and SRC-family kinase pathways. CSF3R mutations were originally reported in a fraction of patients with severe congenital neutropenia (SCN), and these patients exhibited increased risk of developing AML. The variants observed in SCN always took the form of frameshift or nonsense mutations that truncated the cytoplasmic tail of the receptor. Subsequently, it was found that mutation of CSF3R is quite frequent in Philadelphia-negative neutrophilic leukemia with a majority of chronic neutrophilic leukemia (CNL) patients and a minority of atypical chronic myeloid leukemia (aCML) patients exhibiting CSF3R variants. The vast majority of mutations seen in CNL/aCML are point mutations proximal to the transmembrane that result in increased receptor dimerization and ligand-independent signaling, although some patients also exhibit truncating mutations similar to those seen in SCN patients. CSF3R mutations have rarely been observed in AML and, until now, had not been observed to cluster into specific disease subsets.

Lavallée et al examined RNA-Seq and genotypic data from the Leucegene cohort of 415 primary AML patient specimens to identify a gene signature of JAK-STAT pathway activation and sensitivity to JAK kinase inhibitors, and a proportion of atypical CSF3R AML patients, as marked by a gene expression signature, also display JAK-STAT dysregulation and dependence. In numerous patients, this JAK-STAT pathway activation appears to be at least partially driven by mutations in JAK-STAT–regulating genes such as CSF3R or STAT3B, but the etiology of JAK activation in the remaining patients remains to be determined.

From a translational standpoint, these studies suggest the exciting possibility that CSF3R AML patients may benefit from treatment with JAK kinase inhibitors. These studies certainly provide a strong rationale for testing this hypothesis in prospective clinical trials. The authors of both articles are all to be commended for the scope, strategy, and potential impact of their studies, and for their elegant work in establishing complex connections between genotype and phenotype. Much more work of this caliber is needed to improve outcomes for patients with AML.

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**Platelets in sepsis: beyond hemostasis**

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In this issue of Blood, Claushuis et al report on a rigorous evaluation of the associations among baseline platelet counts, biomarkers of host response, and clinical outcomes in a large cohort of critically ill patients with sepsis.

A side from their conventionally recognized function in hemostasis and thrombosis, platelets have received increasing attention for their roles in infectious diseases, innate immunity, and inflammation. To date, it has been unclear whether reduced platelet counts...
Platelet counts (independent of disease severity) associated with increased 30-day mortality, activity (decreased antithrombin). These integrity (increased ratio of angiopoietin molecule 1 and fractalkine), reduced vascular biomarkers (increased intercellular adhesion (IL)-8 and IL-10, elevated endothelial activation with elevated plasma levels of interleukin shock, platelet counts that were classified as very low (<50 × 10^9/L) or intermediate low (50 × 10^9/L to 99 × 10^9/L) were both associated with increased 30-day mortality, independent of disease severity.

The authors also observed that very low platelet counts (<?50 × 10^9/L) were associated with elevated plasma levels of interleukin (IL)-8 and IL-10, elevated endothelial activation biomarkers (increased intercellular adhesion molecule 1 and fractalkine), reduced vascular integrity (increased ratio of angiopoietin [Ang]-2 to Ang-1), and increased coagulation activity (decreased antithrombin). These findings implicate several important host response pathways in the relationship between thrombocytopenia and adverse clinical outcomes. Notably, the associations between platelet counts and host response pathways remained statistically significant after rigorous adjustment using propensity matching to account for baseline differences in disease severity and other factors.

In support of a role for platelets in host response to sepsis, patients with thrombocytopenia had whole-blood leukocyte transcriptome patterns that revealed underexpression of genes encoding proteins involved in leukocyte adhesion, diapedesis, and extravasation signaling. Although these results do not demonstrate a causal association between platelet counts and host response, the findings are consistent with the preclinical animal studies reviewed by the authors in the manuscript’s discussion. The importance of these findings lies in their demonstration of the significance of thrombocytopenia as a risk factor for both dysregulated host response and adverse clinical outcomes.

Platelets can participate with immune cells in immune-driven thrombus formation and play a key role in the formation of neutrophil extracellular traps, which can aid in the capture and killing of bacteria and viruses. In addition, platelets can interact with bacterial pathogens to kill them directly via microbicidal proteins, known as thrombocidins (see figure panel A). Platelets also interact with immune cells, enhancing a number of immune functions via cytokine and chemokine secretion and promoting neutrophil tethering at sites of damaged endothelium (see figure panel B). Finally, activated platelets can increase antigen presentation to T cells, thereby enhancing the activation of adaptive immune responses (see figure panel C).

Endothelial stabilization is another potential mechanism that could account for the effect of platelets on clinical outcomes in sepsis. Over the past 15 years, our understanding of sepsis has evolved beyond rogue inflammation and compensatory anti-inflammatory responses.

Sepsis has been increasingly recognized by a number of investigators as a syndrome of severe infection-related endothelial activation and dysfunction that leads to systemic microvascular leak and multiple-organ failure. The Ang-Tie2 system, in particular, has emerged as an important regulator of
endothelial activation status. In general, Ang-1 stabilizes the endothelium and prevents microvascular leak, and its major sources are pericytes and platelets. Systemic serum or plasma levels of Ang-1 decline precipitously in severe sepsis, as reported previously and in the current study. Therefore, it seems entirely plausible that thrombocytopenia in sepsis could contribute to adverse clinical outcomes by decreasing the delivery of bioavailable Ang-1 to endothelium at risk.

Overall, Claushuis et al have advanced the field by highlighting the importance of platelets in a large cohort of critically ill patients with clinical sepsis. This study was limited by the inability to demonstrate causality inherent to observational clinical studies and by somewhat limited generalizability due to inclusion of only one clinical site in the Netherlands. Further study is needed to identify potential interventions that may be of use in patients with low platelet counts at baseline, using animal models initially. It is unclear whether platelet transfusions or specific interventions to enhance platelet immune function or other innate immune mechanisms would be of value. However, the findings reported by Claushuis et al clearly suggest that the roles of platelets in innate immunity, inflammation, and endothelial stabilization may be as clinically important and multifaceted as their role in hemostasis and thrombosis.

Conflict-of-interest disclosure: W.C.L. is listed as co-inventor on a patent applied for by the University Health Network (Toronto, ON, Canada) to develop point-of-care tests for endothelial activation biomarkers in infectious diseases. S.M.G. declares no competing financial interests.

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THROMBOSIS AND HEMOSTASIS

Comment on Mazepa et al, page 3073

Hemophilia: a cautionary status report

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In this issue of Blood, Mazepa and colleagues analyzed a nationwide US Centers for Disease Control and Prevention registry covering adult patients with severe hemophilia from 1998 to 2011 and identified two major issues: untreated chronic hepatitis C and inadequate control of joint hemorrhages. In reaction to the hideous AIDS epidemic of the 1980s and 1990s, which killed some 80% of infected hemophilia patients, effective anti-HIV drugs were developed and widely employed, and virus-inactivated or recombinant concentrates were introduced, thus gaining control over HIV infection. Fortunately, the virus inactivation methods employed in the 1990s to destroy HIV also killed hepatitis C virus (HCV), which was identified at the end of the 1980s. But how are the survivors of that era getting along? Mazepa et al found that patients who had received earlier clotting factor concentrates were at very high risk of HCV infection: 3490 (71%) of this study’s patients with severe hemophilia, plus 1109 (41.5%) of those with mild hemophilia (whom they also followed.) Only a rough third of infected patients had received any anti-HCV therapy, and liver failure was now the leading cause of death. Today, effective anti-HCV therapy exists but its price makes it inaccessible to most patients. This impasse is an outrage.

The major targets of hemorrhage in persons with hemophilia are, mysteriously, the large joints of the limbs. Repeated bleeding leads to inflammation, erosion, arthritis, and high rates of disability. Ideally, prevention with clotting factor concentrate prophylaxis starts in toddlers, as was pioneered in Sweden and confirmed in a sentinel US study reported in 2007. Prophylaxis has become the standard of care in children, but it is not easy. Concentrates must be frequently administered by IV infusions. Children supervised by concerned parents maintain their schedules reasonably well, but teenagers and young adults are less reliable. Among this study’s youngest subjects (aged 19-28 at end of study), 45% were on prophylaxis, but 35% (not on prophylaxis) had frequent joint hemorrhages. The preservation of joint health in these youths should be a major concern.

Can hemorrhagic bleeding be controlled by anything easier than frequent IV infusions? Never clotting factor concentrates with longer half-lives have extended infusion intervals from 2 to 3 days for hemophilia A, and reduced their frequency from twice weekly to once weekly for hemophilia B. An experimental, subcutaneously administered factor VIII mimetic shows promise as a more easily administered, longer-acting prophylactic agent for hemophilia A. We look forward to the possibility of gene therapy, despite ongoing obstacles.

Roughly 70% of American patients with severe hemophilia now are under the care of the dedicated centers established by Congress some 40 years ago. These leadership centers now need to persuade patients, especially young adults, to continue prophylaxis. They should also encourage patients to build strength in the muscles that support the at-risk joints, an effective but underused preventive effort. All concerned entities, hemophilia centers, and hemophilia organizations need to be committed to the treatment of hepatitis C as universally as we now treat HIV infection.