Table 1. Risk of bleeding in the 796 VWD patients according to clinical and laboratory predictors

<table>
<thead>
<tr>
<th>BS</th>
<th>Crude HRs (95% CI)</th>
<th>Adjusted HRs (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>2.10 (1.10-3.90)</td>
<td>2.05 (1.07-3.91)</td>
</tr>
<tr>
<td>5-10</td>
<td>6.80 (3.80-12.30)</td>
<td>7.27 (3.83-13.83)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>3.27 (1.77-6.06)</td>
<td>1.12 (0.50-2.51)</td>
</tr>
<tr>
<td>VWF:RCo, IU/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;30</td>
<td>1.51 (0.72-3.14)</td>
<td>1.12 (0.50-2.51)</td>
</tr>
<tr>
<td>10-30</td>
<td>3.27 (1.77-6.06)</td>
<td>1.12 (0.50-2.51)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>6.80 (3.80-12.30)</td>
<td>7.27 (3.83-13.83)</td>
</tr>
<tr>
<td>FVIII:C, IU/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;40</td>
<td>2.07 (1.16-3.69)</td>
<td>1.52 (0.80-2.90)</td>
</tr>
<tr>
<td>&lt;20</td>
<td>4.20 (2.43-7.26)</td>
<td>2.20 (1.05-4.62)</td>
</tr>
</tbody>
</table>

*The effect of each predictor on the bleeding risk was adjusted for that of the other ones and for age and sex, in a multivariable Cox proportional hazard model.
†Reference group.
See Table 2 in the article by Federici et al beginning on page 4037.

ISTH-BAT descended directly from it, the results remain meaningful today.

Overall, this study provides critically important knowledge about the utility of BS. Although the results are intuitive, this does not in any way diminish the study impact.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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HEMATOPOIESIS & STEM CELLS

Comment on Mallampati et al, page 4064

Sox4 B-lymphocyte progenitors

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In this issue of Blood, Mallampati et al provide mechanistic insight into the functions of the transcription factor Sox4 in pro-B lymphocytes using both gain-of-function and loss-of-function approaches combined with global gene expression and genome-wide transcription factor binding analysis.1

Lympocyte development is regulated by transcriptional regulatory networks that include cell type–specific, as well as broadly expressed transcriptional regulators. These networks guide the activation of lineage-appropriate genes, a process called lineage specification, and they repress genes for alternative lineages, thereby enforcing commitment to the specified fate.2 The networks controlling B-lymphocyte development are among the best studied and have provided many of the paradigms that shape our thinking on gene regulatory circuits and lymphocyte development. Compelling evidence also indicates that lineage-specifying transcriptional networks form the basis for growth regulatory and differentiation control throughout lymphocyte development because key members are frequently mutated in immature and mature B-cell malignancies.3 Nonetheless, multiple transcription factors whose functions remain enigmatic have an impact on B-lymphocyte development, and their activities are not easily placed into the known regulatory networks.

One enigmatic transcription factor is Sox4, a high-mobility group family transcription factor that is required for early B-cell development and whose expression is a poor prognostic factor in B-cell leukemia.4 In 1996, Sox4 was reported to be essential for B-cell development at the stage of interleukin-7–dependent expansion, and at least a portion of the function of Sox4 involves promoting the survival of pro-B lymphocytes.5,6 However, Sox4 is not a B-lymphocyte–specific transcription factor; it plays critical roles in T-lymphocyte development as well as in nonhematopoietic cells, and therefore, its functions are likely influenced by the context in which it is expressed.7 The essential targets of Sox4 in B lymphopoiesis have been difficult to identify because pro-B lymphocytes are absent in Sox4−/− mice. However, the development of a conditional allele for Sox4 has opened the door for investigation into its functions in vivo and in vitro.8 Mallampati et al created gain- and loss-of-function models in vitro to study the requirements for Sox4 in pro-B lymphocytes. By using a self-excising Cre–producing retrovirus, they deleted conditional alleles of Sox4 in primary and Bcr-Abl–expressing pro-B lymphocytes and subsequently complemented the Sox4 deficiency with Sox4–producing retrovirus. Combining a careful analysis of the developmental requirements for Sox4 with microarray analysis of messenger RNA (mRNA) expression and chromatin immunoprecipitation of biotin-labeled Sox4 followed by high–throughput sequencing (Sox4 chromatin immunoprecipitation sequencing), they revealed some of the critical targets of Sox4 in pro-B lymphocytes. Of note, approximately 35% of the identified binding sites contained the canonical Sox4 binding sequence; however, Sox4 also appeared to bind DNA through potential GABPA sites, and signature motifs for additional transcription factors were also enriched. Reporter assays revealed that Sox4 can activate transcription via GABPA consensus sites. Therefore, Sox4 may regulate a substantial number of genes through unanticipated interactions, either with unique DNA binding motifs or through interactions with the factors that bind to those motifs. Future experiments will be needed to clarify the
mechanisms by which Sox4 regulates gene expression through DNA binding motifs that are not the Sox4 consensus. However, these data raise the intriguing hypothesis that Sox4 function may be highly context dependent because of an ability to regulate genes through interactions with other sequence-specific transcription factors.

The combined analysis performed in the study by Mallampati et al revealed a direct role for Sox4 in expression of the recombination-activating genes Rag1 and Rag2, with a consequent effect on recombination of the B-cell receptor gene loci. In addition, an unexpected effect on the targets of the Wnt signaling pathway was also identified. Sox4 was bound to the promoter of the Csnk1e gene, encoding casein kinase 1 ε (CK1ε), a known kinase in the GSK3β-containing complex that controls the stability of β-catenin and consequently the function of the Tcf1/Lef1 family of transcription factors. Sox proteins and Tcf1/Lef1 proteins can interact and have been implicated as antagonistic regulators of the development of subsets of γδ T cells. The findings in this study raise the possibility that Sox4 may also antagonize Lef1 (Tcf1 is not expressed in pro-B lymphocytes) function by controlling the stability of β-catenin in pro-B lymphocytes. Csnk1e mRNA-directed short hairpin RNA resulted in a decreased number of BPI+ pro-B lymphocytes and increased the activity of a Tcf1/Lef1 reporter, implicating CK1ε-dependent destruction of β-catenin and inhibition of the Wnt pathway in pro-B lymphocyte survival or expansion. However, the kinase activity of CK1ε targets many proteins, and the current experiments do not rule out a role for these other proteins in pro-B lymphocytes. Nonetheless, this study provides a foundation for further understanding of how Sox4 controls B-cell development and how its functions integrate with other critical regulators of B lymphocyte development and transformation.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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Comment on Shi et al, page 4015

Complement, cold agglutinins, and therapy

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In this issue of Blood, Shi and coworkers show that TNT003, a mouse monoclonal antibody targeting complement protein C1s, prevents induction of in vitro hemolysis by cold agglutinins (CA). If successfully transferred into the clinical setting by further studies, these findings may result in a novel therapeutic principle for a frequently difficult problem.

Primary chronic CA disease (CAD) is the “least uncommon” type of cold-antibody autoimmune hemolytic anemia (AIHA), accounting for about 15% of AIHA. Recent studies have shown that CAD is a well-defined clinicopathologic entity, characterized by a probably distinct clonal lymphoproliferative B-cell disorder of the bone marrow. The autoimmune hemolysis in CAD is entirely dependent on complement activation by the classical pathway (see figure). C3b opsonized erythrocytes are phagocytosed by reticuloendothelial cells, predominantly in the liver, resulting in extravascular hemolysis. According to the prevailing view, activation of the terminal complement pathway with intravascular hemolysis is usually not important but does occur in some patients and situations, as evidenced, for example, by the finding of hemoglobinuria in 15% of the patients. Secondary CA syndrome (CAS) associated with specific infections or aggressive lymphoma is a heterogeneous condition, different from CAD in terms of etiology and B-cell clonality but characterized by the same complement-dependent mechanism of hemolysis.

The anemia in CAD is not always mild, as shown by the lower tertile hemoglobin level of 8.0 g/dL. Furthermore, acute-phase reaction in febrile diseases or following major surgery or trauma may result in complement-mediated, sometimes severe exacerbations. In a cool climate, approximately 90% of the patients experience cold-induced circulatory symptoms that are caused by red cell agglutination and are, therefore, complement independent. In descriptive studies from Norway as well as the United States, pharmacologic therapy had been attempted in 70% to 80% of the patients. The relative success in treatment of CAD during the last decade has been achieved through targeting the pathogenic B-cell clone. Rituximab monotherapy has produced approximately 50% response rates, whereas the combination of rituximab and fludarabine has resulted in a 75% response rate, 20% complete responses, and median response duration of more than 66 months.

Shi and coworkers tested the in vitro effects of TNT003 on CA-induced complement activity against human erythrocytes. This monoclonal antibody has specificity for the complement serine protease C1s. Using CA
Sox4 B-lymphocyte progenitors

Barbara L. Kee