The thrombotic microangiopathies

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Historical notes

Thrombotic thrombocytopenic purpura (TTP) was described for the first time by Moschowitz who, in 1924, published a case report of a 16-year old girl with a pentad of symptoms comprising fever, anaemia, thrombocytopenia, focal changes in the central nervous system and in the kidneys. The patient died after two weeks and a post-mortem examination revealed the presence of disseminated hyaline thrombi in the small vessels of the main organs. Thirty-one years later, the nephrologist Gasser described the case of a child affected by haemolytic anaemia and thrombocytopenia, microvascular thrombosis and signs of severe renal damage. This pathologic condition, which was similar to TTP but differed by the prevailing renal symptoms, was called haemolytic uraemic syndrome (HUS). It was clear from the start that the anaemia and the thrombocytopenia were direct consequences of mechanical destruction of red blood cells and platelet consumption in the microcirculation: thrombocytopenia was caused by massive intravascular platelet aggregation, while anaemia was caused by the passage of red blood cells through small vessels partially obstructed by thrombi, leading to their fragmentation and development of intravascular haemolysis.

The subsequent description of numerous cases of patients with TTP and HUS highlighted the difficulty in making a clear distinction between the two syndromes. Although consumption thrombocytopenia

Cenni storici

La porpora trombotica trombocitopenica (TTP) è stata descritta per la prima volta da Moschowitz che, nel 1924, pubblicò il caso di una giovane donna di 16 anni con una pentade sintomatica costituita da febbre, anemia, piastrinopenia, alterazioni focali del sistema nervoso centrale e del rene. La paziente morì dopo due settimane e l’autopsia rivelò la presenza di tromboci getti disseminati nei piccoli vasi dei principali organi. Trentuno anni più tardi, il nefrologo Gasser descrisse il caso di un bambino affetto da anemia emolitica e trombocitopenia, trombosi microvascolare e segni di grave danno renale. Questa patologia, simile alla TTP ma differente per la prevalenza di sintomatologia renale, venne denominata sindrome emolitico-uremica (HUS). Fu chiaro fin dall’inizio che l’anemia e la trombocitopenia erano la diretta conseguenza della distruzione meccanica di eritrociti e del consumo di piastrine a livello del microcircolo: la piastrinopenia era provocata da un’aumentata aggregazione intravascolare, mentre l’anemia era provocata dal passaggio delle emazie attraverso i piccoli vasi parzialmente ostruiti da trombi che ne determinavano la frammentazione con sviluppo di emolisi intravascolare.

La successiva osservazione di numerosi casi di pazienti affetti da TTP e HUS evidenziò la difficoltà di una netta distinzione tra le due sindromi. Sebbene la trombocitopenia da consumo e l’anemia emolitica microangiopatica fossero le principali caratteristiche di entrambe, la prevalenza di sintomi neurologici o renali non era un criterio sufficientemente discriminante per distinguere la TTP dalla HUS o viceversa. Fu così che nel 1952 Symmers propose il
and microangiopathic haemolytic anaemia were the main features of both, whether neurological or renal symptoms prevailed was not a sufficiently discriminatory criterion for distinguishing TTP from HUS or vice versa. Thus in 1952 Symmers proposed the unifying term of thrombotic microangiopathy (TMA): TTP and HUS would therefore represent two different clinical manifestations of TMA. Another term that has been widely used is the all-embracing TTP/HUS.

The pathogenesis of the main feature of TMA, the microvascular thrombosis due to increased platelet aggregation remained unknown until the beginning of the 1980s, when Joel Moake observed that the plasma of patients with TTP contained very high molecular weight, so-called ultralarge (UL) multimers of von Willebrand factor (VWF), a multimeric adhesion glycoprotein contained in endothelial cells, platelets and plasma. Once released from the abnormally stimulated endothelial, these UL forms of VWF, present in the endothelium but not found in the plasma in physiological conditions, directly promote intravascular aggregation of the platelets and the consequent microvascular thrombosis due to increased platelet adhesion glycoprotein contained in endothelial cells, platelets and plasma. Once released from the abnormally stimulated endothelial, these UL forms of VWF, present in the endothelium but not found in the plasma in physiological conditions, directly promote intravascular aggregation of the platelets and the consequent microvascular thrombosis due to increased platelet aggregation remained unknown until the beginning of the 1980s.

The observation of very low levels of ADAMTS13 in the plasma of patients with TTP, but not in those with HUS, challenged Symmer's unifying theory of TMA or TTP/HUS, and led to the paradigm according to which TTP is always associated with a lack of

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ADAMTS13. However, the ongoing debate has been fuelled by observations that not all cases diagnosed as TTP have low or immeasurable levels of ADAMTS13\(^5\)\(^-\)\(^8\), as well as by the fact that the levels of plasma ADAMTS13 are also reduced in a series of other conditions besides thrombotic microangiopathies\(^9\)\(^,\)\(^10\), although in none of these conditions are the levels of ADAMTS13 as low or indetectable as they are in the majority of cases (60-70%) of TTP\(^5\)\(^-\)\(^8\). Furthermore, despite the fact that most cases diagnosed as HUS have normal plasma levels of ADAMTS13 (in particular the cases manifested as haemorrhagic diarrhoea following intestinal infections with *E. coli*), there are incontrovertible cases of HUS, called atypical or diarrhoea-negative, in which the levels of ADAMTS13 are notably reduced or undetectable\(^6\)\(^,\)\(^11\). However, for the purposes of this review, focusing particularly on TTP, we shall maintain the distinction between the two conditions and shall not use the unifying terms TMA and TTP/HUS.

**Thrombotic thrombocytopenic purpura**

TTP is a rare disease, its incidence among the general population being 5-10 cases per year per million people. It affects both sexes, although the incidence is 2-3 times higher among females, as is often the case with autoimmune-type diseases\(^12\)\(^-\)\(^14\). There are two different forms of TTP: congenital TTP, caused by mutations in the *ADAMTS13* gene (which is located on chromosome 9q34 and codes for the metalloprotease), is inherited as an autosomal recessive condition and is often, but not exclusively, manifested at birth or during childhood\(^15\)\(^-\)\(^17\). The congenital cases are extremely rare (incidence 1:1,000,000) and represent a small percentage of the cases of TTP. The acquired forms can basically be distinguished into two types: immune-mediated forms, due to autoantibodies against ADAMTS13\(^3\)\(^,\)\(^3\,\)\(^18\)\(^-\)\(^20\); and those probably secondary to massive endothelial stimulation with consequent release of UL VWF multimers in amounts exceeding the system's ability to degrade them, despite the presence of normal or only slightly reduced levels of ADAMTS13\(^21\). Both these pathogenic situations are usually triggered by concomitant factors which cause widespread endothelial activation. The most common physiological or pathological conditions present in the case epidemiology. The ongoing debate has been fuelled by observations that not all cases diagnosed as TTP have low or immeasurable levels of ADAMTS13\(^5\)\(^-\)\(^8\), as well as by the fact that the levels of plasma ADAMTS13 are also reduced in a series of other conditions besides thrombotic microangiopathies\(^9\)\(^,\)\(^10\), although in none of these conditions are the levels of ADAMTS13 as low or indetectable as they are in the majority of cases (60-70%) of TTP\(^5\)\(^-\)\(^8\). Furthermore, despite the fact that most cases diagnosed as HUS have normal plasma levels of ADAMTS13 (in particular the cases manifested as haemorrhagic diarrhoea following intestinal infections with *E. coli*), there are incontrovertible cases of HUS, called atypical or diarrhoea-negative, in which the levels of ADAMTS13 are notably reduced or undetectable\(^6\)\(^,\)\(^11\). However, for the purposes of this review, focusing particularly on TTP, we shall maintain the distinction between the two conditions and shall not use the unifying terms TMA and TTP/HUS.

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**Porpora trombotica trombocitopenica**

La TTP è una malattia rara la cui incidenza nella popolazione generale è di 2-10 casi all'anno per milione di persone; nonostante colpisca entrambi i sessi, l'incidenza è 2-3 volte maggiore nel sesso femminile, come solitamente si osserva per la patologie di natura autoimmune\(^12\)\(^-\)\(^14\). Esistono due forme diverse di TTP: la TTP congenita è dovuta a mutationi a carico del gene *ADAMTS13* (che è sito sul cromosoma 9q34 e codifica per la metalloproteasi), si trasmette come carattere autosomico recessivo e si manifesta prevalentemente, ma non esclusivamente, alla nascita o durante l'infanzia\(^15\)\(^-\)\(^17\). I casi congeniti sono molto rari (incidenza 1:1.000.000) e rappresentano una minima percentuale (circa il 5%) dei casi di TTP. Le forme acquisite sono sostanzialmente distinguibili in due tipi: quelle immunomediate, dovute alla presenza di autoanticorpi diretti contro il gene *ADAMTS13* (che è sito sul cromosoma 9q34 e codifica per la metalloproteasi), si trasmette come carattere autosomico recessivo e si manifesta prevalentemente, ma non esclusivamente, alla nascita o durante l'infanzia\(^15\)\(^-\)\(^17\). I casi congeniti sono molto rari (incidenza 1:1.000.000) e rappresentano una minima percentuale (circa il 5%) dei casi di TTP. Le forme acquisite sono sostanzialmente distinguibili in due tipi: quelle immunomediate, dovute alla presenza di autoanticorpi diretti contro l'ADAMTS13\(^3\)\(^,\)\(^3\,\)\(^18\)\(^-\)\(^20\); e quelle verosimilmente secondarie ad una stimolazione endoteliale massiva, a cui consegue il rilascio di multimeri di VWF UL in quantità superiore a quella sostenibile dal sistema di degradazione, pur in presenza di livelli normali o modestamente ridotti di ADAMTS13\(^21\). Entrambi queste situazioni patogenetiche sono in genere scatenate da fattori concomitanti che determinano attivazione endoteliale diffusa. Nelle forme immunomediate, spesso associate a livelli molto bassi o assenti di ADAMTS13 (inferiore al 10% del normale), le più frequenti condizioni   122  Blood Transfus 2005; 3: 120-35  PM Mannucci et al.
immune-mediated forms, which are often associated with very low or undetectable levels of ADAMTS13 (less than 10% of the normal), are pregnancy, infections, autoimmune diseases, and the use of drugs such as ticlopidine and clopidogrel. The most frequent concomitant conditions associated with the forms with normal or only slightly reduced levels of ADAMTS13 (levels greater than 10%) are metastatic tumours, organ transplantation (particularly allogeneic bone marrow transplantation and solid organ transplants) and the use of drugs such as cyclosporine, mitomycin and α-interferon.

In most cases TTP occurs as a single, sporadic acute episode, but there are chronic recurrent forms (20-30% of the cases). The chronic recurrent forms may have a genetic basis or be associated with the formation of autoantibodies, whereas the forms associated with malignancy or transplantation present as acute episodes with a low propensity to recur (in part because of the high mortality rate).

Pathogenesis of TTP

In conditions of high shear blood flow, the UL multimers of VWF are anchored in string-like formations by the P-selectin exposed on the surface of activated endothelium (Figure 1)22,23. A lack of ADAMTS13 protease in plasma, either in absolute terms or relative to the increased need to cleave UL VWF5,6, triggers massive intravascular aggregation of platelets caused by the UL VWF, which in turn leads to the development of microthrombi (Figure 2)22,23. As described, the two main mechanisms responsible for the lack of ADAMTS13 in TTP are mutations in the ADAMTS13 gene and the presence of autoantibodies that either neutralise ADAMTS132,3 or bind to the protein causing its removal from the circulation18-20, although there is some evidence suggesting that mechanisms other than VWF and ADAMTS13 can also be involved in the pathogenesis of TTP (Table II). There are, however, numerous observations that contrast with the above described simplified model, such as the fact that a deficiency of ADAMTS13 is not seen in all patients with TTP, despite a clinically unequivocal diagnosis based on the presence of thrombocytopenia, haemolytic anaemia and neurological symptoms. Furthermore, although UL multimers of VWF play a key role in the development of TTP, these multimers are not observed in the plasma of all patients with TTP6,8. Nor
Figure 1 - Mechanisms of interaction at the level of the endothelial cells between ultralarge (UL) von Willebrand factor (VWF), P-selectin and ADAMTS13. Panels A-D show how UL VWF released from the endothelial cells is anchored by P-selectin (this, too, released by endothelial cells) and modelled by the high shear flow into strings. ADAMTS13 in turn binds, mainly through the A1 and A3 domains, to the strings of UL VWF and cleaves the UL forms, preventing the deposition of platelets and the formation of thrombi.

Figure 2 - Mechanisms of thrombocytopenia and anaemia in thrombocytopenic thrombotic purpura
A- In physiological conditions, the endothelial cells are not abnormally activated and therefore the ultralarge (UL) multimers of von Willebrand factor (VWF) are mainly present within the cells themselves. The circulating blood contains platelets, red cells and ADAMTS13 in its inactive form.
B- In pathological conditions the endothelial cells are activated by various stimuli and release greater quantities of UL VWF multimers. In the presence of normal levels of ADAMTS13, these multimers are cleaved efficiently, so only "normal" multimers circulate in plasma.
C- In the absence of ADAMTS13 (or when the concentrations of ADAMTS13 are not sufficient to cleave the increased amounts of UL VWF multimers released by the activated endothelial cells), UL VWF aggregates platelets within the vessels causing thrombi which block blood flow (see also figure 1). The red cells passing through the thrombi become fragmented and form schistocytes.
is it yet clear why patients with a deficiency of ADAMTS13, whether acquired or congenital, only manifest the disease sporadically. Finally, the agents triggering the acute episode have not yet been identified.

**Clinical symptoms and laboratory investigations**

TTP occurs predominantly in healthy individuals (acute idiopathic form), although in many cases it can be associated with some physiological and pathological conditions (Table I). The simultaneous presence of intravascular consumptive thrombocytopenia and Coombs' negative haemolytic anaemia caused by mechanical red cell fragmentation is essential in order to make the diagnosis. The platelet count in the acute phase is very low (<20x10^9/L) and is often, but not always, associated with signs of haemorrhage such as petechiae, ecchymoses and purpura. The signs of haemolytic anaemia due to red cell fragmentation are the presence of schistocytes on a peripheral blood smear, increased serum levels of indirect bilirubin, and reduced or absent haptoglobin, with a direct Coombs' test. A high serum level of lactate dehydrogenase (LDH), as a result of both haemolysis and tissue necrosis caused by ischaemic damage, is an excellent diagnostic indicator and parameter for monitoring the evolution of the disease. There are not usually major alterations in coagulation or fibrinolysis. The most frequently reported neurological symptoms are coma, convulsions and motor deficits, associated with headache, visual disorders and an altered mental state, although neurological symptoms are not always present and tend to occur particularly in the more advanced stages of the disease. There are also changes in renal function, with moderately increased levels of serum creatinine, di ADAMTS13, acquisita o congenita, manifestano la malattia solo sporadicamente, e soprattutto non sono ancora stati identificati gli agenti scatenanti l'episodio acuto.

**Sintomi clinici ed esami di laboratorio**

La TTP si manifesta prevalentemente in soggetti sani (forma acuta idiopatica), ma, come abbiamo visto, in molti casi può essere associata ad alcune condizioni fisiologiche e patologiche (Tabella I). La presenza contemporanea di piastrinopenia da consumo intravascolare e di anemia emolitica Coombs negativa da frammentazione eritrocitaria meccanica è essenziale per porre la diagnosi. La conta piastrinica nella fase acuta è notevolmente ridotta (<20x10^9/L) ed è spesso, ma non sempre, associata alla presenza di segni emorragici, quali petecchie, ecchimosi e porpora. Segni di anemia emolitica da frammentazione eritrocitaria sono la presenza di schistociti nello striscio di sangue periferico, reticolocitosi, incremento dei valori sierici di bilirubina indiretta, riduzione o assenza di aptoglobina. Il test di Coombs diretto è negativo.

Elevati livelli sierici di latticodeidrogenasi (LDH), conseguenti sia all'emolisi che alla necrosi tissutale indotta dal danno ischemico, sono un ottimo parametro per la diagnosi e il monitoraggio della malattia.

Non sono di solito presenti importanti alterazioni della coagulazione e della fibrinolisi. I sintomi neurologici più frequentemente riportati, peraltro non costantemente e soprattutto nelle fasi più avanzate della malattia, sono coma, convulsioni e deficit motori, associati a cefalea, disturbi visivi, atassia e alterazioni dello stato mentale. Sono presenti inoltre alterazioni della funzionalità renale con livelli moderatamente aumentati di creatinina sierica, microematuria e

### Table I - Conditions associated with thrombotic thrombocytopenic purpura

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<th>Condition</th>
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<tr>
<td>Pregnancy and puerperium</td>
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<td>Infections (particularly HIV)</td>
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<tr>
<td>Drugs (quinidine, ticlopidine, clopidogrel, cyclosporine A, interferon α,</td>
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<tr>
<td>statins, mitomycin C, cisplatin, gemcitabine)</td>
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<tr>
<td>Metastatic tumours</td>
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<tr>
<td>Allogeneic bone marrow and solid organ transplants</td>
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<tr>
<td>Autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis,</td>
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<tr>
<td>scleroderma)</td>
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<tr>
<td>Major surgery (particularly heart surgery with extracorporeal surgery)</td>
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### Table II - Possible mechanisms of pathogenesis in TTP other than or complementary to ADAMTS13 and ultralarge (UL) von Willebrand factor (VWF)

<table>
<thead>
<tr>
<th>Mechanism</th>
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<tbody>
<tr>
<td>Activation of endothelial cells</td>
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<tr>
<td>Overexpression of P-selectin</td>
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<tr>
<td>Decrease of prostacyclin</td>
</tr>
<tr>
<td>Apoptosis of endothelial cells</td>
</tr>
<tr>
<td>Activation/aggregation of platelets</td>
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<tr>
<td>Presence of aggregating agents other than UL VWF</td>
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<tr>
<td>Presence of platelet-aggregating proteases such as calpain and cathepsins</td>
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*Blood Transfus 2005; 3: 120-35*
microhaematuria and proteinuria. The renal symptoms are usually less severe than those in HUS, and are more frequently reversible.

**Differential diagnoses**

TTP is a differential diagnosis of HUS and of other syndromes characterised by the presence of thrombocytopenia and microangiopathic haemolytic anaemia. Typical HUS is distinguished from TTP by the former's prodromic diarrhoea and much more severe renal symptoms.

The so-called atypical cases of HUS are not easily distinguished from TTP, although renal symptoms tend to prevail over neurological ones. Other conditions characterised by thrombosis in the microcirculation, which may be considered in the differential diagnosis of TTP, are disseminated intravascular coagulation (DIC), antiphospholipid antibody syndrome (APS), eclampsia, pre-eclampsia and the HELLP syndrome.

DIC is distinguished from TTP by the marked increase in fibrinogen degradation products and/or D-dimer, hypofibrinogenemia and prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT). Pre-eclampsia and eclampsia are distinguishable because of the considerable changes in coagulation and fibrinolysis manifested by very greatly increased levels of PAI-1, reduced protein C and antithrombin, and increased D-dimer.

Finally, very marked rises in serum transaminases allow the HELLP syndrome to be differentiated from TTP. Some cases of so-called "catastrophic" APS present in a way similar to TTP with microvascular thrombosis, haemolytic anaemia and severe neurological symptoms. However the presence of antinuclear and antcardiolipin antibodies and lupus anticoagulant allows the correct diagnosis to be made.

None of the pathological conditions described above is associated with a severe deficiency in plasma levels of ADAMTS13: thus, a complete lack of the protease for VWF clearly directs the diagnosis towards TTP. As noted before, normal or moderately reduced levels of ADAMTS13 do not help in the differential diagnosis, since they can be found in both TTP and in atypical HUS, but also in other pathological and physiological conditions (Table III).
Treatment

The therapeutic strategies used in the management of TTP involve the use of plasma infusions and/or plasma exchange. These treatments, introduced many decades ago in the absence of controlled trials, reduced mortality from about 80-90% to 20-25%. In 1991, the results of a prospective randomised clinical trial demonstrated the greater efficacy of plasma exchange over that of plasma infusion: the response rate to plasma exchange was 78% and the mortality rate was 22%; the corresponding values for plasma infusion were 49% and 37%, respectively. The description of the key role of UL VWF in the pathogenesis of TTP and the subsequent identification of ADAMTS13 clarified the mechanisms leading to the therapeutic efficacy of plasma treatment: this procedure removes the anti-ADAMTS13 antibodies in the secondary, immune-mediated forms and replaces the lack of the protease in the congenital forms. It has been demonstrated that a delay in starting this treatment (beyond 24 hours) can compromise its efficacy. It is, therefore, important to make the diagnosis quickly and start treatment with plasma as soon as a clinical diagnosis of TTP is made because of the presence of haemolytic anaemia, consumptive thrombocytopenia without any other apparent cause and an increase in serum LDH levels. Assays of ADAMTS13 and the identification of autoantibodies are not currently considered requisites for starting treatment, although there are data indicating that cases with normal or moderately reduced levels of ADAMTS13, which are often associated with organ transplantation and metastatic tumours, have a poorer prognosis. Data concerning a relationship between the titre of autoantibodies to ADAMTS and prognosis are contradictory and inconclusive. Table IV describes

### Table III - Physiological and pathological conditions other than those for TTP associated with slightly or moderately reduced levels of ADAMTS13

<table>
<thead>
<tr>
<th>Condition</th>
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<tr>
<td>Old age</td>
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<td>Neonatal period</td>
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<td>Pregnancy</td>
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<td>Tumours</td>
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<td>HELLP syndrome</td>
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<td>Cirrhosis of the liver</td>
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<tr>
<td>Chronic inflammatory states</td>
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<tr>
<td>Post-operative period</td>
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<tr>
<td>Uraemia</td>
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<tr>
<td>Autoimmune disorders (lupus, scleroderma)</td>
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### Terapia

I regimi terapeutici impiegati nella cura della TTP prevedono l’utilizzo di infusione di plasma e/o plasma exchange (PE). Tali terapie, introdotte da molti decenni senza uno studio controllato, hanno ridotto la mortalità da 80-90% a 20-25%. Nel 1991, i risultati di uno studio clinico prospettico randomizzato hanno dimostrato la superiorità dell’efficacia del PE sull’infusione di plasma, con una risposta del 78% e una mortalità del 22% con il PE rispetto ad una risposta del 49% ed una mortalità del 37% con la sola infusione di plasma. La descrizione del ruolo chiave del VWF UL nella patogenesi della TTP e la successiva identificazione dell’ADAMTS13 hanno chiarito i meccanismi dell’efficacia terapeutica del trattamento plasmatico: questa procedura, infatti, rimuove gli anticorpi anti-ADAMTS13 nelle forme secondarie immunomediata e supplisce alla carenza della proteasi nelle forme congenite. È dimostrato che un ritardo nell’inizio di questo trattamento (oltre le 24h) può comprometterne l’efficacia. È dunque importante eseguire una diagnosi precoce ed iniziare il trattamento con plasma non appena ci sia una diagnosi clinica di TTP per la presenza di anemia emolitica, piastrinopenia megacariocitica senza altra causa apparente ed incremento di LDH sierica. Il dosaggio dell’ADAMTS-13 e la ricerca degli autoanticorpi non sono attualmente considerati requisiti necessari per iniziare la terapia, anche se vi sono dati che indicano che i casi caratterizzati da livelli normali o moderatamente ridotti di ADAMTS13, che si associano in genere a trapianti d’organo e a tumori metastatici, hanno una prognosi più sfavorevole. Sono invece contrastanti e non conclusivi i dati riguardanti il rapporto fra il titolo degli autoanticorpi anti-ADAMTS e prognosi.

La tabella IV descrive i passaggi principali nella terapia della TTP. Per quanto riguarda la terapia plasmatica, non esistono indicazioni sul numero di sedute di PE necessarie per ottenere la remissione della fase acuta: è raccomandabile eseguire una seduta giornaliera di PE con lo scambio di 3-5 litri di plasma fino all’ottenimento di remissione della sintomatologia neurologica se questa è presente, di una conta piastrinica stabile superiore a 150 x 10^9/L, di una normalizzazione dei livelli sierici di LDH e della correzione dell’anemia. È inoltre raccomandata la prosecuzione del trattamento giornaliero con PE per
Table IV - Main steps in the treatment of TTP

1- Clinical diagnosis (presence of thrombocytopenia, haemolytic anaemia with schistocytes, raised levels of serum LDH, possible fluctuating neurological symptoms)
2- Plasma infusion (30mL/kg), until the start of plasma exchange
3- Plasma exchange (3-5L/day), until the platelet count exceeds 150x10^9/L for at least 3 days in the presence of normal serum levels of LDH
4- Additional treatments: prednisone 1.0-1.5 mg/kg/day
5- Red blood cell transfusions

the main steps in the treatment of TTP. As far as concerns plasma therapy, there are no indications on the number of sessions of plasma exchange necessary in order to obtain a remission of the acute phase: it is advisable to carry out daily sessions of plasma exchange with an exchange of 3-5 litres of plasma until achieving remission of neurological symptoms, if these are present, a stable platelet count above 150 x 10^9/L, normalisation of serum levels of LDH and correction of the anaemia. It is also recommended that daily treatment with plasma exchange is continued for at least 3 days after the remission has been obtained. There are reports of genetically-based TTP in which prophylactic treatment of recurrent episodes has been given, based on the administration of plasma (30 mg/kg) at regular intervals (every 5-7 days). There are no firm data supporting the efficacy of this strategy, nor are there data on the optimal frequency of administering plasma. Plasma that has been submitted to viral inactivation methods should be preferred for the plasma exchange, in order to reduce the risk of blood-borne infections.

An array of immunosuppressive treatments has been proposed in association with plasma exchange for the treatment of acquired forms of TTP due to autoantibodies. These proposed treatments include corticosteroids, intravenous immunoglobulins, splenectomy, cytotoxic agents and anti-CD20 monoclonal antibodies. Corticosteroids are almost always used in the acute treatment of immune-mediated TTP, although the efficacy of this strategy has not been demonstrated by controlled studies. The recommended dose of prednisone is 1.0-1.5 mg/kg. The efficacy of treatment with lower doses in the prevention of relapses of chronic recurrent forms of TTP has not been demonstrated, although such treatment is often given. The dose used for high-dose intravenous immunoglobulin treatment is 400 mg/kg for 5 days or 1g/kg for 2 days. Splenectomy in TTP, un minimo di 3 giorni, dopo l’ottenimento della remissione. Sono riportati casi di TTP su base genetica in cui viene attuato il trattamento profilattico degli episodi ricorrenti con somministrazione di plasma (30 mg/kg) a intervalli regolari (ogni 5-7 giorni). Non esistono, peraltro, dati solidi che supportino l’utilità di questo approccio, né la frequenza ottimale della somministrazione del plasma. Per le sedute di PE è da preferirsi il plasma trattato con metodi di inattivazione virale, ai fini di ridurre il rischio di infezioni ematogene.

Per le forme acquisite dovute ad autoanticorpi, in associazione a PE sono stati proposti trattamenti immunosoppressivi quali corticosteroidi, immunoglobuline endovenose, splenectomia, agenti citotossici e anticorpi monoclonali anti-CD20. L’utilizzo dei corticosteroidi nel trattamento acuto della TTP immunomediata è pressoché costante, anche se l’efficacia di questa terapia non è stata dimostrata da studi controllati. La dose raccomandata di prednisone è di 1.0-1.5 mg/kg. Non è dimostrata l’efficacia del trattamento steroideo a più basse dosi nella prevenzione delle recidive nelle forme croniche ricorrenti, anche se viene spesso praticato. Per le immunoglobuline endovenose ad alte dosi il dosaggio utilizzato è 400 mg/kg per 5 giorni o alternativamente 1g/kg per 2 giorni. La splenectomia nella TTP, come nelle altre patologie ematologiche su base autoimmune, va considerata con molta cautela nelle forme croniche ricorrenti non rispondenti a altre terapie. La sua efficacia, tutt’altro che costante, è basata sulla rimozione di una sede importante di produzione degli anticorpi anti-ADAMTS13. Diversi studi riportano l’efficacia della vincristina nel trattamento della TTP cronica ricorrente.

Negli ultimi anni, si è assistito all’impiego sempre più frequentemente nella TTP dell’anticorpo monoclonale anti CD-20 (Rituximab), soprattutto in casi caratterizzati dalla mancata risposta alla terapia con PE e da recidive multiple. Scopo del trattamento con Rituximab nella TTP è di ostacolare la produzione di anticorpi anti-ADAMTS13 tramite la deplezione dei linfociti B. Diversi studi riportano l’efficacia della vincristina nel trattamento della TTP cronica ricorrente.
as in other blood diseases with an autoimmune background, can be very cautiously considered in the chronic, recurrent forms refractory to other treatments. Its efficacy, which is by no means constant, is based on the removal of an important site of production of anti-ADAMTS13 antibodies. Various anecdotal studies have reported the efficacy of vincristine in the treatment of chronic, recurrent TTP. In recent years there has been an increase in the use of an anti-CD20 monoclonal antibody (rituximab) in TTP, particularly in those cases not responding to treatment with plasma exchange and characterised by multiple recurrences. The aim of treatment with rituximab in TTP is to block the production of anti-ADAMTS13 antibodies by depleting B lymphocytes. Various studies have reported that this treatment plays an important role in producing prolonged (but rarely sustained) remissions in chronic, recurrent TTP.

The recommended dose is 375 mg/m² every 7 days, repeated for three or four cycles. It remains to be demonstrated that the complete B lymphocyte depletion caused by this drug is without short- or long-term pathological consequences for the patients. Another category of drugs whose use is still controversial is the platelet anti-aggregants, including ticlopidine, clopidogrel, acetylsalicylic acid and dipyridamole. Their use is not based on a pathogenic rationale, since the platelet aggregation induced by UL VWF is not inhibited by these drugs. They must not, in any case, be used when the platelet count is below 50 x 10⁹/L, in order to avoid increasing the risk of haemorrhage. Finally, a new therapeutic approach could be the administration of plasma concentrates or recombinant ADAMTS13. It has in fact been demonstrated that the recombinant protease is able to correct the defective degradation of the high molecular weight VWF in vitro, but the availability of this product and its in vivo efficacy in the treatment of TTP have yet to be demonstrated.

Haemolytic uraemic syndrome

The so-called typical HUS, called "diarrhoea-related HUS [D(+) HUS]", is an acquired disease which manifests predominantly in neonates and children, and less frequently in adults, following a gastrointestinal infection with bloody diarrhoea. There are two different forms of atypical HUS: the first, familial and very rare, is due to a congenital lack of complement factor H or other complement farmaco sia senza conseguenze patologiche, a breve o lungo termine, per i pazienti.

Un'altra categoria di farmaci, il cui uso rimane a tutt'oggi controverso, sono gli antiaggreganti piastrinici, tra cui la ticlopidina, il clopidogrel, l'acido acetilsalicilico e il dipiridamolo. Il loro utilizzo è poco plausibile dal punto di vista patogenetico, perché l'aggregazione piastrinica indotta da VWF UL non è inibita da questi farmaci. Il loro utilizzo va comunque evitato in presenza di una conta piastrinica inferiore a 50 x 10⁹/L, per evitare l'aumento del rischio emorragico.

Infine un nuovo approccio terapeutico potrebbe essere rappresentato dalla somministrazione di concentrati plasmatici o recombinanti di ADAMTS13. È stato infatti dimostrato che la proteasi ricombinante è in grado di correggere la degradazione difettosa del VWF ad alto peso molecolare in vitro, ma la disponibilità di tale prodotto e la sua efficacia in vivo nel trattamento della TTP non sono ancora stati dimostrati.

Sindrome emolitico uremica

La cosiddetta HUS tipica, chiamata "diarrhoea-related HUS [D(+) HUS]", è una patologia acquisita che si manifesta prevalentemente nei neonati e nei bambini, meno frequentemente negli adulti, in seguito ad infezione gastrointestinale accompagnata da diarrea ematica. Esistono, invece, due diverse forme di HUS atipica: la prima, familiare e assai rara, è dovuta alla carenza congenita del fattore H del complemento e si manifesta principalmente nei neonati e nei bambini; l'altra forma di HUS atipica si presenta a tutte le età in associazione a condizioni comuni alla TTP, da cui non è quindi facile distinguervi (Tabella V).

HUS associata a diarrea

L'esordio della D(+)HUS è preceduto di qualche giorno da episodi di diarrea ematica. Sintomi tipici sono l'insufficienza grave renale con oligoanuria, ittero e manifestazioni emorragiche quali petecchie, anemia emolitica e aumento di LDH sierica. La presenza di VWF UL è molto meno frequente che nella TTP. Il coinvolgimento del sistema nervoso centrale è raro, tuttavia coma e convulsioni si osservano spesso in associazione ad uremia e ipertensione grave. I
components and presents particularly in neonates and children; the other form of atypical HUS can present at any age in association with the conditions common to TTP, such that it is not easy to distinguish the two (Table V)\textsuperscript{38}.

**HUS associated with diarrhoea**

The onset of D(+)HUS is preceded by several days by episodes of bloody diarrhoea. The typical symptoms are severe renal failure with oligoanuria, jaundice and haemorrhagic signs such as petechiae, haemolytic anaemia and raised levels of serum LDH. The presence of UL VWF is much less frequent than in TTP. Central nervous system involvement is rare, however coma and convulsions are often seen in association with uremia and severe hypertension. The main aetiological agents, which are responsible for the gastrointestinal prodromal symptoms, are *Escherichia coli* strain 0157:H7 and *Shigella dysenteriae* serotype I. These infective agents produce an exotoxin called verotoxin or shiga toxin, which is absorbed in the gastrointestinal tract and binds to a globotriaosylceramide (Gb3) receptor of the membrane sphingolipids, particularly expressed in the endothelium of glomerular capillaries in neonates and children\textsuperscript{38}. The binding of verotoxin to the Gb3 receptor has a cytotoxic effect on glomerular endothelial cells, causing swelling and desquamation of the endothelium and formation of microthrombi in the kidney. From a pathological point of view, D(+)HUS is characterized by more substantial endothelial damage but little or no deposition of VWF in microthrombi in comparison with TTP. It has been convincingly demonstrated that the plasma levels of ADAMTS13 are normal in patients with typical (D+) HUS\textsuperscript{2,3}.

**Treatment**

Agents reducing intestinal mobility should not be given during the prodromic diarrhoea, since they facilitate the retention of the verotoxin in the intestinal lumen and its absorption into the circulation. The use of the principal agents eziologici, responsabili dei prodromi gastrointestinali, sono l'infezione da *Escherichia coli* ceppo 0157:H7 e da *Shigella dysenteriae* sierotipo I. Questi agenti infettivi producono una esotossina detta verotossina o tossina shiga, che viene assorbita nel tratto gastrointestinale e si lega al recettore globotriaosilceramide (Gb3) degli sfingolipidi di membrana, particolarmente espresso a livello dell'endotelio dei capillari glomerulari dei neonati e dei bambini\textsuperscript{38}. Il legame della verotossina al recettore Gb3 ha un effetto citotossico sulle cellule endoteliali glomerulari, con conseguente rigonfiamento e desquamazione dell'endotelio e formazione di microtrombi a livello renale. Dal punto di vista patologico la D(+)HUS è caratterizzata da un più importante danno endoteliale ma da una scarsa o assente deposizione di VWF nei microtrombi rispetto alla TTP. È stato dimostrato convincentemente che nei pazienti affetti da HUS tipica D(+), i livelli plasmatici di ADAMTS13 sono normali\textsuperscript{2,3}.

**Trattamento**

Durante la diarrea prodromica è sconsigliata la somministrazione di agenti che inibiscono la mobilità intestinale, in quanto favoriscono la permanenza della verotossina a livello del lume intestinale e il suo assorbimento nel circolo. L'utilizzo degli antibiotici è controverso e comunque limitato ai casi di pazienti con documentata infezione da *Shigella dysenteriae*. In presenza di insufficienza renale acuta importante, l'inizio precoce del trattamento dialitico è fondamentale ai fini della riduzione della mortalità e delle sequele. Non vi è alcune prova che la terapia plasmatica sia efficace nei casi di HUS tipica (anche se viene spesso praticata), mentre rimane il trattamento di prima scelta nei casi di HUS atipica, confermando la somiglianza tra queste patologie e la TTP. Un possibile approccio per la prevenzione dei casi di HUS provocati dalla verotossina potrebbe essere la vaccinazione dei bambini nelle aree endemiche.

**HUS familiare**

I casi di HUS familiare rappresentano il 5-10% dei casi totali, si manifestano soprattutto nei bambini e giovani adulti e sono associati a grave insufficienza renale con un tasso di mortalità intorno al 30%. I pazienti che sopravvivono all'episodio acuto...
of antibiotics is controversial and should, in any case, be limited to patients with a documented *Shigella dysenteriae* infection. In cases of significant acute renal failure, early institution of dialysis is essential in order to reduce sequelae and death.

There is no proof that plasma therapy is effective in cases of typical HUS (although it is often performed), while the therapy is the treatment of first choice in cases of atypical HUS, again confirming the similarities between these conditions and TTP. A possible approach to the prevention of cases of HUS caused by verotoxin could be to vaccinate children in endemic areas.

**Familial HUS**

Five to ten percent of all cases of HUS are familial. This condition presents predominantly in children and young adults and is associated with severe renal failure with a mortality rate of about 30%. The patients who survive an acute attack usually require permanent haemodialysis. In 30-40% of the cases, familial HUS is caused by a lack or dysfunction of complement factor H, with consequent activation and deposition of complement component C3 in the kidney; it is inherited as an autosomal dominant condition and caused by missense mutations in the gene coding for factor H.

Assaying plasma levels of C3 could be a simple method for confirming the diagnosis of familial HUS. There is, however, a rarer form of familial HUS which presents particularly in childhood and has an autosomal recessive pattern of inheritance; it, too, is associated with low plasma levels of factor H. The treatment of choice consists in administering factor H by infusing fresh plasma, in association with haemodialysis to control the uraemia and oligoanuria. In most cases chronic renal failure is a serious sequel of familial HUS. Kidney transplantation is not an appropriate therapeutic strategy because the congenital deficiency of plasma factor H is not corrected by transplantation and the transplanted kidney will become damaged.

**Atypical HUS (non-diarrhoea-related)**

This form of HUS can be distinguished from TTP exclusively by a lack of neurological symptoms and the presence of renal ones.

There are cases of atypical HUS associated with severe deficiency of ADAMTS136,11, albeit less frequently than in TTP. Atypical HUS frequently presents during the post-partum period and following the intake of various drugs (Table V). Plasma therapy, richiedono solitamente un trattamento emodialitico permanente. Questa forma di HUS è provocata nel 30-40% dei casi da carenza plasmatica o disfunzione del fattore H del complemento, con conseguente attivazione e deposizione della componente complementare C3 a livello renale; si trasmette come carattere autosomico dominante ed è provocata da mutazioni missense a livello del gene codificante il fattore H. La misurazione dei livelli plasmatici di C3 potrebbe essere un metodo semplice per confermare la diagnosi di HUS familiare. Esiste, tuttavia una forma più rara di HUS familiare che si manifesta soprattutto durante l’infanzia, si trasmette come carattere autosomico recessivo ed è associata anch’essa a bassi livelli plasmatici di fattore H. Il trattamento di scelta consiste nella somministrazione del fattore H attraverso l’infusione di plasma fresco, in associazione al trattamento emodialitico per il controllo dell’uremia e dell’oligoanuria. Nella maggior parte dei casi l’insufficienza renale cronica è una grave conseguenza della HUS familiare ma il trapianto di rene non rappresenta il corretto approccio terapeutico. Ciò è dovuto alla persistenza della carenza congenita del fattore H plasmatico, che non viene corretto dal trapianto e provocherebbe, quindi, il danno del rene trapiantato.

**HUS atipica (non diarrhoea-related)**

Questa forma di HUS può essere distinta dalla TTP esclusivamente dall’assenza di sintomatologia neurologica e dalla prevalenza di quella renale. Anche se meno frequentemente che nella TTP, vi sono casi di HUS atipica associati a carenza grave di ADAMTS13. La HUS atipica si manifesta spesso durante il periodo post-partum e in seguito all’assunzione di diversi farmaci (Tabella V). La terapia plasmatica, secondo il protoccolo descritto per la TTP, è il trattamento di scelta in questa forma di HUS.

**Considerazioni conclusive**

Le microangiopatie trombotiche TTP e HUS sono malattie rare, anche se sembra che vi sia un recente incremento della loro incidenza. La diagnosi non è semplice, e la possibilità che vengano confuse con altre malattie associate ad occlusione trombotica del
using the protocol described for TTP, is the treatment of choice for this form of HUS.

**Concluding remarks**

The thrombotic microangiopathies TTP and HUS are rare diseases, although their incidence seems to be increasing. They are not easy to diagnose and the possibility that they are mistaken for other disorders associated with thrombotic occlusion of the microcirculation is considerable. Much progress has been made in recent years in understanding the aetiopathogenetic mechanisms of TTP, through the demonstration of the role of UL VWF and ADAMTS13 in the pathogenesis of many immune-mediated or genetic cases. There are, however, many cases of TTP with normal or only slightly reduced levels of ADAMTS13, in which there are obviously pathogenic mechanisms other than those of the interaction between the protease/VWF, although these have not yet been identified. The pathogenesis of HUS is also heterogeneous; while there are well-characterised cases in paediatric age associated with diarrhoea and those linked to genetic defects in factor H, there is a borderline condition resembling TTP whose pathogenetic mechanisms remain to be understood. The laboratory methods for assaying ADAMTS13 are currently too complex to be able to be used routinely on a wide scale. The laboratory diagnosis of TTP (and also of HUS) is, therefore, based on the concomitant presence of thrombocytopenia (due to increased destruction of platelets) and anaemia (due to mechanical damage to red cells), supported by a marked increase in serum LDH. Nevertheless, we advise those clinical centres which have suspected cases of TTP but are unable to assay ADAMTS13 (and factor H in suspected cases of HUS) to collect a sample of plasma to send it for subsequent analysis in a specialised laboratory. Even though normal levels of ADAMTS13 do not exclude a diagnosis of TTP (as in the cases associated with bone marrow transplantation and neoplastic metastases), very low values (less than 10%) seem to have a more favourable prognostic value. It seems that there also therapeutic implications, because patients with normal or slightly reduced levels of ADAMTS13 seem to respond poorly to plasma exchange and have a high mortality rate. On the other hand, given the lack of alternatives, no
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one would suggest not carrying out plasma exchange in patients with a diagnosis of TTP, even in the presence of normal levels of ADAMTS13. The significance of the level of the protease and variations in its plasma levels during treatment with plasma exchange have not been established either in the acute forms or in the periods between relapses in the chronic, recurrent forms. The higher frequency of autoimmune forms (compared with congenital forms or those due to an imbalance between production and inactivation of UL VWF) has made the use of high doses of corticosteroids biologically plausible in acute episodes, even if there is no objective evidence that this treatment adds benefit to that of treatment with plasma exchange. Other immunomodulatory treatments are of even less well demonstrated efficacy in the acute phase, are associated with more side effects and are more expensive than corticosteroids.

One important unresolved therapeutic problem is that of secondary prevention of the chronic recurrent forms. Various different treatments have been tried, from chronic low-dose corticosteroids or other immunomodulatory agents (vincristine, immunoglobulins) to the more recent and promising use of anti-CD20 monoclonal antibodies. There are anecdotal reports of the efficacy of all these agents, but their use is based on small series and the reports are affected by a publication bias. The prophylactic administration of plasma is plausible in the genetic forms, but much less so in the immunomediated ones. The much awaited plasma concentrates or recombinant ADAMTS13 could avoid risks of infections and volume overload associated with the administration of large amounts of plasma.

There are numerous problems left to solve and research on TTP and HUS must necessarily be multicentre, given the rarity of these conditions. What is the pathogenic mechanisms of cases with normal or slightly reduced levels of ADAMTS13? What are the factors in the genetically based or autoimmune chronic recurrent forms that periodically trigger the episodes of thrombocytopenia and anaemia, in the presence of constantly reduced levels of ADAMTS13? The behaviour of the levels of autoantibodies following plasma treatment and therefore exposure to antigen (anamnestic response) is not known. Likewise, the value of inducing immune tolerance by regular administrations of ADAMTS13, which will only be possible when plasma concentrates become available, has not been investigated.
References


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