

duced by interferon- γ in human macrophages (7).

The model system of activated PBMCs has been well established in clinical immunology for several decades and allows standardization of T-cell activation and T-cell/macrophage interaction. It is certainly more informative than the myelomonocytic tumor cell line THP-1 and more relevant for in vivo testing. Our approach has already been used for testing antiinflammatory drugs for ~5 years with very reproducible results, even between assays of blood from different donors (3–6). Dose-dependent effects were detected for compounds such as resveratrol (5), drugs such as atorvastatin (4), and beverages with well-described anti-atherogenic potential, such as green and black tea (3) and beer (6).

A model system that would measure products of stimulated macrophages as a read-out is clearly relevant to atherosclerosis, in which inflammation is pivotal. However, the PBMC model draws more attention to the role of T-cell/macrophage interplay, which is highly relevant in the pathogenesis of atherosclerosis, and acknowledges the existing data on the role of the proinflammatory cytokine interferon- γ in the process of atherogenesis and other inflammatory conditions. By inducing the depletion of antioxidant systems and causing oxidative stress, interferon- γ is probably the most important trigger for the production of reactive oxygen species in macrophages (8), considered to be of utmost relevance in atherogenesis. Our in vitro PBMC system is fully in accordance with the notion that macrophage products should be measured, and it is valid both in vitro and in vivo. Both neopterin production and tryptophan degradation are induced by interferon- γ in macrophages; thus, both reflect macrophage activity. In coronary heart disease, increases in neopterin concentrations not only correlate with tryptophan degradation (9) and with the activity of the disease (9), they also predict coronary events more sensitively than do methods such as the more widely used C-reactive protein measure-

ments (10,11). By contrast, studies on the involvement of cytokines such as IL-1, IL-6, and TNF- α in atherogenesis stem almost exclusively from in vitro experiments.

In summary, the combined study of effects on T cells and macrophages from healthy donors appears superior to using only the myelomonocytic THP-1 cell line. The alternative approach with PBMC preparations provides insight into signaling cascades, especially those initiated by T cells. The monitoring of biochemical effects such as neopterin formation and tryptophan degradation reveals more stable results in quantitative terms than does monitoring of cytokine production. Moreover, this strategy monitors the net effect of various pro- and antiinflammatory cascades initiated during stimulated immune response in vitro and in vivo and provides data on the influence of tested compounds on the whole cascade of events. Finally, both read-out systems seem particularly suited to testing for antiinflammatory effects of compounds because enhanced production of neopterin and accelerated degradation of tryptophan are closely related to the pathogenesis of various diseases in which inflammatory processes are involved.

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Serum Amyloid A Is Not Useful in the Diagnosis of Severe Acute Respiratory Syndrome

To the Editor:

In our present study, we aimed to investigate whether the serum concentration of serum amyloid A (SAA), as measured by the surface-enhanced laser desorption/ionization (SELDI) ProteinChip technology or by ELISA, is useful in differentiat-

ing the patients with severe acute respiratory syndrome (SARS) from the non-SARS patients who were suspected cases during the SARS outbreak period.

In a recent report from Kang et al. (1), the mean intensity of the protein peak at m/z 11514, identified by SELDI ProteinChip technology, of the SARS patient groups was 8 times greater than the intensity of the corresponding peak in the control patient group. This SELDI peak was observed previously in another proteomic study, under similar experimental conditions, and was identified as SAA (2). In a recent study by Yip et al. (3), the intensity of a SELDI peak at m/z 11695, which was identified as SAA, was significantly higher in the SARS patient group than in the control group. A similar increased peak was also found in pediatric patients with SARS (4). All of these studies suggested that SAA is useful in the diagnosis of SARS.

In these studies, the control cases were either healthy persons or patients with viral infections from other clinics. Unfortunately, the degree of similarity of the symptoms between SARS and control group participants and the time point of blood collection had not been considered in these

studies. From the perspective of infectious disease diagnosis, we are not trying to differentiate healthy persons from infected patients; rather, we are trying to identify the disease causing the symptoms in patients presenting with similar symptoms (5). Bearing in mind the above issues, we recently attempted to profile and compare the serum proteomes of 39 adult patients in the early stages of SARS infection and 39 adult non-SARS patients who were suspected cases during the SARS outbreak period (6). We found specific SELDI peaks in the sera of the adult SARS patients; however, the peaks corresponding to SAA were not identified as SARS-specific features. This led us to question whether SAA is a useful biomarker for the diagnosis of SARS.

In our study, the non-SARS patients were those who had symptoms similar to SARS patients at admission. They were admitted to the same hospital as the SARS patients and were later shown to be negative for SARS coronavirus (CoV) infection by an anti-SARS-CoV antibody serology test at least 6 weeks after the onset of symptoms. The SAA concentrations in the serum samples (37 non-SARS cases and 29 SARS cases)

that remained from the SELDI study were determined by an anti-SAA ELISA according to the manufacturer's instructions (BioSource International).

Using WCX2 ProteinChip arrays (also called CM10) and pH 4 binding buffer, Tolson et al. (2) showed that 3 peaks, at m/z 11682, m/z 11526, and m/z 11439, were full-length SAA and des-arginine and des-arginine/des-serine variants at the NH₂ terminus, respectively. In our SELDI dataset, obtained with the same ProteinChip type and binding conditions, there were 3 SELDI peaks with similar m/z values (mean values): m/z 11681, m/z 11526, and m/z 11439. Spearman rank correlation analysis showed that the normalized intensities of these 3 peaks correlated highly with the serum concentration values obtained by ELISA (all correlation coefficients >0.9; all P values <0.0005; Table 1). Such high correlations strongly suggested that the SELDI peaks at m/z 11681, m/z 11526, and m/z 11439 were full-length SAA and the des-arginine and des-arginine/des-serine variants at the NH₂ terminus. In contrast to the previous SELDI studies, the normalized intensities of these 3 peaks were significantly lower in the adult SARS patients, instead of higher,

Table 1. Summary of the SELDI peaks corresponding to SAA and serum SAA concentrations in adult SARS patients and adult non-SARS patients who were suspected cases during the SARS outbreak period.

A. Results of SELDI analysis

Mean (minimum–maximum) m/z of the SELDI peak	Theoretical average mass	Protein identity ^a	Mean (SD) normalized intensity of proteomic feature relative to total sum of proteomic features, ^b %		P^c	Correlation (r^d) with serum concentration of:	
			Non-SARS	SARS		SAA	CRP ^e
11 439 (11 431–11 448)	11 439	SAA-1 (-RS)	0.25 (0.21)	0.10 (0.10)	0.004	0.912 (<0.0005)	0.444 (0.006)
11 526 (11 514–11 541)	11 526	SAA-1 (-R)	0.78 (0.62)	0.36 (0.43)	0.003	0.923 (<0.0005)	0.345 (0.029)
11 681 (11 657–11 689)	11 862	SAA-1	1.38 (1.19)	0.50 (0.58)	0.001	0.930 (<0.0005)	0.402 (0.013)

B. Serum SAA concentrations

	Non-SARS (n = 37)	SARS (n = 29)	P^c	Correlation (r^d) with serum CRP concentration
Mean (SD) serum SAA concentration measured by ELISA, mg/L	853 (769)	395 (428)	0.003	0.499 (0.011)

^a -RS, des-arginine/des-serine variant; -R, des-arginine variant.

^b Sample size for both non-SARS and SARS patients, n = 39.

^c Mann-Whitney test.

^d Spearman rank correlation test (P in parentheses).

^e CRP, C-reactive protein.

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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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.

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