ICH and 55% for all ICH). Improvements in imaging and significantly longer follow-up could explain the higher incidence of ICH in this study.

Despite the well-designed study, its retrospective nature creates inherent limitations. The enoxaparin cohort included patients who were deemed eligible for anticoagulation. Theoretically, providers' clinical acumen could have identified cancer patients with a lower risk of ICH in whom enoxaparin increased to the level of patients whom providers considered ineligible for anticoagulation. Unfortunately, besides tumor type, the multivariable analysis did not identify other clinical factors to guide clinicians in the assessment of ICH risk. Additionally, only 60 patients with renal cell carcinoma and melanoma (20 treated with enoxaparin and 40 controls) were available; thus, firm conclusions in this high-risk cohort cannot be established. Recognizing these limitations, the data presented suggest that anticoagulation does not increase the risk of ICH in patients with brain metastasis. Replicating this analysis in larger data sets and including patients with brain metastases in prospective studies are warranted.

Although oncologists often make the decisions regarding anticoagulation in cancer patients, hematologists are frequently consulted in scenarios involving a tenuous balance between bleeding and thrombosis. The results presented by Donato and colleagues suggest that cancer patients with brain metastasis have a wider fulcrum than expected to balance the risks of anticoagulation.2 This study further supports the statement from the 2014 American Society of Clinical Oncology Guidelines that brain metastases are not a contraindication to treatment of VTE with low-molecular-weight heparin.2

Conflicts of interest: The author declares no competing financial interests.

REFERENCES

Platelet secretion paves the way

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In this issue of Blood, Sakurai et al3 examine the response of single platelets to fibrinogen- and collagen-coated microdots and show that platelets can orient their release of α-granule cargo to promote spreading beyond the dot’s boundary.

Advances in imaging and microfabrication are increasing our ability to observe individual platelets and thus are expanding our views of what platelets can do and how they do it. Increases in imaging resolution (ie, super-resolution microscopy and total internal reflection microscopy), acquisition speeds, and computational techniques coupled with the production of microscale surfaces and fluids systems are revolutionizing how we dissect platelet function on the micro- and molecular scales. The work of Sakurai et al is one such example of how imaging and microprinting technologies are expanding our understanding of how platelets sense and modify their local microenvironments.

Sakurai and colleagues use microprinting to generate surfaces on which platelets are allowed to spread. Their overall goal is to determine how the biophysical properties of a matrix affect a platelet’s response. Previous work by the Lam group showed that matrix stiffness increased platelet adhesion and spreading as well as αIIbβ3 activation, and both P-selectin and phosphatidyserine exposure.2

The authors also noticed that platelets, when bound to defined, microcontact–printed surfaces (coated with fibrinogen), dynamically extended filopodia in all directions, apparently sampling their microenvironment. Platelets could extend filopodia across uncoated regions of up to 5 μm in width.3 This previous work demonstrated the platelet’s ability to sense and to respond to the spatial constraints of their local microenvironment.

In their present work, Sakurai et al create fibrinogen- and collagen-coated microdots of different diameters to assess how geometric orientation of the matrix and spatial sensing affect platelet exocytosis and spreading. The surface covered by individual platelets increased as the dot’s diameter, reaching a maximum on dots of 7 μm (38.5 μm²). Surprisingly, the area covered by platelets decreased slightly as the microdot diameters increased. Closer examination of the platelets, spread on the smaller dots, showed that they were extending beyond the microdot edges onto the unprinted surfaces. Further imaging analysis showed that there was a concentration of P-selectin at the edges of the smaller microdots, which was not seen as platelets spread on the larger (>7 μm) microdots. Platelets spreading on micropatterns containing uncoated holes also showed a concentration of P-selectin at the coated/uncoated boundaries. Indeed, it appeared that secretion of some granule cargo (ie, fibrinogen, fibronectin, and P-selectin) was directed to these boundary regions and thus was depositing matrix for further platelet extension beyond the boundary. Activated αIIbβ3 was concentrated in these boundary regions, but GP1ib was
not. Platelets on collagen-coated microdots show a similar extraboundary spreading, which required αIIbβ3, and was inhibited by eptifibatide, an αIIbβ3 antagonist. Consistent with the need for α-granule secretion, platelets from a patient with gray platelet syndrome failed to extend beyond the microprinted surface. Additional analysis by Sakurai et al demonstrated the importance of actin cytoskeleton, but not microtubules, to extraboundary spreading and showed a role for Rac1 and myosin light-chain kinase in its regulation. Rho kinase had a negative effect on the process. Platelets from a patient with Wiskott-Aldrich syndrome also failed to spread.

These data are exciting because they show that platelets sense substrate borders and respond by “self-depositing” matrix proteins to alter the boundary and extend their ability to move beyond it. This suggests that platelets can, in response to spatial cues, polarize their secretion to peripheral regions at the edges of the cell. Release from the centralized granulomere may also represent polarized secretion, although that is unclear. Clearly, actin and Rac1/RhoA are important, but what else is involved? Platelet secretion is mediated by membrane proteins called soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), which facilitate granule–plasma membrane fusion for granule content release. There are 2 classes of SNAREs: v-SNAREs from vesicles/granules and t-SNAREs from the target membrane (the plasma membrane). Platelets contain 4 major v-SNAREs (vesicle-associated membrane protein–2, –3, –7, and –8); each contributes to platelet function. VAMP-8 is thought to be the dominant form, mediating the fast secretion events. VAMP-7 mediates secretion from a spatially distinct population of granules. Previous work by the Flaumenhaft group, using time-lapse microscopy, showed that VAMP-7+ granules translocate to the platelet periphery during spreading, in contrast to VAMP-8+ granules, which concentrate in the granulomere. Translocation of VAMP-7+ granules was proposed to provide a membrane reservoir for filopodia and lamellipodia formation. More recent studies, using VAMP-7 knockout mice, have confirmed the importance of VAMP-7–mediated secretion to platelet spreading. These studies also showed interaction between VAMP-7 and key actin cytoskeletal regulators, which may be responsive to the platelet’s boundary detection system.

What does this work tell us about platelet function? First, Sakurai et al suggest that this mode of boundary detection and response may facilitate platelet adhesion to small, subclinical vascular lesions. Platelet adhesion must be sufficiently stable to withstand the shear forces of blood flow, and the ability to “self-deposit” matrix and to polarize P-selectin exposure would strengthen their contacts with neighboring endothelial cells. Similarly, this process could stabilize attachments of platelets at the periphery of a growing thrombus. These data might also explain phenotypes where secretion in suspension is modestly affected but bleeding is significant. Defects in secretion polarization may not be readily detected in suspension assays but could be critical in vivo. The most exciting implication of this work is the realization that platelets are not only detecting biochemical changes in the vasculature, but are also deciphering spatial and physical cues. Understanding how this detection system transduces signals to the platelet secretory machinery will undoubtedly yield new insights into how individual platelets integrate into a growing thrombus.

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Comment on Lindi et al, page 539

Warfarin pharmacogenomics and African ancestry

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In this issue of Blood, Lindi and coauthors demonstrate that racially informed warfarin pharmacogenetic algorithms perform better than traditional algorithms, which previously excluded genetic variants that are unique to patients with African ancestry.

Approximately 34 million warfarin prescriptions are filled annually in the United States to reduce the morbidity and mortality associated with various hypercoagulable states. Warfarin, a vitamin K antagonist, is a potent anticoagulant with proven efficacy when patients reach therapeutic anticoagulation goals. However, warfarin demonstrates significant interpatient variability in dose requirements, and it has a narrow therapeutic index. This makes warfarin difficult to manage despite the use of widespread multidisciplinary anticoagulation clinics dedicated to its management and monitoring. Warfarin’s variability in dosing requirements and narrow therapeutic index contribute to this drug being a leading cause of the adverse drug events requiring
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