# Refractory Thrombotic Thrombocytopenic Purpura (TTP)

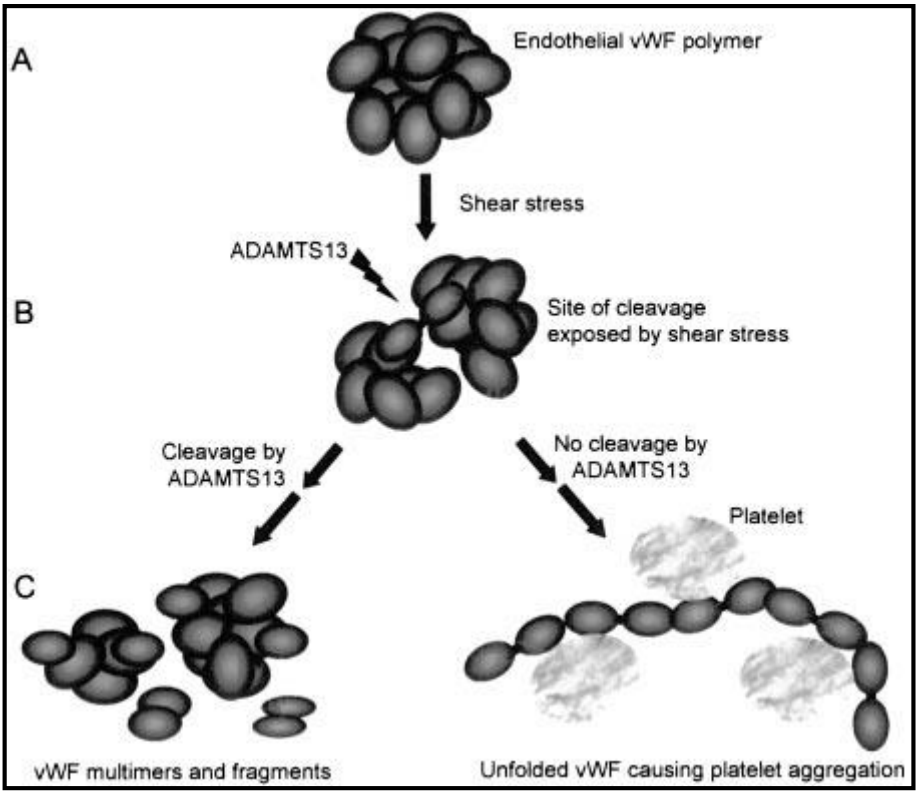
**Dr. Mohammad Abd Allah Shazly, M.D,**
**Lecturer of Hematology, Ain Shams University**

The thrombotic microangiopathies (TMA) are a group of life-threatening disorders sharing the common outcome of microvascular ischaemia and end-organ dysfunction as the end result of a diverse group of pathogenic processes. This broad classification encompasses several disease entities, including thrombotic thrombocytopenic purpura (TTP), haemolytic uraemic syndrome (HUS) (both typical (HUS) and atypical forms (aHUS)), and TMA associated with malignancy, bone marrow transplant (BMT) and pregnancy. **(Blombery et al, 2015)**

It is now recognized that congenital and acute acquired TTP are due to a deficiency of von Willebrand factor (VWF) cleaving protein, also known as ADAMTS1, (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 – von Willebrand factor cleaving protein).

In the absence of ADAMTS13, ultra large multimers of VWF (ULVWF) released from endothelium are not cleaved appropriately, and cause spontaneous platelet aggregates in conditions of high shear, such as in the microvasculature of the brain, heart and kidneys. Congenital TTP is due to an inherited deficiency of ADAMTS13, but acquired immune TTP is due to the reduction of ADAMTS13 by autoantibodies directed against ADAMTS13.

Other clinical forms of thrombotic microangiopathy (TMA) occur in the absence of severe deficiency. Diagnosis can be difficult, as there is clinical overlap with haemolytic uraemic syndrome (HUS), autoimmune disease and a spectrum of pregnancy-related problems **(Scully et al, 2012).**

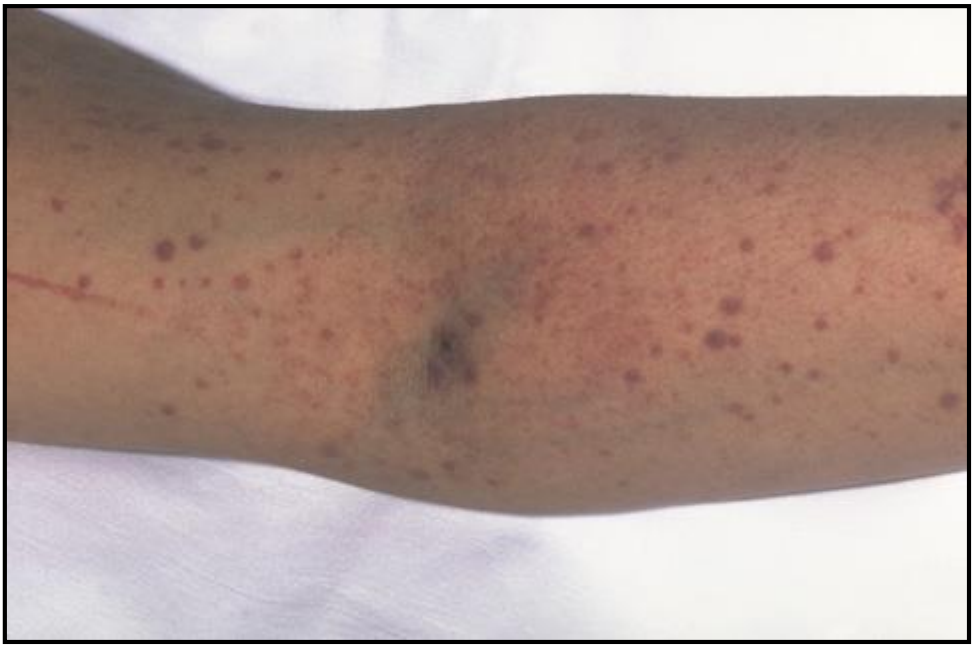



Deficiency of ADAMTS13 causes platelet thrombosis. Endothelial vWF polymer (A), when unfolded by high levels of shear stress in the arterioles and capillaries, becomes susceptible to cleavage by ADAMTS13 (B). This process repeats when vWF travels through the microcirculation, generating a series of multimers (C, left). Deficiency of ADAMTS13 results in the accumulation of unfolded vWF, which facilitates platelet aggregation and microvascular thrombosis (C, right). Increase of shear stress caused by vascular occlusion results in more unfolding of vWF and cycles of further thrombosis characteristic of TTP

## Diagnosis of TTP

Thrombotic thrombocytopenic purpura was originally characterized by a pentad of thrombocytopenia, MAHA, fluctuating neurological signs, renal impairment and fever, often with insidious onset. However, TTP can present without the full pentad; up to 35% of patients do not have neurological signs at presentation and renal abnormalities and fever are not prominent features. The revised diagnostic criteria state that TTP must be considered in the presence of thrombocytopenia and MAHA alone **(Galbusera et al, 2006)**

TTP remains a diagnosis based on clinical history, examination of the patient and the blood film. ADAMTS 13 assays help to confirm the diagnosis and monitor the course of the disease and possible need for additional treatments. Consumption of platelets in platelet-rich thrombi results in thrombocytopenia **(Scully et al, 2008**).

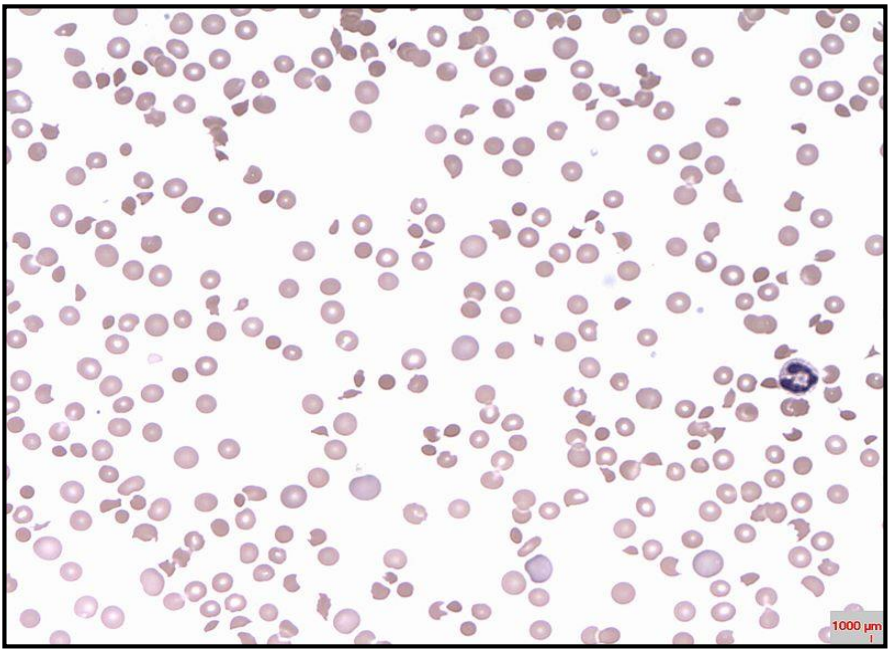


Purpuric eruptions in patient with TTP

Mechanical fragmentation of erythrocytes during flow through partially occluded, high shear small vessels causes a MAHA. Median hemoglobin levels on admission are typically 80–100 g/l, with schistocytes in the film, low haptoglobin levels and raised reticulocyte counts due to hemolysis. The direct Coombs test is negative. The combination of hemolysis and tissue ischemia produces elevated lactate dehydrogenase (LDH) values.

Peripheral blood film in patient with TTP shows fragmented RBC

The clotting screen (prothrombin time, activated partial thromboplastin time and fibrinogen) is usually normal. A virology screen pre-treatment is necessary to exclude human immunodeficency virus (HIV) and other viral-associated TTP, and as a baseline prior to plasma exposure. Troponin T levels are raised in 50% of acute idiopathic TTP cases highlighting that cardiac involvement is common (**Hughes et al, 2009**)




Raised troponin levels are a sinister finding, for coronary artery occlusion is a common mode of early death. The incidence of symptomatic heart failure is increased in patients who have been given a recent platelet transfusion **(Gami et al, 2005**)

## ADAMTS13 assays

Blood must be taken prior to treatment to assess baseline ADAMTS13 activity. Severely reduced ADAMTS13 activity (<5%) ± the presence of an inhibitor or IgG antibodies, confirms the diagnosis **(Ferrari et al, 2007).**

Decreased ADAMTS13 activity (<40% but >5%) has been reported in a wide variety of non-TTP conditions such as uremia, inflammatory states, post-operatively and during pregnancy **(Moore et al, 2001).**

The specificity of severe ADAMTS13 deficiency (<5%) in distinguishing acute TTP from HUS is 90% (**Zheng et al, 2004)**

ADAMTS13 assays currently available include assays of activity, antigen and neutralizing or non-neutralizing anti-ADAMTS13 autoantibodies. Functional assays measuring ADAMTS13 activity are based on the failure of the patient plasma to degrade VWF multimers or synthetic VWF peptides. Inhibitory autoantibodies can be titrated in vitro using classical mixing studies and non-neutralizing antibodies can be detected by Western blotting or enzyme-linked immunosorbant assays **(Peyvandi et al, 2010).**

## Treatment of acquired TTP

PEX (plasma exchange) and corticosteroids are the mainstays of treatment of acquired TTP. Individuals with thrombocytopenia and MAHA, and without secondary causes of TMA, meet the working diagnosis of acquired TTP. These patients should immediately receive PEX with at least 1 plasma volume (PV), and the treatment continued daily until a response is achieved. According to the 2012 American Society of Apheresis Consensus Conference on TTP, response is defined as achieving a platelet count ,150 000/mL for 2 consecutive days, a normal or near normal lactate dehydrogenase (LDH), and stable or improving neurologic deficits.**( Sarode et al, 2014**)

We advocate supplementing PEX therapy with corticosteroids immediately upon diagnosis. PEX is superior to plasma infusion in TTP, and is associated with decreased mortality **(Rock et al, 1991).**

Although frequently intolerable, very large doses (25-30 mL/kg) of plasma infusion might be sufficient until PEX can be initiated **(Coppo et al, 2003)**

In patients with severe disease, or progressive symptoms over the first few days, PEX may be intensified to 1.5 PV and reduced to 1 PV once the clinical situation and laboratory findings stabilize **(Nguyen et al, 2008)**

Once a response has been achieved, PEX can be stopped without a taper while closely monitoring blood counts and LDH. Limited evidence suggests that there is no value to tapering the frequency and/or volume of PEX **(Bandarenko et al, 1998)**

The apheresis catheter should be removed as soon as the patient has stabilized off apheresis, or at the first sign of infection.

## Refractory TTP

Refractory TTP is defined as a failure of platelet response after 4 to 7 days of PEX, or a clinical deterioration in a patient receiving standard therapy. In the case of possible refractory acquired TTP, it is absolutely imperative to reevaluate the clinical scenario in order to identify other causes of thrombocytopenia and MAHA that may require additional therapy. It is in this situation that the ADAMT13 activity and inhibitor analysis is perhaps most helpful. Patients who have very low ADAMTS13 levels and high inhibitor titers most likely have TTP, and should be treated with therapies that will be described in this review. Patients who do not have low ADAMTS13 levels might not actually have TTP, and should be extensively evaluated for other causes of thrombocytopenia and MAHA **(Sayani and Abrams, 2015)**

## Treatment options for TTP refractory to PEX

### Corticosteroids

Steroids are believed to suppress the production of anti-ADAMTS13 autoantibodies. However, the basis for this knowledge has not been demonstrated in clinical trials. For the initial treatment of acquired TTP, we start prednisone 1 mg/kg per day in all patients. However, for acutely ill refractory patients, who are clinically unstable or who have neurologic symptoms, we consider increasing the immunosuppression. A randomized trial of standard dose (1 mg/kg per day) vs a higher dose (10mg/kg per day for 3 days) IV methyl prednisolone revealed significantly improved remission rates for the latter. In patients with clinical deterioration despite standard doses of corticosteroids, we consider the administration of high-dose methyl prednisolone 1 g per day for 3 days **( Balduini et al, 2010)**

**Twice-daily PEX**

Twice-daily PEX is a treatment option in refractory acquired TTP, albeit with limited data on its effectiveness. Twice-daily PEX is sometimes initiated when an acutely ill patient, who initially responded to single-volume-daily PEX, has a sudden decline in platelet count or develops new neurologic symptoms. **(Nguyen et al, 2008)**

**Rituximab**

Rituximab, a monoclonal antibody targeting the CD20 antigen present on B lymphocytes, is often used in treating refractory or relapsed TTP with good response rates **(Caramazza et al, 2010)**

In several case series, treatment of acute refractory and/or relapsing TTP with rituximab alone resulted in clinical remission in 87% to 100% of patients, and platelet recovery within a median of 11 to14 days after the first dose **(de la Rubia et al, 2010)**

Anti-ADAMTS13 antibodies disappear, and ADAMTS13 activity levels significantly improve after the administration of rituximab **(Scully et al, 2007)**

The most frequently used dose is 375 mg/$m^2$ once weekly for 4 weeks. This dose is based on what is used to treat lymphomas. Various groups have tried other dosing regimens for TTP, including giving rituximab 375 mg/$m^2$ on days 0, 3, 7, and 14 **(Froissart et al, 2012)**

Because 65% of rituximab is removed during PEX, it may be more effective to give rituximab more frequently than once a week. However, further study is needed to determine the optimal dose and scheduling of rituximab and PEX. Rituximab has generally been well tolerated, but it can produce infusion reactions. Rare severe complications, including progressive multifocal leukoencephalopathy, herpes zoster transverse myelitis, encephalitis, paraplegia, hepatitis B reactivation, and

cardiac toxicity have been reported in patients treated with rituximab for any indication.

## Treatment options after PEX, steroids, and rituximab do not work

For patients who are still refractory and fail to respond to increased doses of corticosteroids, larger volume PEX, and rituximab, alternative therapies to consider are stronger immunosuppression, such as cyclophosphamide, vincristine, and cyclosporine or splenectomy.

### Cyclosporine

Cyclosporine is an immunomodulating drug that inhibits T-cell activation, thereby inhibiting interleukin-2 receptor expression and interleukin-2 production. In the treatment of TTP, cyclosporine is generally continued for at least 6 months, and in the few cases of relapse, patients have responded when retreated with cyclosporine. In a study evaluating the efficacy of PEX and cyclosporine in the treatment of TTP (included refractory TTP cases), remission was achieved in 89% of all patients**. (Cataland et al, 2007)**

Although this study also showed improvement in ADAMTS13 activity and in the suppression of ADAMTS13 inhibitors, the response rates were closely similar to those achieved with PEX alone.

### Cyclophosphamide and vincristine

Prior to the availability of rituximab, immunosuppressive therapy with cyclophosphamide was considered a reasonable upfront option for treating patients refractory to PEX and steroids. This was based on the results of a few case reports and case series. **(Zappasodi et al, 1999)**

More recently, it has been used in patients who are refractory to rituximab, or used in combination with rituximab for TTP patients who are refractory to steroids and PEX **(Zheng et al, 2003)**

In a recent case series of 5 patients with refractory TTP (refractory to PEX, steroids, along with vincristine or rituximab or both), who were then treated with 3 to 6 pulses of cyclophosphamide (500-750 mg/$m^2$ per pulse), a durable platelet recovery was seen at a median period of 10 days after the first cyclophosphamide pulse **(Beloncle et al, 2012)**

Side effects of cyclophosphamide include bone marrow suppression, Infection risk, infertility, and a potential long-term risk of myelodysplastic syndrome/leukemia. The additional risk of acute infection with the possibility of an exacerbation of TTP makes this a less favorable option. Therefore, now that rituximab is widely used, we only consider cyclophosphamide as a potential salvage therapy in rituximab refractory TTP. In addition, we tend to avoid treating young individuals with cyclophosphamide, due to the risk of infertility or malignancy. Vincristine has also been used in relapsed or refractory TTP with response rates of 50%to 87%. (**Ferrara et al, 1999)**

The side-effect profile includes mild peripheral paresthesias, muscle weakness, paralytic ileus, leukopenia, and transient alopecia. Vincristine may work better

when used in the initial treatment of patients with TTP, rather than for treating refractory patients. **(Ziman et al, 2005)**

A combination of cyclophosphamide and vincristine has also been used successfully in treating refractory TTP. **(Yang et al, 2003)**

In the era of rituximab, we would consider this combination only in those patients who are refractory to rituximab. **(Sayani and Abrams, 2015)**

## Splenectomy

The response rate of refractory TTP to splenectomy has been variable in the limited published literature. In a case series of 6 patients with refractory TTP undergoing splenectomy, 1 patient died immediately post splenectomy, whereas 5 had complicated clinical courses, including worsening hematocrit, thrombocytopenia, and even coma **(Bell et al, 1991)**
On the other hand, in a different case series of 6 patients with refractory TTP, splenectomy induced remission within 6 days or less in every patient **(Aqui et al, 2003)**

Splenectomy presumably removes not only a major site of anti ADAMTS13 antibody production, but it can also remove a major site of clearance of opsonized ADAMTS13. When considering splenectomy, the initial risks of the surgical procedure, and the long-term risk of infections, need to be weighed against the potential benefits in the clinical scenario. With newer modalities of immunosuppression showing potential promise in the management of refractory TTP, splenectomy may be a less favorable option in the future **(Sayani and Abrams, 2015)**

## Emerging roles for other agents

In the last few years, new potential therapies for refractory TTP have shown promise, including bortezomib and N-acetylcysteine (NAC). However, limited data on these treatment options are available, and further study is required.

### Bortezomib

The proteasome inhibitor bortezomib has been shown in several case reports to induce remission and deplete ADAMTS13 autoantibodies in refractory and relapsing TTP. **Shortt et al** reported on a case of a woman with TTP who was refractory to PEX, prednisone, cyclophosphamide, rituximab, and NAC, despite having documented depletion of B cells within her bone marrow **(Shortt et al, 2013)**

Because this patient still had plasma cells that could have been synthesizing ADAMTS13 autoantibodies, she was treated with bortezomib with a good outcome. A similar rationale has driven the use of bortezomib to deplete the alloreactive HLA-matched antibodies encountered during solid-organ transplant rejection. **(Everly et al, 2009)**

The few case reports describing the use of this therapy in patients with refractory TTP have used a dosing regimen that is typical for the treatment of multiple myeloma, in which bortezomib is given at 1.3mg/$m^2$ on days 1, 4, 8, 11, and repeated every 21 days. Further studies to establish the role of bortezomib in the management of refractory and/or relapsing TTP are needed **(Sayani and Abrams, 2015)**

**N-acetylcysteine**

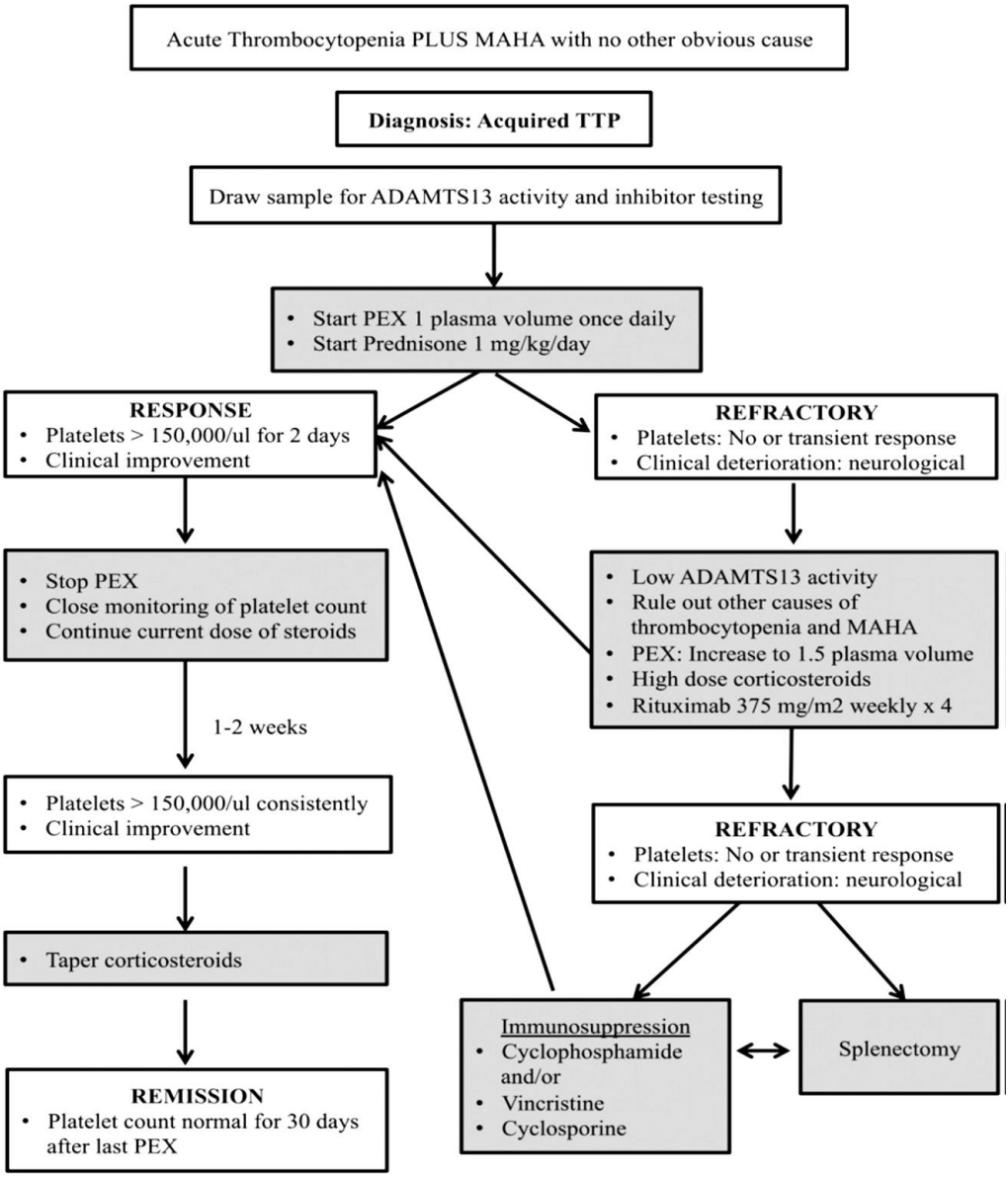
NAC is a widely available and affordable drug used in the treatment of acetaminophen overdose, or to decrease the viscosity of mucous secretions in respiratory disorders. It has recently been tested as a potential adjunct to PEX in the treatment of TTP **(Sayani and Abrams, 2015)**

NAC has a free sulfhydryl group that reduces the disulfide bonds in mucin polymers, thereby decreasing the size and viscosity of mucins **(George et al, 2014)**

VWF, like mucin, is also a polymer of dimers linked by disulfide bonds. Based on the observation that mucin and VWF share structural similarities, Chen and colleagues hypothesized that NAC could substitute for ADAMTS13 and reduce the size of ultra-large-molecular-weightVWF(ULVWF) **(Chen et al, 2011**)

However, there is very little information at this time to state that NAC has any clinical value in the treatment of TTP.

Approach to the management of acute and refractory TTP **(Sayani and Abrams, 2015)** .


Acute Thrombocytopenia PLUS MAHA with no other obvious cause
Diagnosis: Acquired TTP
Draw sample for ADAMTS13 activity and inhibitor testing
• Start PEX 1 plasma volume once daily
• Start Prednisone 1 mg/kg/day
RESPONSE
• Platelets > 150,000/ul for 2 days
• Clinical improvement
REFRACTORY
• Platelets: No or transient response
• Clinical deterioration: neurological
• Stop PEX
• Close monitoring of platelet count
• Continue current dose of steroids
1-2 weeks
• Platelets > 150,000/ul consistently
• Clinical improvement
• Taper corticosteroids
REMISSION
• Platelet count normal for 30 days after last PEX
• Low ADAMTS13 activity
• Rule out other causes of thrombocytopenia and MAHA
• PEX: Increase to 1.5 plasma volume
• High dose corticosteroids
• Rituximab 375 mg/m2 weekly x 4
REFRACTORY
• Platelets: No or transient response
• Clinical deterioration: neurological
Immunosuppression
• Cyclophosphamide and/or
• Vincristine
• Cyclosporine
Splenectomy

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