

# Serum immunoglobulin concentrations in preschool children measured by laser nephelometry: reference ranges for IgG, IgA, IgM

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**SUMMARY** Serum immunoglobulin concentrations were determined on sera from 298 healthy children aged six months to six years using the Hyland laser nephelometer PDQ system. Age-specific 95% reference ranges for serum IgG, IgA and IgM are presented; considerable care has been taken to ensure statistical validity of the reference ranges. The wide range of values in children under two years suggest that measuring immunoglobulin concentrations in this age group is of little value in diagnosing immunodeficiency.

The determination of reference ranges for serum immunoglobulins in childhood has often been frustrated by the ethical difficulties of taking blood from healthy subjects. Many previous studies have therefore used sera from hospital in-patients who cannot legitimately be considered a normal population. In other studies too few subjects have been tested, leading to unreliable reference ranges.<sup>1-3</sup> Other dubious methods include the assumption of a constant variability of log values. Adult serum immunoglobulin values are generally accepted as being lognormally distributed and several authors have assumed the same for immunoglobulin values in childhood.<sup>1,4,5</sup> Under this assumption of a log-normal distribution reference ranges are calculated by taking the antilogarithms of the mean  $\pm 2$  SD of the logarithms of the values. In some papers, however, arithmetic means and standard deviations were calculated from the raw data.<sup>2,6</sup> Unless the data have a Gaussian (Normal) distribution, if necessary after logarithmic transformation, reference ranges obtained by these conventional statistical methods will be incorrect.

There are two controversial "clinical entities" in infants and young children which seem to be associated with low immunoglobulin concentrations. Firstly, it has been suggested that a low serum IgA in

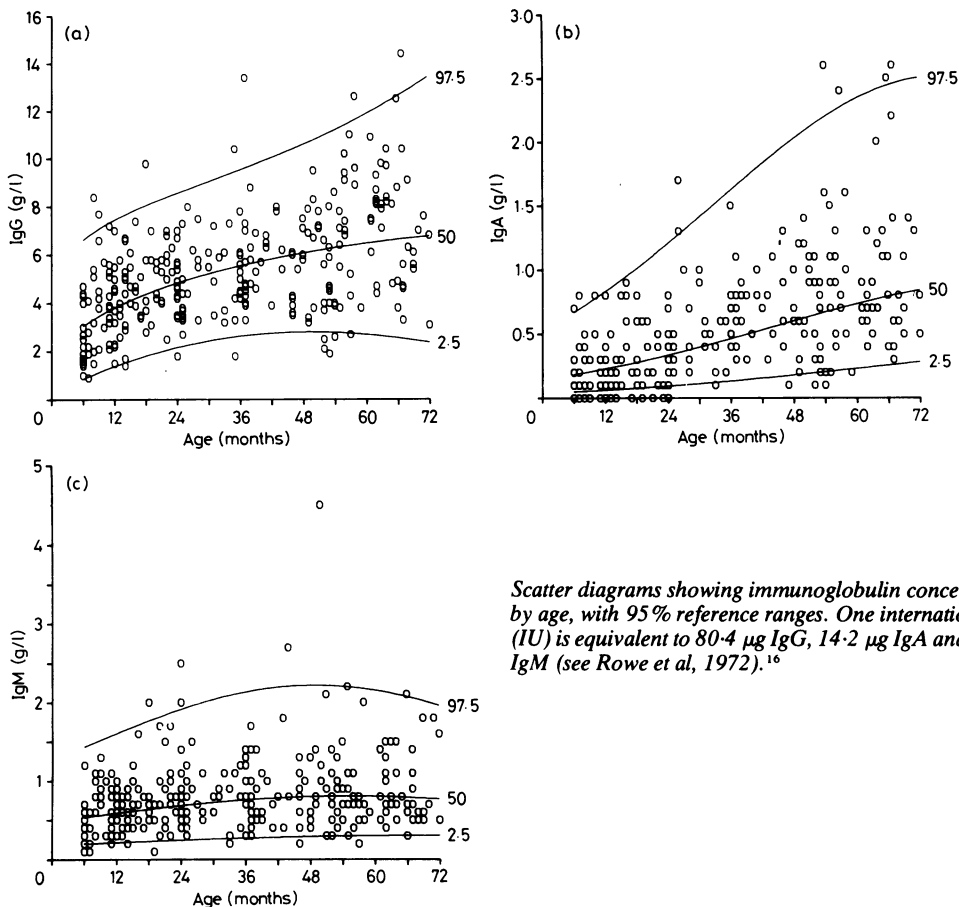
infants from atopic families predisposes to infantile eczema and possibly other allergic phenomena.<sup>7</sup> Secondly, there is confusion about the definition of "transient" hypogammaglobulinaemia of infancy. Some workers define this condition by a "lower than normal" serum IgG, despite the finding that many of these children can make normal amounts of functional IgG antibody.<sup>8</sup> The aim of the present study was to produce carefully validated reference ranges for serum IgG, IgA and IgM and to question whether these concentrations are likely to be of use in the diagnosis of antibody deficiency in young children.

## STUDY POPULATION

One of us (DI) attended infant welfare clinics and school medicals in the Harrow area. The primary purpose of the study was to obtain information on the antibody response to pertussis vaccine in children (to be published elsewhere). The study was approved by the Harrow District Ethical Committee. The nature of the research was fully explained to the parents and permission was sought to obtain a single sample of venous blood from their child. Permission was also obtained from children over three years of age, and when possible also in younger children. A surprising number agreed to give blood "to help other children." Children with a history of recurrent infection, allergic disease or any intercurrent infection were excluded.

Venous blood (2 ml) was obtained using a size 23 butterfly needle (Venisystem, Abbott Ireland Ltd,

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Scatter diagrams showing immunoglobulin concentrations by age, with 95% reference ranges. One international unit (IU) is equivalent to 80.4  $\mu\text{g}$  IgG, 14.2  $\mu\text{g}$  IgA and 8.47  $\mu\text{g}$  IgM (see Rowe et al, 1972).<sup>16</sup>

Sligo). A total of 298 specimens were collected (141 boys and 157 girls) and there were at least 30 serum specimens for each age range, 6–9 months; 10–12 months; 13–18 months; 18–24 months; 25–36 months; 37–48 months; 49–60 months and 61–72 months. There were 224 of European origin, 72 were Asian and two were West Indian.

#### Material and methods

All sera were analysed using a Hyland laser nephelometer PDQ system.<sup>9</sup> The standard serum used was calibrated from the WHO standard human serum (Reference Code No 67/97)<sup>16</sup>. The sera were stored at +4°C for up to 4 days before being tested; freezing and thawing were avoided as they may alter immunoglobulin concentrations.<sup>10</sup> Serum IgA concentrations under 0.1 g/l were recorded as <0.1 g/l, since this is the lower limit of accurate detection of IgA by nephelometry. However, sera from children with nephelometric serum IgA <0.1 g/l were also

tested by a double antibody radioimmunoassay previously described<sup>11</sup> and all such sera were found to have an IgA >0.03 g/l by this method.

#### STATISTICAL METHODS

There are two main desirable properties of reference ranges. Firstly, they should be a valid reflection of the raw data, and secondly specific reference ranges should change *smoothly* with age. Very few studies have demonstrated the correctness of their statistical methods. Also, most published reference ranges are implausibly erratic, where a smoothly-changing range is much more credible. In the present study great care was taken to achieve valid and plausible reference ranges, although the end result must still be largely dependent upon the representativeness of the children in the study. The general strategy adopted here is briefly summarised:

- (i) The data were transformed so that the transformed data values were reasonably Normally distributed at all ages. This was achieved not

by considering statistical significance alone, but by finding the transformation for which the transformed values were nearest to a Normal distribution, using Shapiro and Wilk's W test.<sup>12</sup>

- (ii) A polynomial regression model was fitted to the transformed data.
- (iii) Another polynomial regression model was fitted to the standard deviations of the residuals about the previous regression. For this purpose the age range was subdivided into eight. (Note that step (i) does not imply that the transformed variables were equally variable at all ages.)
- (iv) The reference range was calculated as

$$f_1(\text{age}) \pm 1.96 f_2(\text{age})$$

where  $f_1$  and  $f_2$  are the fitted functions of age derived in steps (ii) and (iii), and these values were backtransformed as appropriate.

- (v) The goodness-of-fit of the models was carefully assessed.

Steps (ii) to (iv) were repeated until a satisfactory result was obtained.

Values of serum IgA which were recorded as <0.1 g/l necessitated the use of statistical estimation procedures appropriate for censored data.

## Results

The standard deviation of the nephelometric method, as determined in 19 replicates of a normal serum, was between 7–10% of the mean for all three immunoglobulin classes. Logarithmic transformation was found to be highly appropriate for both IgA and IgM; but for IgG the square root transformation was superior. The equations fitted to the means and standard deviations, which are given in the Appendix, were combined as previously described and appropriately back-transformed to give 95% reference ranges.

The raw data for the serum immunoglobulins and the 95% reference ranges are shown in Figs. a–c. No difference was observed between boys and girls, nor between European and Asian children so the reference ranges were derived from all the data. There were a large number of healthy children in the first two years of life with serum IgA concentrations below 0.1 g/l. Fig. (b) shows that the lower limit of the reference range for IgA is below 0.1 g/l until 26 months of age.

## Discussion

We have shown that serum immunoglobulin values in children are not all lognormally distributed and

have produced smooth age-specific reference ranges for IgG, IgA and IgM. The care taken in this study is important because many of the previously reported ranges are either of dubious validity because of the statistical methods used or the small number of subjects tested in each age range studied, or are less than ideal because no smoothing was performed. The very low concentrations of serum IgG found in apparently healthy children in the first year of life calls into question the usefulness of measuring such concentrations in children with suspected antibody deficiency. Tiller and Buckley<sup>8</sup> have recently suggested that transient hypogammaglobulinaemia of infancy is extremely uncommon, since they found only 11 cases amongst 10 000 whose sera were sent for immunoglobulin studies over a 12-year period. Their reference range is based on sera from 201 normal individuals, but only about 10 subjects were tested in each three-month age range from 0–2 yr; and there were less than 10 subjects in each subsequent year up to the age of 5 yr.<sup>4</sup> These results have been questioned by Siegel and colleagues<sup>13</sup> who studied T helper activity in 17 children with transient hypogammaglobulinaemia of infancy. One of their criteria for the diagnosis of transient hypogammaglobulinaemia was a serum IgG concentration three or more standard deviations below the geometric mean for the patient's age; but again their reference range was based on sera from less than 15 healthy subjects in each year. Although it is not strictly legitimate to compare results from two different laboratories, it is interesting that only 8 of 17 abnormal serum IgG values in their study fall below our 95% reference range.

We also found that our lower limit of normal for serum IgA was considerably lower than is generally accepted. Although laser nephelometry in our hands was unable to detect serum IgA concentrations under 0.1 g/l, this has been allowed for in the statistical analysis and there were too few such concentrations to have a great effect on our reference range. Much of the present controversy over the clinical significance of low serum IgA in childhood is based on data analysed on the assumption that the data were Normally distributed. Using our rigorously derived reference ranges, it appears unlikely that low IgA in infancy will show a significant association with atopic eczema<sup>7</sup> cow's milk allergy,<sup>14</sup> or febrile convulsions.<sup>15</sup>

This study demonstrates that the measurement of serum immunoglobulins is of limited value in the diagnosis of antibody deficiency in the first two years of life. We clearly need different criteria for the diagnosis of antibody deficiency, and a better test might be to measure specific antibody responses after test immunisation.

## Appendix

The polynomial regressions fitted to the age-specific mean and standard deviation of the transformed serum immunoglobulin values are given below. Note that age is in months.

### *log IgA*

$$\text{Mean} = -1.92 + 0.03975 \text{ Age} - 0.0002155 \text{ Age}^2$$

$$\text{SD} = 0.658 + 0.0003166 \text{ Age} - 0.00002374 \text{ Age}^2$$

### *Square root of IgG*

$$\text{Mean} = 1.16 + 0.2715 \text{ Age} - 0.01195 \text{ Age}^2$$

$$\text{SD} = 0.45 - 0.00550 \text{ Age} + 0.0000945 \text{ Age}^2$$

### *log IgM*

$$\text{Mean} = -0.74 + 0.01899 \text{ Age} - 0.0001728 \text{ Age}^2$$

$$\text{SD} = 0.494 + 0.002064 \text{ Age} - 0.00003191 \text{ Age}^2$$

The 95% reference ranges were calculated by taking the age-specific values of mean  $\pm 1.96$  SD and back-transforming as appropriate.

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