Thrombotic thrombocytopenic purpura and the hemolytic uremic syndrome: much progress and many remaining issues

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There is currently a lot of interest among clinical hematologists for the thrombotic microangiopathies called thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS). These diseases are rare – their combined incidence is one annual case per 100,000 people in the general population[1] – so that we sometime have the impression that there are more scientists and publications in this field than patients. Why do TTP and HUS engender so much interest? Perhaps because they are life-threatening conditions, difficult to diagnose and treat. What do TTP and HUS have in common? They are both due to disseminated thrombosis in the microcirculation, resulting in ischemic damage of multiple organs. In both, thrombi are mainly composed by platelets, even though fibrin is also present in HUS. Thrombocytopenia is due to the consumption of platelets within the disseminated thrombi, whereas anemia is caused by mechanical damage of red cells that circulate through the partially occluded microcirculation. Thrombocytopenia and mechanical hemolytic anemia also occur in other conditions characterized by thrombosis in the microcirculation. Among them, the most frequent are eclampsia and disseminated intravascular coagulation, that can usually be distinguished from TTP and HUS by marked laboratory signs of coagulation activation and secondary fibrinolysis, with very high plasma levels of D-dimer. Consumptive thrombocytopenia may also occur in other thrombotic conditions, such as the so called catastrophic antiphospholipid syndrome and heparin-induced thrombocytopenia, but in them thrombosis mainly involves large arteries and veins. Moreover, serology can help in the diagnosis through the positivity of anti-platelet and antiphospholipid antibodies.

Until recently, the distinction of TTP from HUS was almost exclusively based on clinical grounds, after having excluded, as outlined above, other thrombotic microangiopathies. With consumptive thrombocytopenia and mechanical hemolytic anemia as common features, HUS was distinguished from TTP by the presence of more severe and refractory renal insufficiency (serum creatinine of 3-4 mg/dL or more) and the prodromic occurrence of hemorrhagic diarrhea, particularly in the forms affecting children. On the other hand, TTP was distinguished from HUS by the presence of signs of focal ischemia in the central nervous system (CNS), whereas signs of renal damage are less consistent and severe and more reversible (common findings are abnormal urine-analysis with albuminuria and microscopic hematuria, but serum creatinine rarely exceeds 2-3 mg/dL). However, distinction based upon the main organs affected by ischemia (the CNS in TTP, the kidney in HUS) is sometime difficult, because signs of CNS involvement may also be present in HUS (particularly in uremic patients) and renal failure is occasionally more severe than mentioned above in TTP. The need for an accurate distinction between TTP and HUS goes beyond theoretical interest, because plasma exchange, the mainstay of therapy in TTP, is not as efficacious in HUS, at least in the typical enterohemorrhagic form that occurs more frequently in children.

The birth of a paradigm

The complexity of the differential diagnosis between TTP and HUS was apparently simplified at the end of the second millennium, when two seminal studies, independently designed and carried out but jointly published, reported that patients clinically diagnosed with TTP were severely deficient in a plasma protein called ADAMTS13, while those with HUS and other thrombotic microangiopathies had normal or only modestly reduced levels.[2,3] ADAMTS13 is a plasma metalloprotease that cleaves the most thrombogenic, ultralarge forms of von Willebrand factor, the multimeric glycoprotein that plays a pivotal role in platelet plug and thrombus formation in the microcirculation.[4-6] When ADAMTS13 activity is deficient, endothelium-derived, ultralarge multimers of von Willebrand factor, endowed with a heightened reactivity with platelets, remain uncleaved in the circulation[7] and determine, in the conditions of high shear forces of terminal arterioles and capillaries, intravascular platelet aggregation and disseminated thrombus formation, resulting in end-organ ischemia and failure.[8] In TTP ADAMTS13 deficiency is due to two distinct mechanisms: in a small proportion of familial cases (no more than 2-3%), to the reduced synthesis of the protease, in turn due to defects in the corresponding gene, while in the great majority of cases, ADAMTS13 deficiency is acquired and explained by inactivation or removal of the protease from plasma due to the development of neutralizing or non-neutralizing autoantibodies.[2,3] Hence, ADAMTS13 deficiency was proposed as a highly specific and sensitive beacon of TTP, the latter diagnosis being excluded in thrombotic microangiopathies characterized by normal or moderately reduced levels of ADAMTS13.[2,3] The ADAMTS13 deficiency paradigm had therapeutic implications, because the well-established efficacy of plasma therapy acquired a proof of principle, acting through the replacement of the deficient protease and/or the removal of

anti-ADAMTS13 autoantibodies. By the same token, it become clearer why plasma therapy is usually not effective in cases with HUS (with the exception of the rare familial cases due to the deficiency or dysfunction of complement factors).

The paradigm is challenged

In clinical medicine, paradigms are seldom long-lasting. When confirmation of the original findings was looked for, several investigators reported cases of TTP that, as acceptably diagnosed on clinical and laboratory grounds as those included in the two original studies, had normal or moderately reduced levels of ADAMTS13.[9-16] This pattern of normal or slightly reduced ADAMTS13 was present not only in secondary TTP (associated with cancer, allogeneic bone marrow transplantation, HIV infection, use of antitumoral drugs, pregnancy) but also in apparently primary cases. Moreover, several cases clinically diagnosed with HUS, on the basis of the severity of signs of renal insufficiency, had very low or undetectable levels of ADAMTS13,[9,10,16] giving support to the views of those clinicians who maintain that the two diseases cannot be distinguished and should be better identified as TTP-HUS13, like Swisher *et al.* who report in this journal five cases that developed after acute pancreatitis.[17]

Where do we stand?

These varied results generated a heated debate and did increase dramatically the already abundant flow of literature on these fascinating diseases. Which message can be actually conveyed to practicing hematologist? Finding very low or undetectable ADAMTS13 can confidently lead to a bona fide diagnosis of a thrombotic microangiopathy called TTP. But the some diagnosis can be appropriately made in those patients who have thrombocytopenia and mechanical hemolytic anemia, when other causes can be reasonably excluded and yet ADAMTS13 levels are normal or only moderately reduced (20 to 40%). One can expect this pattern mainly in cases secondary to a number of severe diseases, but also in apparently primary cases (albeit more seldom).[13] Diarrhea- and enterotoxin-associated HUS is usually characterized by normal levels of ADAMTS13. On the other hand, there are cases with thrombocytopenia and hemolytic anemia accompanied by severe and refractory renal insufficiency in which ADAMTS13 is severely deficient. It is a matter of semantics whether these cases should be called atypical HUS or TTP.

Which are the clinical implications of these findings? At the moment the laboratory assays of ADAMTS13 and anti-ADAMTS13 autoantibodies are cumbersome, artificial and, most importantly, not suitable to provide the clinician with results in real time.[18,19] Hence, their diagnostic and prognostic value is uncertain, and the practicing hematologist must still diagnose and handle these patients on the basis of the results of such simple laboratory tests as complete blood counts and serum lactate dehydrogenase, a non-specific but sensitive index of tissue ischemia and necrosis. Plasma infusion, followed by plasma exchange, should be promptly started on the basis of these results without waiting for additional laboratory evidence, because it is well established that plasma therapy reduces from 80-90% to 10-20% the mortality rate of TTP. Plasma samples should be collected from these patients before and during plasma therapy and subsequently sent to laboratories equipped to assay ADAMTS13 and anti-ADAMTS13. These data will mainly be used to improve our knowledge on the disease through clinical research, but their usefulness to take therapeutic and prognostic decisions is still uncertain. In patients with diarrhea-associated HUS, the first and most important action is directed to control renal insufficiency, often requiring dialysis. Plasma therapy is not recommended by the majority of clinicians, because there is not solid evidence of its efficacy at variance with TTP13. However, cases with atypical, non-diarrhea associated disease should be tentatively managed with plasma therapy when ADAMTS13 is severely deficient. Again, it is recommended to collect from these patients plasma samples and clinical data, that can be offered to international electronic registries, an essential weapon to cope with the rarity of these cases.[20]

The future

The assays of ADAMTS13 and anti-ADAMTS13 should be become not only more facile but also more suitable to explore the interaction between VWF and ADAMTS13 in conditions mimicking more dosely the en-flow conditions that are needed for the optimal interplay between VWF and its cleaving protease. Cases of TTP characterized by normal ADAMTS13 in the presence of ultralarge forms of VWF in plasma are particularly interesting for research purposes (are other proteases of VWF involved?).[19,15] There is a need to monitor patients with TTP with a prolonged follow-up, particularly those who have chronic recurrent disease, and to see whether or not the markers of outcome and recurrence that are being proposed from the observation of small series of patients are validated in larger, prospective series.[21-26] In cases of familial TTP with ADAMTS13 deficiency it remains to be firmly established that prophylaxis with plasma or ADAMTS13 containing plasma fractions prevents recurrences, and to establish more accurately the trough levels of the protease that attain this goal. During the acute phase it should better understood whether or not the widely adopted practice of associating to plasma exchange immunosuppressive drugs is warranted, and which is the most effective agent among corticosteroids, cyclosporin or chimeric anti-CD20 monoclonal antibodies.[27,28] The issue of recurrence prevention in autoimmune TTP with these and other weapons (for instance, splenectomy) is also

looming large. Finally, it is necessary to revise the classification and nomenclature: for instance, it may be appropriate to distinguish TTP and HUS with and without ADAMTS13 deficiency, and those with and without antibodies, and to establish more accurately than now whether or not prognosis and outcome are different. This goal can only be achieved through the implementation of international registries[20] and collaborative research, the only way to circumvent the rarity of these diseases.

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