assay–type binding assays to investigate the affinity of the A and B subunit interaction and calculated affinity constants within an order of magnitude of each other between $4 \times 10^{-7} \text{ M}$ and $8 \times 10^{-8} \text{ M}$. This degree of consistency is typically regarded as an acceptable level of scientific validation, and these values have stood for 10 or more years. However, Katona et al noted discord between these values and empiric measurements of the FXIII-A3 subunit concentration in plasma. Given the plasma concentrations of the A and B subunits, these affinity constants imply that most FXIII-A2 should circulate in the free form, whereas in reality, almost all plasma FXIII-A2 is present in complex with FXIII-B2. Thus, these values must substantially underestimate the affinity of the A and B subunits. The revised affinity constant determined in the present work ($\sim 10^{-10} \text{ M}$) is significantly tighter than previous values and is supported by independent and creative tests provided by the natural biodistribution of FXIII. Using the newly determined affinity constant, Katona et al predict and then confirm experimentally that 99% of plasma FXIII-A2 is present in complex with FXIII-B2 and only ~1% of circulating FXIII-A2 is present as free homodimers. In contrast, in 2 nonplasma sources of FXIII-A2 (cerebrospinal fluid and tears) in which FXIII-subunit concentrations are 3 orders of magnitude lower than in plasma, >80% of FXIII-A2 is present in the free form. Use of these 2 biological pools of FXIII not only validates the newly calculated affinity measurement but also shows that FXIII is uniquely regulated in different fluids. The present study also sheds light on a second unresolved question regarding the identity of FXIII-B subunit residues that mediate its association with the FXIII-A subunit. In a 2-step sequence of experiments, Katona et al first demonstrate that a monoclonal antibody recognizing sushi domains 1 and 2 of the FXIII-B subunit prevents complex formation with the FXIII-A subunit, consistent with previous data implicating the first FXIII-B sushi domain in FXIII-A subunit binding. Surprisingly, however, their subsequent experiments localize the epitope to amino acid residues 90 to 103 on the second sushi domain (see figure). As the authors note, these findings are not necessarily contradictory to the earlier study and may reflect complex interactions between the first and second sushi domains of the FXIII-B subunit. Additional studies using peptides and constructs expressing amino acid mutations within this region are needed to resolve the relationship between these residues and FXIII-A$_2$B$_2$ complex formation.

In addition to providing fundamental information about mechanisms regulating FXIII-A$_2$B$_2$ heterotetramer assembly, the observation that FXIII concentration and quaternary structure differ in intra- and extravascular fluids raises new questions about its regulation and function in these spaces. Why is FXIII-B$_2$ present in excess in these fluids? Does free FXIII-B$_2$ have a function(s) apart from its role in the FXIII-A$_2$B$_2$ complex? Is FXIII-A$_2$ in tears and cerebrospinal fluid prone to spontaneous activation, and is basal constitutive FXIII-A$_2$ activation beneficial in tears and cerebrospinal fluid? The thoughtful approach taken by Katona et al for the determination and confirmation of their measurements is a model for the type of well-designed and executed biological investigations needed to resolve these outstanding questions.

### Conflict-of-interest disclosure

The author declares no competing financial interests.

## REFERENCES


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### CLINICAL TRIALS & OBSERVATIONS

**Comment on Jiang et al, page 1674**

**TTP and pregnancy**

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In this issue of *Blood*, Jiang et al use the Oklahoma Thrombotic Thrombocytopenic Purpura-Hemolytic Uremic Syndrome (TTP-HUS) Registry to demonstrate that in women with a previous history of TTP, associated with severe ADAMTS13 deficiency, the frequency of TTP recurrence is low and pregnancy outcomes are positive.1

**T**TP is a rare, life-threatening disorder characterized by microangiopathic hemolytic anemia and thrombocytopenia often associated with renal failure and neurologic manifestations.2 Previously, survival rates for patients with TTP were ~10%. With the intervention of plasma exchange, however, it has now increased to >80%.3 At the center of the deficiency is severe depletion of a von Willebrand factor cleaving protease termed ADAMTS13 protease. There are both congenital and acquired forms of this disorder both of which are classified based on diminished levels of ADAMTS13. The

<table>
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<th>Table 1. Summary of data on TTP recurrence and pregnancy outcomes</th>
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<tr>
<td><strong>Oklahoma Registry data</strong></td>
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<tr>
<td>Total no. of women</td>
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<td>Total no. of pregnancies included</td>
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<tr>
<td>Number of pregnancies associated with recurrent TTP</td>
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<td>Number of pregnancies associated with preeclampsia</td>
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Oklahoma TTP-HUS Registry represents a database of patients in which data have been collected dating back to 1995. In this registry, >70% of the cases of TTP occur in women, 45% of whom are of childbearing age.4

Although it is well established that pregnancy may predispose to TTP, little is understood regarding pregnancy outcomes in women with a history of acquired TTP who subsequently become pregnant.5 Hematologists caring for these women are often asked to estimate the risk of recurrence of TTP during pregnancy and also the negative impact on the pregnancy itself. Because this is a rare disorder, and because few studies have been conducted on pregnant women with a past history of TTP, physicians have limited anecdotal studies to consult when advising these patients. Because congenital TTP behaves differently in pregnancy, experience with those patients cannot be extrapolated to women with the acquired form of the disorder.6 In women with hereditary ADAMTS13 deficiency, it is known that TTP can occur during pregnancy and is associated with fetal loss.7 Prophylaxis with plasma exchange can be initiated in those pregnancies, especially in women with documented low levels of ADAMTS13 in the peripartum period. Whether acquired forms of TTP are also associated with poor pregnancy outcomes is not well understood. In addition, there are no data regarding ADAMST13 levels and pregnancy outcome in women with a history of TTP.

Jiang and colleagues specifically address the risk of TTP recurrence and pregnancy outcomes in pregnant women.1 The authors build on their original report regarding recurrent TTP in pregnancy published in 2004.4 The previous report focused on the frequency of recurrent TTP with subsequent pregnancy in 19 women in the Oklahoma TTP-HUS Registry. In this new manuscript, the authors specifically use the Oklahoma TTP-HUS Registry, focusing primarily on women who had TTP associated with acquired, severe ADAMTS13 deficiency (level <10%). No data regarding the level of ADAMTS13 in the population were discussed in the previous manuscript.

The primary outcomes of the new study were not only risk of recurrence of TTP but also development of pregnancy complications. The results presented in the manuscript are based on 10 women who recovered from TTP and went on to have a total of 16 pregnancies. Nine of the 10 women in the study had TTP associated with inhibitors that were measureable at the time of their first TTP episode. Of the 10 women, 3 had TTP associated with pregnancy or postpartum, 2 women had systemic lupus erythematosus and the other 5 women were “idiopathic.” As shown in Table 1, 2 patients had recurrent episodes of TTP associated with pregnancy. Interestingly, these cases did not occur in the women who initially had pregnancy-associated TTP. In addition to the recurrence of TTP, 2 patients had preeclampsia. There were also 2 cases of pregnancy loss. The majority of the rest of the pregnancies (10) were not associated with any poor pregnancy outcomes.

Because there were such small patient numbers in their study, the authors also performed a literature search and pooled information from other studies to identify all reported cases of pregnancies in women following recovery from TTP associated with acquired, severe ADAMTS13 deficiency. In their literature search that included 10 additional pregnancies, 6 pregnancies were associated with recurrent episodes of TTP. Although the episodes of recurrent TTP were minimal, the frequency of poor pregnancy outcomes was increased. This included risk of preeclampsia and severe preeclampsia. These data, however, are less transparent than that from the Oklahoma registry, as 3 of the patients had a preexisting history of preeclampsia, which may have accounted for the increased frequency of preeclampsia in this study population.

The authors acknowledge that an obvious limitation of their data is the small number of women and pregnancies as is expected based on the low frequency of this disorder. This manuscript, however, represents data on more women and pregnancies than all the previously published reports and therefore represents the most comprehensive body of data to date. Also, because these patients were followed closely through the Oklahoma TTP-HUS Registry, it provides a comprehensive evaluation of these women for up to 18 years. For these reasons, this manuscript provides a compendium of data to guide decisions regarding pregnancy in women with a past history of TTP.

Still, there are many questions left unanswered. More information is needed to fully understand the risk of recurrent TTP in this population as well as the risk of poor pregnancy outcomes. The utility of ADAMTS13 measurement is not discussed in this manuscript, although it may represent a means of surveillance for women at risk of TTP recurrence in pregnancy. Perhaps serial measurement of ADAMTS13 levels in pregnancy could represent a monitoring mechanism for risk of recurrence. In patients at risk, therapeutic plasma exchange could be initiated as a preventative measure. Further studies using these levels during pregnancy are needed.

The findings of this study, therefore, albeit based on small patient numbers, demonstrate good pregnancy outcomes for over 80% of women with a history of TTP. It should provide reassurance to physicians caring for these women that healthy pregnancies can occur with diligent observation and careful monitoring.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES
TTP and pregnancy

Elisabeth M. Battinelli