Thrombotic thrombocytopenic purpura: a review of the literature in the light of our experience with plasma exchange

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Introduction

Thrombotic thrombocytopenic purpura (TTP), a disease characterised by disseminated thrombotic microangiopathy associated with haemolytic microangiopathic anaemia, was described for the first time by Eli Moscowitz in 1925 as an "acute febrile pleiochromic anaemia with hyaline thrombosis of the terminal arterioles and capillaries"[1]. The disease is now better understood from a pathophysiological point of view even though its rarity (annual incidence of 11.3 cases per 1,000,000 population)[2] and the lack of specificity of the signs, symptoms and laboratory findings make its management difficult. The symptoms, as stated, are non-specific: fever, renal dysfunction (to the point of acute renal failure in some cases), fluctuating neurological disorders (mild headache, onset of behavioural anomalies, transient sensory and motor deficits, coma), possible ischaemic gastrointestinal complications (abdominal pain) and retinal detachment. More than 35% of patients do not have neurological symptoms at onset; fever and renal dysfunction are present in only a small minority of cases. The diagnosis can, therefore, be made in the presence of a microangiopathic haemolytic anaemia (with schistocytes in a peripheral blood smear), thrombocytopenia (from platelet consumption) and increased levels of lactate dehydrogenase (LDH) not due to other identifiable causes[3,4].

In 1982, as a result of the need to aid the differential diagnosis in these cases, Moake *et al.*[5] observed that the pathogenesis of TTP is closely related to von Willebrand factor (VWF) since there were unusually large or ultralarge multimers of VWF (ULVWF) in the circulation of patients with TTP whereas these multimers were not present in patients in remission or in healthy controls. Moake hypothesised that the patients with TTP lacked a protease capable of cleaving the ULVWF multimers to prevent the intravascular formation of thrombi. Only in 1996, drawing on the independent observations of Tsai[6] and Furlan[7], there was an understanding of the relationship with a metalloprotease whose lack or inhibition plays a key role in the pathology of TTP[8,9]: ADAMTS 13, "a disintegrin-like and metalloprotease with thrombospondin repeats".

In conditions of high shear stress in the blood, the ULVWF multimers secreted by activated endothelial cells are anchored as filaments to the molecules of P-selectin exposed on the surface of the activated endothelium. ADAMTS13 regulates the length and, therefore, the thrombogenic potential of the VWF by binding to the accessible A3 domains of the VWF and breaking down the ULVWF multimers by cleaving the peptide bond between the Tyr1605-Met1606 residues in the A2 domain. As a consequence of a lack of ADAMTS13 (activity $\leq$50%), ULVWF multimers are not broken down after being secreted by endothelial cells, but remain anchored to the endothelium. Platelets passing close by adhere through GpIb and the GpIIb/IIIa complex to the A1 and A3 domains of the monomeric subunits of the ULVWF filaments anchored to the P-selectin and form large, occlusive thrombi.

In congenital forms of TTP mutations have been found in the gene for ADAMTS 13 (located on chromosome 9q34). More than 70 mutations have been identified so far: 60% are missense mutations, while the other 40% are nonsense, frameshift or splicing mutations[10,11]. The disease has an autosomal recessive mode of inheritance and is usually, but

not always, manifested at birth or during infancy. The congenital cases are extremely rare (incidence 1:1,000,000) and account for a small percentage (5%) of all cases of TTP. More frequently the disease is manifested in adults as secondary TTP (in haematopoietic stem cell transplant recipients, in pregnant women, in patients with autoimmune diseases, human immunodeficiency virus infection or cancer[12]) or as idiopathic TTP characterised in 70-80% of cases by the presence of IgG autoantibodies (mainly IgG4) capable of inhibiting the enzymatic function of ADAMTS13 in various ways. IgG4 block the proteolytic activity of ADAMTS13 with regards to VWF, increasing the clearance of ADAMTS13 from the bloodstream, or can interfere with the interactions between ADAMTS13 and cells or other plasma proteins. The biological function of the IgG is strongly dependent on their specificity, affinity and subclass: IgG4 are produced mainly after a prolonged period of antigenic stimulation and for this reason the abundant production of IgG4 anti-ADAMTS13 autoantibodies suggests a condition of chronic antigenic stimulation of the immune system when the mechanisms of systemic immune tolerance fail[14].

From a transfusional point of view, the interest in TTP is based on the possibility of treating patients with plasma exchange (PEX) with fresh-frozen plasma. Since the introduction of this treatment in the 1970s, the natural history of TTP has changed radically, with 70-80% of patients obtaining a complete remission (compared to a mortality rate of 90% in untreated patients)[12]. The problem with the treatment of TTP is that although 80% of patients have a complete response to therapy *(responders),* about 20% of patients relapse after successful treatment of an acute episode or are refractory to PEX with persistent thrombocytopenia and high levels of LDH after a complete cycle of treatment (at least 7 days) of PEX (*refractory or non-responders or partial responders*). The Canadian Apheresis group found that 36% of patients with TTP relapsed during a 10-year follow-up[2,4,13].

Our experience

In a 2-month period (May - July 2011) the therapeutic apheresis group at the Service of Transfusion Medicine of "Paolo Giaccone" Hospital in Palermo observed and used PEX to treat four cases of acquired TTP attending the Haematology Unit of the same hospital.

After a careful clinical evaluation, the diagnosis of TTP was made on the basis of signs of microangiopathic haemolysis, a negative Coombs' test, decreases in haemoglobin concentration and platelet count, an increase in the number of reticulocytes, the presence of schistocytes in a peripheral blood smear, and increases in the values of total bilirubin and serum activity of LDH. The diagnosis was not, however, supported by the search for anti-ADAMTS13 antibodies or an evaluation of the activity of this enzyme.

All the patients were treated with the same therapeutic regimen which, in accordance with the guidelines from the American Society for Apheresis[15], consisted of daily PEX with the exchange of at least one plasma volume. The replacement fluid, which was fresh-frozen plasma and physiological saline in a ratio of 2:1, enabled 100% of the plasma volume to be exchanged in all four patients. At least nine exchange sessions were performed before achieving a normal platelet count ($\geq 150 \times 10^9$/L) and LDH levels ($\leq$300 IU/L) on 3 consecutive days. Furthermore, each patient was treated with adjuvant corticosteroids (methylprednisolone 1 mg/Kg/die)[4,15]. The transfusion therapy consisted of infusion of red cell concentrates and platelets to enable the placement of a central venous catheter without running the risk of haemorrhage during its insertion. After normalisation of the platelet count, the PEX was suspended for clinical observation and laboratory studies. The patients were discharged only after their platelet counts and LDH levels had remained stable for 1 week (Table I).

The observation of so many patients in such a short time stimulated our group to evaluate the patients' response to treatment, in terms of normalisation of platelet count, levels of LDH and haemoglobin concentration, during and immediately after the PEX therapy.

Case 1

A 29-year old secundipara (blood group O positive, phenotype Rh CCDee, kk) was referred to us because of a first relapse of TTP. The diagnosis had been made 18 months earlier during the woman's second pregnancy (38th week), when she was admitted to hospital because of anaemia and worsening thrombocytopenia with diffuse petechiae on the abdomen and lower limbs. On that occasion, treatment was started with steroids and an infusion of fresh-

Table I - Clinical and laboratory features of the four patients with TTP.

	Case 1	Case 2	Case 3	Case 4
Age (years)	29	50	54	31
Type of TTP	Relapsed TTP	Acute TTP	Acute TTP	Post-partum TTP
Blood group	O CCDee	A CcDEe	A CcDEe	A CcDEe
Disease onset	Anaemia, thrombocytopenia and petechiae of lower limbs	Anaemia, thrombocytopenia and meno-metrorragia	Anaemia, thrombocytopenia and petechiae of lower limbs	Anaemia, thrombocytopenia and paraesthesia
Laboratory parameters at onset	Hb 8.3 g/dL; Plt $5x10^9$/L; LDH 3669 IU/L	Hb 8.4 g/dL; Plt $20x10^9$/L; LDH 2180 IU/L	Hb 7.4g/dL; Plt $23x10^9$/L; LDH 1274 IU/L	Hb 9 g/dL; Plt $28x10^9$/L; LDH 1588 IU/L
PEX	9 exchange sessions (2,000 mL plasmasafe)	10 exchange sessions (2,200 mL plasmasafe)	9 exchange sessions (2,000 mL plasmasafe)	11 exchange sessions (2,200 mL plasmasafe)
Plasma volume exchanged (%)	100%	100%	100%	100%
Transfusion treatment	4 units of RBC, 23 units of FFP, 2 units of Plt from apheresis	2 units of RBC, 20 units of FFP, 1 unit of Plt from apheresis	1 unit of RBC, 27 units of FFP	2 units of RBC, 16 units of FFP, 2 units of Plt from apheresis
Adjuvant treatment	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone
Start of rituximab treatment (day of admission)	day 26	day 17	no	no
Laboratory parameters at discharge	Hb 10.5 g/dL; Plt $92x10^9$/L; LDH 475 IU/L	Hb 10 g/dL; Plt $163x10^9$/L; LDH 440 IU/L	Hb 11.1 g/dL; Plt $183x10^9$/L; LDH 279 IU/L	Hb 11.4 g/dL; Plt $122x10^9$/L; LDH 241 IU/L

frozen plasma: the woman had a complete response which enabled her pregnancy to be concluded with a delivery by Caesarean section. Following discharge from hospital, the patient had monthly follow-up assessments for 6 months with apparent resolution of the disease. The relapse started with a fever (lasting 1 week) with considerable weakness followed by the appearance of petechiae on the lower limbs and pathognomonic laboratory evidence of recurrent disease (platelet count $5x10^9$/L, Hb 8.3 g/dL) together with increased indices of haemolysis and renal function (total bilirubin 3.91 mg/dL, LDH 3669 IU/L, AST/ALT 48/31 IU/L, creatinine 1.7 mg/dL), a negative Coombs' test and six to seven schistocytes per field in a peripheral blood smear.

A therapeutic protocol of PEX was started immediately and, in relation to her own plasma volume, the patient's plasma was exchanged with 2,000 mL of fresh-frozen plasma/die (percentage of plasma volume exchanged: 100%) for 9 days until her platelet count and haemoglobin concentration reached levels compatible with resolution of the dyscrasia and haemolysis (platelets $\geq 140x10^9$/L, Hb $\geq$10 g/dL, total and direct bilirubin $\leq$1mg/dL).

Eight days after suspension of PEX the disease recurred with a new decrease in the platelet count (platelets $52x10^9$/L), so the patient was treated with rituximab 600 mg (Figure 1).

Case 2

A 50-year old woman (blood group A Rh positive, phenotype CcDEe Kk) with a euthyroid nodular goitre in menopause for 1 year was referred to us because of progressive weakness, ecchymoses and an episode of marked meno-metrorrhagia. Laboratory tests showed anaemia (Hb 9.5 g/dL), thrombocytopenia (platelets $20x10^9$/L) and indices of haemolysis (LDH 2,180 IU/L, total bilirubin 3.36 g/dL), five to six schistocytes per field in a peripheral blood smear and a negative Coombs' test compatible with the diagnosis of TTP. The patient had no neurolological disorders or neuropsychological alterations.

The patient started treatment with PEX, in which her plasma was exchanged with 2,200 mL of fresh-frozen plasma/die (percentage of plasma volume exchanged: 100%) in ten sessions at the end of which she had a good recovery of her blood count and normalisation of indices of haemolysis. After the sixth session of plasmapheresis the patient

suddenly developed fever (39 °C), high levels of transaminases (ALT 224 IU/L) and a new decrease in platelet count, so she was started on treatment with rituximab 600 mg. After a few hours high levels of transaminases associated with a fever were again recorded levels and for this reason the treatment was suspended. Only 6 days later was reactivation of a previous cytomegalovirus (CMV) infection clearly demonstrated (CMV IgM negative, CMV IgG positive, CMV pp65 positive, CMV DNA positive) and specific antiviral treatment was started (valaciclovir hydrocholoride) (Figure 2).

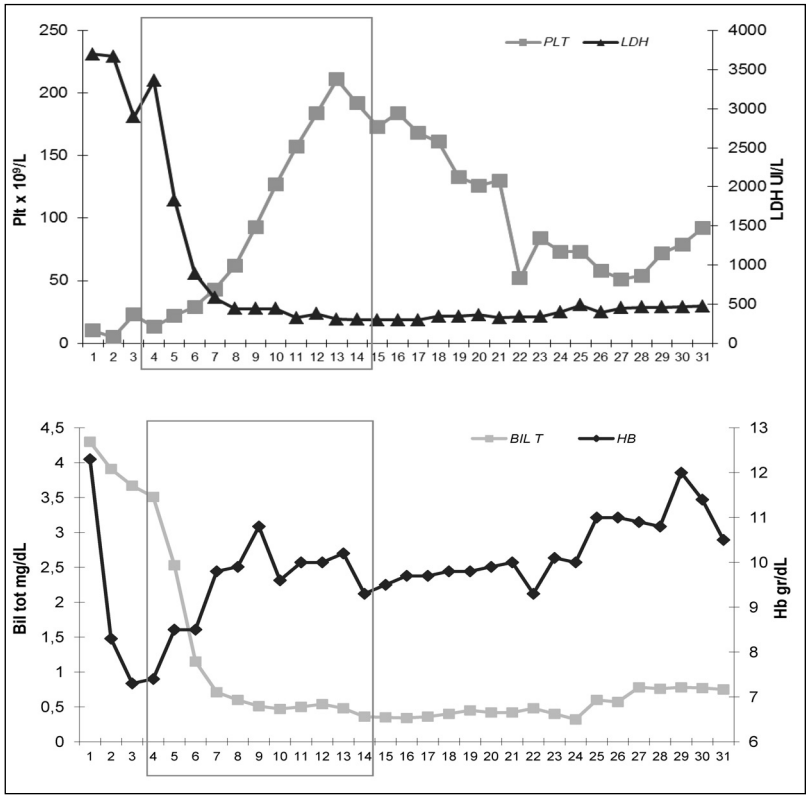

Figure 1 - First relapse of TTP: trend in platelet count (Plt), haemoglobin concentration (Hb) and levels of total bilirubin (Bil Tot) and lactate dehydrogenase (LDH) during treatment with plasma exchange (box): the lack of a platelet response is clear.

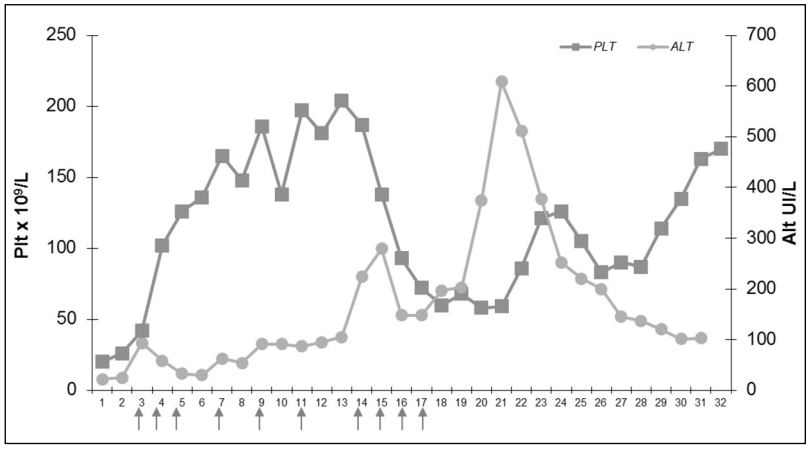

Figure 2 - Trends in platelet count (Plt) and transaminase concentration (ALT) from the day of admission and in relation to the plasma exchange sessions (arrows) in a patient with cytomegalovirus infection.

Case 3

The third case was a 54-year old woman (blood group A Rh positive CcDEe kk) with an approximately 20-year history of Raynaud's phenomenon for which she is regularly treated in the Day Hospital with iloprost, a synthetic analogue of prostacyclin I with vasodilating, antioxidant and anti-aggregant activity. During the woman's last planned admission to the angiology ward, in which she should have received her treatment and follow-up, she was seen to have evident manifestations of cutaneous bleeding (petechiae on the lower limbs, ecchymoses in the gluteal region, haematuria) with clear laboratory evidence of anaemia (Hb 7.4 g/dL), thrombocytopenia (platelets 23x10^9/L) and haemolysis (total bilirubin 1.39 g/dL; LDH 1,274 IU/L). There were six to seven schistocytes per field in the peripheral blood smear. The Coombs' test was negative. These findings were immediately compatible with a diagnosis of TTP, despite the absence of neurological disorders or neuropsychological disturbances, and PEX treatment was started with the aim of exchanging the patient's plasma with 2,000 mL of fresh-frozen plasma/die (percentage of plasma volume exchanged: 100%). At the end of the cycle of nine sessions, the patient had a good blood count recovery and complete resolution of haemolysis (Hb 11.1 g/dL; platelets 183x10^9/L; LDH 279 IU/L) (Figure 3).

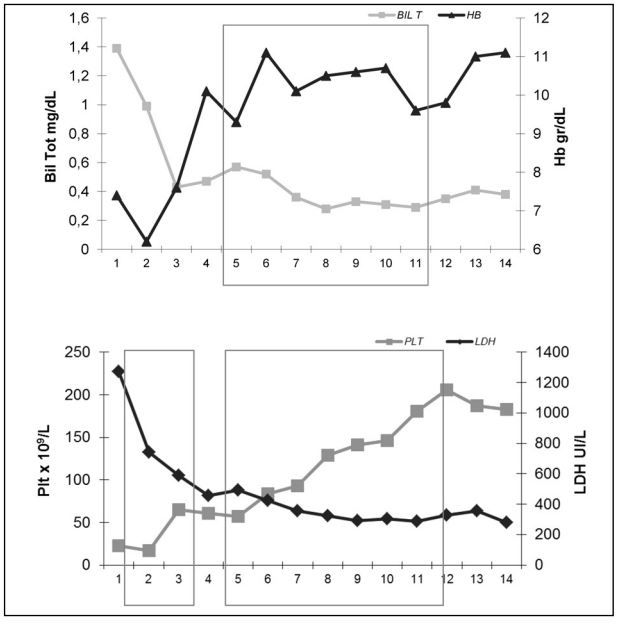

Figure 3 - Trends in indices of haemolysis, thrombocytopenia and anaemia in a patient with Raynaud's phenomenon who responded to treatment with plasma exchange (sessions of plasma exchange carried out on the days shown in the box).

Case 4

A 31-year old pluripara (blood group A Rh positive with phenotype CcDEe kk) was referred to us because 1 month after her last delivery she developed notable weakness with even modest efforts often associated with recurrent paraesthesia of the upper limbs. At the time of her admission the clinical findings and laboratory results -anaemia (Hb 9 g/dL), thrombocytopenia (platelets 28x10^9/L), signs of haemolysis (total bilirubin 2.13 g/dL; LDH 1,588 IU/L)- together with the presence of three to four schistocytes per field in a peripheral blood smear suggested a diagnosis of post-partum TTP. The patient was treated with 11 sessions of PEX in which her plasma was exchanged with 2,000 mL of fresh-frozen plasma/die (percentage of plasma volume exchanged: 100%); she was also given adjuvant cortisone therapy. During the first sessions of PEX the woman had an evident neurological disorder (dysarthria) which resolved completely as treatment was continued.

At the end of the treatment the patient had a clear recovery of platelet count (platelets 154x10^9/L), an improvement of the picture of haemolysis (total bilirubin 0.30 g/dL; LDH 230 IU/L) and resolution of anaemia (Hb 11 g/dL) (Figure 4).

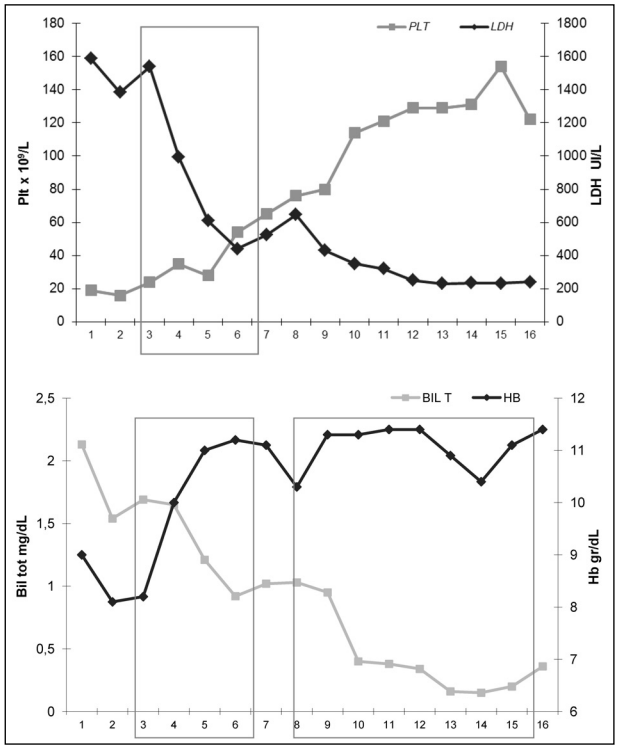

Figure 4 - Trends in laboratory parameters in a patient with post-partum TTP who responded to treatment with plasma exchange.

Discussion

The cases observed highlighted some particularly interesting clinical aspects. During a careful evaluation of the pharmacological history of case 1, it emerged that 2 months prior to the onset of the relapse, the patient had spontaneously assumed dietary supplements containing chitosan, a non-acetylated or partially deacetylated linear polysaccharide derived from chitin (which is found in nature in the exoskeleton of crustaceans, in the cuticle of insects and in the cell walls of fungi) associated with succinic acid[16]. Although a strong relationship between these molecules and the appearance of TTP has not been described in the literature, it is known that chitosan acts *in vitro* as a modulator of the activation and adhesion of platelets such that already a few years ago its haemostatic effect in heparinised mice was noted, confirming the fact that its activity does not depend on the interference with the coagulation cascade, but rather from an interaction with the elements of primary haemostasis[17]. Subsequent studies demonstrated that chitosan-dependent platelet activation causes the release of growth factors contained in the α-granules of platelets and this stimulus could, therefore, be potentially useful for activating platelet-rich plasma, by-passing the allergic and prothrombotic effects of the thrombin normally used for this purpose[18]. In addition, a recent study indicated that succinate could also be an independent platelet activator[19]. It can, therefore, be hypothesised that, in the presence of a pathological condition predisposing to the formation of thrombi, a stimulus of this type could trigger relapse of the disease.

Case 2, in contrast, showed an interesting association between TTP and CMV infection. As recently observed[20] there may be relationship (albeit not well defined) between infections (including those by CMV) and TTP, particularly as regards response to therapy and the possibility of relapses or exacerbations. The mechanism of action of this is unclear but it is possible that infections together with other inflammatory stimuli could alter the delicate equilibrium between VWF and the activity of ADAMTS13. Infection-induced vascular activation could increase the release of ULVWF through the effects of interleukin-8 and tumour necrosis factor-α, while interleukin-6 inhibits the cleavage of ULVWF[21]; on the other hand, sepsis causes a decrease in the activity of ADAMTS13 both through its cleavage by proteolytic enzymes in the bloodstream and through its reduced synthesis in the liver[22]. Furthermore, it is possible that, as for other micro-organisms, the immunogenic stimulation of the infection could cause a cross-reaction with anti-ADAMTS13 antibodies, reducing the activity of the protein and thereby limiting the response to therapy[21,23]. Furthermore, in the specific case of CMV, this virus can infect megakaryocytes directly and, therefore, reduce the production of platelets[24]. Indeed, we observed a proportional, inverse relationship between platelet count and transaminase levels in our patient (Figure 2).

As far as concerns case 3, Raynaud's phenomenon, the clinical expression of a transitory ischaemic crisis caused by vasoconstriction of the digital arteries, precapillary arterioles and cutaneous arteriovenous shunts in response to cold or emotion, can present as a primary condition or secondary to systemic sclerosis. The pathogenesis of this phenomenon, although not completely understood, may involve dysregulation of mechanisms of neuroendothelial control and the presence of intravascular anomalies such as platelet activation, reduced fibrinolysis, activation of white blood cells, reduced deformability of red blood cells, high oxidative stress due to accumulation of free radicals and the possible presence of anti-endothelium antibodies[25]. In particular, platelet activation plays a key role in the natural history of the disease. Indeed, during systemic sclerosis, the α-granules of platelets release greater quantities of mediators with pleiotropic activities able to influence the natural history of the disease in various ways because they act on vascular tone and pro-inflammatory mechanisms as well as stimulating fibrogenesis and angiogenesis. In particular, high levels of vascular endothelial growth factor (VEGF) have been found in the serum of patients with systemic sclerosis. This growth factor, besides being involved in endothelial cell regeneration (stimulating their survival, growth, permeability and migration) is also involved in the regulation of inflammatory processes. The angiogenic activity and stabilisation of the vessel wall carried out by VEGF are expressed through interacvtions with other mediators present in the endothelium such as platelet-derived growth factor, transforming growth factor-β and angiopoietin[26]. Between 1966 al 2010, 16 cases of systemic sclerosis associated with TTP were reported in the literature, one of which in a pregnant woman[27-40]; although the pathophysiological

mechanism underlying this association is not known, based on the foregoing, it can be hypothesised that the platelet activation present in systemic sclerosis could be a valid substrate for exposing a latent thrombotic state related to a functional or quantitative lack of ADAMTS13; furthermore, the presence of IgG autoantibodies against ADAMTS13[14] is indicative of a relationship with autoimmune diseases, of which systemic sclerosis is one.

The last case observed (case 4) focused our attention on the association between TTP and pregnancy. The incidence of pregnancy-associated TTP is 1:25,000 pregnancies and this form of TTP accounts for about 10% of all cases of TTP. The disease can appear *de novo* during the pregnancy or as a reactivation of a previously known TTP triggered by the pregnancy because of the presence of placental proteins in the bloodstream capable of inducing the production of anti-ADAMTS13 antibodies[41]. Unfortunately, these clinical pictures often overlap with pre-eclampsia/eclampsia and HELLP and so the diagnosis is missed. From a careful review of published data on pregnancy-associated TTP (from which cases of pre-eclampsia/eclampsia and HELLP were excluded) from 1955 to 2006, Martin *et al*. showed that the mean gestational age of onset of TTP is late: among the patients affected by ante-partum TTP, 55.5% developed the disorder in the second trimester of pregnancy (28.9±8.3 weeks), 32.8% during the third trimester (38.5±1.9 weeks) and only 11.7% in the first trimester. TTP in the puerperium is less common (12.7%) and occurs a mean of 4 days after delivery, although the range is from 0 to 42 days. However, there are no significant differences in the outcome of patients who develop TTP ante-partum or post-partum with regards to either laboratory findings or maternal mortality (ante-partum 25.8%, post-partum 23.8%)[42] and in both cases PEX (better if associated with adjuvant cortisone therapy) is the treatment of choice (Figure 4).

The type of response in the days immediately following suspension of PEX can be evaluated in the light of the cases observed. Patients with a first episode of disease are defined "responders" if they achieve normalisation of platelet count ($\geq 150x10^9$/L) and LDH ($\leq$300 IU/L), an increase in haemoglobin ($\geq$9.5 g/dL) and clinical resolution of neurological symptoms (when present) and signs of microangiopathy (ecchymoses, petechiae, haematomas) 3 days after the last treatment; as "partial responders" if they have a clear improvement in clinical and laboratory findings but a platelet count between $75x10^9$/L and $150x10^9$/L; and as "non-responders or refractory" if the platelet count remains $\leq 75x10^9$/L with or without neurological disturbances and/or persistence of signs of microangiopathic damage. The patient with a relapse of TTP was evaluated using the same criteria but extending the time of observation to 8 days after the last treatment in order to assess whether a relapse occurred after suspension of treatment (Figures 5 and 6).

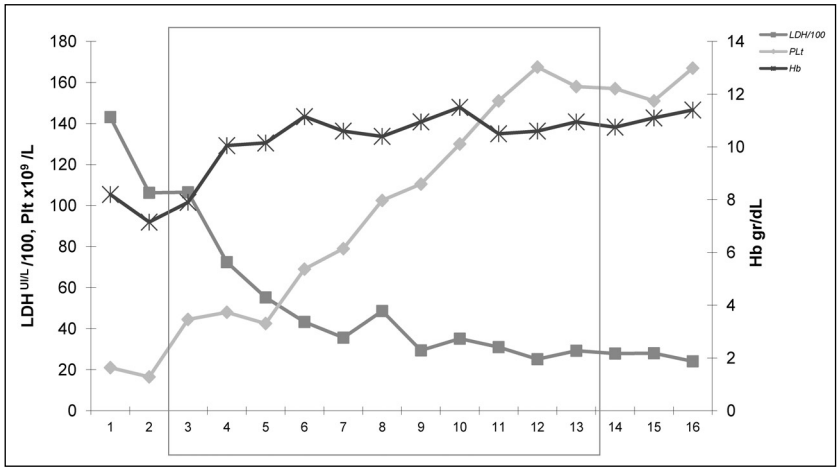

Figure 5 - Mean trends in laboratory parameters - mean platelet count (Plt), mean lactate dehydrogenase concentration (LDH) and mean haemoglobin concentration (Hb) - in patients responding to treatment (plasma exchange sessions in the box).

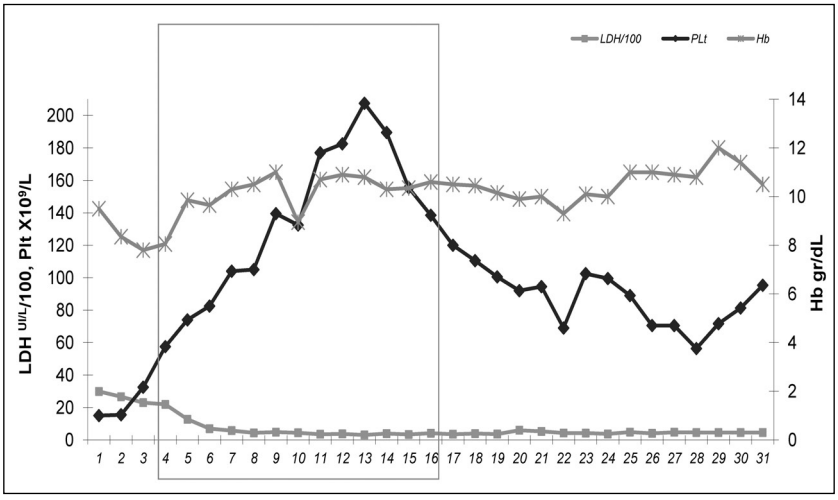

Figure 6 - Mean trends in laboratory parameters - mean platelet count (Plt), mean lactate dehydrogenase concentration (LDH) and mean haemoglobin concentration (Hb) - in patients not responding to treatment (plasma exchange sessions in the box). There is a clear drop in platelet count immediately after suspension of the treatment.

In only two patients (cases 1 and 2) thrombocytopenia returned brusquely, after significant increases in platelet counts (reaching values ≥200x10^9/L) during PEX treatment, such that the counts fell again to values considerably below those established as the cut-of for defining non- responders.

As mentioned, the underlying causes of these decreases in platelet count differed in the two patients. In case 1, after the first 7 days in which the platelet count was stable, the count began to decrease rapidly probably due to an early relapse of the disease. During this period the patient started treatment with rituximab (on day 26) which produced a slow recovery of the platelet count and level of haemoglobin (Figure 7). In contrast, case 2 showed an abrupt drop in circulating platelet count already during the last sessions of PEX contemporaneously with a brusque, fast rise in ALT due to CMV-associated hepatitis (Figure 2). In reality, this patient, rather than being a true non-responder, should be considered to have had a complicated form of TTP in which the normalisation of the platelet count occurred not only in response to

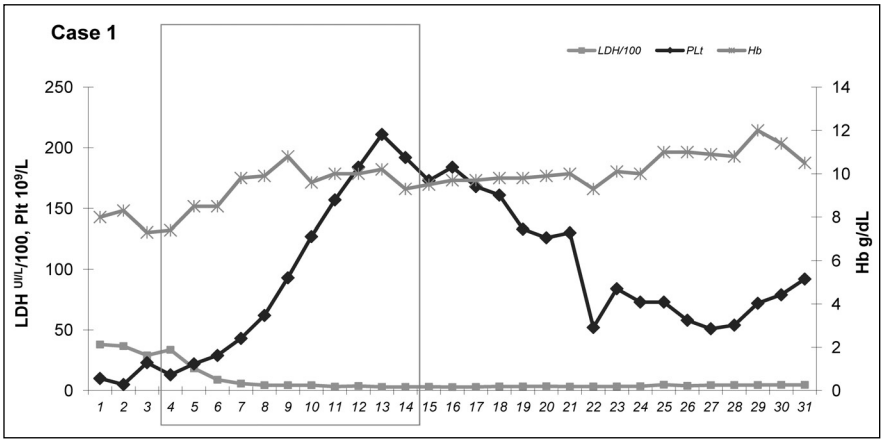

Figure 7 - Trends in platelet count (Plt), lactate dehydrogenase concentration (LDH) and haemoglobin concentration (Hb) in a patient with a relapse of TTP not responding to treatment (plasma exchange sessions in the box) (case 1). There is clear drop in the platelet count a few days after suspension of treatment and a persistently low count until the patient's discharge from hospital.

the administration of rituximab (on day 17) but also because of the subsequent introduction of antiviral therapy (valaciclovir hydrochloride administered on day 23) (Figure 8).

The possibility of classifying the patients' outcome as a complete response or relapse will depend on their clinical wellbeing and laboratory results in a follow-up lasting at least 12 months.

One further consideration should be made concerning the patients' blood groups. In recent studies on the correlation between TTP and blood group[43] it has been seen that group O patients are underrepresented among patients with TTP, leading to the hypothesis that such individuals are partially protected against the development of TTP. This hypothesis is based on various observations made during comparisons of O group and non-O group individuals: subjects with blood group O have lower levels of VWF, faster clearance of VWF, faster proteolysis of VWF by ADAMTS13 and an inverse relationship between levels of plasma activity of ADAMTS13 and VWF. All these factors make group O subjects better protected against the development of TTP because they have less VWF available (the key protein in the aetiopathogenesis of TTP) and what is present is broken down more quickly. This protection from TTP is in line with previous observations that group O individuals have a degree of protection from myocardial, cerebral and peripheral vascular thrombosis[44].

Terrel *et al.*[45], who studied a larger cohort of patients (281 cases of TTP from 1995 to 2009 recruited through the Oklahoma TTP Registry) relating blood group with circulating ADAMTS13 levels, showed that in actual fact when there was a severe deficiency of ADAMTS 13, the frequency of blood group O was significantly higher than expected, whereas when the reduction of ADAMTS 13 was not severe, the frequency of group O individuals was lower than that of subjects with non-O groups (as previously found). The blood group characteristics are, therefore, probably only significant if ADAMTS13 function is not severely compromised.

The series of patients we studied was very small and did not allow us to make hypotheses on statistically significant correlations with the ABO system since quantitative, qualitative and genetic assays of the ADAMTS 13 profile were not conducted. However, we note that 75% of our patients had a non-O blood group and the only patient with group O was treated for TTP in relapse, an indicator of greater aggressiveness of the disease. This observation is in line with the previous considerations.

Conclusions

TTP, which is characterised by thrombocytopenia and microangiopathic haemolytic anaemia, was a fatal condition until the introduction, in 1970, of PEX, a treatment which has radically changed the natural history of this disease, reducing the mortality from

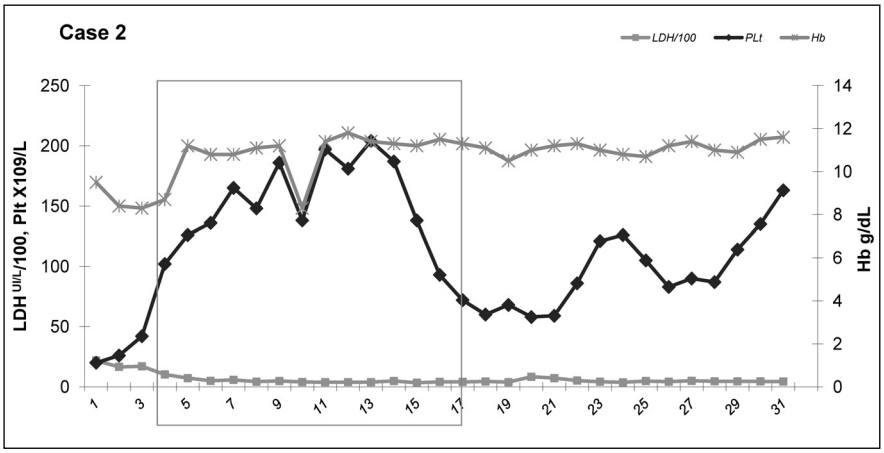

Figure 8 - Trends in platelet count (Plt), lactate dehydrogenase concentration (LDH) and haemoglobin concentration (Hb) in a patient with cytomegalovirus-associated hepatitis and TTP not responding to treatment (plasma exchange sessions in the box) (case 2). The platelet count fell during treatment and returned to optimal levels after successful antiviral therapy.

more than 90% to about 10-20%[12]. The mainstay of the treatment of acute TTP is daily PEX, exchanging the patient's plasma with fresh-frozen plasma or plasma lacking cryoprecipitate, which should be started within 24-48 hours of the appearance of the disease because it has been shown that a delay in initiation of therapy could be one of the factors responsible for treatment failure[4]. The duration of treatment required to obtain a remission varies greatly: the mean number of sessions necessary is about nine, since premature suspension or a single session of PEX can be associated with an exacerbation of the disease[4]. Furthermore, it is empirically recommended that exchange treatment is continued for at least 2 days after the achievement of complete remission, in accordance with guidelines that call for treatment continuation until platelet counts reach values $\geq 150x10^9$/L and the levels of LDH return to within the normal range for 2-3 consecutive days. However, LDH, which is rapidly removed during PEX, does not reflect the response to treatment and even the possible persistence of some schistocytes in the peripheral blood smear, in the absence of other clinical and laboratory features, is not a contraindication to treatment suspension[4]. The guidelines of the American Society of Apheresis propose daily PEX treatment in TTP with a grade 1A recommendation (*strong recommendation, high-quality evidence*)[15].

The value of plasma therapy was already demonstrated some years ago in a prospective, randomised trial comparing PEX and plasma infusions in the treatment of adults with TTP[46]: the 6-month survival rate was 78% in the group treated with PEX and 63% in that given plasma infusions, with this difference in favour of PEX being statistically significant (p=0.036). Nowadays the standard treatment for TTP is PEX with fresh-frozen plasma at a dose of 40 to 60 mL/Kg daily, started within 24 hours of the diagnosis of the disease and continued for at least 2 days after the complete remission in which the patient achieves a normal platelet count and LDH level in the absence of neurological symptoms. Only in the case that PEX cannot be performed is treatment with plasma infusions indicated: the dose of the fresh-frozen plasma infusion is at least 30 mL/Kg daily[4]. In secondary forms, patients who are refractory to PEX and relapse are candidates for second-level therapy with splenectomy, vincristine, azathioprine and also rational use of immunosuppressant drugs (corticosteroids, cyclophosphamide and cyclosporine) but above all with rituximab, a monoclonal chimeric antibody directed against CD20 (expressed on the surface of B lymphocytes), which rapidly depletes blood, lymph nodes and bone marrow of B cells. The rationale for using this drug in the treatment of TTP is its capacity to destroy the CD20+ B lymphocytes which produce anti-ADAMTS13 antibodies even if, in autoimmune diseases, the mechanism of action is not limited to depletion of B cells, as demonstrated by recent clinical studies in which the levels of autoantibodies were not significantly reduced during the treatment. In addition to the direct effect of the drug on B lymphocytes (depletion and consequent reduced function of cells presenting the antigen, release of inflammatory and immunomodulatory cytokines and co-stimulation signals), it has been proposed that the bond between rituximab and opsonised B cells blocks the function of the Fc receptor of macrophages. This block is able to reduce the splenic sequestration of platelets in TTP[13,47,48].

More recently another drug, defibrotide, has acquired growing importance. This single-stranded polydeoxyribonucleotide derived from porcine mucosa (by controlled depolymerisation) has potent antithrombotic, anti-ischaemic and anti-inflammatory effects with thrombolytic properties without systemic anticoagulant effects. Defibrotide interacts with the receptor for adenosine and leads to the selective release of prostaglandins I2 and E2 with consequent inhibition of platelet function and activation, reduces the activity of plasminogen activator inhibitor-1 and potentiates the function of tissue plasminogen activator[49,50].

Patients with idiopathic TTP generally respond better to treatment with PEX than do patients with secondary TTP.

Keywords: thrombotic thrombocytopenic purpura, ADAMTS 13, plasma exchange therapy.

Acknowledgments

Claudia Rizzo is PhD student at the Molecular Medicine PhD course (directed by Calogero Caruso) at Palermo University and this work is submitted in partial fulfillment of the requirement for her PhD degree.

The Authors declare no conflicts of interest.

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Arrived: 4 October 2011 - Revision accepted: 5 December 2011
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