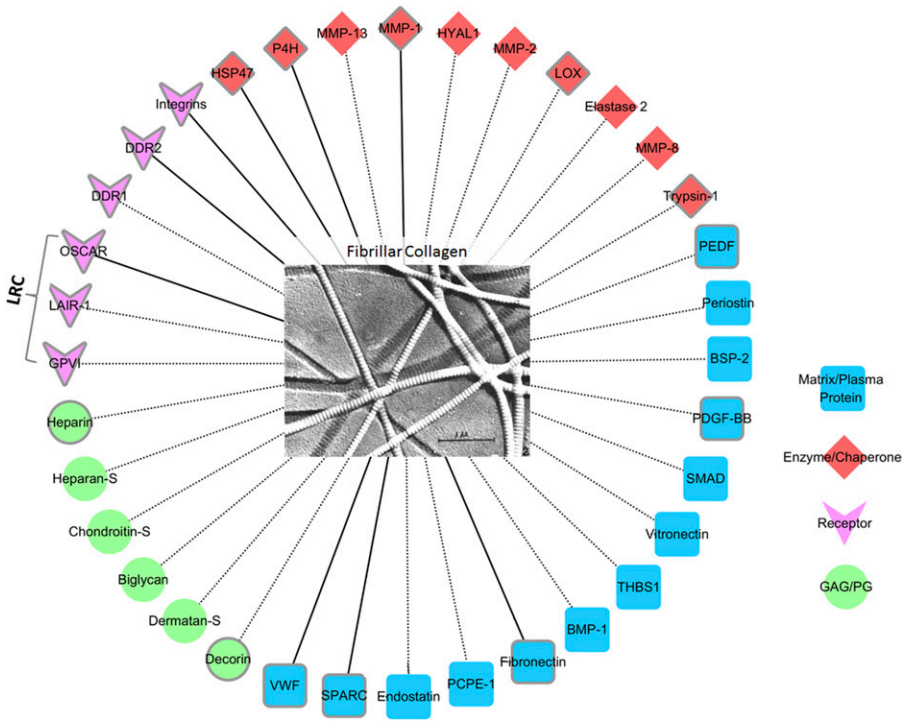

A schematic diagram of the interaction network of major fibrillar collagens showing the classes of molecules that interact with them, together with representative examples of each class (plotted with Cytoscape 3.2.1, based on multiple sources including MatrixDB [http://matrixdb.ibcp.fr/]). A gray outline around the molecule name indicates the crystal structure of the molecule has been determined. Molecules connected to collagen by solid lines indicate that the structures of cocrystals of that molecule (or part of that molecule) with a collagen-like peptide have been solved. BMP, bone morphogenetic protein; BSP, bone sialoprotein; DDR, discoidin domain receptor; GAG, glycoaminoglycan; GPVI, glycoprotein VI; HSP, heat shock protein; HYAL, hyaluronidase; LAIR-1, leukocyte-associated immunoglobulin-like receptor-1; LOX, lysyl oxidase; LRC, leukocyte receptor complex; MMP, matrix metalloproteinase; PCPE, procollagen C-proteinase enhancer; PDGF, platelet-derived grwoth factor; PEDF, pigment epithelium-derived factor; PG, proteoglycan; SPARC, secreted protein acidic and rich in cysteine; THBS, thrombospondins; VWF, von Willebrand factor. The central electron micrograph of collagen fibrils is from Gross and Schmitt.[2]

a number of diseases including osteoporosis, atherosclerosis, chronic obstructive pulmonary disease, and rheumatoid arthritis.[9-11] For example, OSCAR expression by monocytes is inversely correlated with disease activity in rheumatoid arthritis.[9] OSCAR expression is highly specific to osteoclasts and their precursors, compared with other immune system modulators such as receptor activator of nuclear factor κB, triggering receptor expressed on myeloid cells 2, and DNAX-activating protein of 12 kDa, making OSCAR a promising therapeutic target for common diseases with elevated osteoclast bone resorption activity such as osteoporosis and rheumatoid arthritis. Therapies that target osteoclast maturation and inhibit inflammatory osteoclastogenesis may be possible through regulation of OSCAR function, using collagen-like peptides, anti-OSCAR antibodies, or recombinant soluble OSCAR. The findings by Zhou et al[1] provide important new insights into the molecular mechanism of OSCAR-collagen interactions and create a foundation for potential therapies.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● CLINICAL TRIALS AND OBSERVATIONS

Comment on Nagler et al, page 546

Does my patient have HIT? There should be an app for that

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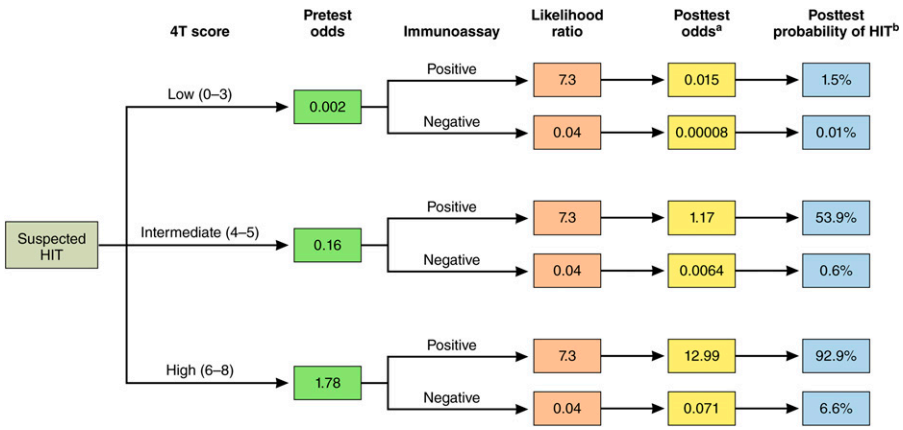
In this issue of *Blood*, Nagler et al present a systematic review and meta-analysis on the diagnostic accuracy of immunoassays for heparin-induced thrombocytopenia (HIT). Their data, when combined with the 4T score, provide an easy-to-use, evidence-based framework for estimating the probability of HIT.[1]

The hematology fellow's phone rings. A call from the surgical intensive care unit (SICU). Another consult for thrombocytopenia, rule out HIT. The fellow looks down at the floor and follows the well-worn path to the SICU. She has done so many



Bayesian approach to estimating the probability of HIT. The 4T score in conjunction with an immunoassay can be used to estimate the posttest probability of HIT. The pretest odds of HIT, based on the 4T score, is derived from Cuker et al.[3] For likelihood ratios for various immunoassays, see Table 2 in the article by Nagler et al that begins on page 546.[1] The likelihood ratios for the polyspecific ELISA (low threshold) are shown as an example. These values can be used to calculate the posttest odds and posttest probability of HIT using the equations below. [a]Posttest odds = pretest odds × likelihood ratio; [b]Posttest probability = posttest odds/(posttest odds + 1). Professional illustration by Patrick Lane, ScEYEnce Studios.

consults like this before and yet still finds them difficult. Should she recommend cessation of heparin and initiation of a nonheparin anticoagulant while awaiting confirmatory laboratory testing? Or is the probability of HIT sufficiently low that heparin may be continued? She recognizes the high stakes inherent in this decision. Delays in implementing appropriate therapy in patients with serologically confirmed HIT are associated with an initial 6.1% daily risk of thromboembolism, amputation, and death.[2] Misdiagnosis, conversely, may result in exposure of postoperative, thrombocytopenic patients without HIT to costly alternative anticoagulants and their attendant ~1% daily risk of major bleeding. She wishes for a diagnostic tool that will help her make the right call.

The 4T score, a clinical or pretest scoring system with well-characterized operating characteristics, is one such tool. In a systematic review and meta-analysis, the negative predictive value of a low probability 4T score was 99.8% (95% confidence interval, 97-100). The positive predictive value of an intermediate and high probability 4T score was 14% (9-22) and 64% (40-80), respectively.[3] Although helpful, the 4T score is hampered by interobserver variability[4] and modest positive predictive value, and may be difficult to calculate or unreliable if clinical information is missing.[5]

In light of these limitations, clinicians rely heavily on laboratory testing to assist with diagnosis. Laboratory assays fall into 2 categories: platelet factor 4/heparin immunoassays and washed platelet functional assays. The latter are more specific than immunoassays and are considered the "gold standard" among HIT laboratory tests, but are highly specialized and are performed only at select reference laboratories, often with turnaround times of several days. Immunoassays, by contrast, are the mainstay of HIT laboratory testing in most centers. The prototypical immunoassay, the polyspecific enzyme-linked immunosorbent assay (ELISA), is encumbered by frequent false-positive results and slow turnaround time. Recent years have witnessed a proliferation of immunoassays designed to overcome these limitations. Modifications to the ELISA including immunoglobulin G-specific detection, addition of a high heparin confirmatory step, and increases in the optical density cutoff have been implemented to enhance specificity. No less than 6 rapid immunoassays, which provide results in 30 minutes or less, have entered the market to shorten turnaround time and provide laboratory diagnostic information at the point of care.[6]

The operating characteristics of many of these immunoassays have been described only in small single center studies and have not been compared with one another. Nagler et al present the results of a well-designed systematic review and meta-analysis on the diagnostic accuracy of immunoassays for HIT. Their analysis provides the best available estimates of the performance of these assays. They observed important differences between tests based on type of assay, antibody specificity, and cutoff. Only 5 of the 20 tests they investigated met criteria for high sensitivity (>95%) and specificity (>90%).[1]

The results of Nagler et al provide clinicians with critical information to assist in test interpretation, patient evaluation, and management. The authors calculated positive and negative likelihood ratios for each of the immunoassays that they investigated. These likelihood ratios may be combined with the pretest odds of HIT, as determined by the 4T score, to estimate the posttest odds of HIT (see figure).[6-8] This Bayesian approach provides clinicians with a systematic framework for estimating the probability of HIT in their patients, information that can be used to guide management decisions. In the example highlighted in the figure, a positive polyspecific ELISA (low threshold) in a patient with an intermediate 4T score would increase the probability of HIT to 53.9%, supporting empiric treatment of HIT while awaiting confirmation with a functional assay. Conversely, a negative ELISA in the same patient would reduce the posttest probability of HIT to 0.6%, suggesting that heparin could be safely continued. Similar analyses can be conducted for other immunoassays by inserting their corresponding likelihood ratios, as determined by Nagler et al.[1]

The approach illustrated in the figure has limitations. Estimates of the diagnostic accuracy of both the 4T score and immunoassays were derived, in part, from small studies of low or moderate methodologic quality.[1,3] Sound clinical judgment remains central to evaluation of the patient with suspected HIT. Nevertheless, a Bayesian strategy that combines information from the 4T score and laboratory assay result provides a much-needed, evidence-based structure to this evaluation.

Nagler et al have provided the key data. A logical next step is the development of an app to facilitate estimation of the posttest probability of HIT at the point of care. Such a tool could improve clinical decision-making, outcomes, and cost-effectiveness of care, and perhaps diminish the sense of dread instilled in the hematology fellow the next time the SICU calls.

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

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LYMPHOID NEOPLASIA

Comment on Nagata et al, page 596

Opposite RHOA functions within the ATLL category

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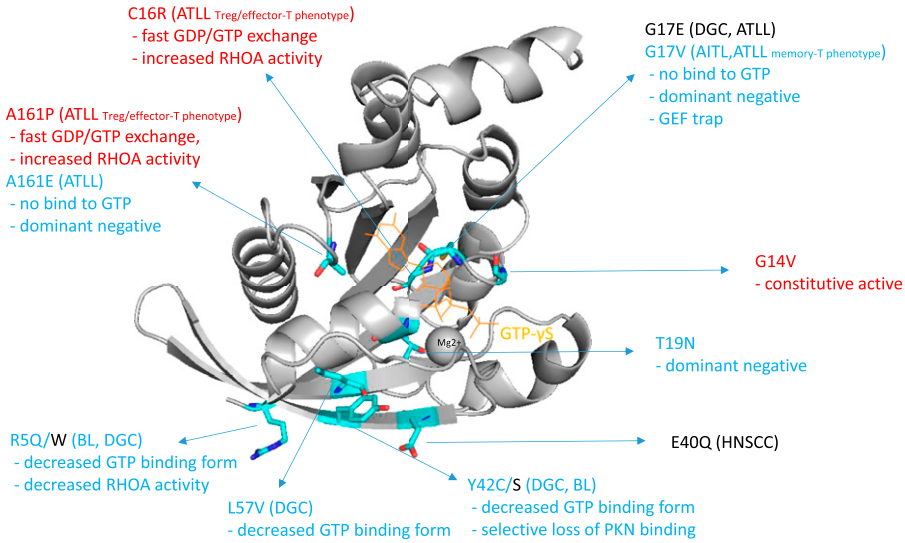
In this issue of *Blood*, Nagata et al reported that different Ras homolog gene family, member A (*RHOA*) hotspot mutations among the adult T-cell leukemia/lymphoma (ATLL) category have opposite biochemical activities, which are linked to different T-cell phenotypes.[1]



The RHOA hotspot mutations reported so far in various tumor subtypes.[1-9] The disease categories, mutations, and biochemical properties linked to increased and decreased RHOA activity are indicated by red and blue color, respectively. Image of 1A2B[10] created with PyMOL (Schrödinger, LLC). GDP, guanosine diphosphate; GEF, guanine nucleotide exchange factor; Treg, T-regulatory cell.

RHOA is a well-known protein which was extensively characterized in the context of cell movement, cytokinesis, and stress fiber formation. Although RHOA's involvement in cancer has been reported by many researchers so far, its critical role as a tumor driver gene has recently attracted attention through cancer genome sequencing. Several groups reported recurrent somatic *RHOA* mutations in angioimmunoblastic T-cell lymphoma (AITL), a subtype of lymphoma with follicular helper T-cell phenotype.[2,3] The mutation distribution among AITL cases constitutes a clear hotspot G17V. Rohde et al also reported recurrent mutations of *RHOA* in pediatric Burkitt lymphoma (BL), and they found that the mutation distribution is biased with several hotspots, like R5W.[4] *RHOA* somatic mutations have also been identified in solid tumors. Large-scale pan-cancer genome analysis identified recurrent hotspot E40Q mutations in head-and-neck squamous cell carcinoma (HNSCC).[5] Independent groups discovered *RHOA* mutations in diffuse-type gastric carcinoma (DGC), and, among several hotspots, Y42C/S mutations are identified recurrently across independent cohorts in these studies.[6,7]

The recurrent hotspot nature of the mutations gives rise to a simple notion that these mutations increase the biochemical RHOA activity, like typical tyrosine kinase mutations, therefore, small-molecule inhibitors targeting the RHOA mutant should be effective. However, the story is unlikely to be so simple. In the report about AITL and BL, G17V and R5Q mutations reduce the guanosine triphosphate (GTP)-binding form of RHOA, and the downstream effector activities like serum response factor transcription and the stress fiber formation significantly decreased.[2,3,8] In addition, it has been shown that the loading of GTP does not occur by G17V mutation. In the case of Y42C mutations in gastric cancer, it has also been shown that the adenosine triphosphate–bound active form has decreased, whereas another report showed selective loss of binding of RHOA to protein kinase N (PKN) effector protein.[7,9] It is inferred from these facts that, unlike typical kinase mutations, RHOA mutants are not biochemically activated and so-called "RHOA inhibitor" would not be effective.

There are 2 major questions about RHOA functions. First, why are the sites of hotspot mutations different depending on the tumor