

## References

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## To the editor:

### Misclassification of amyloidosis is unwarranted

The article by Comenzo et al<sup>1</sup> addresses a seminal issue; namely, the therapeutic necessity of establishing the identity of the protein contained in amyloid deposits. They found, as did Lachmann et al,<sup>2</sup> that certain patients presumed to have immunoglobulin light-chain (AL) amyloidosis based on clinical and laboratory criteria (including the presence of a monoclonal gammopathy) in fact did not, but rather had a different form that resulted from a mutation in a gene encoding amyloidogenic precursor proteins (eg, transthyretin [TTR]). Given that individuals diagnosed with AL amyloidosis could be subjected to high-dose melphalan and stem cell transplantation<sup>3</sup>—an intensive and potentially lethal therapy—it is essential that the nature of the amyloid-forming protein be unequivocally established.

The aforementioned reports emphasize the fallacy of relying on indirect evidence to ascertain if an individual has AL amyloidosis or some other kind of amyloid disease. Notably, the presence of a monoclonal protein, an altered serum  $\kappa/\lambda$  ratio, or a mutated gene may represent confounding factors that do not necessarily reveal the true composition of the congophilic deposit, thus making a precise diagnosis impossible. Further, although the type of amyloid may be inferred immunohistochemically, it is well known that

results gained using this technique can be misleading or negative.<sup>4</sup> Thus, to obtain a true diagnosis, the amyloid contained in biopsy-derived specimens must be extracted and analyzed chemically.<sup>5</sup> Indeed, contrary to the statement “innovative and improved techniques are needed for typing amyloid from tissue biopsies,”<sup>1(p3491)</sup> we previously had reported that such procedures have been developed and are routinely used in our laboratory.<sup>6</sup> More recently, we also have used tandem mass spectrometry (MS/MS) to gain this information from formalin-fixed, paraffin-embedded sections, as well as subcutaneous fat aspirates.<sup>7</sup> We can perform such studies upon request.

The ability to identify precisely the nature of the amyloid present in pathologic deposits may avoid diagnostic errors, especially in the case of AL amyloidosis, where patients could be subjected to inappropriate and costly therapy that can have untoward and possibly legal consequences.

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## Response:

### Constraints on the pursuit of diagnostic confidence

We agree with the critique offered by Solomon and colleagues but hasten to add that there are a series of constraints on the effort to obtain a “true diagnosis” in patients with amyloidosis and 2 possible fibril-precursor proteins. The proximal constraints are specimen related, assay related, and clinical. The overarching constraint is that our knowledge of the fundamental processes of amyloid fibril formation and amyloid disease remains so limited.<sup>1,2</sup>

Specimen-related constraints involve the accessibility of biopsy material containing amyloid and the amount of amyloid such biopsies contain. Surrogate biopsies from abdominal, rectal, or

gingival tissue are negative a significant fraction of the time, and involved organ biopsies in patients with isolated cardiac or peripheral nervous system amyloidosis frequently contain minimal amounts of amyloid. The possibility that oligomeric intermediates on the path to fibril formation may be toxic, particularly to the heart and nerves, is consistent with these findings.<sup>3</sup>

Admittedly, sophisticated techniques for amyloid protein extraction and identification have been described by Solomon and colleagues, but the constraint on these assay techniques is that they have not been validated in large numbers of samples in multiple

centers.<sup>4</sup> 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.<sup>5</sup> 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<sup>1</sup> 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<sup>2</sup> 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.