Reprint requests: Alyna T. Chien, MD, MS, Department of Pediatrics, 21 Autumn St, Room 130.2, Boston, MA 02115. E-mail: Alyna.chien@childrens.harvard.edu

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New Insights into Platelet Dysfunction in Kawasaki Disease Using a Microfluidic Model of Thrombosis

In this volume of *The Journal*, Tsujii et al report their findings of platelet activation characteristics and the effect of antiplatelet therapy in 33 Japanese children with acute Kawasaki disease using a newly automated flow chamber system to evaluate platelet aggregate formation under high shear rates (1000 s^{-1} and 2000 s^{-1}) in a type I collagen-coated chip.[1]

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The study involved assessment of hirudin-anticoagulated room temperature whole blood samples taken immediately before, 1 week after, and 1 month after the initiation of treatment (intravenous immune globulin and either aspirin or flurbiprofen) for Kawasaki disease in 33 pediatric patients (range, 3-149 months of age; median, 27 months), compared with controls: 19 healthy adults (range, 20-40 years), 11 healthy children (range, 1-146 months; median, 26 months), and 5 febrile children (range, 7-116 months; median, 26 months) without Kawasaki disease. The change in pressure over time in the flow chamber and end points, such as elapsed time before the onset of platelet accumulation, were compared with clinical outcomes, particularly the development of coronary artery lesions.

Overall, the investigators demonstrated that the acute phase of Kawasaki disease was characterized by early onset and weak stability of platelet aggregates. However, no statistically significant differences were observed in time to onset of platelet accumulation between patients with acute Kawasaki disease and febrile child controls. Thus, platelet aggregation characteristics under a high shear condition in children with Kawasaki disease were not specific. Video microscopy confirmed the early initiation of platelet aggregation at 1 minute in patients with Kawasaki disease. However, after 7 minutes, the aggregates embolized, indicating weak stability.

Kawasaki disease may be associated with damage to the vascular endothelial cells, potentially resulting in morbidity and mortality via coronary thrombosis, myocardial infarction, and/or vascular aneurysms. Therefore, antiplatelet therapy with aspirin, aimed at subduing the acute phase of the disease, has been a mainstay of clinical therapy. This practice is based on limited platelet activation data from prior studies under static, no flow conditions. No relevant data exist for flow conditions in the clinical setting of Kawasaki disease. This study by Tsujii et al provides an important new method for the quantitative analysis of platelet aggregation under physiologically relevant shear stresses on thrombogenic surfaces.[1] The authors also demonstrated that patients with acute Kawasaki disease developed early and unstable platelet aggregates regardless of aspirin use, suggesting that this therapy may be unnecessary to decrease coronary artery lesion risk.

This study serves as an example of the potential usefulness of flow-based assays, particularly in microfluidic format that are clinically attractive owing to the small sample volume required along with high throughput capacity. These assays could reveal consistent patterns of hemostatic or thrombotic pathology, and have the potential to aid in assessing and monitoring patient-specific effects of coagulation-modifying therapies, specifically the platelet-based aspects of coagulation.[2,3] Indeed, these aspects have been evaluated in similar prior microfluidic studies with low-medium sized subject cohorts of subjects with von Willebrand disease (VWD). The same group that authored this study in Kawasaki disease, used their flow chamber to assess the clinical severity of type 1 VWD.[4] Similarly, Brazilek et al found that the platelet aggregation growth in a stenotic microfluidic device directly correlates with von Willebrand factor levels and could detect platelet aggregation defects associated with VWD subtypes with non-inferior, if not superior,

VWD von Willebrand disease

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sensitivity when compared with a traditional platelet function analysis (PFA-100).[5] Lehmann et al also demonstrated that platelet accumulation in a microfluidic assay correlates directly with levels von Willebrand factor, allowing for discrimination between type 1 VWD from healthy controls, as well as response to therapeutic intervention.[6] Tsujii et al have demonstrated the usefulness of this technique in a physiologic (under shear conditions) evaluation of aspirin as a therapeutic intervention in Kawasaki disease, highlighting the opportunity for broader clinical application beyond VWD.[1]

Despite these promising proof-of-concept studies, key issues remain that require additional study before the mainstream use of flow assays to evaluate disorders of platelet function or platelet-supported coagulation. It will be essential to determine normal ranges for these assays to identify patients with disordered platelet aggregation. Prelinical variables like the choice of anticoagulant, source and amount of thrombogenic proteins, shear rates, and channel dimensions all need to be systematically studied for any given flow chamber.[7-9] For large vessel injuries such as those in Kawasaki disease, the hemodynamics cannot be faithfully replicated in microfluidic channels because inertial forces are small. Additionally, it is crucial to further identify the specific impact of changes in the proportion of various cellular components of blood when these changes may simultaneously fall within normal ranges, but also affect the kinetics of thrombus formation, such as variations in hematocrit that impact rheology.[10,11] Finally, the assimilation of coagulation and fibrinolysis into the platelet aggregation component of these assays may allow for more direct comparison with global assays of thrombus formation such as thromboelastography or rotational thromboelastometry that also are influenced by myriad factors (blood cell counts, lipid profile, inflammatory status [eg, platelet activation/exhaustion], and drugs).[12]

The featured study marks an important expansion of the clinical applications of a microfluidic platform. The major usefulness of whole blood flow assays will likely come from their use as screening tools for abnormalities of hemostasis or thrombosis, and response to therapy. Larger clinical studies will be needed to determine whether they can be used to generate clinically relevant measures of hemorrhagic or thrombotic risk. ■

Brian R. Branchford, MD
Medical Sciences Institute, Thrombosis Hemostasis & Vascular Biology Program
Versiti Blood Research Institute
Milwaukee, Wisconsin

Keith Neeves, PhD
Department of Pediatrics
Division of Hematology/Oncology/Bone Marrow Transplant
University of Colorado School of Medicine
CU Anschutz Medical Campus
Aurora, Colorado

Reprint requests: Brian R. Branchford, MD, Versiti Blood Research Institute, 8727 W Watertown Plank Rd, Milwaukee, WI 53226. E-mail: bbranchford@versiti.org

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