(see figure panel C),[9] although in many patients, anti-ADAMTS13 autoantibodies with epitopes in other ADAMTS13 domains are also present. Anti-ADAMTS13 S domain autoantibodies likely interfere with the ADAMTS13 binding to the unfolded VWF A2 domain, resulting in strong inhibition of the ADAMTS13 activity. An ADAMTS13 S domain exosite variant with 5 amino acid substitutions, Arg568Lys, Phe592Tyr, Arg660Lys, Tyr661Phe, and Tyr665Phe, showed resistance against the inhibitory function of autoantibodies from iTTP patients and expressed even higher proteolytic activity (a so-called gain-of-function variant).[10] For therapeutic use of ADAMTS13, the inhibition of the ADAMTS13 activity by autoantibodies is undesired, and the autoantibody-resistant ADAMTS13 variants are beneficial and promising.

Ercig et al undertook an elegant and sophisticated strategy, using prediction guided by structural bioinformatics to produce novel ADAMTS13 variants resistant to anti-ADAMTS13 S domain autoantibodies. First, they generated docking models of the ADAMTS13 C-S domains and the variable fragments from patient-derived monoclonal anti-ADAMTS13 autoantibodies. This enabled them to identify a larger epitope, consisting of 11 amino acid residues, than previously identified for autoantibodies on the S domain. Then, they produced novel single Ala ADAMTS13 variants based on the new models. Unfortunately, all of the Ala variants failed to escape autoantibody bindings. They further produced 6 ADAMTS13 variants containing the N-glycan attachment sequences based on the models, and finally, identified 1 N-glycosylated variant (NGLY3-ADAMTS13 with a p.Lys608Asn substitution) that showed autoantibody-resistant properties.

The NGLY3-ADAMTS13 variant thus identified showed low binding (<50%) against the sera in 10 out of 13 iTTP patients. The NGLY3 variant showed activity similar to the wild-type ADAMTS13 using the peptidyl VWF substrate as well as the VWF multimer under flow conditions. The variant retained the peptidyl VWF substrate-cleaving activity in the presence of an excess of patient autoantibodies, indicating that it had an autoantibody-resistant property. The presence of N-glycan on the variant was confirmed using the mass spectrometry analysis. Based on these findings, they concluded that a newly introduced N-glycan on Asn608 in ADAMTS13 hindered the autoantibody binding to the variant that conferred the autoantibody resistance (see figure panel D). They proposed this novel variant as a therapeutic option for treatment of iTTP.

There are several caveats to be considered. As the NGLY3-ADAMTS13 variant is engineered in the central S domain, it can bind to the autoantibodies against the C-terminal domains. For treatment of iTTP patients, studies on the risk for immunization against the NGLY3 variant will be needed.

Ercig et al showed that an approach using bioinformatics-guided prediction is valid and important for producing autoantibody-resistant ADAMTS13 variants that advance the field of iTTP. The predictions of the introduced N-glycan sites on the S domain should be particularly stimulating and informative for researchers. The concept that artificially inserted N-glycans interfere with the binding of inhibitory autoantibodies while retaining normal levels of proteolytic activity is also valuable. The study by Ercig et al has high therapeutic potential and is a welcome advance in the field.

Conflict-of-interest disclosure: The author is a member of the Clinical Advisory Board for Takeda and received a speaker fee from Shino-Test. He has a patent for specific substrate and activity measurement method for ADAMTS13 issued in Japan and the United States. ■

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TRANSFUSION MEDICINE

Comment on Magid-Bernstein et al, page 2699

Rethinking platelet transfusion practices

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In this issue of *Blood*, Magid-Bernstein et al examine the question of the significance of ABO-incompatible platelet transfusion.[1]

Like red blood cells, platelets express ABO antigens, and the general practice is to provide ABO-compatible platelets whenever possible. The rationale for this has primarily been concern over the infusion of ABO-incompatible plasma with the platelets and the associated risk of hemolysis in the recipient. Because of the

pressure on platelet availability and/or lack of knowledge of the blood group of the recipient, platelets are not infrequently transfused across the ABO blood group as an incompatible transfusion. Historically, less attention has been paid to the fate of platelets transfused into an ABO-incompatible recipient. The PATCH trial reported that the use of platelet transfusion in intracranial hemorrhage (ICH) patients receiving antiplatelet drugs is associated with an increase in mortality and disability.[2,3]

Magid-Bernstein et al have now investigated the role of ABO-incompatible platelet transfusion in the treatment of patients with ICH, a factor that was not examined in the original PATCH trial. Their analysis shows that patients who received ABO-incompatible transfusions had significantly lower platelet recovery, increased odds of in-hospital mortality, and poorer neurological outcomes. Although the study is somewhat limited by its size, nearly 40% of patients received ABO-incompatible platelet transfusions. Interestingly, there was no significant change in the progression of ICH between ABO-compatible and -incompatible platelet transfusions. Although the mechanisms contributing to these clinical outcomes remain to be determined, the observation is important for the management of ICH patients.

There have been other reports of suboptimal transfusion outcomes from ABO-incompatible platelet transfusion; however, the literature is inconsistent. ABO-incompatible transfusions may result in lower platelet count increments than ABO-compatible transfusions, although at least for prophylactic transfusions, these are not associated with detrimental clinical effects.[4] Literature on bleeding patients contains reports of ABO-incompatible transfusions where patients did worse as well as reports of no discernable effect of incompatible transfusion, sometimes in the same patient population.[5,6] As a result, we are left with a lack of clarity on appropriate use, and day-to-day practice may require pragmatism based on which platelets are available.[7] It is clear that additional clinical trials are needed to understand which patient populations are at risk for adverse outcomes from ABO-incompatible platelet transfusions. The new information in the Magrid-Bernstein et al study is an important contribution to move toward improving transfusion practice.

Some issues require further exploration. The platelets in the Magid-Bernstein et al study were exclusively apheresis platelets, meaning that each dose came from a single donor, reflecting >90% of the platelet inventory in the United States. The expression of ABH antigens on platelets is complex. The amount of A antigen on platelets varies widely in donors whose red cell type is A1, ranging from 0% to 87%, whereas donors with A2 red cells express no A antigen on their platelets.[8] However, the amount of A antigen on the platelets of a given donor is quite consistent over time.[8] The expression of B antigen is less variable, but both A and B are influenced by the Lewis and secretor statuses of the donor, because some fraction of the antigen is adsorbed to the platelet surface from the plasma.[9]

The recognition that the ABO compatibility of platelets matters to outcomes for at least some patients argues for a more sophisticated understanding of the characteristics of platelet donors and an appreciation that not all doses of platelets are equivalent. This is complicated by the ongoing pressure on the platelet supply in the United States, as plateletpheresis donors become more difficult to retain in the donor pool, and platelet demand continues to increase. Approximately 30% of platelet transfusions in the United States may be major incompatible, which may shorten the intertransfusion interval, particularly for prophylactic platelet use, resulting in increased platelet demand.[10] For ICH patients and others for whom incompatible platelet transfusions may matter, consideration must be given to alternative strategies that maintain the best platelet inventory to ensure platelet transfusions are not performed that fail to improve patient outcomes because of issues of compatibility.

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