HEMOSTASIS AND THROMBOSIS

*Original article*

# Validation of PLASMIC score in a cohort of patients with suspected thrombotic microangiopathy in an academic medical centre

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**Background** - The PLASMIC score is a rapid and inexpensive clinical assessment tool for predicting severe ADAMTS13 deficiency (<10% activity) in patients with suspected thrombotic thrombocytopenic purpura (TTP). The score includes 7 parameters: absence of active cancer, patient not having received stem cell transplant or organ transplant, platelet count <$30\times10^9$/L, hemolysis, mean corpuscular volume <90 fl, International Normalized Ratio <1.5, and serum creatinine <2 mg/dL.

**Materials and methods** - In this retrospective study, we evaluated a cohort of 59 consecutive patients with suspected thrombotic microangiopathy who had been referred to the Hemostasis and Thrombosis Center of the "Federico II" University of Naples, Italy, for measurement of ADAMTS13 activity. Relevant clinical and laboratory information were collected for all patients.

**Results** - The PLASMIC score was calculated in 52 of the 59 patients included in the study. In the high-risk group (PLASMIC score 6 or 7), 12 out of 20 patients (60%) had ADAMTS13 <10%. Interestingly, all 6 patients (100%) with PLASMIC score 7 had ADAMTS13 <5%. In the intermediate risk group (score 5), only one case out of 17 (5.9%) had ADAMTS 13 <10%. In the low-risk group (score 0-4), none of the patients had severe ADAMTS13 deficiency. The collected data enabled the sensitivity and specificity of PLASMIC score in TTP to be calculated, achieving 92% (95% CI: 0.80-0.98) and 79% (95% CI: 0.66-0.89), respectively. The PLASMIC score was seen to be a very efficient tool in distinguishing between patients with severe ADAMTS13 deficiency from those without, with an AUC of 0.92 (95% CI: 0.82-1.0; p<0.001).

**Discussion** - In our cohort, a high-risk PLASMIC score successfully predicted patients with severe ADAMTS13 deficiency, allowing the clinician to quickly define the best therapeutic approach, especially useful for those clinicians not used to the diagnosis and treatment of thrombotic microangiopathies.

**Keywords:** *thrombotic thrombocytopenic purpura, PLASMIC, ADAMTS13, thrombotic microangiopathy.*

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## INTRODUCTION

Thrombotic microangiopathies (TMA), including thrombotic thrombocytopenic purpura (TTP) and atypical hemolytic uremic syndrome (aHUS), are different rare disorders,

but with common precipitating conditions, such as malignancy, drugs, pregnancy, and inflammatory disorders. The clinical features include microangiopathic hemolytic anemia (MAHA), thrombocytopenia, and various degrees of ischemic organ injury[1,2].

The classic signs/symptoms of TTP (hemolytic anemia, severe thrombocytopenia, fever, neurologic abnormalities, and renal abnormalities), also known as "Raynaud's Pentad", are present in only 40% of patients. Therefore, the unexplained presence of severe thrombocytopenia and MAHA should prompt a suspicion of TTP[3,4]. The distinction between TTP and aHUS relies on the evaluation of the level of activity of plasma ADAMTS13, a disintegrin and metalloproteinase, with a thrombospondin type 1 motif, member 13. Indeed, TTP is caused by a deficiency of this von Willebrand factor (VWF) cleaving enzyme. ADAMTS13 activity levels below 10% are pathognomonic of TTP. ADAMTS13 deficiency is most commonly due to anti-ADAMTS13 autoantibodies (immune-mediated TTP; iTTP) and is rarely hereditary/congenital (cTTP)[3,4].

Unfortunately, tests for ADAMTS-13 are not available in many hospitals, so usually blood samples are sent to reference laboratories, requiring many hours/days to obtain the diagnosis, and a subsequent delay in treatment[3]. Clinical risk prediction tools, such as the PLASMIC score or the French score, may predict the presence of severe ADAMTS13 deficiency (<10%) in patients with suspected TTP, facilitating treatment decisions in those patients in whom timely results of ADAMTS13 testing are unavailable[5-7].

In this retrospective study, we analysed the data collected on a cohort of consecutive patients with suspected TMA, who had been referred to the Hemostasis and Thrombosis Center of the "Federico II" University of Naples, Italy, for measurement of ADAMTS13 activity. Using the medical records of each patient, we applied the PLASMIC score to verify if the model is able to predict severe ADAMTS13 deficiency in this cohort.

## MATERIALS AND METHODS

### Study population

We evaluated data from 59 patients with suspected TMA from the "A. Cardarelli" Hospital and Federico II University Hospital of Naples, Italy, observed in a period between 2015 and 2019. The ADAMTS13 activity testing was performed in the Hemostasis and Thrombosis Center of the "Federico II" University. ADAMTS13 inhibitor was also determined.

### Methods

Clinical and laboratory information was collected and evaluated for all patients. The ADAMTS13 activity was detected by a quantitative chromogenic ELISA assay (TECHNOZYM® ADAMTS13 Activity ELISA, Technoclone GmbH), while the search for the inhibitor was carried out by the Bethesda method. The concentration of the IgG towards ADAMTS13 was also determined with an ELISA chromogenic assay (TECHNOZYM® ADAMTS13 INH, Technoclone GmbH, Wien, Austria). Severe ADAMTS13 deficiency was defined as activity <10%. The cut-off value for ADAMTS13 score inhibitor was 12 IU/mL.

The PLASMIC was calculated by assigning one point for each of the following parameters: platelet count <30×10$^9$/L, indices of hemolysis (reticulocyte count >2.5%, undetectable haptoglobin or indirect bilirubin >2 mg/dL), absence of active neoplasm (<1 year), the patient not having received a solid organ transplantation or stem cell transplantation, International Normalized Ratio (INR) <1.5, mean corpuscular volume (MCV) <90 fl, serum creatinine <2 mg/dL (**Table I**). The laboratory data and the other PLASMIC score variables were collected at the time of ADAMTS13 testing.

Of the 59 patients included in the study, only in 52 was it possible to obtain the data necessary for the calculation of the PLASMIC score from the medical records. The 52 patients were divided according to the PLASMIC score into 3 groups: low risk (score 0-4), intermediate risk (score 5), and high risk (score 6-7) of having an ADAMTS13 activity <10%.

**Table I** - *Parameters used to calculate the PLASMIC score*

| Component | Points |
|---|---|
| Hemolysis (indirect bilirubin >2 mg/dL, reticulocytes >2.5% or undetectable haptoglobin) | 1 |
| No active cancer | 1 |
| No history of solid-organ or stem cell transplant | 1 |
| MCV <90 fl | 1 |
| INR <1.5 | 1 |
| Serum creatinine <2 mg/dL | 1 |
| Platelet count <30×10$^9$/L | 1 |

INR: international normalised ratio; MCV: mean corpuscular volume.





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## RESULTS

Of the 59 patients enrolled in the study, 17 had ADAMTS13 activity levels <10%, confirming the diagnosis of TTP; 42 had levels >10%. Among the 17 with confirmed diagnosis of TTP, in 2 cases there was an oncological disease, one patient was pregnant, 2 patients had a history of autoimmune disease (respectively systemic sclerosis and systemic lupus erythematosus; SLE), one case was secondary to major surgery (cholecystectomy in the previous 15 days), and in 6 cases fever and flu-like syndrome preceded the onset of symptoms. The remaining 5 patients had no apparent precipitating conditions (**Table II**). In 12 out of the 17 cases of TTP, the anti-ADAMTS13 inhibitor titre was measured with a median of 37 IU/mL (4.78-97 IU/mL). In 5 cases, the inhibitor titre was not assessed.

In the group with ADAMTS13 >10%, 7 patients had a diagnosis of aHUS, 3 sepsis, 5 pancytopenia (B12 and folate deficiency), one HELLP syndrome, one post-partum, 2 autoimmune diseases (respectively undifferentiated connective tissue and SLE-like), one histiocytosis and HIV-HBV-CMV co-infection, 4 solid cancers, and 4 hematological diseases (one relapse of acute myeloid leukemia, 2 myelodysplastic syndromes, and one multiple myeloma). The remaining 14 cases with ADAMTS13 >10% showed no potential risk factors.

Among patients with TTP (n=17), 47% (n=8) had neurological disorders ranging from confusion, dysarthria, ischemic stroke (n=1) to coma (n=1), 5.8% developed acute myocardial infarction (n= 1), 23.5% acute renal failure (n=4), 11.8% deep vein thrombosis (n=2), 23.5% skin manifestations such as petechiae and ecchymosis (n=4), 35.3% fever (n=6), and 23.5% gastrointestinal symptoms including abdominal pain, vomiting and diarrhea.

The therapeutic approach for all patients with TTP was plasma exchange and steroids. Rituximab was also used in 6 cases, and vincristine in one case; none of the patients in this study cohort received caplacizumab. Of the 17 patients with confirmed TTP, 4 patients died from multiple organ failure during the acute phase despite 2 of them (50%) receiving high-dose steroids, plasma exchange and rituximab, one (25%) receiving plasma exchange and high-dose corticosteroids, and one (25%) receiving fresh concentrated plasma, high-dose corticosteroids, and immunoglobulins. Application of the score did not influence the management of these cases.

In the high-risk group of patients (score 6-7), 12 out of 20 (60%) had ADAMTS13 <10%. All 6 patients (100%) with PLASMIC 7 had ADAMTS13 <5%. In the intermediate risk group (score 5), only one case out of 17 (5.9%) had ADAMTS13 <10%. None of the patients in the low-risk group (score 0-4) had severe ADAMTS13 deficiency (**Figure 1**). Receiver operator curve (ROC) analysis was used to assess the performance of the PLASMIC score in predicting severe ADAMTS13 deficiency. The sensitivity and specificity of PLASMIC in TTP screening were 92% (95% CI: 0.80-0.98) and 79% (95% CI: 0.66-0.89), respectively. The PLASMIC score showed good discrimination between patients with and without severe ADAMTS13 deficiency with an AUC of 0.92 (confidence interval 0.82-1.0; p<0.001).

**Table II** - *Risk factors/conditions in 17 patients with thrombotic thombocytopenic purpura*

| Condition | N (%) |
|---|---|
| Cancer | 2 (11.7%) |
| Autoimmunity | 2 (11.7%) |
| Pregnancy | 1 (5.9%) |
| Surgery | 1 (5.9%) |
| Fever, infection | 6 (35.2%) |
| No precipitating factors | 5 (29.4%) |

## DISCUSSION

In our study, the PLASMIC score demonstrated a good diagnostic performance with an AUC of 0.92 (CI 0.82-1.0). All patients included in the "low-risk" group (PLASMIC score 0-4) confirmed ADAMTS13 activity >10%. Therefore,

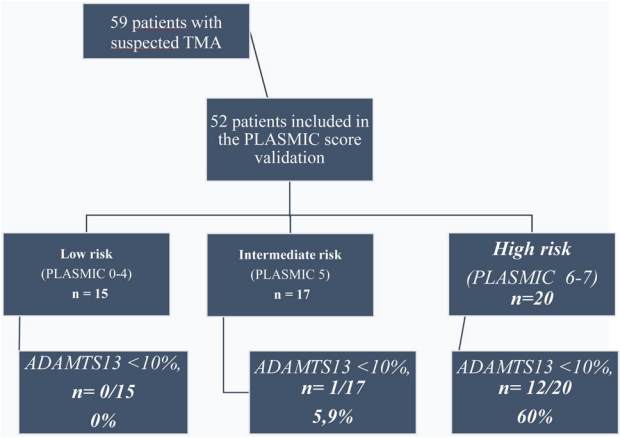


**Figure 1 - Flow chart describing 59 patients observed in a period between 2015 and 2019**

if the PLASMIC score had been applied methodically to all patients, there would have been no need to require measurement of ADAMTS13 activity for the 15 patients classified at "low risk" of TTP.

In the group of patients included in the "high-risk" group, only 60% actually had severe ADAMTS13 deficiency, a discrepancy which was mostly due to an inappropriate request for ADAMTS13 testing. In fact, of our 8 high-risk PLASMIC score patients in whom severe ADAMT13 deficiency was not confirmed, 3 had no signs of hemolysis and one had a platelet count >100×10$^9$/L.

On the other hand, a further consideration should be made regarding those more complex patients in whom pre-existing liver or kidney failure results in INR or serum creatinine values higher than the cutoffs required by PLASMIC; in these patients, the information provided by application of the PLASMIC score may not be sufficiently accurate, even though these patients have TTP. In fact, in one patient, not included in the PLASMIC analysis due to incomplete data, although an ADAMTS13 activity of 2% was found, the presence of concomitant renal insufficiency with serum creatinine >2 mg/dL and an INR of 1.75 would, in any case, have prevented classification as "high risk". This is a limitation of our study, and highlights the concept that age, renal insufficiency, cancer, or increased MCV with age reduce the sensitivity of the PLASMIC score, in particular in patients aged 60 or over[8].

However, one of the merits of this score is the identification of clinical and laboratory data that should be considered in the context of TTP, information which is often overlooked by doctors who are not experts in coagulation diseases. The high sensitivity of the test (92%) achieved from the data collected would seem to recommend the routine use of the PLASMIC score in those patients with a suspicion of TTP or in order to exclude it. Referring to the recent guidelines drawn up by ISTH on the initial management of TTP, this score can be useful in directing the clinician to providing rapid treatment with plasma exchange, steroids, and caplacizumab[9].

Our study has other limitations: the first is the retrospective nature of the study. Furthermore, the number of patients enrolled is limited, given the low incidence of this pathology, but in line with other studies in the literature. However, it is one of the largest studies performed for external validation of the PLASMIC score in the literature, including previous studies reporting conflicting results[10-16].

## CONCLUSIONS

In our cohort, a high-risk PLASMIC score successfully predicted patients with severe ADAMTS13 deficiency, allowing the clinician to quickly define the best therapeutic approach, especially useful for those clinicians not used to the diagnosis and treatment of TMA.

## INFORMED CONSENT

All subjects gave their informed consent to participate to the study.

## AUTHORSHIP CONTRIBUTION

MP, BM and FP selected the patients and analyzed the clinical records, AT and GC analyzed and interpreted data, FC and RM performed the laboratory assays, AT wrote the manuscript, GDM made revision of the manuscript.

*The Authors declare no conflicts of interest.*

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