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haematologica
the hematology journal

Early Release Paper

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Haematologica 2012 [Epub ahead of print]

Citation: Benhamou Y, Assie' C, Boelle PY, Buffet M, Grillberger R, Malot S, Wynckel A, Presne C, Choukroun G, Poullin P, Provôt F, Gruson D, Hamidou M, Bordessoule D, Pourrat J, Mira JP, Le Guern V, Pouteil-Noble C, Daubin C, Vanhille P, Rondeau E, Palcoux JB, Mousson C, Vigneau C, Bonmarchan G, Guidet B, Galicier L, Azoulay E, Rottensteiner H, Veyradier A, and Coppo P. Development and validation of a predictive model for death in acquired severe ADAMTS13 deficiency-associated idiopathic thrombotic thrombocytopenic purpura: the French TMA Reference Center experience. Haematologica. 2012; 97:xxx doi:10.3324/haematol.2011.049676

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DOI: 10.3324/haematol.2011.049676

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DOI: 10.3324/haematol.2011.049676

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Key words: thrombotic thrombocytopenic purpura, ADAMTS13, prognostic factor, plasma exchange, rituximab.

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DOI: 10.3324/haematol.2011.049676

Abstract

Background. Acquired thrombotic thrombocytopenic purpura is still associated with a 10-20% death rate. So far, early prognostic factors of death could not be clearly identified. To identify prognostic factors associated with 1-month death in thrombotic thrombocytopenic purpura patients with acquired severe (< 10% of normal activity) ADAMTS13 deficiency.

Design and Methods. Prospective cohort of patients included between October, 2000, and August, 2010. A validation cohort of patients was set up from September, 2010 to August, 2011. 281 (analysis cohort) and 66 (validation cohort) consecutive adult thrombotic thrombocytopenic purpura patients with acquired severe ADAMTS13 deficiency. 30-day mortality after treatment initiation according to characteristics at inclusion.

Results. Non-survivors (11%) were older ($P=10^{-6}$) and presented more frequently arterial hypertension ($P=5.10^{-4}$) and ischemic heart disease ($P=0.013$). Prognosis was increasingly poor with age ($p=0.004$). On presentation, cerebral manifestations were more frequent in non-survivors ($P=0.018$) and serum creatinine level was higher ($P=0.008$). The most significant independent variables for determining death were age, severe cerebral involvement and LDH level ≥ 10N. A 3-level risk score for early death was defined and confirmed in the validation cohort using these variables, with higher values corresponding to increased risk of early death.

Conclusions. A risk score for early death was defined in patients with thrombotic thrombocytopenic purpura and validated on an independent cohort. This score should help to stratify early treatment and intensify patients with a worse prognosis.

DOI: 10.3324/haematol.2011.049676

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a severe form of thrombotic microangiopathy (TMA) characterized by profound thrombocytopenia, erythrocyte fragmentation and organ failure of variable severity. This rare disease results from an excessive systemic platelet aggregation caused by the accumulation of unfolded high molecular weight von Willebrand factor (VWF) multimers in plasma. This failure to degrade the endothelium-derived hyper-reactive VWF multimers into less adhesive forms is related to a severe deficiency in ADAMTS13 (A Disintegrin And Metalloproteinase with ThromboSpondin-1 like motifs), a protease specifically involved in this process (1). Severe ADAMTS13 deficiency results from biallelic mutations of the encoding gene in hereditary forms, or from polyclonal autoantibodies in acquired forms (2). These pathophysiological findings account for the efficiency of plasma-based treatment in TTP, which allows supplying ADAMTS13 deficiency. Immunomodulatory drugs aimed at depleting anti-ADAMTS13 antibody-producing B-lymphocytes are associated to standard treatment in an increasing number of cases (3, 4). Those therapeutical strategies allowed to improve outstandingly TTP prognosis, with overall survival rates which may reach 80-85% (5). Despite this significant progress, death still occurs in up to 20% of patients according to most large multicentre reports, usually within the first days of management. Interestingly, this incidence apparently did not improve for more than ~ 20 years according to some authors (6). Therefore, it is becoming essential to understand accurately the factors associated with a fatal outcome at the acute phase of the disease from large series of patients to better tailor initial treatment and further improve those results. So far however, the risk factors associated with death remain unclear. Severe clinical presentation on diagnosis, including cerebral involvement, renal failure (7), very low platelet count and profound anemia, was variably associated with a higher mortality rate by some authors (8, 9), but were not confirmed by others (7, 10-13). Patients at relapse were also reported as having a disease of better prognosis (14). Studies assessed the prognostic value of anti-ADAMTS13 antibodies, and inhibitory

DOI: 10.3324/haematol.2011.049676

anti-ADAMTS13 antibodies were associated with a more severe thrombocytopenia (15), a delayed response to standard treatment (16, 17) or an increased mortality rate (5, 6, 18).

So far, the identification of accurate prognostic factors in TTP was hampered by the limited series of homogeneous patients. However, the increasing number of patients included prospectively in national registries should now help addressing this question. In this regard, we conducted a study from a large, homogeneous group of patients included in our registry. We report here original independent risk factors associated with 30-day death, and provide a prognostic score aimed at identifying at diagnosis patients at risk of fatal outcome who could possibly benefit from more intensive first-line therapies.

Design and methods

Study Design

To assess prognostic factors associated with early death at the acute phase of TTP, we studied the characteristics of adult (≥ 18 year-old) patients who died during the 30-day period starting from treatment initiation (non-survivors group). These features were compared to those of patients who survived from their episode (survivors group). Patients of both groups were included during the same period of time from October, 2000 (date at which our registry was set up) (19) to the date of the study design (August, 2010). **All patients were studied until death or durable remission.** From this study group (learning group), we established a prognostic score to identify patients at risk of death during the episode of TTP using information at diagnosis. To validate our results on a prospective series of patients, a second group of patients (validation group) **managed with the same conditions** was set up from the same centers during the analysis period of the study group, i.e., from September, 2010 to August, 2011. All patients of the whole study were included nonselectively from intensive care units and departments of internal medicine, hematology, and nephrology in 39 French centers. The study

DOI: 10.3324/haematol.2011.049676

protocol was reviewed and approved by the institutional review board and ethical committee. An informed consent was obtained from all patients.

Patients and Treatment

All adult patients with a first episode of TTP associated with a severe acquired ADAMTS13 deficiency (< 10% of normal activity) were enrolled in the study (6, 19 ; Supplemental appendix). To avoid the influence of inadequate plasma volumes in survival and to focus on the prognostic value of patient characteristics on diagnosis, we excluded patients who were not managed according to standard recommendations (Supplemental appendix and (4, 6)).

Analysis

Data were collected from diagnosis and before treatment (19), with a particular emphasis for a past history of cardiovascular risk factors (including arterial hypertension and diabetes) and a pre-existing history of ischemic heart disease and ischemic stroke. Cerebral involvement assessment on diagnosis included headache, stupor, seizure and/or focal deficiency. Renal involvement was assessed by serum creatinine level and the estimated glomerular filtration rate (eGFR) by the Modification of the Diet in Renal Disease (MDRD) method. Measurement of ADAMTS13 activity, ADAMTS13 plasma inhibitor and anti-ADAMTS13 antibodies were performed as previously described (supplemental appendix and (20)).

Statistical Analysis

Quantitative variables were summarized by mean (standard deviation) and compared by the Wilcoxon rank-sum test; categorical data was summarized as count (%) and compared by the chi-squared test or Fisher's exact test. Risk factors of early mortality were investigated by logistic regression. A univariable analysis was first carried out, and variables associated with mortality at the *P*

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< .20 level were included in a backward multivariable analysis. Only variables associated at the *.05* level were kept in the final model. Multiple imputation was used for missing values. Using rounded coefficients from the linear predictor of the multivariable analysis, we designed a score for early death taking discrete values from 0 to 4. The score was validated by testing association of score with early death by TTP in a logistic regression. A cutoff value was obtained by maximizing the Youden score (sensitivity + specificity – 1), and the sensitivity, specificity, positive and negative predictive values and their respective 95% confidence intervals (95% CI) were determined. The area under the ROC (Receiver Operating Characteristic) curve was used to evaluate the discriminating power of our score. **The difference in discriminating power between tests was tested using deLong procedure (21).** Data were analyzed using the R software v2.9.

Results

1. Study group

From October, 2000 to August, 2010, the Registry included 944 adult patients with a diagnosis of TMA and available data. Among them, 281 were considered as having a TTP with an acquired severe ADAMTS13 deficiency fulfilling all inclusion criteria of the study (Supplemental figure S1); all were followed during at least 30 days after treatment initiation. Thirty-three of those patients died during the 30-day period of observation, corresponding to 12% (95% CI [8%-15%]) death rate. The other patients were discharged alive.

2. Univariable analysis for prognostic factors

Table 1 details the clinical features of patients of the learning cohort on diagnosis according to outcome. Non-survivors were older ($\boldsymbol{P = 10^{-6}}$) and presented more frequently arterial hypertension ($P = 5.10^{-4}$) and ischemic heart disease ($P = .013$). On presentation, cerebral involvement including stupor and seizure were more frequently observed in non-survivors ($P = .018$, $P = .014$ and $P = .004$,

DOI: 10.3324/haematol.2011.049676

respectively). Serum creatinine level was higher (***P = .008***) and eGFR was lower (***P = 5.10***$^{-5}$) in non-survivors. The incidence of inhibitory anti-ADAMTS13 antibodies and the titers for IgG anti-ADAMTS13 antibodies were comparable between survivors and non-survivors.

Age clearly showed the greatest impact on survival in our series. To get further insight on this feature, we analyzed the number of non-survivors according to age class (Table 2). We found that prognosis was increasingly poor with age, particularly in patients > 60 year-old (test for trend, *P = .004*). **By comparing patients > 60 year-old with younger patients**, we confirmed that elderly patients had a distinct presentation with more frequent cardiovascular risk factors and pre-existing comorbidities; organ injury was predominant (i.e., cerebral manifestations were more frequent and renal involvement was more severe), whereas anemia was less profound (supplemental Table 1).

Most deaths (77%) occurred in the first 2 weeks following diagnosis **(mean time from admission to death: 9.1±8.1 days). Deceased patients received a plasma volume of 346±303 ml/kg.** Death occurred in a context of one or multiple organ failure in relation with an uncontrolled TTP. Platelet count between diagnosis and death did not significantly increase (20±24x10^9/L versus 25±40x10^9/L, respectively, *P = .97*). Non-survivors received steroids less frequently than survivors (***P = .007***). **The use of salvage therapies (i.e., rituximab, vincristine, cyclophosphamide and splenectomy) was not different between survivors and non-survivors** (*P = NS* for all). To assess whether rituximab could have influenced TTP prognosis in the more recent years, we compared the number of deceased patients before and after the rituximab era. We found that between those 2 periods (from 2001 to 2004 and from 2005 to 2010, respectively), the number of deceased patients was comparable (15/112 [13.4%] patients and 18/169 [10.7%] patients, respectively, *P = .57*), which apparently does not support a major role for rituximab in the improvement of TTP survival. Survivors achieved a durable complete remission within 23.7±23 days, and required a plasma volume of 787±670 ml/kg. Platelets were infused in patients of both groups mainly for severe thrombocytopenia

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soon after plasmatherapy was started, without apparent worsening of clinical condition. Dialysis was more frequently performed in non-survivors than in survivors (*P = .001*) (Table 3).

3. Multivariable analysis

Variables tested for inclusion in the model were age, arterial hypertension, cerebral involvement, LDH, serum creatinine and eGFR. Arterial hypertension was not retained in the model as it was strongly correlated with increasing age. Finally, increasing age, cerebral involvement and LDH level ≥ 10N were retained as risk factors in the multivariable model (**Supplemental Table 2 and** Table 4). On the basis of the coefficients of the multivariable regression, the following score was developed: cerebral involvement or LDH level ≥ 10N scored 1 point, an age between 41-60 years scored 1 point and an age above 60 years scored 2 points (Table 4). Table 5 reports mortality for patients by increasing score groups. The cutoff maximizing the Youden score was 3. Using this threshold, the sensitivity (% score ≥ 3 among non survivors) was 52% (95%CI, [35%, 67%]) and specificity (score < 3 among survivors) 90% (95%CI [86%, 93%]). The positive predictive value (mortality among those with a score ≥ 3) was 41% [95% CI, 28%, 57%] and negative predictive value (survival among patients with a score < 3) was 93% [95% CI, 89%, 96%]. The AUC was 0.77, showing that the score had discriminating ability (*P < .0001*) (Figure 1).

4. Accuracy and validation of the prognostic score

In the 66 patients included for validation **(Clinical features on diagnosis are depicted in supplemental Table 3)**, 12 died in the 30 days after initial diagnosis. The scores calculated for this sample ranged from 0 to 3. **There was increasing mortality with increasing score in the validation cohort (*P < .025*). The calibration of the model was also reasonable, as the observed number of deaths in each risk group compared with the expected number of deaths calculated using the predicted mortality from the model** (Table 5). The mortality in the 9 patients with score ≥3 in the

DOI: 10.3324/haematol.2011.049676

validation sample was 33%, which was not different from the PPV calculated above (*P = .36*). Survival in the validation sample patients with score <3 was 84%, a little smaller than the 93% expected according to PNV (*P = .01*).

Discussion

We report herein a simple and reliable prognostic score for TTP regarding critical factors related to clinical presentation in a large group of homogeneous patients on diagnosis. The main finding of our work is the emphasis that age-related categorization is important for evaluation of survival. Indeed, we provide here strong evidence that TTP in the elderly has a more aggressive presentation, with a more severe organs dysfunction (as illustrated here by a higher incidence of cerebral involvement and a more severe renal involvement, with more stupor and seizure and a higher serum creatinine level, respectively) and, consequently, a higher death rate. Previous retrospective studies with a more limited number of patients also emphasized that non-survivors were older than survivors (13, 22-24). A more severe neurologic and/or renal involvement was also reported in non-survivors, in accordance with our findings (7, 8, 13, 22, 24, 25). By contrast, we were unable to correlate the severity of cytopenias and the presence of fever with prognosis (13, 25). Despite those inconsistencies, which may be due to differences in inclusion criteria and in the size of the cohorts, an increasing number of studies including ours support clear evidence that age strongly impacts TTP prognosis.

The more severe prognosis of TTP in older patients can be explained by different ways. First, most patients in our study had a history of arterial hypertension, which results in chronic endothelial and vascular dysfunction that worsens with ageing (26). In line with those features, patients in non-survivors group, which included a large number of old patients, had more frequently a history of ischemic heart disease. Second, ageing is physiologically associated with vascular senescence and loss of vascular compliance (27) that may result in a higher shear stress and to more severe vascular wall constraints and more organ injury during TTP. Moreover, the very high LDH level we identified as a

DOI: 10.3324/haematol.2011.049676

factor of worse prognosis reflects a severe multiple organ involvement (28) which may include not only brain and kidney, but also heart, digestive tract, adrenal glands, pancreas and liver (29). In particular, cardiac involvement was associated with a high incidence of morbidity and mortality (30-32), and it is likely that the evaluation of this latter should improve the accuracy of our prognostic score.

Despite standard treatment, the mortality of TTP in high risk categories may range from 30% to 60%. Therefore, our score should be of help in the early management of these patients to discuss whether or not additional therapies should be added to standard treatment soon after diagnosis. Among these, rituximab could be a promising strategical therapy in this context, though its efficiency is not immediate (4). Some reports suggested that the administration of very high volumes of plasma could improve the prognosis of the more severe patients (33). As a perspective point of view, old patients with a diagnosis of TTP should benefit from more intensive supportive care and should be monitored more closely in intensive care units with more aggressive attention to cardiac and renal function. Also, forthcoming studies should evaluate the role of promising adjunctive therapies in those patients (34), and our score should help to identify accurately the more suitable patients for such studies.

One limitation of our study is some variation in the treatment of patients throughout the inclusion period; particularly, there were an increasing number of patients with a suboptimal response to standard treatment treated with rituximab, which may have influenced TTP prognosis in the more recent years. However, the number of deceased patients was comparable throughout the inclusion period, and particularly before and after the rituximab era. Similarly, steroids were administered more frequently in survivors, though this difference was not significant by multivariable analysis. Therefore, whether steroids impacted prognosis in our study remains unknown and it is likely that steroids were less frequently administrated in non-survivors because this group included a large number of old patients for whom steroids are usually prescribed with reluctance.

In conclusion, we provide here a reliable score predictive of death in TTP with acquired severe ADAMTS13 deficiency, which should help to tailor treatment on admission. We provide strong

DOI: 10.3324/haematol.2011.049676

evidence that age is an important prognostic factor. In patients with a severe score, more intensive therapies should be considered and evaluated on prospective trials.

DOI: 10.3324/haematol.2011.049676

Acknowledgments

Patients were recruited with the help of the members of the Reference Center for Thrombotic Microangiopathies (CNR-MAT) (listed in the appendix). We thank S. Thouzeau, S. Savigny (Laboratoire d'Hématologie, Hôpital Antoine Béclère, AP-HP, Clamart), A. de Labarthe (Laboratoire d'Hématologie, Hôpital Saint-Louis, AP-HP, Paris), I. Hauchard (Service de Réanimation, Hôpital de Rouen), M. Baragay (Laboratoire d'Immunologie, Hôpital Saint-Louis, AP-HP, Paris) for technical assistance. This work was funded by grants from the Etablissement Français du Sang (CS/2002/009) and the GIS-Institut des Maladies Rares (GIS MR0428).

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References

1. Sadler JE. Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura. Blood. 2008;112(1):11-8.
2. Klaus C, Plaimauer B, Studt JD, Dorner F, Lammle B, Mannucci PM, et al. Epitope mapping of ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura. Blood. 2004;103(12):4514-9.
3. Scully M, McDonald V, Cavenagh J, Hunt BJ, Longair I, Cohen H, et al. A phase II study of the safety and efficacy of rituximab with plasma exchange in acute acquired thrombotic thrombocytopenic purpura. Blood. 2011;118(7):1746-53.
4. Froissart A, Buffet M, Veyradier A, Poullin P, Provot F, Malot S, et al. Efficacy and safety of first-line rituximab in severe, acquired thrombotic thrombocytopenic purpura with a suboptimal response to plasma exchange. Experience of the French Thrombotic Microangiopathies Reference Center. Crit Care Med. 2012;40(1):104-11.
5. Franchini M, Mannucci PM. Advantages and limits of ADAMTS13 testing in thrombotic thrombocytopenic purpura. Blood Transfus. 2008;6(3):127-35.
6. Hovinga JA, Vesely SK, Terrell DR, Lammle B, George JN. Survival and relapse in patients with thrombotic thrombocytopenic purpura. Blood. 2010;115(8):1500-11; quiz 662.
7. Pereira A, Mazzara R, Monteagudo J, Sanz C, Puig L, Martinez A, et al. Thrombotic thrombocytopenic purpura/hemolytic uremic syndrome: a multivariate analysis of factors predicting the response to plasma exchange. Ann Hematol. 1995;70(6):319-23.
8. Rose M, Eldor A. High incidence of relapses in thrombotic thrombocytopenic purpura. Clinical study of 38 patients. The American journal of medicine. 1987;83(3):437-44.
9. Rock G, Kelton JG, Shumak KH, Buskard NA, Sutton DM, Benny WB. Laboratory abnormalities in thrombotic thrombocytopenic purpura. Canadian Apheresis Group. British journal of haematology. 1998;103(4):1031-6.
10. Patton JF, Manning KR, Case D, Owen J. Serum lactate dehydrogenase and platelet count predict survival in thrombotic thrombocytopenic purpura. Am J Hematol. 1994;47(2):94-9.
11. Rock GA, Shumak KH, Buskard NA, Blanchette VS, Kelton JG, Nair RC, et al. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. Canadian Apheresis Study Group. The New England journal of medicine. 1991;325(6):393-7.
12. Bobbio-Pallavicini E, Gugliotta L, Centurioni R, Porta C, Vianelli N, Billio A, et al. Antiplatelet agents in thrombotic thrombocytopenic purpura (TTP). Results of a randomized multicenter trial by the Italian Cooperative Group for TTP. Haematologica. 1997;82(4):429-35.
13. Wyllie BF, Garg AX, Macnab J, Rock GA, Clark WF. Thrombotic thrombocytopenic purpura/haemolytic uraemic syndrome: a new index predicting response to plasma exchange. British journal of haematology. 2006;132(2):204-9.
14. Lotta LA, Mariani M, Consonni D, Mancini I, Palla R, Maino A, et al. Different clinical severity of first episodes and recurrences of thrombotic thrombocytopenic purpura. British journal of haematology. 2010;151(5):488-94.
15. Zheng XL, Wu HM, Shang D, Falls E, Skipwith CG, Cataland SR, et al. Multiple domains of ADAMTS13 are targeted by autoantibodies against ADAMTS13 in patients with acquired idiopathic thrombotic thrombocytopenic purpura. Haematologica. 2010;95(9):1555-62.
16. Zheng XL, Kaufman RM, Goodnough LT, Sadler JE. Effect of plasma exchange on plasma ADAMTS13 metalloprotease activity, inhibitor level, and clinical outcome in patients with idiopathic and nonidiopathic thrombotic thrombocytopenic purpura. Blood. 2004;103(11):4043-9.
17. Coppo P, Wolf M, Veyradier A, Bussel A, Malot S, Millot GA, et al. Prognostic value of inhibitory anti-ADAMTS13 antibodies in adult-acquired thrombotic thrombocytopenic purpura. British journal of haematology. 2006;132(1):66-74.

18. Tsai HM. High titers of inhibitors of von Willebrand factor-cleaving metalloproteinase in a fatal case of acute thrombotic thrombocytopenic purpura. Am J Hematol. 2000;65(3):251-5.
19. Coppo P, Schwarzinger M, Buffet M, Wynckel A, Clabault K, Presne C, et al. Predictive features of severe acquired ADAMTS13 deficiency in idiopathic thrombotic microangiopathies: the French TMA reference center experience. PLoS One.2010; 5(4):e10208.
20. Ferrari S, Scheiflinger F, Rieger M, Mudde G, Wolf M, Coppo P, et al. Prognostic value of anti-ADAMTS 13 antibody features (Ig isotype, titer, and inhibitory effect) in a cohort of 35 adult French patients undergoing a first episode of thrombotic microangiopathy with undetectable ADAMTS 13 activity. Blood. 2007;109(7):2815-22.
21. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988;44(3):837-45.
22. Dervenoulas J, Tsirigotis P, Bollas G, Pappa V, Xiros N, Economopoulos T, et al. Thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS): treatment outcome, relapses, prognostic factors. A single-center experience of 48 cases. Ann Hematol. 2000;79(2):66-72.
23. Scheiflinger F, Knobl P, Trattner B, Plaimauer B, Mohr G, Dockal M, 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(9):3241-3.
24. Eymin G, Andrade M, Andresen M, Pereira J. [Thrombotic thrombocytopenic purpura: experience in 18 cases and literature review]. Rev Med Chil. 2008;136(12):1518-27.
25. Lara PN, Jr., Coe TL, Zhou H, Fernando L, Holland PV, Wun T. Improved survival with plasma exchange in patients with thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. The American journal of medicine. 1999;107(6):573-9.
26. Thorin E, Thorin-Trescases N. Vascular endothelial ageing, heartbeat after heartbeat. Cardiovasc Res. 2009;84(1):24-32.
27. O'Rourke MF. Arterial aging: pathophysiological principles. Vasc Med. 2007;12(4):329-41.
28. Cohen JA, Brecher ME, Bandarenko N. Cellular source of serum lactate dehydrogenase elevation in patients with thrombotic thrombocytopenic purpura. J Clin Apher. 1998;13(1):16-9.
29. Berkowitz LR, Dalldorf FG, Blatt PM. Thrombotic thrombocytopenic purpura: a pathology review. Jama. 1979;241(16):1709-10.
30. Patschan D, Witzke O, Duhrsen U, Erbel R, Philipp T, Herget-Rosenthal S. Acute myocardial infarction in thrombotic microangiopathies--clinical characteristics, risk factors and outcome. Nephrol Dial Transplant. 2006;21(6):1549-54.
31. Hawkins BM, Abu-Fadel M, Vesely SK, George JN. Clinical cardiac involvement in thrombotic thrombocytopenic purpura: a systematic review. Transfusion. 2008;48(2):382-92.
32. Hughes C, McEwan JR, Longair I, Hughes S, Cohen H, Machin S, et al. Cardiac involvement in acute thrombotic thrombocytopenic purpura: association with troponin T and IgG antibodies to ADAMTS 13. J Thromb Haemost. 2009;7(4):529-36.
33. Clark WF, Forzley BR, Sontrop JM, Kadri A, Moist LM, Suri RS, et al. TTP/HUS: observational studies generate hypotheses that lead to randomized controlled trials. Kidney Int Suppl. 2009;112:S50-1.
34. Noris P, Balduini CL. Investigational drugs in thrombotic thrombocytopenic purpura. Expert Opin Investig Drugs. 2011;20(8):1087-98.

DOI: 10.3324/haematol.2011.049676

Table 1. Clinical characteristics of patients on diagnosis according to outcome.

	Survivors (N=248)	**Non-survivors (N=33)**	**P-value**
Ethnicity			
Caucasians	209 (86)	29 (85)	0.70
Afro-Caribbeans	26 (11)	3 (9)	
Others	9 (4)	0	
Age (year-old)	39.2 ± 15.7	56.5 ± 19.4	**10^{-6}**
Females	167 (67)	23 (70)	0.79
Cardiovascular risk factors and pre-existing comorbidities			
Arterial hypertension	25 (10)	11 (33)	**5.10^{-4}**
Diabetes	16 (6)	3 (9)	0.57
Ischemic stroke	6 (2)	0 (0)	0.37
Ischemic heart disease	11 (4)	5 (15)	**0.013**
Cerebral involvement	145 (59)	27 (82)	**0.018**
Headache	62 (25)	5 (15)	0.21
Stupor	34 (14)	10 (30)	**0.014**
Seizure	16 (6)	7 (21)	**0.004**
Focal deficiency	41 (17)	10 (30)	0.94
Fever	60 (25)	9 (28)	0.69
Hemoglobin level (g/dL)	7.9 ± 2.0	7.9 ± 2.5	0.73
Reticulocyte count (N=179)	202 ± 126	157 ± 91	0.15
LDH level (xN) (N=232)$^{\$}$	5.8 ± 4.2	8.3 ± 4.8	0.06
LDH ≥ 10N (%) (N=232)	22 (9)	6 (21)	**0.05**
Platelet count ($x10^9$/L) $^{\$}$	19.1 ± 19.0	20.0 ± 23	0.97
Serum creatinine (µmol/L) $^{\$}$	116 ± 88	172 ± 118	**0.008**
Estimated glomerular filtration rate (mL/min) $^{\$}$	72.1 ± 31	50.8 ± 33	**5.10^{-5}**
ANA (N=253)	114 (50)	13 (52)	0.84
APLA (N=187)	18 (11)	2 (10)	0.86
ADAMTS13 inhibitor (N=177)	118 (74)	13 (72)	0.92
IgG anti-ADAMTS13 Abs (U/mL) (N=90)	97 ± 106	183 ± 366	0.28

Abbreviations: xN: number of times the upper normal value; ANA: antinuclear antibodies; APLA: antiphospholipid antibodies; Abs: antibodies. $^{\$}$: Test on the Log scale. In case of missing values, the number of patient tested is specified in parentheses in the left column.

DOI: 10.3324/haematol.2011.049676

Table 2. Mortality rate in patients according to age class.

Age (years) (N)	Deceased patients	% [CI 95%]*
< 30 (92)	**4**	4 % [2% - 11%]
30-40 (51)	**2**	4% [1% - 13%]
40-50 (53)	**6**	11% [5% - 23%]
50-60 (42)	**7**	17% [8% - 31%]
60-70 (22)	**5**	23% [10% - 43%]
> 70 (21)	**9**	43% [24% - 63%]

Abbreviation: N: number of patients; CI: confidence interval.

* Test for trend, $P = .004$.

DOI: 10.3324/haematol.2011.049676

Table 3. Characteristics of patients' management according to outcome.

	Survivors (N=248)	**Non-survivors (N=33)**	**p-value**
Steroids (N=32)	199 (80)	19 (59)	**0.007**
Rituximab	75 (30)	5 (15)	0.07
Splenectomy	12 (5)	1 (3)	0.64
Vincristine	43 (17)	6 (18)	0.90
Cyclophosphamide	10 (4)	0 (0)	0.24
Platelets infusion (N=267)	100 (43)	15 (47)	0.64
Hemodialysis **(N=263)**	6 (2.4)	4 (27)[$]	**0.001**

Data are given as number of cases (%). In case of missing values, the number of patient tested is specified in parentheses in the left column.

[$] The analysis was performed from 15 patients.

DOI: 10.3324/haematol.2011.049676

Table 4. Association between patients' characteristics and outcome by multivariable analysis.

	Odds Ratio	**95% CI**	**p-value**	**Score**
Cerebral involvement	2.6	[1.0, 6.9]	**0.05**	**+1**
Age			**8.10^{-6}**	
≤40	1	-		**+0**
41-60	3.4	[1.2, 9.7]		**+1**
> 60	10.6	[2.0, 32.0]		**+2**
LDH level ≥ 10N	3.0	[1.3, 11.6]	**0.014**	**+1**

Abbreviations: CI, confidence interval; N: number of times the upper normal value.

DOI: 10.3324/haematol.2011.049676

Table 5. Prognostic score of thrombotic thrombocytopenic purpura mortality in the learning cohort and in the validation cohort.

Risk group	Score	Learning cohort		Validation cohort		
		N	% death [95% CI]	N	% death [95% CI]	Observed/Expected*
Low	0	62	3 [0.9, 11.0]	12	0 [0, 25]	**0 / 0.2**
	1	99	5 [2.1, 11.3]	23	13 [5, 32]	**3 / 1.1**
Intermediate	2	79	11 [6.1, 20.3]	22	27 [13, 48]	**6 / 3.3**
High	3	38	39 [25.6, 55.3]	9	33 [12, 65]	**3 / 3.2**
	4	3	66 [20.8, 98.3]	-	-	-

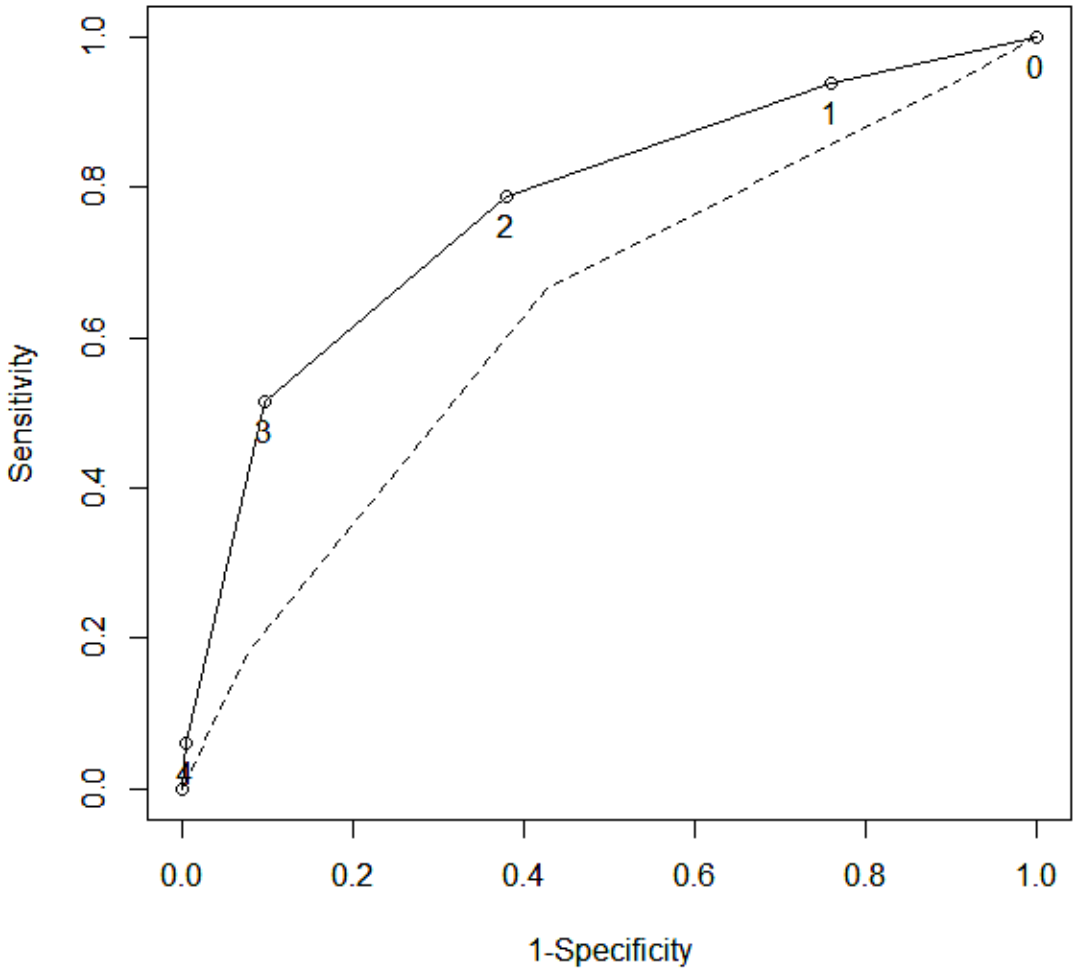
Abbreviations: N: number of patients; CI: confidence interval.

* Expected deaths are based on model predicted mortality.

Figure 1. ROC (Receiver Operating Characteristic) curve. The AUC (area under the curve) was 0.77, showing that the score had discriminating ability (P<0.0001). The present score (full line) had better discriminating ability than the Wyllie score (broken line) (deLong test, P<0.0035).[13]

DOI: 10.3324/haematol.2011.049676

Figure 1



DOI: 10.3324/haematol.2011.049676

Supplemental Table 1. Clinical characteristics of patients on diagnosis according to age.

	Age ≤ 60 (N=241)	**Age > 60 (N=40)**	**P-value**
Ethnicity (%)			0.386
Caucasians	86	90	
Afro Carribeans	11	5	
Others	3	5	
Females (%)	68	65	0.718
Cardiovascular risk factors and pre-existing comorbidities (%)			
Arterial hypertension	6	52.5	**9.10^{-12}**
Diabetes	4	33	**4.10^{-5}**
Ischemic stroke	1	7.5	**6.10^{-5}**
Ischemic heart disease	4	17.5	**4.10^{-5}**
Cerebral involvement			
Headache	27	5	**0.001**
Stupor	13	32.5	**0.004**
Seizure	6	20	**0.008**
Focal deficiency	27	60	0.185
Fever	23	37.5	0.075
Hemoglobin level (g/dL)	7.8 ± 2	8.4 ± 2.5	0.073
Reticulocyte count (N=179)	204 ± 127	154 ± 89	0.067
LDH level (xN)	6.0 ± 4.2	5.5 ± 3.6	0.597
LDH > 10N (%)	13	5	0.274
Platelet count ($x10^9$/L)	18 ± 17	25 ± 30	0.355
Serum creatinine (µmol/L)	116 ± 90	164 ± 104	**0.002**
Estimated glomerular filtration rate (mL/min)	73 ± 32	47 ± 25	**2.10^{-6}**
ANA (N=253)	52	37.5	0.135
APLA (N=187)	9	21	0.147
ADAMTS13 inhibitor (N=177)	113 (74)	18 (72)	0.809
IgG anti-ADAMTS13 Abs (N=126) (U/mL)	109 ± 169	97 ± 70	0.751
Death (%)	8.3	32.5	**0.0001**

DOI: 10.3324/haematol.2011.049676

Abbreviations: xN: number of times the upper normal value; ANA: antinuclear antibodies; APLA: antiphospholipid antibodies; Abs: antibodies. In case of missing values, the number of patient tested is specified in parentheses in the left column.

DOI: 10.3324/haematol.2011.049676

Supplemental Table 2. Full model of logistic regression.

	Odds Ratio	**95% CI**	**p-value**
Cerebral involvement	2.7	[1.0, 7.7]	**0.042**
Age			**0.024**
≤40	1	-	
41-59	2.2	[0.7, 6.7]	
≥ 60	3.5	[1.3, 18.4]	
Serum creatinine (µmol/L)	0.998	[0.95, 1.05]	**0.93**
Estimated glomerular filtration rate (mL/min)	0.84	[0.68, 1.04]	**0.10**
LDH level ≥ 10N	3.1	[1.0, 9.6]	**0.047**

Abbreviations: 95% CI: 95% confidence interval; xN: number of times the upper normal value.

DOI: 10.3324/haematol.2011.049676

Supplemental Table 3. Clinical characteristics of patients on diagnosis according to outcome in the validation cohort.

	Survivors (N=54)	**Non-survivors (N=12)**	**P-value**
Ethnicity (N=47)			
Caucasians	30 (65)	12 (100)	0.07
Afro-Caribbeans	10 (21)	0 (0)	
Others	7 (19)	0	
Age (year-old)	38.7 ± 13.1	49.6 ± 14.7	**0.027**
Females	46(85)	8 (67)	0.21
Cardiovascular risk factors and pre-existing comorbidities (N=63)			
Arterial hypertension	8 (15)	1 (10)	1
Diabetes	2 (4)	0 (0)	1
Ischemic stroke	2 (4)	1 (10)	0.43
Ischemic heart disease	1 (2)	0 (0)	1
Cerebral involvement	37 (68)	10 (83)	0.48
Headache (N=64)	31 (60)	2 (20)	0.01
Stupor	7 (13.5)	5 (42)	**0.039**
Seizure	4 (8)	1 (8)	1
Focal deficiency	17 (33)	7 (58)	**0.013**
Fever (N=54)	5 (11)	3 (30)	0.15
Hemoglobin level (g/dL) (N=58)	8 ± 2.0	8.3 ± 1.5	0.54
Reticulocyte count (N=33)	213 ± 158	228 ± 110	0.33
LDH level (xN)	4.8 ± 2.8	8.1 ± 4.6	**0.008**
LDH > 10N (%)	4 (7.4)	3 (25)	0.11
Platelet count ($x10^9$/L) (N=61)	21 ± 18	14 ± 10	0.12
Serum creatinine (µmol/L) (N=51)	117 ± 102	130 ± 68	0.43
Estimated glomerular filtration rate (mL/min) (N=51)	71.3 ± 29	56.7 ± 27	0.27
ANA (N=43)	19 (53)	5 (71)	0.44
APLA (N=18)	1 (7)	0 (0)	1
ADAMTS13 inhibitor (N=59)	43 (88)	8 (80)	0.61
IgG anti-ADAMTS13 Abs (U/mL) (N=46)	77 ± 54	111 ± 73	0.16

DOI: 10.3324/haematol.2011.049676

xN: number of times the upper normal value; ANA: antinuclear antibodies; APLA: antiphospholipid antibodies; Abs: antibodies. In case of missing values, the number of patient tested is specified in parentheses in the left column.

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Supplemental figure 1. Flow diagram of the study.

From the 944 patients of our registry, 610 were not included in the present study because of an associated condition (327 cases) or a detectable ADAMTS13 activity (283 cases). From the remaining patients with an undetectable ADAMTS13 activity and considered as having a TTP, 38 had a past history of TTP and 12 were not treated according to our standard guidelines (in most cases, TPE were not performed daily), leaving 281 patients for analysis.

DOI: 10.3324/haematol.2011.049676

Supplemental figure S1