Reliable Measurement of Circulating Immune Complex Depends on Stable, Accurate Reference Material

To the Editor:

Accurate quantification of circulating immune complexes (CIC) in serum is essential for the diagnosis, estimation of prognosis, and monitoring of antigen–antibody–complement-mediating diseases (1). At least 40 different assays for quantifying CIC have been reported in the past 20 years, but no single one can be used exclusively because results generally correlate poorly with the disease state (2). Attempting to standardize these assays of CIC, an expert panel under the auspices of the IUUIS/WHO investigated the suitability of two international reference preparations (IRP), based on either heat-aggregated human IgG (hA-IgG) or solubilized tетanus toxoid;anti-tetanus (Tea’T) complexes (3). They suggested that Tea’T be used as the IRP for standardization, but some manufacturers (e.g., Sigma Diagnostics, Cytotech, and Immunomedics) continue to use the IRP hA-IgG.

At Hartford Hospital, we have complied with the recommendations of the IUUIS/WHO by offering two different assays to assist in the diagnosis and monitoring of immune complex-related diseases: the Raji cell RIA (performed by Nichols Laboratory, San Juan Capistrano, CA 92675), and the CIC-C1q solid-phase EIA (performed in-house with a kit from Cytotech, San Diego, CA 92121). Evaluation of the CIC-C1q EIA and our six months of experience with it have been satisfactory, with results that supported the patients’ clinical presentations and substantial agreement with Raji cell RIA results, i.e., 65% agreement in results for 51 patients. This agreement increased to 74.5% when we used the Cytotech confirmatory procedure (4) on samples with above-normal results by the CIC-C1q EIA, but normal by Raji cell assay (n = 5), finding all such increases to be attributable to nonspecific factors (5). Confirmatory procedures do not exist for the Raji cell assay, so we cannot investigate nonspecific increases when results are normal by CIC-C1q EIA but above-normal by the Raji cell assay (n = 12).

A recent discrepancy in the CIC-C1q EIA values for our two in-house control materials ("RG," 2.60 mgEq/L, and "32," 10.70 mgEq/L) led us to re-evaluate the assay. The CVs for within-run and day-to-day runs—15.9% and 37.0% for RG, and 7.2% and 21.5% for the 32 controls—were within the manufacturer’s specifications and are representative of the present state of the art in CIC assays. However, examination of the CVs for inter-assay lots gave 194.5% and 39.0% for the RG and 32 controls, respectively. As Figure 1 shows, the curves for concentration vs absorbance for the different standards in each lot depart from linearity for lots A, E, and F, although the regression analyses still met the manufacturer’s stipulation for acceptable slope and intercept. This nonlinearity of the standard curves caused negative or positive biases in CIC-C1q EIA results for test samples.

This discrepancy was attributable to incorrect value assignment to the standards, because use of standards from lot B in the assays of lots E or F gave us acceptable linear curves. Evidently, accuracy and an acceptable reference standard material are still major problems in CIC assays. The instability of the materials in the longitudinal study by Nydegger and Svahag (3) had also been previously noted by other investigators (6). We therefore urge the IUUIS/WHO to re-evaluate the problem of international Reference Material for CIC assays. All forms of reasonable materials should be included: heat-treated, sodium sulfate-precipitated, alkali-treated, or glutaraldehyde-polymerized human IgG, and the solubilized Tea”Te complexes. The effects of lyophilization and long-term storage conditions should also be thoroughly addressed.

References

A. O. Okorodudu2
R. Gillmor
M. Onoroski
L. Parker3
R. E. Moore

Dept. of Pathol., and 3 Rheumatol. Div.
Hartford Hosp.
Hartford, CT 06115

2 Present address: AHS, Univ. of Texas Health Science Center at San Antonio, San Antonio, TX 78284.
5 Address correspondence to this author.

The "Fecatwin Sensitive" Test for Fecal Occult Blood Is Not Intended for Use without Testing Positives with the FECA-EIA Assay

To the Editor:

Recently a latex-agglutination test for immunological detection of fecal occult blood was described in this journal (1). We believe that the comparison of this test with three guaic tests as carried out by the investigators does not give a true picture of the performance of the various tests. Furthermore, the article contains a few minor errors, deserving comment because they could mislead the readers.
These authors compared their "Hemolex" test (Orion Diagnostica, Espoo, Finland) with two relatively insensitive guaiac tests (Hemocult, Smith-Kline Diagnostica, Sunnyvale, CA) and "Hemofec" (Boehringer Mannheim F.R.G.) and one very sensitive test ("Fecatwin Sensitive"; Labsystems, Helsinki, Finland). In the text and in Table 3 the authors, when referring to Fecatwin Sensitive use the name "Fecatwin," another test from Labsystems with a sensitivity similar to that of the Hemocult and Hemofec tests (2). This may cause confusion.

Since the introduction of the immunological test for hemoglobin, Fecatwin Sensitive has not been recommended by us for use without testing positives with the FECA-EIA assay (Labsystems). This quantitative enzymimmunoassay is carried out in duplicate by removing from the Fecatwin Sensitive plastic case two small discs, which are then impregnated with fecal fluid. This test, specific for human hemoglobin, is essential for excluding nonspecific positive results due to dietary and other causes, which occur with rather high frequency because of the high sensitivity of Fecatwin Sensitive (2). The FECA-EIA has been available in Europe for more than five years (3, 4), and it is therefore somewhat surprising that the authors have not compared their new immunological test with this earlier immunological method. If only guaiac tests are used for comparison, the Fecatwin test should have been used instead of the Fecatwin Sensitive, because its sensitivity is similar to that of the two other tests.

There is also another problem with the comparison of the tests as performed by Vännänen and Tenhunen. Only samples primarily positive with "Hemostest" (Ames Division of Miles Labs., Elkhart, IN) were compared, indicating that subjects not following the appropriate diet also were included. This should have been stressed in the Discussion. According to our experience, even patients allegedly on an appropriate diet may consume bananas or other food products (2), which they get from visitors or buy in the cafeteria, interfering with the guaiac tests.

It is stated in the Discussion that Fecatwin Sensitive will detect 1–4 mL of blood per 100 g. The correct figure is 0.4–0.9 mL/100 g (2). In our hands the detection limit for Fecatwin is in the same range as for Hemocult and Hemofec: about 0.7 to 2 mL/100 g (2). On page 1765 the authors state that seven specimens were positive with Hemocult and Fecatwin (should be Fecatwin Sensitive), whereas in Table 3 there are seven specimens positive with Hemolex and Fecatwin Sensitive. These positives are of particular interest, because in six of these cases there was no bleeding from the lower gastrointestinal tract. The authors do not tell us anything about possible pathological findings in the upper gastrointestinal tract in these subjects. In the next paragraph they show that Fecatwin Sensitive detects more subjects with upper gastrointestinal bleeding than does Hemolex, and therefore these positives may belong to this particular group of patients.

For the above reasons we think that it was inappropriate to compare Fecatwin Sensitive with Hemolex without including the FECA-EIA test, because the strategy of using the combination of Fecatwin Sensitive and FECA-EIA seems to perform well in field studies (4).

References

H. Adlercreutz
K. Liewendahl
Department of Clinical Chemistry
University of Helsinki
Meilahti Hospital
SF-00290 Helsinki
Finland

Fructosamine and IgA: No Correlation in Non-Diabetics

To the Editor:

In describing the effects of various serum proteins on fructosamine activity, Rodriguez-Segade et al. (1) state that only IgA concentration is related to fructosamine activity, and they ascribe this to either IgA being highly reactive with respect to nitroblue tetrazolium or the IgA molecule being highly glycated. Although the data produced by Lemon and Forrest (2) do show that, of the serum proteins, the gamma-globulins are the most readily glycated in vitro, Zoppi et al. (3) have demonstrated that, in vivo, IgM shows the highest proportion of glycated molecules, which they suggest accounts for the observations of Lemon and Forrest. They (3) found IgA to be slightly less glycated than albumin. It would seem unlikely that IgA is disproportionately reactive towards nitroblue tetrazolium, because Seng and Staley (4) have reported that, with respect to the proportion of plasma protein fractions (the gamma-globulins included), nitroblue tetrazolium reduction is similar to both [14C]glucose incorporation and Ponceau S staining with scanning densitometry.

We studied 51 non-diabetic patients and found no correlation between serum fructosamine and IgA (Table 1). In those patients with IgA concentrations >5.0 g/L the mean fructosamine activity was slightly higher than in those with IgA <3.0 g/L. However, our group of patients included 13 with acute liver disease, and if we excluded the results from these patients from the data, those patients with IgA >3.0 g/L had lower values for fructosamine than those with IgA <3.0 g/L. The mean values for both fructosamine and IgA in the patients with liver disease exceeded the mean values of their respective normal reference intervals, but it seems probable that this increase in fructosamine activity is produced by positive interference by bilirubin and decreased albumin synthesis, leading to reduced turnover and increased glycation.

Could it therefore be possible that the data reported by Rodriguez-Segade et al. are attributable to the inclusion in their study of a group of patients similar to our liver-disease group who had co-elevated, though not necessarily causal-related concentrations of fructosamine and IgA?

References