Supplementary Appendix

Supplementary Methods

ADAMTS13 Germline Genetic Sequencing

Next-generation sequencing (NGS) was used to detect single nucleotide changes and small insertions and deletions (<20 bp) in the *ADAMTS13* gene, including the complete coding region and splice junctions. Regions with coverage less than 50x, fewer than 50 sequencing reads, or low quality were supplemented with Sanger sequencing. Array comparative genomic hybridization (aCGH) was used to detect copy number variation due to deletions or duplications in *ADAMTS13* using custom targeted arrays from OGT consisting of probes approximately 60 bp in length with a minimum density of coverage of 4 probes per 500 bp in exonic regions. Human genome build GRCh37.p13 and reference sequence NM_139025.4 were used for this analysis. Deletions or duplications larger than 20 bp and smaller than 500bp and balanced chromosomal rearrangements (e.g., translocations, inversions) may not be detected using these methods. Variant classification was performed in accordance with ACMG guidelines.[1]

ADAMTS13 Activity Assay[2,3]

ADAMTS13 activity was measured using a laboratory developed ADAMTS13 activity assay. Citrated patient plasma was diluted and incubated with an ADAMTS13 substrate peptide labeled with a fluorophore and quencher (FRETS-VWF73). Fluorescence emitted due to cleavage of the substrate peptide by ADAMTS13 was serially measured using a fluorescence plate reader.

ADAMTS13 Inhibitor Assay[2,3]

Functional ADAMTS13 inhibitors were detected using a Bethesda-like assay. Endogenous patient ADAMTS13 was inactivated by heat-treatment of citrated patient plasma at 56°C. The

heat-treated patient plasma was mixed 1:1 with calibrator plasma and incubated at room temperature for one hour. Residual ADAMTS13 activity in the 1:1 mix was measured using the ADAMTS13 activity assay described above. One inhibitor unit is defined as the concentration of inhibitor able to reduce the ADAMTS13 activity in the 1:1 mix by half (i.e., 50% residual activity).

Von Willebrand Factor Antigen and Activity Assays

Von Willebrand Factor antigen level (VWF:Ag) was determined by enzyme-linked immunosorbent assay (ELISA) as described previously.[4] The activity assay (VWF:GPIbM) was performed using an enzyme-linked immunosorbent assay to detect patient vWF binding to recombinant gain-of-function human GPIb immobilized in microtiter wells.[5]

Von Willebrand Factor Multimer Analysis

Quantitative vWF multimer analysis was performed via western blot by the Versiti Hemostasis Laboratory as described previously.[6] vWF multimers were separated by sodium dodecyl sulfate agarose gel electrophoresis. Loading volumes were adjusted to approximately normalize the vWF antigen level across samples. vWF multimers were transferred to a nitrocellulose membrane by electroblotting and membranes were incubated with anti-vWF monoclonal antibodies produced by the Versiti Blood Research Institute Hybridoma Core Laboratory. Chemiluminescent detection and densitometry analysis were performed and the percentages of low, intermediate, and high molecular weight multimers were calculated and compared to the normal plasma control.

Anti-ADAMTS13 Antibody ELISA[3]

The anti-ADAMTS13 IgG assay was performed by sandwich ELISA. Diluted citrated patient plasma was incubated in microwell plates coated with full-length recombinant human ADAMTS13. Unbound antibodies were washed away, and antibodies bound to recombinant ADAMTS13 were detected using HRP-conjugated anti-human IgG and TMB substrate. Color production was measured using a spectrophotometer and results were calculated using a calibration curve.

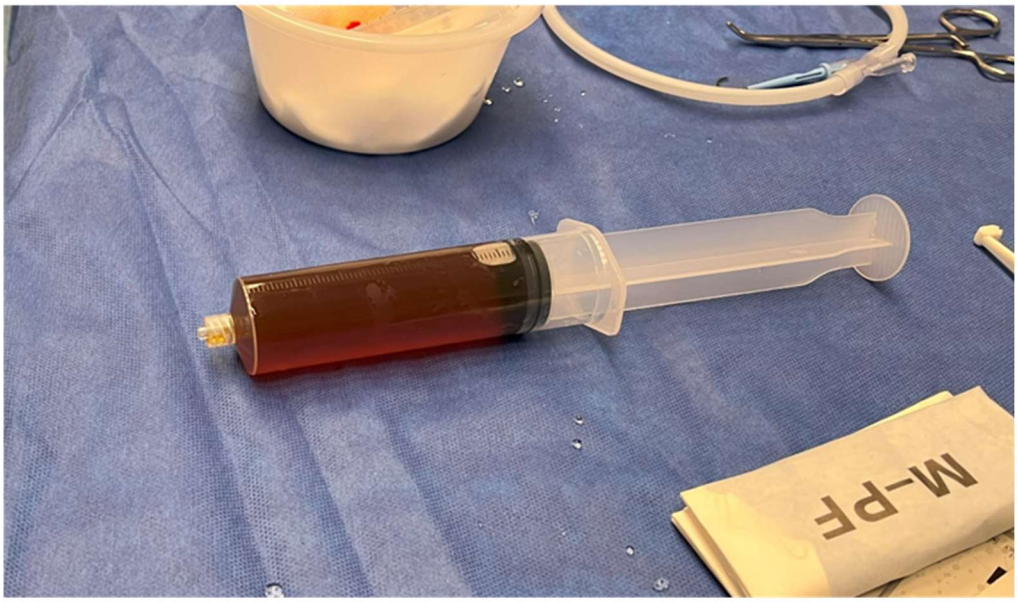
ADAMTS13 Circulating Immune Complex ELISA[7]

A 96-well flat bottom polystyrene ELISA plate (Maxisorp, from Thermo Fisher cat#: 442404) was coated with 120 µL/well of anti-ADAMTS13 monoclonal antibody clone 5C11 at a concentration of 2 µg/mL in 1X PBS buffer (Gibco, from Fisher Scientific cat#: 10010-023). The plate was then incubated overnight at 4°C on an orbital shaker. The following day, the primary antibody solution was decanted, and the plate was washed three times in 300 µL PBS + 0.1% Tween (Sigma Aldrich cat# P1379-500) per well, after which the plate was blocked with 200 µL/well of PBS + 3% non-fat dry milk powder (Blotto, Santa Cruz Biotechnology cat#: sc-2325) for 2 hours at room temperature on an orbital shaker. The blocking buffer was then decanted and the plate washed as described above. The control and TTP plasma samples were prepared at a 1:2 dilution in PBS + 1% non-fat dry milk power, which was then added at 100 µL per well in triplicate for each sample condition. The plate was then incubated for 1 hour at room temperature on an orbital shaker. After incubation, the samples were decanted and the plate washed as described above. A solution of anti-human IgG-HRP antibody (Jackson ImmunoResearch cat# 709-035-098) was prepared in a 1:10,000 dilution in PBS + 1% non-fat

dry milk power, and 100 μL of solution was added to each well. The solution was incubated for 1 hour at room temperature on an orbital shaker. The plate was then washed as described above, and 100 μL of 1-Step Ultra TMB-ELISA Substrate Solution was added to each well for 15 min, after which 1 N HCl was added to each well to stop the reaction. Finally, absorbance was measured at 450 nm.

Supplementary Figures

FIGURE S1



Variable	Pericardial Fluid
Red Blood Cells per µl	6000
Nucleated Cells per µl	331
Hematocrit (%)	Not done
Albumin (g/dL)	2.2
Amylase (U/L)	52
Glucose (mg/dL)	124
LDH (U/L)	1,127
Total Protein (g/dL)	3.4

Figure S1. Fluid Removed During Emergent Pericardiocentesis. Selected fluid chemistries and cell counts are shown in the accompanying table.

FIGURE S2

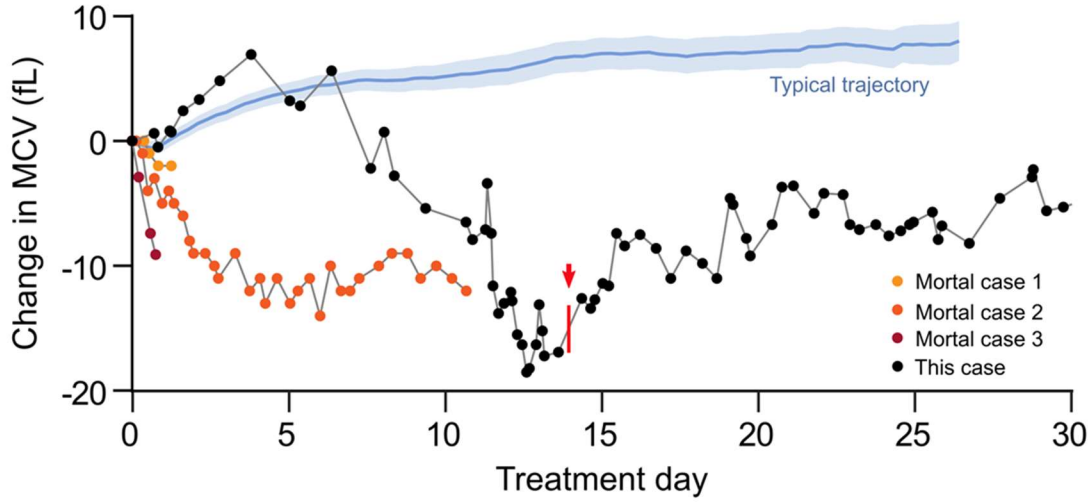


Figure S2. Trajectory of MCV in Mortal Cases of iTTP. Serial daily measurements of red blood cell mean corpuscular volume (MCV) in our patient (black) are shown alongside three consecutive cases (yellow, orange, red) from the Harvard TMA Research Collaborative registry in which iTTP patients expired due to refractory disease despite daily plasma exchange treatments. The blue line and shaded region represent the mean ± 95% CI MCV trajectory for the remaining 99 consecutive cases in the Harvard dataset. The red arrow and mark indicate initiation of treatment with rADAMTS13 in our patient.

FIGURE S3

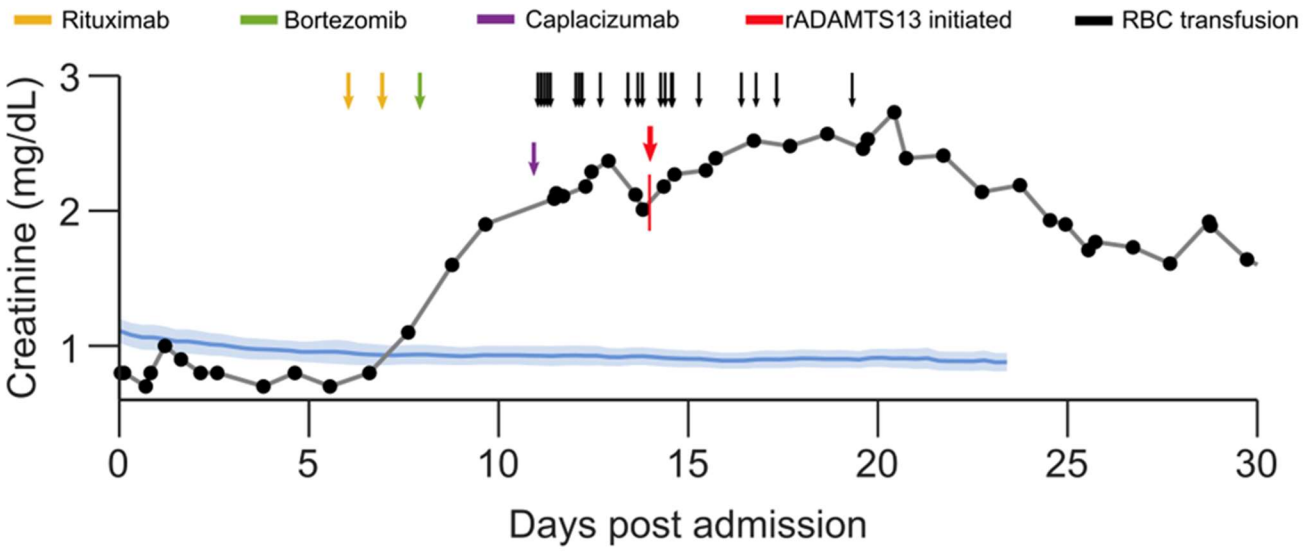


Figure S3. Creatinine Trend in our Patient. Serial daily measurements of serum creatinine in our patient are shown (black), with the timepoints of key therapies depicted as described in the legend. The blue line and shaded region represent the mean ± 95% CI creatinine trajectory for 102 consecutive iTTP cases in the Harvard TMA Research Collaborative dataset.

FIGURE S4

Days since Admission	12	16	17	18	32	36	39	46	51	72	126	193	230
On rADAMTS13	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No
VWF Activity	49	139	104	42	163	143	143	162	158	184	167	156	136
VWF Antigen	68	159	146	82	169	144	138	167	164	175	169	149	132
Ratio	0.72	0.87	0.71	0.51	0.96	0.99	1.04	0.97	0.96	1.05	0.99	1.05	1.03

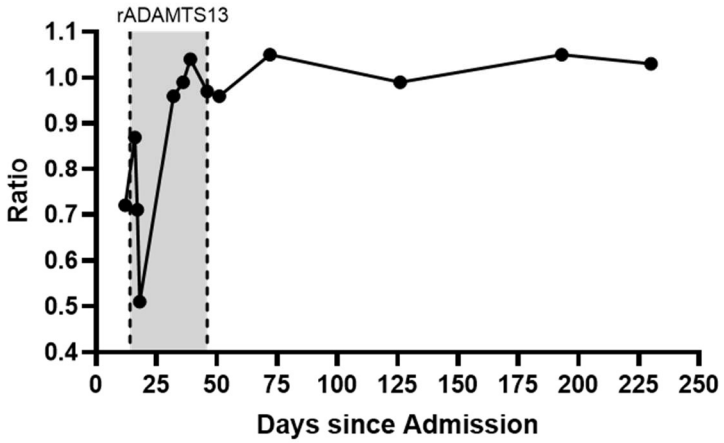


Figure S4. von Willebrand Factor (vWF) Testing in Relation to Therapy with rADAMTS13. The table (top) depicts results of vWF testing during and after therapy with rADAMTS13. The ratio of vWF activity to vWF antigen was graphed according to the day since admission, with the period of rADAMTS13 therapy shaded in gray (bottom). We measured vWF antigen and activity using the ELISA and vWF:GPIbM assays, respectively, as described in the Supplementary Methods.

FIGURE S5

Period	No. of Samples	Day(s)	Description
1	1	12	High disease activity, off rADAMTS13
2	3	16-18	High disease activity, on rADAMTS13
3	4	32-46	Low disease activity, on rADAMTS13
4	5	51-230	Low disease activity, off rADAMTS13

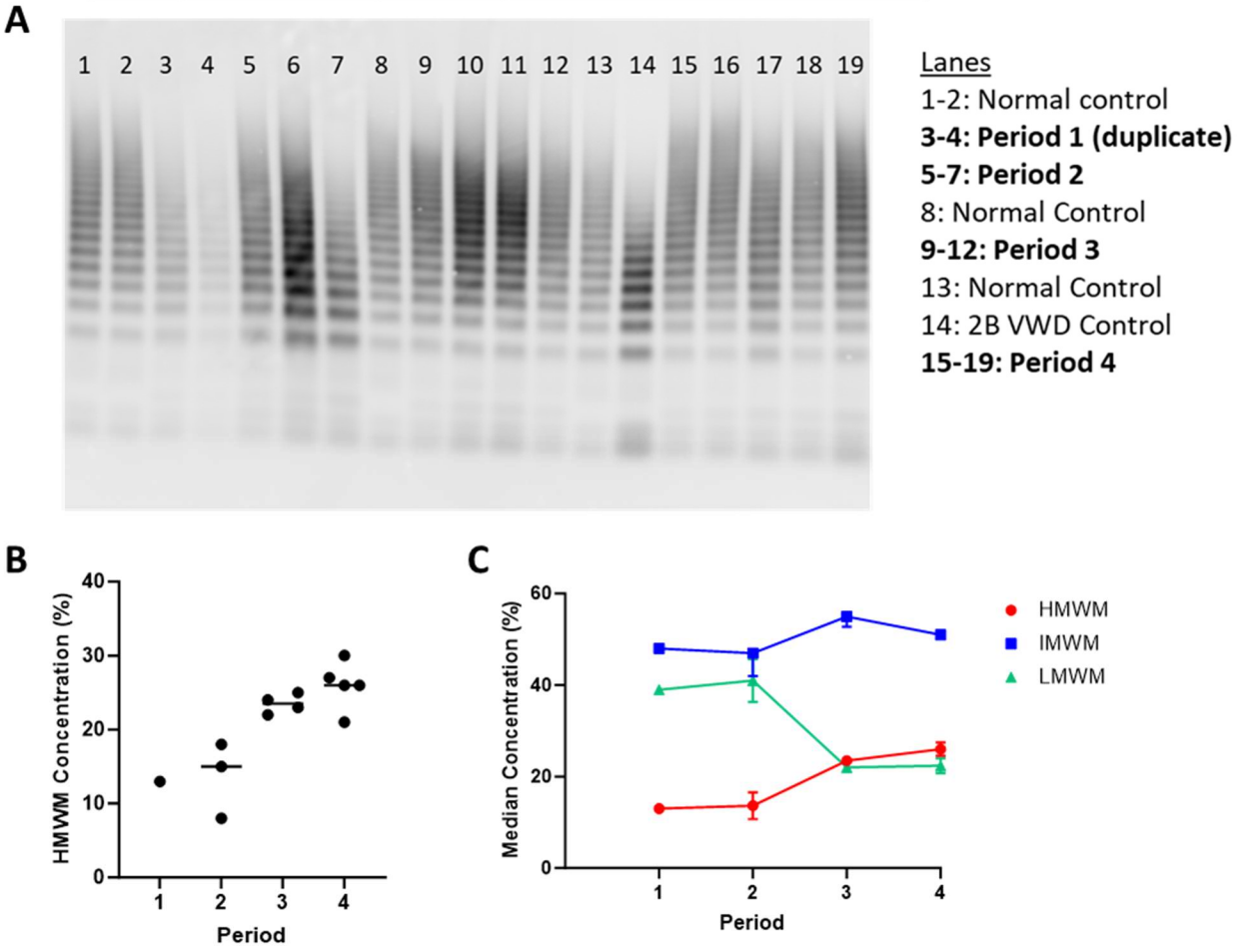


Figure S5. Plasma von Willebrand Factor Multimer Analysis. The patient's plasma von Willebrand factor multimers were evaluated at selected time points by western blot on non-reducing SDS agarose gel electrophoresis. Samples were divided into four broad categories based on the level of apparent disease activity and rADAMTS13 treatment status as shown in the table. **Panel A** depicts the raw chemiluminescent image of the western blot demonstrating the presence of vWF multimers of varying sizes in all samples. **Panel B** shows the quantification of

high-molecular-weight multimers (HMWM) as a percentage of the total vWF present across Periods 1-4. The horizontal lines represent median values. In **Panel C**, trends in the concentrations of high-, intermediate- (IMWM), and low- (LMWM) molecular-weight multimers are shown by Period as a percentage of the total vWF present. Data are depicted as median ± IQR.

FIGURE S6

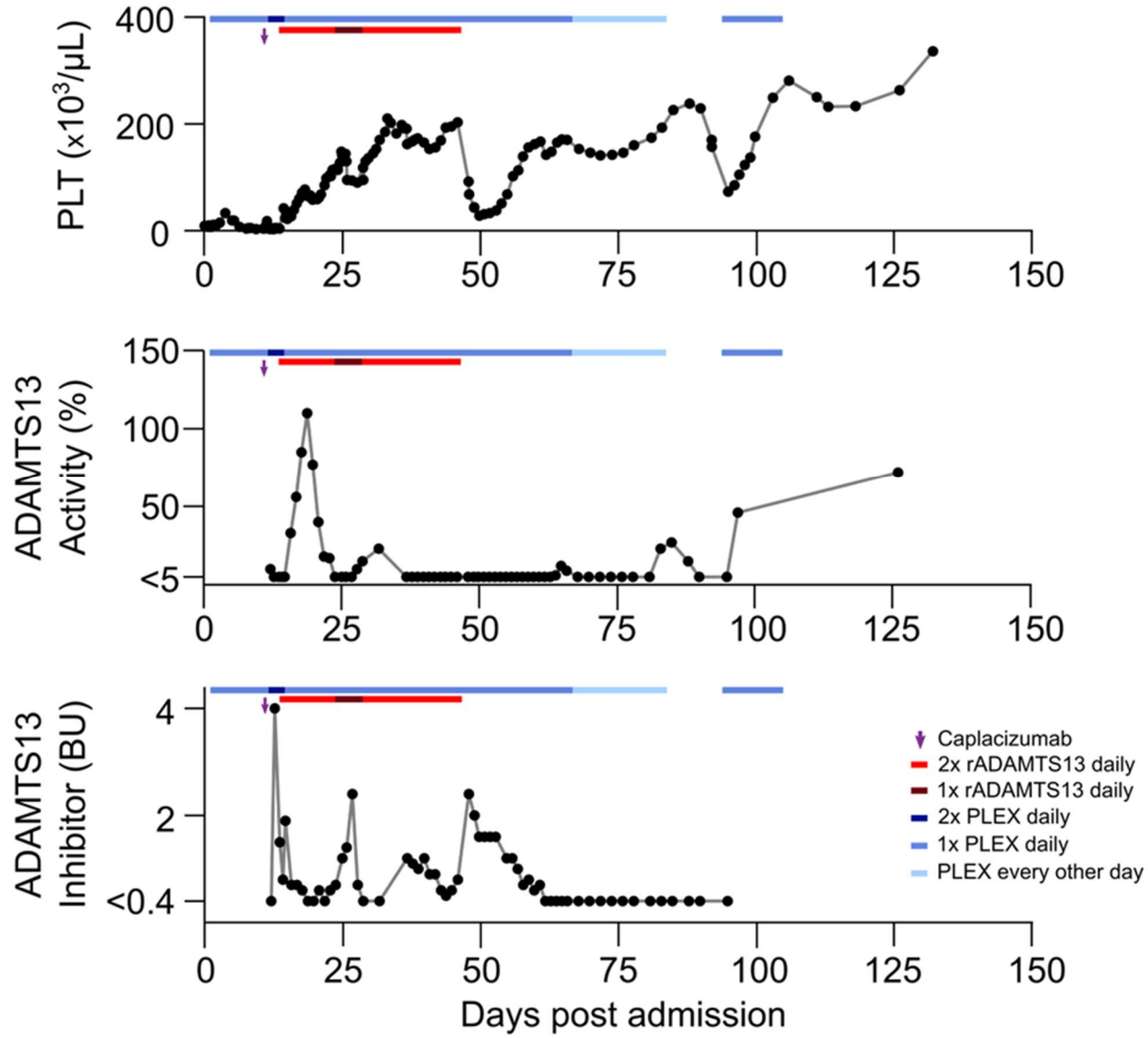


Figure S6. Relationship between ADAMTS13 Activity and Inhibitor Levels, Platelet Count, and iTTP Therapies. ADAMTS13 activity and inhibitor values represent trough levels drawn at the conclusion of a 12-hour rADAMTS13 dosing interval and immediately prior to daily plasma exchange. Once daily platelet counts drawn with morning phlebotomy are shown.

Supplementary Tables

TABLE S1

Variable	Reference Range, Adults	Admission Labs
White-cell count (x10^3 per µl)	4.0-11.0	13.1
Hemoglobin (g/dl)	11.7-15.5	6.9
Hematocrit (%)	35.7-45.8	20.8
Red-cell count (per µl)	4.20-5.4	2.14
Platelet count (per µl)	150-460	9
Mean cell volume (fl)	80-100	97.2
Total bilirubin (mg/dl)	0-1.2	3.3
LDH (units/L)	94-250	2089
Reticulocyte count (%)	0.9-2.1	16.6
Haptoglobin (mg/dl)	30-200	<10
PT (seconds)	9.2-11.4	13.3
PTT (seconds)	23.4-33.1	23.3
INR	0.9-1.1	1.3
Sodium (mmol/liter)	133-145	136
Potassium (mmol/liter)	3.6-5.2	4.1
Chloride (mmol/liter)	98-107	100
Bicarbonate level (mmol/liter)	22-29	24
Urea nitrogen (mg/dl)	6-20	31
Creatinine (mg/dl)	0.5-1.0	0.8
Glucose (mg/dl)	70-99	138
Alanine aminotransferase (U/liter)	0-33	15
Aspartate aminotransferase (U/liter)	0-32	66
Alkaline phosphatase (U/liter)	35-104	64
Direct antiglobulin test, Anti-IgG	Negative	Negative
Direct antiglobulin test, Anti-C3b,-C3d	Negative	Negative
ADAMTS13 Activity (%)	>66.8%	<2
ADAMTS13 Antibody (AU)	<12	49

Table S1: Laboratory Parameters at the Time of Presentation.

References

1. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17(5):405-24. DOI: 10.1038/gim.2015.30.
2. Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. British journal of haematology 2005;129(1):93-100. (In eng). DOI: 10.1111/j.1365-2141.2005.05420.x.
3. Froehlich-Zahnd R, George JN, Vesely SK, et al. Evidence for a role of anti-ADAMTS13 autoantibodies despite normal ADAMTS13 activity in recurrent thrombotic thrombocytopenic purpura. Haematologica 2012;97(2):297-303. DOI: 10.3324/haematol.2011.051433.
4. Flood VH, Gill JC, Morateck PA, et al. Common VWF exon 28 polymorphisms in African Americans affecting the VWF activity assay by ristocetin cofactor. Blood 2010;116(2):280-6. DOI: 10.1182/blood-2009-10-249102.
5. Flood VH, Gill JC, Morateck PA, et al. Gain-of-function GPIb ELISA assay for VWF activity in the Zimmerman Program for the Molecular and Clinical Biology of VWD. Blood 2011;117(6):e67-74. DOI: 10.1182/blood-2010-08-299016.
6. Flood VH, Gill JC, Friedman KD, et al. Collagen binding provides a sensitive screen for variant von Willebrand disease. Clinical chemistry 2013;59(4):684-91. DOI: 10.1373/clinchem.2012.199000.
7. Lotta LA, Valsecchi C, Pontiggia S, et al. Measurement and prevalence of circulating ADAMTS13-specific immune complexes in autoimmune thrombotic thrombocytopenic purpura. Journal of thrombosis and haemostasis : JTH 2014;12(3):329-36. DOI: 10.1111/jth.12494.