Review

Management of TTP – the main options for diagnosis

Roxana Cătălina FEREA[1,2], Stejara Nicoleta MIHAI[1,2], Cristina Tatiana ENACHE[1,2]

[1]Carol Davila Medical and Pharmacy University, Bucharest
[2]Hematology Department, University Emergency Hospital, Bucharest, Romania

Keywords: *TTP, ADAMTS13, assay, activity, antigen, antibody*

Abstract

Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy caused by severe deficiency of ADAMTS13, a von Willebrand factor (VWF)-specific protease.[1] TTP is a severe condition and a medical emergency in hematology. Prolonged time from presentation to diagnosis and therapeutic actions is life-threatening. Establishing a clear diagnosis of TTP is essential, as the therapeutic measures for TTP may include invasive procedures that have risks of their own.[1]

Therefore, the discovering and production of a synthetic ADAMTS13-specific VWF fragment (VWF73) allowed for quantification of ADAMTS13 activity, the basis for TTP diagnosis. Further advances led to the development of faster and reliable technologies[2]. One of these widely used methods is fluorescence resonance energy transfer (FRET).

Recently, several rapid ADAMTS13 activity assays have become available to potentially fill this void. One is a semi-quantitative screening assay from Technoclone[3], and the other is a fully automated, quantitative chemiluminescence-based immunoassay (CLIA; HemosIL AcuStar ADAMTS13 Activity, Instrumentation Laboratory, Bedford, MA).[4]

The ELISA based activity assay, manufactured by Technoclone (Vienna, Austria) and named Technozym ADAMTS-13 Activity, is the most widely used according to ECAT data.[5] The ELISA based Antigen assay is also possible.[5]

Further advances in diagnostic methods led to identifying immune-mediated TTP (iTTP) via the presence of anti-ADAMTS13 immunoglobulin G (IgG) autoantibodies. Methodologies used include enzyme-linked immunosorbent assays (ELISAs) and/or functional inhibitor assays based on mixing studies.[6] The most common approach here is the assessment for functional inhibitors (ie. autoantibodies that block ADAMTS13 activity) using a Bethesda-like assay.[7]

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening thrombotic microangiopathy defined by severe deficiency of a von Willebrand factor (VWF)-specific protease, ADAMTS13.[1]

The primary idiopathic form is more frequent and associates anti ADAMTS13 antibodies, while the congenital form (Upshaw-Shulman Syndrome) is rare, <1% of all TTPs. A secondary TTP was described, following certain clinical conditions, such as neoplasms, sepsis, medication, and does not associate a drop in ADAMTS13 activity.[8]

Von Willebrand factor is an adhesion glycoprotein important in coagulation process, that circulates in plasma as a heterogenous mix of multimers. VWF is also a carrier for factor VIII and has a role in platelet adhesion and aggregation at endothelial cell surface. VWF multimers, synthetized by megakaryocytes and endothelial cells, are stocked in α granules and Weibel-Palade corpuscles in endothelial cells. Their size varies from 600.000 D to 20 million D. Ultra large multimers have higher adhesion effect then the smaller multimers, predisposing to microthrombi formation when present in circulating plasma, thus determining TTP.[9]

VWF binds collagen fibers exposed by vascular injury and then suffers a conformational change in response to the blood flow. In a rapid flux, a unfolded form of VWF will expose the platelets

Corresponding author: Stejara Nicoleta Mihai
email: stejaramihai@yahoo.com S. M.

binding sites 1b/IX/V and αIIbβ3 and promote their adhesion to endothelial cells.[9]

ADAMTS13 breaks ULVWF produced by endothelial cells at the A_2 central domain, Tyr[1605]-Met[1606] before their activation in circulating plasma. Activation of ADAMTS13 is dependent on conformational changes of VWF and itself`s.[8,10,11,12]

ADAMTS13 is a Ca, Zn dependent metalloproteinase produced by hepatic cells, endothelial cells, megakaryocytes and platelets and its genetic information is located on chromosome 9 (9q34). It is formed of 1427 AA residues and has 4 distinct regions: catalytic region, C terminal proximal region, containing a disintegrin, the first repeating thrombospondin region (TSP1-1) rich in Cys and a spacer domain, medial C terminal region, with repeating thrombospondin regions 2-8 (TSP2-8) and distal C terminal region, with 2 CUB domains (c1r/ c1s urinary epidermal growth factor, bone morphogenetic protein).

Proteolytic activity of ADAMTS13 is dependent on its conformational change and that of VWF. Interaction between CUB and spacer domain ensures the normal globular conformation, prevents exposure of the functional site and the interaction with VWF A2 domain. In a rapid vascular blood flow, VWF`s C-terminal domain cleaves the CUB-spacer bond and determines the unfolding and conformational change of ADAMTS13.[11,13,14,15]

Acute TTP, congenital or acquired immune form, is characterized by a <5% or <10% ADAMTS13 activity, the main physio pathological mechanism.[12,14]

Metode

2. Hereditary TPP

Hereditary TTP is a result of a genetic mutation, homo or heterozygous. To present, over 140 mutations of ADAMTA13 gene are known, 60% of them are missense, 20% are deletions and insertions. Nonsense or splicing regions mutations have also been described.

Insertion of a single base at exon 29 (4143insA) has been described in the geographic area of Baltic Sea, Scandinavia and Moravia, while the missense mutation Arg1060Trp at exon 24 has been mainly identified in USA population and in some countries from Europe. Homozygotes mutation have been identified mostly in consanguine families.[14]

TTP is a severe condition and a medical emergency in hematology. Prolonged time from presentation to diagnosis and therapeutic actions is life-threatening. Establishing a clear diagnosis of TTP is essential, as the therapeutic measures for TTP may include invasive procedures that have risks of their own.[1] Therefore, the discovering and production of a synthetic ADAMTS13-specific VWF fragment (VWF73) allowed for quantification of ADAMTS13 activity, the basis for TTP diagnosis. Further advances led to the development of faster and reliable technologies[2] Most widely used tests for TTP diagnosis are summarized in Table 1.

Table 1

ADAMTA13 Activity	FRET	AnaSpec, PeptaNova, Mybiosource etc.
	CLIA	HemosIL
	ELISA	Technocolne
	Immunochromatography (semi-quantitative)	Technoscreen
ADAMTA13 Ag	ELISA	Technocolne
Anti ADAMTS13 antibodies	ELISA	Technocolne
	Bethesda- like assay	Based on FRET or CLIA

2.1 ADAMTS13 activity:

a. FRET

One of these methods is fluorescence resonance energy transfer (FRET). The FRET method utilizes a VWF73 fragment, marked with a pair formed by fluorophore and quencher.[16] ADAMTS13 metalloproteinase from patients' plasma cleaves the VWF73 fragment and thus separates the fluorophore from its quencher, determining fluorescence emission that can be quantified as MFI (Measured fluorescence intensity) at a specific wavelength; the fluorescent signal is proportional to ADAMTS13 activity. This is an efficient diagnostic method, with low rate of false positive but is has the disadvantage of a longer turnaround time.[17]

Hence, therapeutic treatment consisting of plasma exchange is usually initiated before the diagnosis is confirmed. However, plasma exchange in itself comes with a risk of adverse events, including transfusion and allergic reactions.[17]

b. CLIA

An alternative to this method is the HemosIL AcuStar, a fully automated method for

quantification of ADAMTS13 activity with an analysis time of 33 minutes. It is an immunoassay performed in two steps: the first step requires incubation of patient`s plasma with magnetic particles coated with VWF73, while step two represents incubation with an isoluminol-labeled antibody against the cleavage site of VWF73.[18] Only when ADAMTA13 has cleaved the VWF73 on the magnetic particles, the cleavage site is exposed, and a chemiluminescent signal is produced. Emitted light is proportional to the enzymatic activity of ADAMTS13, is quantified as MFI and then converted in IU/mL against a standard curve.

Dimopoulos K et al study describes the performance of an ample clinical study for validation of both methods` ability to differentiate TTP from other TMAs. There are a variety of assays that can be used for measuring ADAMTS13, inclusive of antigen (level) and activity. However, measurement of ADAMTS13 activity is generally considered more clinically meaningful. Original ADAMTS13 assays utilized plasma derived VWF multimers as substrate and required time-consuming steps of conformational unfolding involving various agents. Currently, most of the commonly utilized assays for measuring ADAMTS13 activity are based on enzyme linked immunosorbent assay (ELISA) or fluorescence resonance energy transfer (FRET)-based technologies utilizing recombinant VWF substrates.[17]

Data on HemosIL AcuStar ADAMTS13 is now available in several publications.[5] In the latest multicenter study evaluating this method, a large sample number was co-assessed against the Technozym assay. There are three other reports on the HemosIL AcuStar ADAMTS13 Activity assay published in the literature, albeit using smaller sample sizes.[5]

Dimopoulos K et all study compared the HemosIL assay, a new, fully automated method, and the current widely used assay FRET in quantifying ADAMTS13 activity using plasma samples collected from a large lot over a time span of 14 years[17].

The CLIA is accurate and very rapid compared to standard commercial or other inhouse based testing, taking only 33 m to derive a test result as opposed to 3–5 h for ELISA, and may therefore be regarded as a valuable option for supporting urgent/emergency testing[17].

For the FRET method, patient plasma was mixed with VWF73 and loaded into a 96-well plate together with 7 samples with known ADAMTS13 activity used to produce a calibration curve. The plate was read for 60 minutes at 5 minutes intervals at an emission detection wavelength of 440 nm. ADAMTS13 activity is directly proportional to the detected light signal and by the use of the calibration curves the fluorescence can be transformed to ADAMTS13 activity units. Values under the lower limit of detection were translated to 0.01 kIU/L.[17]

For the HemosIL method the sample is loaded together with 2 VWF controls inside a preconstructed cassette. Patient`s plasma is mixed with magnetic particles coated with a GST-VWF73 peptide. ADAMTS13 from plasma, if present, will cleave the peptide. Magnetic separation is used for a wash step, then an isoluminol-conjugated antibody against the cleavage site is added. The emitted chemiluminescent signal is quantified in RLU (relative light units) and converted to ADAMTS13 activity using the calibration curve. Duplicate samples were tested for a randomized cohort of samples, to test the method variability.[17] A strong correlation and a high degree of agreement was observed for the two methods.[17]

TTP patients in remission phase are hard to evaluate by the use of only one of either method. It is generally known that RF can interfere with chemiluminescent methods because of the antigen-antibody reaction and can lead to underestimation of ADAMTS13 activity.[17] We cannot exclude a possible false positive, equivalent of a falsely low activity by this method as RF was not quantified in these patients.[4] Moreover, chemiluminescence measurement is affected by lipemic and icteric plasma. A week point of the FRET method is that it has a low detection point of 0.04 kIU/L.[17]

c. ELISA

One of the ELISA based activity assay, manufactured by Technoclone (Vienna, Austria) and named Technozym ADAMTS-13 Activity, is the most widely used according to ECAT data.[5] The ELISA assay for ADAMTS13 activity has a high sensitivity for ADAMTS-13 and an inferior limit of detection of 0.003 IU/ml, turnaround time of 3h and no interference with icteric, hemolytic or lipemic plasma. In is also possible to determine ADAMTS13 Inhibitor using the Bethesda assay[19]. The recombined VWF fragment that contains the A2 domain and the cleavage point Tyr^{1605}-Met^{1606} is marked with GST-His and fixed at the base membrane of the testing plate via anti-GST monoclonal antibodies. These fragments are then incubated with the patient's plasma. The plasmatic ADAMTA13 (if present) will act on the fragment

and the cleavage site will be marked with monoclonal antibodies HRP conjugated. Chromogenic substrate is then added, and the color intensity is evaluated spectrophotometrically at 450 nm wave length.[20] Fluorogenic ELISA can also be used to measure ADAMTS13 antigen and activity.[19]

While all the above test approaches provide valid results, most do not easily accommodate urgent testing for many reasons, including assay time (typically 4–5 h) and technical skill required. Also, for cost/labor efficiency, most current assays are best performed in batches and not for individual patients. These factors compromise timely testing for ADAMTS13 activity despite clinical urgency. It is widely accepted that timely testing (i.e. test results within the same day as patient management decisions are required) would assist rapid identification or exclusion of TTP, with patients thereby better managed, with potentially reduced risk and cost burden by avoiding unnecessary plasma exchange.[17]

d. Immunochromatography

Recently, several rapid ADAMTS13 activity assays have become available to potentially fill this void. One is a semi-quantitative screening assay from Technoclone[3], and the other is a fully automated, quantitative chemiluminescence-based immune-assay.[4] Data on the semi quantitative screening assay has recently been published by Moore et al.[3] Plasma from a cohort of patients with thrombotic microangiopathy suspicion were tested with TECHNOSCREEN ADAMTS13 activity screening test. Plasma was also tested by an alternative method, ELISA or FRET.[3] Technoscreen ADAMTS13 activity screen is a useful test that can be used for screening but needs to be confirmed though another method. It is a semiquantitative immunochromatographic test that estimates ADAMTS13 activity levels in human citrate plasma. Assay principle: the patient`s plasma is incubated with VWF fragment. ADAMTS13 specific cleavage of this fragment is then detected with a cleavage site specific antibody by the amount of color development. The color intensity directly corelates with the amount of cleaved substrate and thus with ADAMTS13 activity level in the plasma sample.[21] The test has four level-indicator points: 0, 0.1, 0.4, or 0.8 IU/mL and it shows good sensitivity (88.7%), specificity (90.4%), positive predictive value (74.6%) and negative predictive value (96.2%).[3]

2.2 ADAMTA13 Antigen:

a. ELISA

ADAMTA13 antigen can also be determined by various methods. Chromogenic ELISA for determination of ADAMTS13 antigen concentration measures Ag values between 0.0 and 1.0 U/ml, has a turnaround time of approximately 3.5h and detection is independent of enzyme activity.[19] This method measures all states of the enzyme: free, bound to inhibitor antibodies or bout to transporting or other proteins. ELISA uses plates coated with anti ADAMTD13 antibodies that will bound all forms of the enzymes. The enzyme ADAMTS13, if present, will fix the next set of antibodies, HRP conjugated that interacts with a chromogenic substrate, resulting in a colored substance. Optical density of this substance is measured by spectrophotometry at 450 nm/620 nm wavelength and is directly proportional to the antigen ADAMTA13 concentration. Optical density measurements may be affected by lipemic or hemolyzed samples.[20,22] Fluorogenic ELISA useful in measurement of ADAMTS13 antigen and activity is also available.[19]

Starke R et all study aims to evaluate the potential use of ADAMTS13 activity, antigen, and antibodies-Ig G anti-ADAMTS13, using ELISA. Plasma samples from several TTP confirmed patients were analysed.[23]

Measurement of ADAMTS13 antigen together with an activity and antibody assay makes it possible to distinguish between neutralizing and nonneutralizing antibodies. In congenital TTP ADAMTS13 antigen and activity levels are both decreased. In the acquired TTP cases, ADAMTS13 antigen levels can be normal or slightly decreased, while ADAMTS13 activity level is significantly lower. Some patients don`t show a good correlation between the ADAMTA13 antigen and activity probably due to either activity-neutralizing antibodies or immune complexes that influence binding in the antigen assay. Antigen assay is not efficient in predicting ADAMTS13 activity, especially when autoantibodies are involved. Antigen values are not reliable in patients in relapse phase and it is indicated to be quantified in sample series. Also, after treatment, measurement of ADAMTS13 is not beneficial.[23]

On the other hand, Alwan F et al studies the correlation between the presence of anti-ADAMTS13 IgG antibodies, ADAMTS13 antigen level and patients` mortality. A large cohort of patients were tested, some of them multiple times,

over 7 years and 3 months. The study concluded that higher anti-ADAMTS13 antibody values and decreased ADAMTS13 antigen corelates with higher mortality.[7]

3.Acquired TTP

Circulating anti ADAMTS13 antibodies are present in 94-97% of TTP idiopathic/ acquired form patients together with severely reduced enzyme activity. Most antibodies have an inhibitory effect on enzymatic activity but in 10-15% of cases, antibodies increase enzymatic clearance.

Antibodies are mostly IgG_4 and 97-100% target amino acids from the spacer domain. 64% of cases associate antibodies targeting other regions.[14]

3.1 Anti ADAMTS13 antibodies:

Further analysis in the diagnostic protocol for TTP requires identification of acquired immune-mediated TTP (iTTP) and thus identification of Ig G anti-ADAMTS13. Methods used so far are ELISA and functional inhibitor assays based on mixing studies.[6] The most common approach here is the assessment for functional inhibitors (i.e., autoantibodies that block ADAMTS13 activity) using a Bethesda-like assay.[7] However, some patients with acquired TTP may have nonfunctionally inhibiting antibodies that act to accelerate the clearance process for ADAMTS13 from circulation. Such antibodies can be assessed by alternative assays, such as ELISA.[6]

a. ELISA

One commonly used ELISA assay is also available from Technoclone and detects human immunoglobulin (Ig) G against ADAMTS13.[5] One significant limitation of this assay, however, is the potential to detect non-ADAMTS13 antibodies that may be present in patients with general autoimmune conditions, particularly if high levels of such antibodies are present. It is also important to note that Bethesda-like using the AcuStar method compared to the reference (Technoclone) assay have shown very similar results, according to Favaloro E.J et al`s review.[5]

In almost all cases of iTTP, autoantibodies to ADAMTS13 can be identified[24,25] together with ADAMTS13 activity levels <10%. Most antibodies were catalogued as inhibitory and therefore can be quantified using mixing studies.[26,27] Non inhibitory antibodies can be identified by a simplified ELISA that uses recombinant fragments of ADAMTS13.[28,29] Non inhibitory antibodies reduce circulating ADAMTS13 from plasma by other mechanisms, such as antibody-mediated clearance.[29]

b. Bethesda-based assay

To elucidate the presence of inhibitory antibodies, a Bethesda-based assay can be used. This is very similar to the Bethesda assay in hemophilia, but it is not officially standardized for TTP diagnosis.[7] The aim of Vendramin C et all study was to assess this method and weather a 2-hour incubation time, similar to anti F VIII antibodies, as currently published, is absolutely necessary.[30,31] When coagulation factors are deficient, such incubation tine is usually not required. The study also aims to establish if the Bethesda assay has any benefits over the ELISA method.[6] For this study, acute TTP was characterized by ADAMTS13 activity <10% and detectable anti-ADAMTS13 IgG. The plasma used were first drown samples, from the initial presentation, and the presence of inhibitor antibodies were tested by 50:50 mixing studies.[6] ADAMTS13 activity was assessed using the FRETS assay[16] and antibodies were also quantified using an ELISA technique.[29,32] The Bethesda-based assay requires multiple steps: measurement of ADAMTS13 activity, mixing studies that will confirm the presence of inhibitor antibodies, mixing patient`s plasma with PNP (pooled normal plasma) as to acquire a ATAMTS13 activity of 50% and finally quantification of antibodies titer in the plasma mix. The aim of this method is to standardize measurement of antibodies` titer for all laboratories, similar to methods used in hemophilia.

c. Mixing studies

In mixing tests, the added normal plasma should correct protease activity by more than 50%; Lack of correction at this test indicates the presence of a strong inhibitor.[7] The inhibitor titer was quantified by mixing normal ADAMTS13 activity plasma with the patient`s plasma, containing the antibodies. Several measurements were made for different incubating periods of time and it has been observed that there is a dependency of antibody titer after more than 2-hour incubation.[6,33] Following the study, not all specimens had concordance between Bethesda assay and mixing study. It was hypothesized that the nonconcording specimens were in an early stage of TTP as they had positive mixing study, negative Bethesda assay and ADAMTS13 antigen moderately reduced.[6]

Mixing studies are a useful screening test that can hint to an inhibitor without discriminating between neutralizing and non-neutralizing, whereas Bethesda assay has specificity for neutralizing antibodies. Data from this study confirms the presence of non-neutralizing autoantibodies as described by Scheiflinger and colleagues[29] and contribute to understanding the pathophysiology of TTP.[6] Inhibition is not the exclusive effect of the antibodies. A previous study showed that antibodies are identified more frequently using ELISA than the inhibitor assay, in patients with acquired TTP.[24]

d. Inhibitor assay

Inhibitor assay was used in older studies, before the consensus that ELISA is a more effective assay. In starts with incubation of purified VWF with plasma ADAMTS13, followed by quantification by quantitative immunoblotting, residual ristocetin cofactor activity or residual collagen-binding activity.[24]

Results

4. Conclusion

In conclusion, many assay are available for a correct and complete diagnosis of TTP but none of them are infallible. The optimal combination of a screening or an exclusion rapid assay, with a high negative predictive value, combined with a confirmatory assay, even with a prolonged turnaround time, checks all the boxes for the purpose of a correct and fast diagnosis, initiating treatment as soon as possible and reducing unnecessary plasmapheresis for patients with TTP suspicion. Simultaneous quantification of ADAMTS13 activity and antigen may be useful in differentiating between the hereditary and the acquired form. Furthermore, for the acquired TTP, a simple mixing study can screen for autoantibodies, while adding measurements by ELISA method will differentiate between neutralizing and non-neutralizing antibodies. The large variety of assay ensure the possibility of a complete and correct diagnosis.

Synopsis

The paper "**MANAGEMENT OF TTP – THE MAIN OPTIONS FOR DIAGNOSIS**" puts into focus a life-threatening pathology and the difficulties in its diagnosis. Currently many assays are available for a correct and complete diagnosis of TTP but none of them are infallible. Knowledge of TTP`s physiopathology and the diagnostic methods makes for a better judgement of a clinical case, in the patient`s benefit.

References

1. **Joly BS., Coppo P., Veyradier A.** Thrombotic thrombocytopenic purpura. Blood 2017;129:2836–46.
2. **Kokame K., Matsumoto M., Fujimura Y., Miyata T.** VWF73, a region from D1596 to R1668 of von Willebrand factor, provides a minimal substrate for ADAMTS-13. Blood 2004;103:607–12.
3 **Moore GW., Meijer D., Griffiths M. et al.** A multi-center evaluation of TECHNOSCREEN® ADAMTS-13 activity assay as a screening tool for detecting deficiency of ADAMTS-13. J Thromb Haemost. 2020;18 (7):1686-1694.
4 **Favaloro EJ., Mohammed S., Chapman K. et al.** A multicentre laboratory assessment of a new automated chemiluminescent assay for ADAMTS13 activity. J Thromb Haemost. 2021;19(2):417-428.
5. **Favaloro EJ., Pasalic L., Henry B., Lippi G.** Laboratory testing for ADAMTS13: Utility for TTP diagnosis/exclusion and beyond. Am J Hematol. 2021 Aug 1;96(8):1049-1055. doi: 10.1002/ ajh.26241. Epub 2021 May 31. PMID: 33991361.
6. **Vendramin C., Thomas M., Westwood JP., Scully M. Bethesda.** Assay for Detecting Inhibitory Anti-ADAMTS13 Antibodies in Immune-Mediated Thrombotic Thrombocytopenic Purpura. TH Open. 2018 Sep 26;2(3):e329-e333. doi: 10.1055/s-0038-1672187. PMID: 31249957; PMCID: PMC6524890.
7. **Alwan F., Vendramin C., Vanhoorelbeke K. et al.** Presenting ADAMTS13 antibody and antigen levels predict prognosis in immune-mediated thrombotic thrombocytopenic purpura. Blood 2017;130(04):466–471.
8. **Laboratory Corporation of America. Directory of Services and Interpretive Guide.** ADAMTS13 Activity Reflex Profile. www.labcorp.com. 2016. Ref Type: Internet Communication.
9. **Hoffbrand V., Moss P.** Hoffbrand's Essential Haematology, ed. 7, Wilwy Blackwell, 2016.
10. **Saifan C., Nasr R., Mehta S., Acharya PS., El-Sayegh S.** Thrombotic Thrombocytopenic Purpura. In *J Blood Disord Transfus*, 2012, S3:001. doi: 10.4172/2155-9864.S3-001.
11. **South K., Luken, B.M., Crawley JTB., Phillis R, Thomas M, Collins R., Deforche L, Vanhoorelbeke K. and Lane DA.** Conformational activation of ADAMTS 13.

In PNAS 2014, vol. 111, no.52, 18578-18583, http://www.pnas.org/content/111/52/18578.full.

12. **Tsai HM" Thrombotic**. Thrombocytopenic Purpura, Hemolytic-Uremic Syndrome and Related Disorders" In *Wintrobe's Clinical Hematology,* Greer J, Foerster J, Lukens J, Rodgers G, Pareskevas F, Glader B, Arber D, Means R. jr, 12th ed, Lippincott Williams & Wilkins, 2009, 1314-1325.

13. **Ai J., Smith P., Wang S., Zhang P. and Zheng XL**. The Proximal Carboxyl-terminal Domains of ADAMTS-13 Determine Substrat Specificity and Are All Required for Cleavage of von Willebrand Factor. In *J.Biol.Chem*. 2005,280, 29428-29434.

14. **Kremer Hovinga JA. and Lämmle B.** Role of ADAMTS13 in the pathogenesis, diagnosis and treatment of thrombotic thrombocytopenic purpura. In ASH Education Book, 2012 vol. 2012 no. 1. 610-616. http://asheducationbook.hematologylibrary.org/content/2012/1/610.full.pdf+html.

15. **Scully M., Hunt BJ., Benjamin S., Liesner R., Rose P., Peyvandi F., Cheung B., Machin SJ.** Guidelines on the diagnosis and management of thrombotic thrombocytopenic purpura and other thrombotic microangiopathies. In *British Journal of Haematology,* 2012, 158(3):323-35

16. **Kokame K., Nobe Y., Kokubo Y., Okayama A., Miyata T.** FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. Br J Haematol 2005;129:93–100.

17. **Dimopoulos K., Philips M., Goetze JP.** Diagnosis of Thrombotic Thrombocytopenic Purpura by ADAMTS13 Activity Quantification. J Appl Lab Med. 2022 May 4;7(3):637-649. doi: 10.1093/jalm/jfab148. PMID: 34977929.

18. **Mirabet M., Blanch S. Josep Puig JS.** Highly sensitive fully automated chemiluminescent immunoassay for rapid quantification of ADAMTS13 activity. XXVI Congress International Society Thrombosis Haemostasis 63rd Annual Scientific Standardization Committee Meeting. 2017: PB 703. doi:10.3252/pso.eu.ISTH2017.

19. https://www.technoclone.com/en/product/adamts13-chromogenic-elisas

20. Laborator Synevo. Referintele specifice tehnologiei de lucru utilizate, 2016. Ref Type: Catalog.

21. https://www.technoclone.com/en/product/adamts13-screening-assay

22. https://www.technoclone.com/en/product/adamts13-screening-assay

23. **Starke R., Machin S., Scully M., Purdy G., Mackie I.** The clinical utility of ADAMTS13 activity, antigen and autoantibody assays in thrombotic thrombocytopenic purpura. Br J Haematol. 2007 Feb;136(4):649-55. doi: 10.1111/j.1365-2141. 2006.06471.x. PMID: 17367414.

24. **Rieger M., Mannucci PM., Kremer Hovinga JA. et al.** ADAMTS13 autoantibodies in patients with thrombotic microangiopathies and other immunomediated diseases. Blood 2005; 106(04): 1262–1267.

25. **Tsai H-M., Raoufi M., Zhou W. et al.** ADAMTS13-binding IgG are present in patients with thrombotic thrombocytopenic purpura. Thromb Haemost 2006;95(05):886–892.

26. **Tsai HM., Lian EC.** Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med 1998; 339(22):1585–1594.

27. **Peyvandi F., Ferrari S., Lavoretano S., Canciani MT, Mannucci PM.** Von Willebrand factor cleaving protease (ADAMTS-13) and ADAMTS-13 neutralizing autoantibodies in 100 patients with thrombotic thrombocytopenic purpura. Br J Haematol 2004;127(04):433–439.

28. **Klaus C., Plaimauer B., Studt J-D. et al.** Epitope mapping of ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura. Blood 2004;103(12):4514–4519.

29. **Scheiflinger F., Knäbl P., Trattner B. et al.** Nonneutralizing IgM and IgG antibodies to von Willebrand factor-cleaving protease (ADAMTS-13) in a patient with thrombotic thrombocytopenic purpura. Blood 2003;102(09):3241–3243.

30. **Kasper CK., Aledort L., Aronson D. et al.** Proceedings: a more uniform measurement of factor VIII inhibitors. Thromb Diath Haemorrh 1975;34(02):612.

31. **Verbruggen B., Novakova I., Wessels H., Boezeman J., van den Berg M., Mauser-Bunschoten E.** The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. Thromb Haemost 1995;73(02):247–251.

32. **Scully M., Cohen H., Cavenagh J. et al.** Remission in acute refractory and relapsing thrombotic thrombocytopenic purpura following rituximab is associated with a reduction in IgG antibodies to ADAMTS-13. Br J Haematol 2007;136(03):451–461.

33. **Mancini I., Valsecchi C., Palla R., Lotta LA., Peyvandi F.** Measurement of anti-ADAMTS13 neutralizing autoantibodies: a comparison between CBA and FRET assays. J Thromb Haemost 2012;10(07):1439–1442.