Dr. Shruti Chaturvedi:

Hello everybody, and welcome to the CME program on thrombotic thrombocytopenic purpura. My name is Dr. Shruti Chaturvedi. I am an associate professor of medicine in the division of hematology at Johns Hopkins University, and today I'll be covering some TTP clinical research highlights presented at the exciting ISTH 2025 meeting in June. Here are my disclosures. This program is also supported by an independent medical education grant from Sanofi. Now, TTP or thrombotic thrombocytopenic purpura, as we all know, presents with acute life-threatening episodes of systemic microvascular thrombosis. Its incidence is about 1.7 to three per million per year, so it's truly one of our medical zebras.

It's caused by the deficiency of ADAMTS13, the Von Willebrand factor cleaving protease, and the vast majority of TTP is acquired or autoimmune in nature due to an antibody against ADAMTS13, while 5% is due to a genetic deficiency of ADAMTS13 called congenital or hereditary TTP. Pregnancy, as well as other stimuli, may precipitate episodes. And untreated acute episodes are nearly universally fatal. Now, to contextualize the abstracts presented this year, it's kind of helpful to understand where we have evolved in our understanding of TTP.

The first case was described in 1924. It wasn't until 1991 that plasma exchange was established as a standard of care, as a life-saving therapy that improved outcomes of immune TTP. Prior to plasma exchange being recognized as a standard of care treatment, mortality was over 90%. It's also interesting that plasma exchange was discovered to be effective before the pathogenesis was really understood, because it wasn't until the late 1990s that the function of ADAMTS13, as well as the antibodies against certain TTP, was recognized.

In the early 2000s, Rituximab was first used for relapsing and refractory TTP and later for all patients with TTP. In 2019, the first targeted treatment for TTP was approved by the FDA. This was Caplacizumab, and more recently, recombinant ADAMTS13 has become available for congenital TTP, as being studied for immune TTP. Now, with all of these advances, mortality has also improved. Plasma exchange for the mortality from over 90% to less than 10%, and with all of the other advances, were around 5% mortality of each acute episode right now. In addition to an improvement in mortality from acute episodes and preventing relapse, there really is now a focus on the non-thrombotic microangiopathy, chronic or long-term sequelae of TTP and how to improve morbidity in the long term.

When this happens, our goals shift. In the beginning, it was all about survival and understanding the mechanisms of disease. The 2000s brought targeted therapy and a focus on relapse prevention with immunosuppression. And we are now moving towards treatment without plasma as well as improving long-term outcomes. Now, it's helpful also to understand the pathophysiology of Von Willebrand factor and ADAMTS13 before we dive into TTP. What you're seeing here is this pink endothelium releasing ultra-large multimers of Von Willebrand factor.

ADAMTS13, this protease is like a pair of scissors that snips these ultra-large multimers into smaller-sized multimers that can circulate without causing trouble. In TTP, unfortunately, we have a lack of ADAMTS13, commonly due to antibodies, as shown here, but sometimes due to a genetic deficiency. So we now have these ultra-large multimers of TTP that are circulating. They aggregate along with platelets and form microthrombi, which are the end cause of the manifestations of TTP. The microthrombi caused tissue ischemia, leading to the clinical manifestations.

The platelet count goes down because platelets are used up in these clots, and when red blood cells pass through this mushy clot, they shred, and these are the schistocytes that we see on the blood smear that are characteristic of TTP. ADAMTS13 levels less than 10% are diagnostic in the correct clinical setting. In terms of clinical management for a very long time, there were two pillars of treatment. There was a plasma exchange that removes the autoantibody against ADAMTS13 and replaces ADAMTS13.

And there was immunosuppression to take care of this antibody that was causing an ADAMTS13 deficiency.

Earlier, it was predominantly steroids, but mid 2010 onwards Rituximab was also used to suppress the inappropriate immune response and improve relapse-free survival. The goal of these treatments is really preventing progression of thrombotic microangiopathy through plasma exchange and preventing recurrence, whether it's early or late, for the immunosuppression. Around 2019, Caplacizumab came on the scene. Now, this is a nanobody, and I'm going to talk a little bit about how it works. You're seeing here in blue the strand of Von Willebrand factor with the A1 domain marked out, and this is important because the A1 domain is where platelets bind.

Caplacizumab is a nanobody that binds to the A1 domain and competitively blocks the VWF-platelet interaction. Now, when platelets cannot bind to Von Willebrand factor, there's faster resolution of thrombocytopenia, and it also prevents end-organ ischemia. That has now been evaluated in both phase two and phase three trials. Shown here is the ischemia of the Hercules trial or the phase three trial that led to approval of this agent, where patients with immune TTP were randomized to receive either Caplacizumab or placebo, in addition to plasma exchange and standard of care immunosuppression.

Caplacizumab or placebo was continued for 30 days after the last plasma exchange. At which point in time, the investigator was asked to check their ADAMTS13 level and continue Caplacizumab if the level was less than 10%. Recognizing that if you stop Caplacizumab when ADAMTS13 levels are still very low, recurrences occur, and a maximum extension period was 28 days after that. The primary endpoint here was time to platelet count response.

But secondary endpoints, perhaps more meaningful, were the composite of TTP-related death, recurrence and thromboembolic events. The study did meet its primary endpoint with a platelet normalization rate ratio of 1.55. Now, that's hard to interpret clinically, but what that truly means is at any given point of time, there was a 55% higher chance of achieving platelet count normalization in the Caplacizumab group versus the placebo group, which is really what this graph is also showing that there is faster platelet count normalization with the blue line, which is the Caplacizumab-treated patients.

More exciting to me is the secondary endpoint that shows a reduction in the composite of TTP-related death recurrence and thromboembolism by 74%. This is heavily driven by a reduction and early recurrences, which are about 40% with placebo and about 4% with Caplacizumab. This 38.4 or 40% rate is really similar to the exacerbation rate that has been reported in other cohorts. As expected, this agent causes Von Willebrand disease, which is functional by inhibiting the interaction of platelets with Von Willebrand factor. So mild mucocutaneous bleeding is a predominant side effect; all the more severe bleeding can also occur.

So in 2024 or 2025, we have Caplacizumab added to the paradigm of TTP treatment in addition to plasma exchange and immunosuppression. The goal of Caplacizumab is actually very similar to the goal of plasma exchange to prevent progression of thrombotic microangiopathy and ischemic injury. It also provides us time where we don't have to do plasma exchange but can still work on suppressing the immune response with corticosteroids, Rituximab and other immunosuppression, to prevent both early and late recurrence.

So we had the first abstract here that really looks at clinical response and refractoriness in immune thrombotic thrombocytopenic purpura patients treated with Caplacizumab. This is the REACT 2020 study, and the analysis and focus on identifying whether delayed platelet count recovery in patients who are receiving Caplacizumab indicates true refractoriness. Now, TTP refractoriness is defined as a lack of platelet count response or a worsening in symptoms by day four to seven.

This is a retrospective analysis of 203 patients treated in the REACT 2020 registry from Germany and Austria. In this cohort, two patients or 0.9% met criteria for refractoriness. Both patients had either missed doses of Caplacizumab or had infections. They also looked at common causes of delayed response, and I will show you the pattern in just a little bit, but found that essentially all patients who had a delayed response beyond five days had another cause going on.

In the graph in the top right here, you're seeing the median time to platelet count normalization, which is three days, which is really what has been seen in most real-world evidence studies as well as trials. And they examined what happened five years after Caplacizumab started, very few individuals here in white had no response. The majority had platelet count normalization by day five, which is nearly 84% while 15% had at least double their platelet counts. Now, let's look at these patients who had a response beyond day five.

What the studies really found here was that for the patients who had delayed normalization, you're looking at the panels below over here. So, the first panel normalization after the first dose of Caplacizumab, you're seeing it generally happens on the top side of the graph fairly quickly within the first five days, with some individuals having a really slow and delayed response, which is also reflected in the time to platelet doubling in the second group. Now, the top panel here that's recovering quickly is individuals who do not have a concomitant comorbidity along with TTP.

While the darker blue line here or the darker blue graph with delayed platelet count normalization or doubling are really individuals with other causes. These are infections such as CMV and EBV, or Epstein-Barr virus. These are autoimmune diseases like uncontrolled lupus, as well as malignancy. What this suggests to me is that for patients who are really not achieving a quick response, it's worth examining whether there is a concomitant condition causing persistent thrombocytopenia. For individuals who do not respond after several days, it's also worth looking at the ADAMTS13 activity to see if it is truly severe ADAMTS13 deficiency driving.

The prolonged type response or whether it is another comorbidity, in which case it should be treated. They concluded that TTP refractoriness is extremely rare in individuals treated with Caplacizumab, and observing prolonged times of later recovery should prompt further diagnostic work to identify concomitant diseases and factors, potentially counterfeiting uncontrolled immune thrombocytopenic purpura instead of primarily intensifying TTP treatment. So in contrast, going twice a day plasma exchange, increasing Caplacizumab dose, increasing immunosuppression, it's worth exploring if there's something else that needs to be treated.

Now moving on from the acute episode, what's been recognized over the past decade is that TTP survivors don't really go back to their baseline state of health. They certainly have a lifelong risk of relapse, which is about 30 to 50%, but in addition to that, they have multiple other adverse outcomes. Predominant among them is recognizing that, in addition to relapse risk, that is, short survival and reduced quality of life, they have a high rate of comorbidities such as lupus and other autoimmune diseases.

Many patients report that they don't quite go back to the way they were feeling before with cognitive impairment, depression symptoms, or post-traumatic stress, but also many individuals have an increased risk of stroke and silent cerebral infarction, which is defined as white matter changes or MRI changes that look like a stroke in the absence of overt neurodeficits. And there's also a higher rate of cardiovascular disease, like myocardial infarction, and hypertension, which is really the focus of the abstracts to come.

Now, this first abstract evaluated whether a persistent inflammatory and prothrombotic state persists during clinical remission in immune TTP and whether this is associated with stroke down the line. Now talking about stroke and vascular disease and survivors of TTP, we know now that immune TTP survivors

are at nearly fivefold increased risk of stroke and cardiovascular events compared to a reference population, and that reduced ADAMTS13 levels during clinical remission are associated with stroke risk.

You're seeing in the right-hand side panel the stroke rates of patients of TTP in remission at about 13% versus about two and a half percent of a reference population matched for age, sex and race. And in the panel below, you see that the majority of these strokes are occurring in the dotted line, which represents individuals who do not maintain ADAMTS13 levels in the normal range in remission. And secondary to all of this, cardiovascular disease is the leading cause of death in survivors of TTP.

Now, while we've just talked about the fact that ADAMTS13 deficiency is a risk factor for stroke, it really does not entirely account for all of the increased risk in survivors of TTP. How do we know this? We know this because in an abstract presented at ASH last year, it was seen that the rate of stroke was quite high in TTP cohorts, even when close to normal ADAMT13 activity is maintained in remission. So in this cohort, which is reflected in the panel on the right-hand side, you see that the cumulative incidence of stroke is over 20% at about four years.

But the median age is only about 48 years, which is really quite young. And the median average ADAMTS13 activity is 73%, which you will recognize by most metrics, is in the normal range. Now, we also know that there is more going on with people with TTP. Traditional cardiovascular risk factors such as obesity, hypertension, diabetes, et cetera, are quite prevalent in TTP cohorts at much higher rates than the general population. But even these do not explain all of the increased risk, as shown here. So in this analysis, Ahuja et al looked at standard cardiovascular risk prediction scores to see if they accurately estimate risk in TTP survivors.

They use two scores, with one being the American College of Cardiology or American Heart Association atherosclerotic cardiovascular risk disease score, and the other one they used was the Framingham score. What you're seeing in both of these graphs is the predicted risk by these scores in gray and the observed risk in a TTP cohort in black. And you see in all categories, whether they are categorized as low, moderate or high risk, the observed risk in TTP survivors is significantly higher than predicted by standard risk prediction scores.

So this study, which was presented at ISTH, evaluated whether a persistent pro-inflammatory or pro-thrombotic state persists in TTP remission and whether this contributes to the risk of stroke. This is a cross-sectional analysis comparing 48 patients with immune TTP and remission to 22 healthy controls and 38 acute TTP samples. The total cohort was 108 samples from the Johns Hopkins TTP registry. They measured 41 biomarkers on a multiplexed chemiluminescent amino assay from MSD USA. These are the primary results here.

Now, I know this looks kind of complicated, but I'm just going to highlight major differences here. In pink, we are highlighting the markers that are elevated in remission versus controls, pointing out again, we expect that in remission patients go back to normal and should be similar to controls, but they really are not. And these are markers such as thrombomodulin, placental growth factor, P-selectin, the vascular endothelial growth factors, et cetera. To highlight again, things like thrombomodulin and placental growth factor are endothelial injury markers.

P-selectin indicates both endothelial and platelet activation. You also have some markers that are low in remission versus controls, which are IL-1 alpha, TNF beta, and Eotaxin-3. Overall, this suggests that the pro-inflammatory, pro-thrombotic signature and remission are different from controls. Now, we also see here that many P-values are very small compared to the acute versus control samples, and we'll look into that a little bit more. Now, what this study really found is that there appears to be a dose-dependent relationship between many markers, whether they are in acute TTP remission or control.

For example, here, serum-associated amyloid. An inflammatory marker is significantly higher in acute TTP compared to remission, but that still remains higher than controls, and this pattern is recapitulated with other markers like Thrombomodulin and an endothelial entry marker, where remission TTP samples have lower levels than acute, but again, nowhere close to control samples. Similarly, for P-selectin and percentile growth factor, which has actually been associated with vascular disease. Now, as the next step, they identified certain markers that are associated with stroke.

The authors found that levels of IL-6, IL-16, Thrombomodulin and serum VCAM-1 were all significantly higher in the individuals who went on to develop stroke versus those who did not develop stroke. These markers, as a matter of interest, IL-16 and 16 have previously been associated with vascular disease, including stroke and heart attack, in the general population. Now, they looked at the performance of models trying to predict cardiovascular risk using these candidate biomarkers alone. And I just want to highlight here, IL-6 had a really high area under the curve of 0.72.

With reasonably high sensitivity and specificity of 66 and 76% respectively, which is higher than was seen in the paper that I showed you earlier, where standard risk prediction models do not accurately predict risk in individuals with TTP, the predictive performance actually improved significantly to nearly 80% accuracy when we combine both IL-6 and IL-16. This abstract leads us to a more comprehensive model of vascular disease in TTP. We already know that low remission of ADAMTS13 activity is a risk factor for stroke and heart attacks.

We understand that ischemic events occur during acute TTP, and the circular may persist beyond remission. There are also treatments that we give individuals. Many are on corticosteroids for months, which can increase the risk of vascular disease, but in addition to that, we have comorbidities like hypertension and diabetes, as well as chronic stress and inflammation, which can lead to an increased risk. So in conclusion, markers of inflammation and endothelial activation remain elevated in TTP remission.

Inflammatory markers like IL-6 and IL-16 and endothelial markers like thrombomodulin and VCAM are associated with incident stroke after the time of sampling. The clinical implications are that these biomarkers may help us improve cardiovascular risk stratification in TTP. For example, IL-6 levels are often available clinically and targeting inflammation with drugs like Colchicine or Canakinumab may actually reduce morbidity from stroke in TTP survivors, but this needs to be tested in clinical trials.

The next abstract here looked at the burden of cerebral small vessel disease in ITTP survivors. This abstract focuses really on small vessel disease, which in other papers has been described as silent infarcts, which is evidence of small vessel disease without overt stroke and has been shown to be quite prevalent in remission of TTP, being seen in over 50% of individuals. What's also been shown is that these small vessel disease lesions or silent infarcts are associated with significant cognitive impairment.

What's really unclear but has been addressed by this abstract is whether these lesions occur only during the acute episode or are they progressive during clinical remission. Now, this cohort looked at the prevalence as well as progression of cerebral small vessel disease and immune TTP. They evaluated how common these lesions are, and they examined two time points. One was an MRI done at the median of seven days from admission, so during an acute episode, and then one year later. They had 20 individuals who were evaluated acutely, and 10 had a one-year follow-up.

The majority, as expected, were female at 65%. The median age of the episode was 52 years, and for 80%, this was their first episode. 85% had some neurologic signs or symptoms at presentation, and they had a moderate rate of comorbidities such as hypertension, smoking, and hyperlipidemia. At about 30 to 35%, none of them had had previous major adverse cardiovascular events. Now, during the acute phase, 85% had some evidence of small vessel disease, suggesting that DTP is affecting the small vessels

of the brain. They used something called a cerebral small vessel disease score, and the median score was two.

Now, after one year, two of 10 patients had actually progressed in terms of the cerebral small vessel disease burden at one-year follow-up. One of them had hypertension and one at the fourth TTP event, but overall, the score increased from their first assessment to one year later. This supports this proposed model for neurovascular morbidity and TTP, where you have acute TTP that leads to some ischemic lesions. However, even in remission, patients appear to accumulate additional cerebral small vessel disease lesions or silent infarcts, and this has been shown in other studies to be a risk factor for overt stroke.

What this model really does for us is present opportunities for intervention. Perhaps what's needed is to study whether anti-VWF therapy like Caplacizumab or recombinant ADAMTS13 during acute TTP may help reduce the lesions accumulated during acute TTP and in remission. Perhaps we need to be focusing on things like remission at ADAMTS13 with more immunosuppression targeting higher ADAMTS13 levels. And also, evaluating interventions such as antiplatelet therapy or improving other vascular risk factors to reduce neurovascular morbidity in TTP survivors.

So the clinical pearls that are takeaways from the ISTH meeting are that in patients who have been treated for TTP in a regimen that includes Caplacizumab, prolonged time to platelet count recovery is rarely due to active TTP and is commonly due to other comorbidities such as infections or autoimmune disease, and those should be evaluated before intensifying treatment. Cerebral small vessel disease, also termed silent cerebral infarction, is common and very prevalent during immune TTP remission. Finally, increased levels of inflammatory markers like IL-6 and 16 or endothelial injury markers like thrombomodulin during clinical remission of TTP correlate with stroke risk and may enhance risk prediction beyond our conventional clinical risk factors.

And may help us stratify our patients better so that we can reduce cardiovascular morbidity, which is the leading cause of death in this population. Thank you so much for joining me, and have a great day.