2. Lawrence HJ, Rozenfeld S, Cruz C, et al. Frequent co-expression of the HOXA9 and MEIS1 homeobox genes in human myeloid leukemias. Leukemia. 1999;13:1993-1999.

3. Nakamura T, Largaespada DA, Shaughnessy JD, Jenkins NA, Copeland NG. Cooperative activation of *Hoxa* and *Pbx1*-related genes in murine myeloid leukaemias. Nat Genet. 1996;12:149-153.

4. Kroon E, Krosl J, Thorsteinsdottir U, Baban S, Buchberg AM, Sauvageu G. Hoxa9 transforms primary bone marrow cells through specific collaboration with Meis1a but not Pbx1b. EMBO J. 1998;17:3714-3725.

5. Hisa T, Spence SE, Rachel RA, et al. Hematopoietic, angiogenic and eye defects in *Meis1* mutant animals. EMBO J. 2004;23:450-459.

CLINICAL OBSERVATIONS

Comment on Solomon, page 978

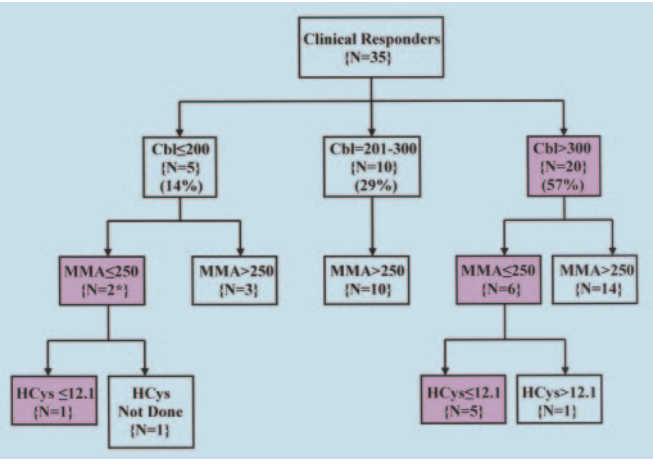
Unreliability of current assays to detect cobalamin deficiency: "nothing gold can stay"

Ralph Green UNIVERSITY OF CALIFORNIA, DAVIS

Measurement of vitamin B_{12}, homocysteine, and methylmalonic acid may not be the ultimate "gold standard" for diagnosis of cobalamin deficiency.

Measurement of the serum vitamin B_{12} level has been widely used as the standard screening test for cobalamin deficiency. It has, however, long been recognized that because of sensitivity and specificity problems, the test has poor positive and negative clinical predictive value.[1,2] In essence, a low serum vitamin B_{12} level does not always connote cobalamin deficiency, nor does a "normal" serum vitamin B_{12} level reliably indicate normalcy. Cobalamin is a necessary cofactor for 2 metabolic reactions. In cobalamin deficiency, resulting bottlenecks cause 2 substrates in those reactions, methylmalonic acid and homocysteine, to accumulate. When clinical diagnostic assays to measure these compounds in the serum became available,[3] they replaced serum B_{12} as the "gold standard" for diagnosing cobalamin deficiency. However, some concerns about the use of these assays and the interpretation of their results soon began to emerge[4] and some of the gloss began to fade.

In this issue, Solomon reports the results of a retrospective review of the records of a large group of patients in an ambulatory setting at a staff model HMO over a 10-year period. These patients were evaluated for possible cobalamin deficiency using the standard laboratory tests of serum B_{12}, methylmalonic acid, and homocysteine. The startling and disturbing findings in this study are that all assays showed considerable variability before any treatment was initiated and that the results of these assays taken singly or in combination often did not reliably predict or preclude a response to specific treatment with vitamin B_{12}. Taken at face value and given the general reliance placed on the clinical reliability of these tests for identification of cobalamin deficiency, these findings are extremely troubling. Overdiagnosis of cobalamin deficiency is one thing, carrying with it the negative consequences of possible unnecessary, but essentially innocuous, treatment as well as burdensome cost; missed diagnosis is, quite clearly, a matter of greater gravity, particularly since the risk of formidable devastation from neurologic damage that results from uncorrected cobalamin deficiency is preventable. In 37 of the patients reported by Solomon who responded to pharmacologic doses of vitamin B_{12}, pretreatment values of serum B_{12} and homocysteine were normal in approximately 1 of every 2 and methylmalonate in 1 of every 4 patients (Figure 1).



Pattern of Cbl, MMA, and HCys values in patients with clinical responses to Cbl therapy. See the complete figure in the article beginning on page 978.

Solomon's provocative findings convey an apt and timely caution and may shake up present complacencies. Many have come to accept at face value the glib messages and mantras conveyed by assay kit manufacturers and the enshrined dogmas that permeate the literature that the laboratory identification of clinically significant cobalamin deficiency is a "cake walk." Indeed, it may be time to carefully reassess a field that is perhaps in a state of confusion and disarray not dissimilar to what existed when radioassays were introduced to replace microbiologic assays for measuring B_{12}. The situation may now be aggravated since the introduction of folic acid fortification of the diet in North America and elsewhere is giving rise to the potential risk of undetected cobalamin deficiency, through "masking" by folate. Moreover, with the virtual disappearance of folate deficiency, cobalamin deficiency has become the most common modifiable cause of hyperhomocysteinemia, with its potential attendant risks of atherothrombosis.

At this stage, it would be prudent to conclude that the currently available assays for identifying or excluding cobalamin deficiency, though potentially useful, should be used with full awareness of their possible limitations, at least until unresolved issues have been settled. Serious questions have been raised about the clinical dependability of the trio of tests variously considered as gold standards for suspicion or confirmation of cobalamin deficiency with a possible devaluation in the currency of all. Nothing gold can stay.[5] ■

REFERENCES

1. Green R. Metabolite assays in cobalamin and folate deficiency. Baillieres Clin Haematol. 1995;8:533-566.

2. Carmel R, Brar S, Agrawal A, Penha PD. Failure of assay to identify low cobalamin concentrations. Clin Chem. 2000;46:2017-2018.

3. Allen RH, Stabler SP, Savage DG, Lindenbaum J. Diagnosis of cobalamin deficiency I: usefulness of serum methylmalonic acid and total homocysteine concentrations. Am J Hematol. 1990;34:90-98.

4. Green R. Screening for vitamin B12 deficiency: caveat emptor. Ann Intern Med. 1996;124:509-511.

5. Frost R. *Nothing gold can stay*. 1926.

HEMOSTASIS

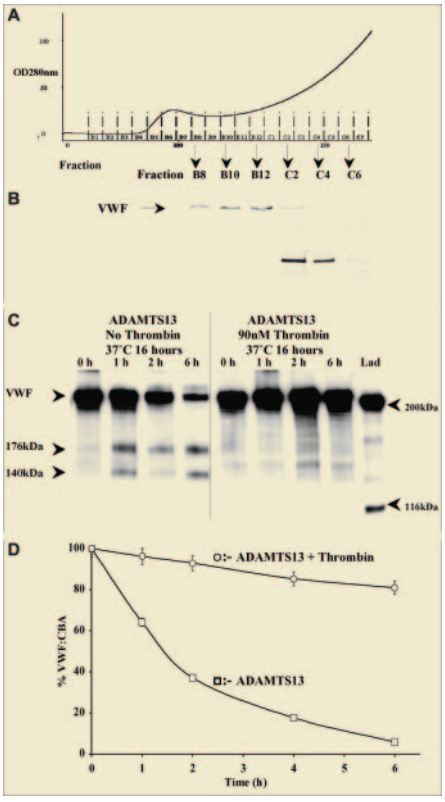
Comment on Crawley et al, page 1085

Down-regulation of ADAMTS13 activity by serine proteases

Yoshihiro Fujimura NARA MEDICAL UNIVERSITY

Left unchecked, ADAMTS13 activity may inhibit platelet adhesion at sites of vascular injury under high shear stress. Crawley and colleagues present evidence that the proteolytic cleavage of ADAMTS13 by coagulation proteinases inhibits its ability to cleave von Willebrand factor.

Platelet adhesion to sites of vascular injury under high shear stress occurs as a 2-step process. Initial platelet rolling is mediated by transient interactions between the platelet membrane glycoprotein Ib-IX-V complex and surface-immobilized von Willebrand factor (VWF), and subsequent firm adhesion occurs following the stable binding of platelet integrin αIIbβ3 or collagen receptors to surface-immobilized VWF or collagens in the vascular subendothelium.[1] In vitro, the interplay of VWF and fibrinogen during thrombus growth on a collagen surface under physiologic high shear stress has been extensively studied using a perfusion chamber and confocal laser microscopy. From these studies, it is now known that during the initial stages of thrombogenesis, platelet thrombi are entirely composed of plasma VWF, and platelet-derived fibrinogen accumulates predominantly inside the growing thrombi and acts as a core adhesive ligand. However, the thrombus surfaces exposed to flow are constantly occupied with VWF.[2] The ability of VWF to bind forming thrombi is dependent on its multimeric size, ranging from 500 to 15 000 kDa. The multimeric state of VWF is regulated by the number of N-terminal intersubunit disulfide bonds formed in the Golgi complex as well as by proteolytic cleavage by ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13) in the circulation.[3]



Proteolysis of ADAMTS13 by thrombin abolishes ADAMTS13 enzymatic activity toward purified human VWF. See the complete figure in the article beginning on page 1085.

ADAMTS13 is a Zn^{2+}-binding metalloproteinase produced in the liver, and its 1427 amino acids form a multidomain structure. Recent studies have revealed that the catalytic or substrate recognition site resides on the metalloproteinase or cysteine-rich/spacer domain. Thus, the structure/function relationship of ADAMTS13 is becoming clear but the regulation of ADAMTS13 activity is entirely unknown. Possible clues to this regulation have been provided by 2 recent reports that showed that interleukin-6 abolishes ADAMTS13 activity solely under flow conditions[4] and that a relatively high concentration of hemoglobin inhibits enzyme activity in a static assay system.[5] The physiologic role of these proteins in normal hemostasis remains undetermined.

In this issue of *Blood*, Crawley and colleagues suggest a role for proteolytic cleavage in the inactivation of ADAMTS13. The 3 serine proteases (thrombin, Xa, and plasmin) used are ubiquitously involved in normal hemostasis. Using purified recombinant ADAMTS13 fused with a myc epitope and polyhistidine tag at the C-terminus, the authors used 2 monoclonal antibodies to the ADAMTS13 metalloproteinase domain as well as an anti-myc antibody to follow the proteolytic degradation of ADAMTS13 by Western blot. The ADAMTS13 fragments generated by these serine proteases were similar in size but not identical. Interestingly, ADAMTS13 activity decreased uniformly with increased proteolysis. Perhaps most interesting, thrombin-mediated proteolysis of ADAMTS13 was entirely inhibited following preincubation with soluble thrombomodulin (TM) but not heparin, implicating thrombin exosite I as a possible site of interaction with ADAMTS13. These data lead to a model in which TM, expressed on normal endothelium, inhibits the thrombin-mediated proteolysis of ADAMTS13. On denuded endothelium, however, the absence of TM would allow thrombin to inactivate ADAMTS13, facilitating the initial platelet adhesion process. Finally, because 3-dimensional models of mural thrombus formation suggest that the outer platelet surface is VWF rich,[2] increased ADAMTS13 activity at sites distant from thrombin generation may ultimately control thrombus size. ■

REFERENCES

1. Savage B, Saldivar E, Ruggeri ZM. Initiation of platelet adhesion by attachment onto fibrinogen or translocation on von Willebrand factor. Cell. 1996;84:289-297.

2. Matsui H, Sugimoto M, Mizuno T, et al. Distinct concerted functions of von Willebrand factor and fibrinogen in mural thrombus growth under high shear flow. Blood. 2002;100:3604-3610.

3. Zheng X, Chung D, Takayama TH, et al. Structure of von Willebrand factor cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. J Biol Chem. 2001;276:41059-41063.

4. Bernard A, Ball C, Nolasco L, et al. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. Blood. 2004;104:100-106.