than in the adult non-SARS patients (all $P$ values $<0.005$; Table 1).

When analyzing the ELISA data, we found that the serum SAA concentrations were greatly increased in both the SARS and non-SARS patient groups. The mean serum SAA concentrations of the SARS and non-SARS patient groups were 40- and 85-fold higher than the upper limit of the reference interval ($<10$ mg/L), respectively. Consistent with the SELDI data, the serum SAA concentrations were significantly lower in the SARS patient group ($P < 0.005$; Table 1). The results from both the SELDI ProteinChip assays and ELISA indicated that serum SAA by itself was not useful in differentiating the SARS patients from the non-SARS patients who were suspected cases during the SARS outbreak period. Because serum SAA was increased in the SARS patients, however, we could not exclude the possibility that it could be used in combination with other serum markers to develop a classification model for SARS diagnosis.

Serum SAA is an acute-phase reactant (7) that has been shown to increase in various types of viral and bacterial infections (8). Regardless of the types of infection, serum SAA concentrations can increase up to 2000 mg/L. The degree of increase may reflect only the severity of the illness and does not indicate the cause. In the SARS patient group, we found that the SAA peaks and the serum concentration correlated significantly with the serum C-reactive protein concentration (Table 1), as in other infectious diseases (8). This suggests that the increases in serum SAA were caused mainly by the inflammatory response to SARS infection.

In conclusion, data from both the SELDI ProteinChip profiling study and an ELISA study do not support the contention that increased serum SAA is indicative for SARS. In contrast, our results strongly suggest that the serum SAA concentration is not useful in differentiating the SARS patients from the non-SARS patients who are suspected cases during the SARS outbreak period.

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References

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Uncertainty Intervals Based on Deleting Data Are Not Useful

To the Editor:

Dimech et al. (1) point out that uncertainty intervals are required for assays by many regulatory agencies, and the authors provide a method for calculating uncertainty intervals for serologic assays. Krouwer (2) has critiqued the use of uncertainty intervals based on GUM (Guide to the Expression of Uncertainty in Measurement) for commercial diagnostic assays. The method proposed by Dimech et al. (1) is based on the EURACHEM/CITAC guide (3), which is itself based on GUM.

One of the first steps in the Dimech method is to delete outliers. It is hard to imagine why an uncertainty interval should not include all data and what such an uncertainty interval means when it is not based on all of the data. Maybe the authors assume that outliers are caused by blunders and that they wish to limit their uncertainty interval to the analytical process. Perhaps, but one cannot know that this assumption is true. Moreover, in the EURACHEM/CITAC guide, there is a specific example in which an outlier is deleted because of an analytical root-cause error (an instrumentation problem).

By use of nonparametric methods based on empirical distributions, uncertainty intervals can be estimated without deleting data (4). If these intervals are too large, one should try to discover root causes, not delete data.

References
One of the authors of the article cited in the above letter responds:

To the Editor:

Krouwer, in his letter, has raised an important point about whether outliers should be removed from a dataset before estimating uncertainty of measurement. At the time of original submission of the example provided, no data were removed. On review, it was apparent that the standard deviations of the results of laboratory 12 did not show Gaussian distribution and that the analysis used would be inappropriate for such a set. In response to a reviewer’s comments, the data were analyzed further. A single result submitted by laboratory 12 was, indeed, grossly aberrant compared with the other results from that laboratory, and this result affected the summary statistics. The aberration was caused by an entry error that resulted from the laboratory staff entering the value of the negative control instead of the cutoff for plasma homocysteine (tHcy) concentration.

Outlying results should be thoroughly investigated to determine whether they are truly a reflection of the test system output or whether they were caused by a mistake. If they were caused by a mistake, it is appropriate that these be removed from the dataset before analysis.

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Folic Acid, Vitamin B12, MTHFR Genotypes, and Plasma Homocysteine

To the Editor:

We read with interest the article by Pastore et al. (1), who demonstrated that the effect of folate and vitamin B12 treatment in reducing total plasma homocysteine (tHcy) concentration is dependent on methylenetetrahydrofolate reductase (MTHFR) genotype in patients with end-stage renal failure. Their findings may partially explain the difficulty in demonstrating a benefit of vitamin supplementation in reducing cardiovascular end-point, as treatment may have to be tailored to subgroups of patients such as those with the MTHFR T allele (2, 3). Although tHcy concentrations are much lower in individuals with normal renal function, they still predict subsequent cardiovascular events (4).

We investigated the value of MTHFR genotype for potentially differentiating vitamin therapy groups in patients presenting with peripheral vascular disease. A total of 217 patients, presenting with abdominal aortic aneurysm (n = 80), carotid artery disease (n = 72), or symptomatic lower limb ischemia (n = 65), were screened for atherosclerotic risk factors, as described previously (5). MTHFR genotype was assessed by the homogeneous MassEXTEND (hME; Sequenom) assay, which uses primer amplification in combination with matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry to identify extension products (6). tHcy values in our patients were low (range, 1–40 μmol/L) and not significantly affected by MTHFR genotype (Table 1). Interestingly, patients with the TT C677T MTHFR genotype had lower serum HDL concentrations. The primary determinants of tHcy were folate and vitamin B12 status. The mean (SD) concentrations of tHcy by folate and vitamin B12 tertiles were 15.34 (5.60), 12.10 (4.46), 11.17 (4.09) (P = 0.003) and 14.99 (5.98), 12.39 (3.27), and 11.24 (4.90) (P = 0.02), respectively. Our findings are in keeping with those from a recent study (7) and suggest that assessment of vitamin B12 and folate status may best predict those patients with peripheral vascular disease requiring intervention to reduce tHcy concentrations.

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