centers.[4] Nevertheless, Solomon and colleagues are to be applauded for developing assay techniques for protein identification that are at least available for problematic cases and for offering their help with characteristic generosity.

Needless to say, the clinical constraints are most daunting. Patients will often get sicker while waiting for a diagnosis. Therefore, on occasion, the need arises to make the best guess as to the cause of amyloid disease in a patient with 2 possible amyloid proteins. Discussion with the patient and with colleagues, as well as sharing of material in pursuit of the diagnosis, should be part of this process. Transthyretin tissue staining is often helpful, but as Solomon et al rightly note, the results of immunohistochemical staining for amyloid type can be "misleading or negative."

Finally, with respect to the innovative techniques needed, I had in mind the development of small reactive peptides specific for amyloid from different types of protein and validated for use in appropriately prepared tissue specimens.[5] The pursuit of such reagents clearly follows both the dreams and footsteps of alchemists.

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To the editor:

The quest for the normal hemoglobin concentration

Beutler and Waalen[1] undertake the Herculean task of tackling the nearly 40-year-old World Health Organization (WHO) definition of anemia. The authors mention several reasons why these old parameters cannot be taken as valid, and we fully agree with all of them. Furthermore, they describe 2 newer large epidemiologic studies, the NHANES-III (the third US National Health and Nutrition Examination Survey) and Scripps-Kaiser databases, which demonstrate very similar hemoglobin concentrations among the different segments of the US population. Both of these studies should be used as a platform to establish the lower limit of normal blood hemoglobin concentration. However, we disagree with the authors that drawing the line of normality should be done by choosing an arbitrary 95%, 97.5%, or any other percentage value out of the Gaussian distribution. We should not be afraid to raise the bar to a level where possibly 22% or more of supposedly healthy men or women in the US would be anemic. The question that needs to be asked is where we start to define what is healthy and what is not. An approach that might help us out of this eternal dilemma and lead us to define the lower limit of normal hemoglobin was presented by Zakai et al[2] in their prospective epidemiologic study, where they take into account the effect of hemoglobin concentration as an independent mortality risk predictor. We are well aware that using 133 g/L and 145 g/L (13.3 g/dL and 14.5 g/dL) for women and men 65 years or older, respectively, could create a tremendous public-health burden. However, we should face it, just as we have come to understand in the last 25 years the value of establishing a healthy low-density lipoprotein (LDL) cholesterol level, rather than the previous normal levels by our population standards. Even now, the appropriate LDL cholesterol level is being readjusted for some high-risk populations to ever-lower limits. The other lesson, therefore, that the LDL cholesterol example should give us when we search for a new lower normal limit of hemoglobin concentration is to stratify the population by meaningful underlying comorbidities, rather than simply aiming for a universal value according to age, sex, and race.

Peter A. Boehringer and Ivy L. Darden

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Response:

Establishing normal limits for blood hemoglobin concentration

We agree with Boehringer and Darden that establishing normal limits is fraught with difficulty and that cutoff values are, of necessity, always somewhat arbitrary. If physicians choose to employ the 95% cutoff they are merely allocating a probability to the patient being a part of the normal distribution of hemoglobin values. As Boehringer and Darden state, it is entirely possible that it is better for hemoglobin levels to be higher. In the game of basketball, being of average height is a disadvantage even though it is clearly normal; perhaps average hemoglobin levels are not optimal in the game of life, either.

We also agree that it is important to try to arrive at some type of functional definition of normal. In the case of their example of LDL cholesterol and heart disease, the data required to establish functional cutoffs have taken decades to acquire and continue to be modified with additional data. In the case of anemia, we are only beginning to accumulate the necessary data.

Although the study of Zakai et al,[1] which shows increased mortality among subjects 65 and older with hemoglobin levels even in the "normal range," is intriguing, in other studies[2,3] confounding comorbid conditions accounted for most or all of the difference between anemic and nonanemic groups. Furthermore, even if 22% of the population were at risk for early demise because their hemoglobin levels were found to be low, it would remain to be shown that therapy directed at the hemoglobin level would have any effect on survival. In addition, given that high hemoglobin levels also have deleterious effects on function, it is likely that the normal distribution of hemoglobin represents a balance struck by evolutionary forces. Future studies, some of which we hope to be able to perform, may clarify some of these issues. In the meantime, we believe that physicians will have to content themselves with reference standards based on population norms, as we do with most other clinical laboratory measurements.

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To the editor:

Does liver biopsy overestimate liver iron concentration?

Cappellini and colleagues[1] claim that the use of a superconducting quantum interference device (SQUID) biosusceptometer underestimates liver iron concentration (LIC) in their phase 3 study of deferasirox (DFX). LIC was measured either in deparaffinized liver samples excised by various biopsy techniques (Menghini with saline flushing, cutting needles) or in an anterior position above the right liver lobe by biomagnetic liver susceptometry (BLS) using low T_C-SQUID biosusceptometers. In vivo wet-weight LICs measured by BLS were converted by a factor of 3.33 into dry-weight values. This approximate conversion factor[2] has been uncritically adopted throughout the literature, even by ourselves, although there were strong data available supporting a higher factor for the ratio of wet to dry weight[3,4] and a significant difference between LIC from fresh tissue and from deparaffinized samples.[5,6] Thus, the conversion factor between LIC as determined by BLS and from deparaffinized liver samples would have been at least 5.5 ± 1.0 (calculated factor $\pm$ uncertainty).[7] Related to activities around this phase 3 study program of DFX, the authors have developed more direct knowledge of ratios of wet to dry weight by various biopsy processing techniques (eg, a conversion factor of 5.8 ± 0.6 for deparaffinized liver samples) and their "paramount importance" for comparison of LICs.[8]

Consequently, the authors should have corrected their LICs measured by SQUID-BLS in order to analyze their data more accurately in this important publication on a novel oral chelator. We think that it is allowed to correct an initially false study assumption in a scientific paper. Measurements by BLS would have the highest impact especially in the LIC group of 7 mg Fe/g dry weight or less, although the final outcome may not change significantly. Moreover, we would hope to avoid giving potential readers the wrong impression that BLS underestimates LIC per se. One could, in fact, claim the opposite, as in our title, particularly in the case of deparaffinized samples.

As part of this discussion, it should be emphasized that the different conversion factors also have a strong impact on the LIC safety thresholds in iron-overloaded patients with thalassemia. These recommended thresholds were based in part on LICs measured by BLS with an approximate conversion factor of 3.33. For example, the threshold for increased risk of cardiac failure of LICs equaling 80 μmol/g wet weight (about 15 mg/g dry weight)[1(p 3455)] would convert to 26 ± 5 mg/g dry weight using the conversion factor of 5.8 for deparaffinized liver biopsies. Thus, dry-weight LICs could be very different depending on the selected biopsy techniques and processing methods.[7]

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