

anti-inflammatory activity and therapeutic utility of leukotriene receptor antagonists.

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Determination of IgG subclasses: A need for standardization

To the Editor:

Measurement of IgG subclasses is widely performed as part of the laboratory evaluation of immunologic deficiencies. In clinical laboratories, IgG subclass measurements are generally performed by automated nephelometry. There is, however, no international reference preparation, and the 2 major providers of IgG subclass kits use different calibration strategies. In the Sanguin (Amsterdam, The Netherlands) assay (PeliClass human IgG subclass kit), the IgG subclass levels in the calibrator are derived from the

World Health Organization 67/97 reference preparation (using IgG subclass values ascertained by Klein et al¹), whereas in the assay from The Binding Site (TBS; Birmingham, United Kingdom), IgG subclass levels in the calibrator are determined from the Certified Reference Material 470 (CRM 470) (using IgG subclass values ascribed to Schauer et al²). The latter is the Reference Preparation for Proteins in Human Serum, released from the College of American Pathologists and the Bureau Communautaire de Référence.

The concentration of IgG subclasses is strongly age-dependent, and most clinical laboratories rely on reference ranges made available by the manufacturer. The reference ranges provided by Sanguin were obtained by compiling several studies.³ In all of the studies, the World Health Organization 67/97 standard was used, and the majority of the studies applied radial immunodiffusion. The reference ranges provided by TBS were established with 312 healthy children by nephelometry using CRM 470 as calibrator.²

We evaluated whether Sanguin and TBS give comparable results and whether interpretation of the results is identical. IgG subclasses were determined in 94 children with increased susceptibility for respiratory tract infection. Each of the children had at least 5 episodes of upper respiratory tract infections complicated by otitis media or chronic (longer than 3 weeks' duration) draining ears and/or had at least 3 lower respiratory tract infections with radiographic evidence of pneumonia in at least 2 of these episodes. The age distribution was as follows: 8, 30, 17, 10, 20, 6, and 3 for 2, 3, 4, 5, 6-10, 11-15, and 16-23 years, respectively (50 boys, 44 girls). All assays were performed on an Immage nephelometer (Beckman-Coulter, Brea, Calif). The Wilcoxon signed rank test was used for statistical comparison. The precision was determined by analyzing 2 aliquots of a serum pool on 20 different days.⁴

For Sanguin and TBS, respectively, total precision values were 3.19% and 5.05% for IgG1, 3.84% and 3.62% for IgG2, 2.38% and 6.24% for IgG3, and 6.42% and 4.50% for IgG4; within-run precision values were 2.45% and 3.1% for IgG1, 3.32% and 2.49% for IgG2, 2.4% and 4.7% for IgG3, and 3.94% and 4.18% for IgG4; between-day precision values were 2.05% and 3.99% for IgG1, 1.93% and 2.63% for IgG2, 0 and 4.1% for IgG3, and 5.07% and 1.66% for IgG4.

The results of the comparison are presented in Fig 1. For IgG1, the respective median (95% CI) values for Sanguin and TBS were 5.84 g/L (5.45 to 6.69) and 6.045 g/L (5.43 to 6.65) ($P = .1037$). For IgG2, the respective values were 1.1 g/L (0.96 to 1.27) and 1.1 g/L (0.95 to 1.33) ($P = .5943$). For IgG3, significantly higher values ($P < .0001$) were found with TBS (median value, 0.508 g/L [95% CI: 0.425 to 0.554]) than with Sanguin (median value, 0.315 g/L [95% CI: 0.266 to 0.36]). For IgG4, significantly lower values ($P < .0001$) were found with TBS (median value, 0.143 g/L [95% CI: 0.104 to 0.193]) than with Sanguin (median value, 0.2 g/L [95% CI: 0.16 to 0.25]). For IgG4, only 71 samples were included in the statistical analysis because many samples had IgG4 values

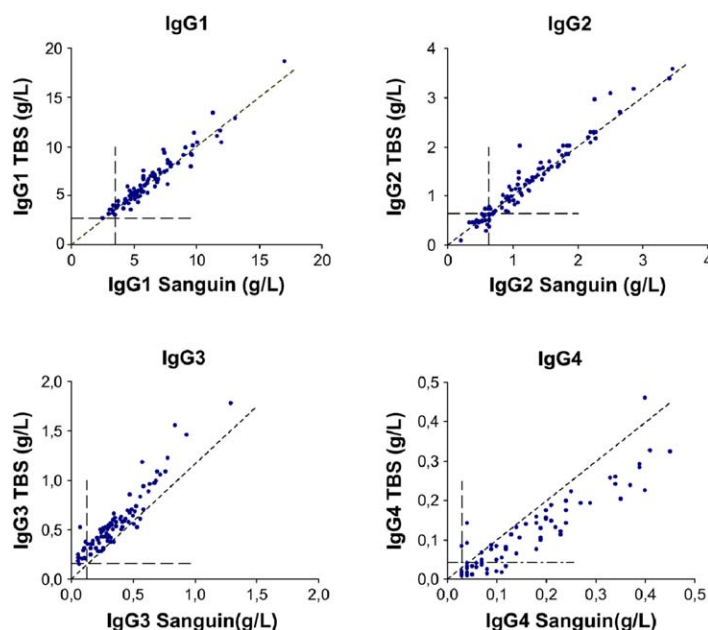


FIG 1. Ninety-four samples from children with increased susceptibility to respiratory infections were analyzed for IgG subclasses through use of reagents from Sanguin and from TBS. The line of identity is indicated, as is the assay-specific lower reference value for the age group 3 to 4 years old with respect to IgG1, IgG2, and IgG3 and for the age group 9 to 12 years old with respect to IgG4.

below the lower detection limit with Sanguin. Table EI in the Journal's Online Repository (at www.mosby.com/jaci) shows the Pearson correlation coefficient, the Altman-Bland bias, and the Deming intercept and slope. A good correlation between the 2 assays was found for all subclasses except for a proportional bias for IgG4 and a proportional bias and constant bias for IgG3. For IgG2, a small proportional bias and constant bias were observed.

We next investigated whether the calibrator affects IgG3 and IgG4 determinations. For IgG3, results obtained with TBS (median, 0.521 g/L [50% CI: 0.358 to 0.716]; $n = 14$) and with the standard from TBS with reagents from Sanguin (median, 0.578 g/L [50% CI: 0.399 to 0.756]; $n = 14$) were higher ($P = .0001$) than results obtained with Sanguin (median, 0.356 g/L [50% CI: 0.241 to 0.463]; $n = 14$). For IgG4, results obtained with TBS (median, 0.247 g/L [50% CI: 0.154 to 0.383]; $n = 18$) and with the standard from TBS with reagents from Sanguin (median, 0.295 g/L [50% CI: 0.192 to 0.383]; $n = 18$) were lower ($P < .001$) than results obtained with Sanguin (median, 0.344 g/L [50% CI: 0.215 to 0.448]). This suggested that the calibrator, but not the reagent, influences the results for IgG3 and IgG4.

Finally, we determined the number of samples with values below the lower age-dependent, assay-specific reference range. The number of samples with such values for IgG1 was 8 for Sanguin and 1 for TBS; the number of samples with IgG2 deficiency was 20 for Sanguin and 21 for TBS; the number of samples with IgG3 deficiency was 13 for Sanguin and 2 for TBS. For IgG4, Sanguin was unable to detect IgG4 deficiency in children aged <9 years; accordingly, no data are given for IgG4. However,

it should be mentioned that we identified 4 samples for which TBS gave IgG4 values below the lower reference range, whereas results from Sanguin were within the reference range.

These data show that application of assay-specific reference ranges to a population of children with increased susceptibility to infection results in marked differences in result interpretation between Sanguin and TBS for IgG1, IgG3, and IgG4. For IgG2, the 2 methods were in agreement with respect to absolute values and reference ranges. For IgG1, absolute values matched but the reference ranges did not; for IgG3 and IgG4, neither absolute values nor reference ranges were in accordance.

We conclude that standardization of IgG subclass assays is unsatisfactory and that there is no consensus with regard to assessment of abnormal values. The international scientific community and the manufacturers should agree on a reference material (eg, CRM 470), and efforts should be undertaken to establish reliable age-dependent reference values.

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Missing parvalbumin: Implications in diagnostic testing for tuna allergy

To the Editor:

Parvalbumin is a 12-kd protein found in abundance in the white muscle of lower vertebrates. It is considered a major fish panallergen.¹ However, there have been conflicting reports regarding parvalbumin in tuna. Two previous studies reported the absence of parvalbumin in this fish.^{2,3} This was also our experience with our initial studies on tuna. However, others have reported the presence of parvalbumin in tuna, and it was also found to be allergenic.⁴ This study set out to evaluate the reasons for these inconsistent findings.

A fresh whole tuna, *Thunnus tonggol* (Bleeker) of about 400-mm fork length (FL) was skinned and cut transversely into sections along the length, beginning about 20 mm posterior to the edge of the operculum, with each section

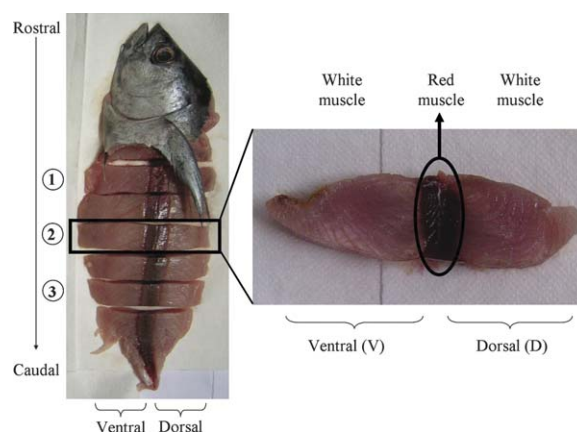


FIG 1. Dissection of *Thunnus tonggol* (Bleeker) for evaluation of parvalbumin. The fish was skinned and torso sectioned transversely into 6 portions (left). Three of these sections, the (1) rostral, (2) mid, and (3) caudal portions, were further divided into 3 parts, dorsal (D) and ventral (V) white muscle and middle red meat (right).

measuring about 25 mm in thickness (Fig 1). Three sections (at about 0.4 FL, 0.6 FL, and 0.8 FL) were chosen for analysis. For each section, the ventral and dorsal portions of the white muscle were separated from the red muscle (Fig 1), and extracts made in PBS (pH 7.4) containing 1 mmol/L phenylmethylsulfonylfluoride. Each extract (5 μ g) was separated in 8% TRIS-tricine gel and electroblotted onto Hybond-C Extra nitrocellulose membranes (Amersham, Uppsala, Sweden). Purified Gad c 1 (cod parvalbumin; kind gift from Pharmacia Diagnostics, Uppsala, Sweden) was used as a positive control. The membranes were blocked with 5% skimmed milk powder in 0.05% PBS-Tween 20 for 1 hour at room temperature

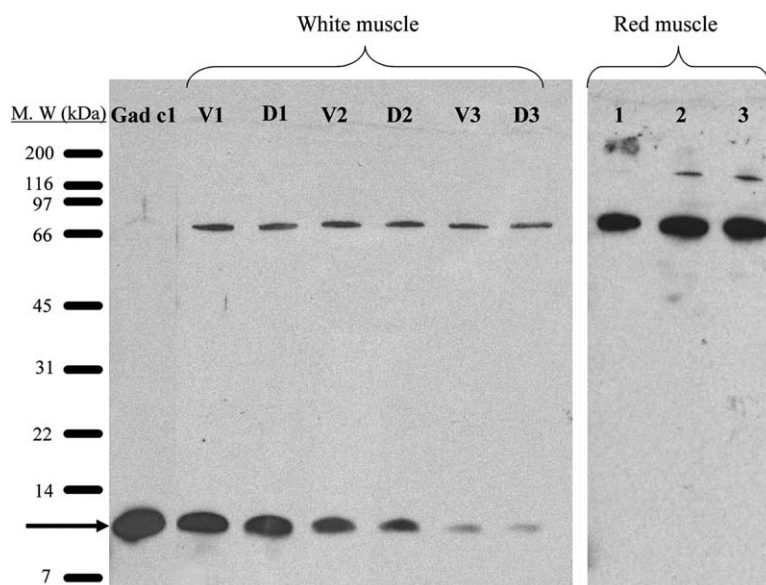


FIG 2. Immunoblot detection of parvalbumin in extracts of *Thunnus tonggol* (Bleeker). Left panel lanes, Gad c 1 (cod parvalbumin), ventral (V), and dorsal (D) white meat portions of sections 1, 2, and 3 (see Fig 1). Right panel lanes, Red meat portions of sections 1, 2, and 3. The 12-kd specific parvalbumin band is shown with an arrow. M. W., Molecular weight.