80 percent to plasma exchange. Response was better in 23 patients who presented with idiopathic TTP. Response was poor in patients with TTP secondary to underlying metastatic carcinoma. Two patients relapsed and one of the relapsed patients died. Plasmapheresis is mandatory and effective for primary TTP. Plasmapheresis may not be effective in all instances, especially if TTP is secondary to underlying disseminated cancer.

Key words: Thrombotic thrombocytopenic purpura, therapeutic plasmapheresis

1. Introduction

Thrombotic thrombocytopenic purpura (TTP) is a severe microvascular occlusive thrombotic microangiopathy characterized by systemic platelet aggregation, organ ischemia, profound thrombocytopenia and fragmentation of erythrocytes (1). TTP has a female predominance and a peak incidence in fourth decade. The disorder is uncommon. However, in recent decades its incidence has been increased (2,3). The presence of microangiopathic hemolytic anemia and thrombocytopenia is sufficient for the diagnosis of TTP, as not all other features are always present from the outset (4). In a study carried out by Ridolfi et al 74% of 258 patients with TTP had the triad consisting of anemia, thrombocytopenia, and neurologic disorders and only 40% of patients had the full pentad of features (5). Prior to the availability of effective treatment with plasma exchange, TTP had 90% fatal outcome. Plasma exchange had achieved a response rate of 81-96% thus the prompt diagnosis of TTP is vital (6). Although many cases are idiopathic; pregnancy, autoimmune diseases, infection, drugs, and bone marrow transplantation may be the underlying causes (7). Von-Willebrand factor-cleaving metalloprotease

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that degrades the multimers by cleaving peptide bonds in monomeric subunits of von-Willebrand factor at position 842-843 normally prevents the entrance of unusually large multimers of von-Willebrand factor into circulation. This metalloprotease named as ADAMTS 13 is a member of a family of zinc and calcium dependent proteases (1). Congenital absence of ADAMTS 13 is the main pathogenetic mechanism of familial TTP. In contrast to, patients with nonfamilial, idiopathic TTP have acquired absence of ADAMTS 13 activity, usually associated with IgG type autoantibody against the protease (8). Therefore, replenishing the missing ADAMTS 13 metalloprotease or removing the inhibitory antibodies is the vital role of the plasma infusion or therapeutic plasma exchange.

There is currently no published data from our region on therapeutic plasmapheresis for TTP. We want to share our experience of therapeutic plasmapheresis for the treatment of TTP in our hospital for the last eight years and we wish to make clinicians more alert of the presenting characteristics and treatment outcome of TTP.

2. Materials and methods

We performed an analysis of the adult patients with TTP that were admitted to Hospital of Dicle and Yuzuncu Yil University Medical Faculty. Diagnostic criteria for TTP were: 1- Microangiopathic hemolytic anemia (hemoglobin level < 12.5g/dL, direct antiglobulin test negative, 3 or more fragmented red cells or helmet cells per high-power field in the peripheral blood smear); 2- Thrombocytopenia (platelet count < $150x10^9$/L or 50% drop from the previous count); 3- No requirement for neurologic symptoms, renal function abnormalities, or fever. Idiopathic TTP was defined as TTP that occurred in patients with no apparent pre-existing illness. All patients were specifically asked about use of drugs reported to be associated with TTP. A clinical diagnosis of TTP was made by hematologist. Plasma exchange was initiated as soon as possible. Metil prednisolone 1 mg/kg was added all of the patients therapy. Administration of plasma exchange therapy was to initiate daily exchange of one plasma volume, using with fresh frozen plasma. Response to treatment was defined as the achievement of a platelet count higher than $150x10^9$/L during plasma exchange treatment or within 1 week after stopping treatment. Daily plasma exchange was typically ordered until the platelet count $150x10^9$/L or more for 3 consecutive days, after which plasma exchange was reduced in frequency or terminated. Lactate dehydrogenase (LDH) values in most patients had decreased to normal or nearly normal. Remission was defined as normal platelet count and no plasma exchange treatment for 30 days or more. Relapse was defined as the recurrence of TTP following a remission (9).

A total of 25 cases were diagnosed during the period of study. The parameters of hemoglobin and platelet counts were analyzed at presentation, as well as the number of plasmapheresis sessions and adjunctive treatment given.

3. Results

Twenty five patients were included into the study. Seventeen (68%) patients were women and other 8 (32%) were men. Female/male ratio was 2.1/1 and mean age of the patients was 38.5±11.3 years (range 16-67 years). Thrombocytopenia, antiglobulin test-negative hemolytic anemia, and schistocytosis in the peripheral blood smear examination were detected in all patients. The average serum hemoglobin and platelet count on initiation of plasmapheresis were 7.3 g/dL (range 4.9-9.7 g/dL) and $27x10^9$/L (range 11-68/L), respectively. Fifteen (60%) patients had renal impairment determined by raised serum creatinine and two patients subsequently required renal replacement therapy including dialysis support. Fever was present in all patients at admission or follow up, and the highest temperature at presentation was 39˚C. Abdominal pain and nausea and/or vomiting were detected in 11 (44%) and 8 (32%) patients respectively at the time of presentation. None of the patients had diarrhea or a history of suspected medication. Neurologic findings including headache, cranial nerve palsies, dysphasia or aphasia, paresis, confusion, stupor, coma and seizures were present in 21 (84%) patients. TTP secondary to malignancy was detected in two patients. TTP was diagnosed at the 20^{th} day subsequent to chemotherapy in one patient. The other one had no history of chemo or hormonal therapy. The remaining 23 cases were idiopathic. Association of TTP and hyperthyroidism was observed in one patient. Diagnosis of hyperthyroidism due to Graves' disease was established on the basis of physical examination and laboratory findings including fine tremor, moisty skin, exopthalmos, diffuse goitre on thyroid ultrasonography and low thyroid stimulating hormone and high free thyroid hormone levels.

Average performed sessions of plasmapheresis was 11 (range 3-30). All patients were supported with fresh frozen plasma (FFP) before the initiation of plasma exchange. Two patients with

Table 1. Demographic, clinical and laboratory characteristics of patients

Mean age, year (min, max)	38.5±11.3 (16-67)
Sex % Female, % Male	F 68%, M 32%
Female / Male ratio	2.1/1
Triggering factors	2 patients with disseminated adenocarcinoma, 23 cases were idiopathic
Drug usage before 20 days	1 patient used combination chemoterapy (CEF)*
Median platelet count	27 X 10^9/L (range 11- 68)
Median hemoglobin	7.3 g/dL (range 4.9- 9.7)
Neurologic abnormalities**	21 (84%)
Fever	25 (100%)
Renal dysfunction	15 (60%)
Abdominal pain	11 (44%)
Nause and vomiting	8 (32%)
Full Pentad	13 (52%)
Clinical outcomes	
Response	20 (80%)
Median number of plasma exchange	11 (3-30)
Relapse	2 (0.8%)
Death	6 (24%)

*CEF Cyclophosphamide, Epirubicin, 5 Fluorouracyl
**Neurologic abnormalities Headache, cranial nerve palsies, dysphasia, or aphasia, paresis, confusion, stupor, coma, and seizures.

breast cancer died during their initial hospitalization. Three patients with idiopathic TTP had no response to plasmapheresis and steroid therapy. Complete remission was achieved in 20 (80%) patients. Remission was sustained in 18 of 20 patients. Two patients relapsed. The first one was in the 22nd month and the second in 49th month of the remission. The first case died despite plasmapheresis. Immunosuppressive drugs were not used or splenectomy was not performed. Demographic, clinical and laboratory characteristics of patients were shown in table-1.

4. Discussion

TTP is a rare, life threatening disseminated thrombotic microangiopathy characterized by a microangiopathic hemolytic anemia, thrombocytopenia, fever, neurologic symptoms, and renal abnormalities (1). The annual incidence in United States estimated at 4 to 11 cases per million people and has a peak incidence in fourth decade (2). TTP is more common in women than men, and among adults; female gender, black race and obesity are associated with an increased risk of TTP (10). In our population, it was 2,1 times more frequent in females. Case series of patients with TTP consistently report a female predominance of 60 to 70 percent. The significance of this entity is unknown. This female predominance may be consistent with an autoimmune etiology (11). Before the availability of effective therapy, the diagnosis of TTP was based on the progressive appearance of the following pentad of clinical features: microangiopathic hemolytic anemia, thrombocytopenia, neurologic and renal abnormalities, and fever. However, recognition of the efficacy of plasma exchange therapy meant that less stringent diagnostic criteria were required to allow a more rapid initiation of treatment (10). The diagnosis of TTP is appropriately suspected in patients who were presented with acute and severe thrombocytopenia and microangiopathic hemolytic anemia, without any another explanation (4-12). Mainstay of TTP treatment is rapid and aggressive plasmapheresis. Prompt plasmapheresis is vital because a response rate varies with the initiation time of therapy. Higher rate of mortality was observed when the plasmapheresis initiated subsequent to the first 24 hours (13). In our small series, 13 patients (52%) developed the classic pentad. The clinical outcomes tend to be poorer for the patients presented with full pentad of manifestations. Treatment is now strongly advocated, even when

patients present with only the aforementioned dyad of manifestations (14).

The pathophysiological mechanisms for TTP were further elucidated recently. Until the 1980s, the pathogenesis for TTP remained very unclear although numerous candidates like endothelium, platelets or plasma proteins were suggested to participate in the triggering of the disease. The role of von Willebrand (vWf) factor, a plasma multimeric protein essential for platelet adhesion and aggregation at the high shear rates of blood flow present in the micro vessels. There is abnormally large vWf multimers in the plasma of TTP patients. These hyper adhesive ultra large multimers of vWf were suspected to be directly responsible for spontaneous platelet clumping in the microcirculation leading to ischemic visceral dysfunction. A plasma protease cleaves and decreases the size of these large multimers to their normal size. This enzyme was identified as the 13th member of the ADAMTS family of metalloproteases. The most adult patients with acute TTP had a severe functional deficiency of ADAMTS 13 in plasma (< 5% of the activity of a normal pooled plasma), and most often related to inhibitory IgG autoantibodies (7, 15). TTP usually occurs in previously healthy people. Infections, malignancies, medications and autoimmune diseases are known to precipitate TTP (3). In a significant number of cases, the syndrome is associated with autoimmune disorders, including systemic lupus erythematosus (16), scleroderma (17), mixed connective tissue disease (18), polymyositis (19), antiphospholipid syndrome (20). In our study consisting of 25 patients with TTP, one patient had Graves' disease (GD) of whom the remission was achieved by 15 sessions of plasmapheresis. In our screening, no other report mentioning to the association of GD and TTP was detected in English literature. Both of the disorders have an autoimmune basis and usually accompany to other autoimmune disease, thus it was suggested that this association was not accidental. Although immune thrombocytopenic purpura and pernicious anemia must be initially considered in the event that thrombocytopenia accompanies to Graves' disease, TTP is likely in the presence of microangiopathy. Our two patients with TTP had bone marrow metastasis of breast cancer. TTP was detected in the 20th day of initiation of chemotherapy (cyclophosphamide and epirubicine) in one subject. The other one had no history of chemo or hormonal therapy. Chang et al. reported a case of chemotherapy (cyclophosphamide plus doxorubicine) associated TTP (21). However, leukoerytroblastosis and bone marrow metastasis at the presentation cause the confusion of whether the thrombotic microangiopathy due to chemotherapy or widespread carcinoma metastasis. Their second case had irregular and short duration of tamoxifen therapy.

Appearance of thrombotic microangiopathy consisting of leukoerytroblastosis and diffuse fragmented erythrocytes attributed to metastatic carcinoma. In cancer patients, at least two causes for thrombotic microangiopathy have been identified. One of them is the complication of chemotherapy, and the other is the manifestation of cancer itself without any relationship to chemotherapy (21). Chemotherapy associated thrombotic microangiopathy is presumed to result from direct toxicity to the endothelium (8). The mechanism of cancer associated TTP is not clear. Bone marrow metastasis of cancer with myelofibrosis is suspected to be the main feature associated with the development of TTP. In the literature, reported cases of TTP in cancer have been associated with severe anemia and advanced cancer (21). The pathogenesis of cancer associated TTP remains poorly understood. Probably, 1- tumor cell emboli could generate endothelial damage with platelet agregation. TTP is considered to be caused by mechanical fragmentation of red blood cells traversing the injured microvasculature (22); 2- immune complexes with platelet aggregating properties were found (23); 3- Since TTP has been seen mostly in adenocarcinoma, this pathology could be another contributing factor, perhaps related to the production of mucin, which may exert a direct detrimental effect on the pathologic endothelial cell to change endothelial function (21); 4- In some patients, in the presence of very high vWf levels, mild deficiency of protease may be a pathogenetically relevant factor for the onset of the tumor associated TTP (24). Treatment of malignancy associated TTP is arduous and usually does not respond to plasma exchange. The contribution of ADAMTS 13 deficiency in TTP associated with cancer is controversial. Literature review indicates that only three of eight cases of TTP related to disseminated cancer have a suboptimal level of ADAMTS 13, which may explain the poor response to the plasma exchange (25). Two of our TTP patients associated with breast cancer. Both of these did not respond to plasma exchange therapy, and died.

Plasma exchange has dramatically improved the survival in TTP. Before plasma exchange treatment was used, 90% of patients with TTP died. Now, approximately 80% of patients

survive (7). This figure is comparable to our local response rate of %80. It was more than 80% when only our patients with idiopathic TTP were considered. Longer delay in initiating plasma exchanges, presence of stupor or coma, and higher creatinine levels at the beginning of plasma exchanges were independent predictors of treatment failure (13). The rationale is that plasma exchange will have only a temporary effect on the presumed autoimmune basis of the disease and additional immunosuppressive treatment may cause a more durable response. The use of glucocorticoids in such patients is based on clinical experience and case series, although other case series have reported similar outcomes without the use of glucocorticoids. For patients who require additional treatment to have remission, small case series have suggested a benefit with more intensive immunosuppressive therapy with rutiximab, cyclophosphamide, vincristine, or cyclosporine. Clinical trials are lacking to guide the use of immunosuppressive agents (10).

Sign and symptoms of TTP occurring after more than 30 days of complete remission on no plasma exchange treatment are appropriately termed a relapse (7). In our study, two patients relapsed and both of them were female and had no association of pregnancy, autoimmunity or medication. According to the results of Canadian Apheresis Group within 10 years follow up, 36% of patients with TTP relapsed and relapses may occur in the 8th year of remission (26). The risk for relapse is related to clinical category of TTP. Relapses are rare in patients who present during pregnancy or after delivery or who have an autoimmune disorder, suggesting a distinction from idiopathic TTP (7). In patients with severe deficiency of ADAMTS 13 activity; half of such patients may have a relapse, most within a year. In patients with drug associated TTP, relapses occur only when the drug is taken again (7, 10).

In conclusion, to the best of our knowledge, our report is the first to describe the prevalence and response to plasma exchange therapy of patients with TTP. Rapid diagnosis and prompt initiation of plasma exchange is vital for the treatment of TTP. Plasmapheresis may not be effective in all instances, especially in the TTP secondary to underlying disseminated cancer, the response may be minimal or not. It is recommended that all cancer patients presenting with unexplained anemia and thrombocytopenia be evaluated not only for bone marrow metastasis, but also for co-existence with secondary TTP. We hope that this article served to physicians to make an early diagnosis and prompt referral of patients suspected of this disorders.

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