Discrepant Serum Total Protein Results for Serum from Some Myeloma Patients, as Analyzed in the SMAC

To the Editor:

We read with interest recent publications (1, 2) regarding the precipitation of protein from multiple myeloma serum causing spurious results. We noted low results (in some cases 20 g/L) for total protein in serum from three myeloma patients, all IgM type, as analyzed in the SMAC continuous-flow analyzer (Technicon Instruments Inc., Tarrytown, NY) when compared with a manual biuret method (3) involving reagents as used in the SMAC. These sera caused the formation of a precipitate when mixed with pre-dilution fluid (de-ionized water + new wetting agent), probably owing to the low ionic strength of this fluid being unable to support the paraprotein in solution. This explanation was confirmed when the precipitates were isolated, washed, redissolved in 150 mmol/L NaCl, and shown by electrophoresis on cellulose acetate (4) to consist almost entirely of paraprotein.

Initially these discrepant values were difficult to explain, because the precipitate redissolved in biuret and protein blank solutions and so turbidity was not present when the peaks reached the flow-cells. Viscosity was ruled out, because results for most other assays done in the SMAC were not proportionately decreased when compared with alternative methods. Occasionally the precipitate was heavy enough to block the pre-dilution system, but this was rare. We conclude that the error is due to a preferential sampling of fluid rather than precipitation from the SMAC pre-dilution system. Analyzing these specimens diluted (one part specimen to two parts 150 mmol/L NaCl) gave results comparable with those by the manual protein method. We tested a series of 50 sera containing paraproteins of different types by mixing 0.1 mL of serum with 0.5 mL of pre-dilution fluid and looked for turbidity in the resulting solution. Precipitation was peculiar to IgM type paraproteins, although not all reacted in this way.

We investigated the effect of this precipitate on results for other analytes, in particular those without dialysis—total bilirubin, lactate dehydrogenase (EC 1.1.1.27), glutamyltransferase (EC 2.3.2.2), alanine aminotransferase (EC 2.6.1.2), aspartate aminotransferase (EC 2.6.1.1), sodium, potassium, and albumin—and in all cases the precipitate redissolved immediately in the diluent reagents, causing no interference attributable to turbidity. However, some results for lactate dehydrogenase and cholesterol appeared abnormally low, possibly indicating some form of interference; we are still investigating this effect.

Although the proportion affected is small, if specimens containing paraproteins are to be assayed in the SMAC, we recommend that serum from each newly diagnosed paraprotein patient be screened for precipitation with pre-dilution fluid so that follow-up total proteins may be analyzed diluted or by manual techniques. There is also a chance that some IgM type paraproteins may be missed on initial analysis, owing to the SMAC total-protein method giving an apparently normal result.

References

Miles O. Sykes
Stephen P. Harrison
Dept. of Biochem.
Bradford Royal Infirmary
Duckworth Lane
Bradford, BD9 6RJ, U.K.

Must CSF Oligoclonal Bands Be Unique to Cerebrospinal Fluid and Absent from Serum to Support the Diagnosis of Multiple Sclerosis?

To the Editor:

Staley et al., in 1986 (1), reported that 11 of 12 patients with multiple sclerosis (MS) who had oligoclonal bands in their cerebrospinal fluid (CSF) had identical oligoclonal bands present in their serum, as determined by agarose gel isoelectric focusing (IEF) followed by silver staining. These authors then stated that use of the criterion that oligoclonal bands be present only in CSF and not in serum may lead to false-negative conclusions regarding clinical significance. They cited Laurensi (2) as stating that, with polyclonal gel IEF, 74% of MS patients with CSF oligoclonal bands also had bands in the corresponding patterns for serum. This is in fact true but misleading, because Staley et al. omit to mention that in all these cases Laurensi also found bands unique to CSF as well as the bands common to both CSF and serum. This result is quite different from their own, and it gives no support to their contention regarding interpretation of non-unique CSF oligoclonal bands. Recently an abstract has appeared (3) that also suggests that a minority of MS patients may have identical band patterns in both CSF and serum. However, there is insufficient information in the abstract for one to evaluate these findings fully.

Oligoclonal bands are present in the CSF of over 90% of MS patients (4) and, although not specific for this disease, their presence can be very helpful in making the diagnosis. The criteria of the Poser committee (5) are currently the best accepted for a definitive diagnosis of MS for research purposes. From these criteria, if oligoclonal bands are to be used to support a clinical diagnosis of MS, then at least some of the bands must be present in the CSF only. In clinical practice, however, a few patients will be diagnosed by the neurologist as having MS, even though they do not meet all the criteria (5).

Using agarose gel IEF followed by immunofixation and silver staining for both CSF and serum from patients with possible MS, we have found 34 patients with multiple IgG bands in their CSF. These patients could be divided into three groups on the basis of their IEF findings. Group I (eight patients) had no bands in their serum. Group II (17 patients) had some bands common to both serum and CSF but also unique CSF bands. Group III (nine patients) had only bands of identical mobility in CSF and serum. On follow-up of the patients' medical records, nine had a final diagnosis of definite MS, four were probable MS, and 21 not MS. All of the patients with a clinical diagnosis of definite or probable MS were in groups I or II; that is, they had oligoclonal bands unique to the CSF. Patients with diagnoses other than MS could have had any of the three patterns.

For oligoclonal bands to be diagnostically significant in MS, current practice requires that bands unique to the CSF be present. The two recent reports