COMPARISON OF NORMAL VALUES OF IgG SUBCLASSES

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ABSTRACT

The concentrations of subclasses of IgG in sera from children as reported by several groups were analyzed. A 4th degree polynomial regression curve was calculated for the upper and lower ranges of the subclasses at various ages. In addition percentile values were calculated. The calculated ranges were tested for their usefulness when looking for immunoglobulin subclass deficiencies. In relation to the calculated ranges the sera from 85 controls did not show decreased concentrations of IgG1 or IgG3. Eight % of the sera showed decreased IgG2 and no evaluation was possible for IgG4. We conclude that the calculated ranges of the IgG subclasses are useful for further research on IgG subclass deficiencies.

KEYWORDS

IgG subclasses: children: reference values: IgG2 deficiency: interlaboratory comparison.

INTRODUCTION

Decreased concentrations of IgG subclasses are associated with a variety of diseases (Schur, 1987). The association between IgG2 deficiency and recurrent infections of the respiratory tract has been documented by several studies (Schur et al, 1970; Shackelford et al, 1986). These studies have led to an increasing demand for the determination of IgG subclasses in sera from children.

The concentrations of IgG subclasses in serum vary widely with age. Therefore large groups of normal children have to be studied to obtain reliable reference ranges. Most studies in this respect report 10 to 20

individuals per age group, which may result in inaccurate reference ranges.

In this study we have compared the IgG subclass concentrations in sera
from healthy children, reported by different groups. In addition we have
pooled data and calculated in two different ways ranges for IgG subclasses on
the basis of the pooled data. IgG subclass concentrations in sera from a
small group of healthy children were evaluated on the basis of the calculated
ranges.

MATERIALS AND METHODS

Quantitation of IgG subclasses

IgG subclasses were measured in sera from 85 children who visited the
out-patient clinic for minor surgical problems. Children suffering from
recurrent infections were excluded.

The IgG subclasses were measured by radial immunodiffusion following the
instructions given for each batch of antiserum. The results were expressed in
g/l. The serum H-00-02(Central Laboratory of The Netherlands Red Cross Blood
Transfusion Service) was used as the standard. Polyclonal antisera, specific
for the IgG subclasses were obtained from the Department for the Preparation
of Immune Reagents(CLB, Amsterdam, The Netherlands).
Calculations.

Calculations were performed on a WANG PC. The calculation of the fourth
degree polynomial regression line through the values of the upper or lower
range was performed by a computer program written by E.J. Nieuwenhuys, which
used the Gaussian least square method in solving the polynomial
parameters(Scheid, 1968). Other calculations were done with the LOTUS 123
package.

RESULTS

Data for IgG subclasses from the following papers were analyzed: Aucou-
turier et al, 1985; Ferrante et al, 1986; Morell et al, 1972; Oxelius, 1979;
Schur et al, 1979; Van der Giessen et al, 1975; Zegers et al, 1980. These
studies were chosen as they gave information on the values of the lower and
upper ranges of all four IgG subclasses in different age groups. In all
studies the WHO 67/97 standard serum or a derived standard serum was used as
a standard and all except one(Ferrante et al, 1986) applied the radial
immunodiffusion assay. The results of the studies were, if necessary,
converted to g/l with the multiplication factors given in the papers.
The mean of the given age interval was converted to age in days and the ^{10}LOG
was taken from this value. The values for the lower and upper range from
every age group in each study are shown in Figs 1a, 1b, 1c and 1d for IgG1,
IgG2, IgG3 and IgG4, respectively. The results from different papers are in

Fig. 1.

Upper and lower values for IgG subclasses. The letters refer to the name of the first author of the analyzed paper. Uppercase and lowercase letters indicate the upper and lower ranges, respectively. The curves drawn are the calculated 4th degree polynomial regression curves. Note that the age is given in ^{10}LOG of days; thus an X value of 3 corresponds to an age of 1000 days.

reasonable agreement. It can be seen that the IgG1 results for the upper range from one study (Oxelius, 1979) were considerably lower than those from other studies and that the IgG3 results for the upper range from the paper by Schur et al(1979) were considerably higher than the results from other studies.

A fourth degree polynomial regression analysis was performed on the data for the upper and lower ranges, respectively, and the curves obtained are plotted in Fig 1. The coefficients of the parameters of the fourth degree polynomials for the upper and lower ranges of all subclasses are given in TABLE I. Thus for example at the age of 100 days the value for the lower range of IgG3 can be calculated as follows: IgG3(g/l) = $0.07716 * 2^4 - 0.7623 * 2^3 + 2.731 * 2^2 - 4.189 * 2 + 2.459$. The coefficients of the correlations calculated(R, TABLE II) were between 0.83 and 0.95 except for the lower range of IgG3 (R=0.74) and the lower range of IgG4 (R=0.41).

TABLE I

Coefficients of the parameters of the fourth degree polynomial to calculate the range concentrations, [IgG subclass] (g/l) = $A * X^4 + B * X^3 + C * X^2 + D * X + E$; X is the ^{10}LOG of the age in days.

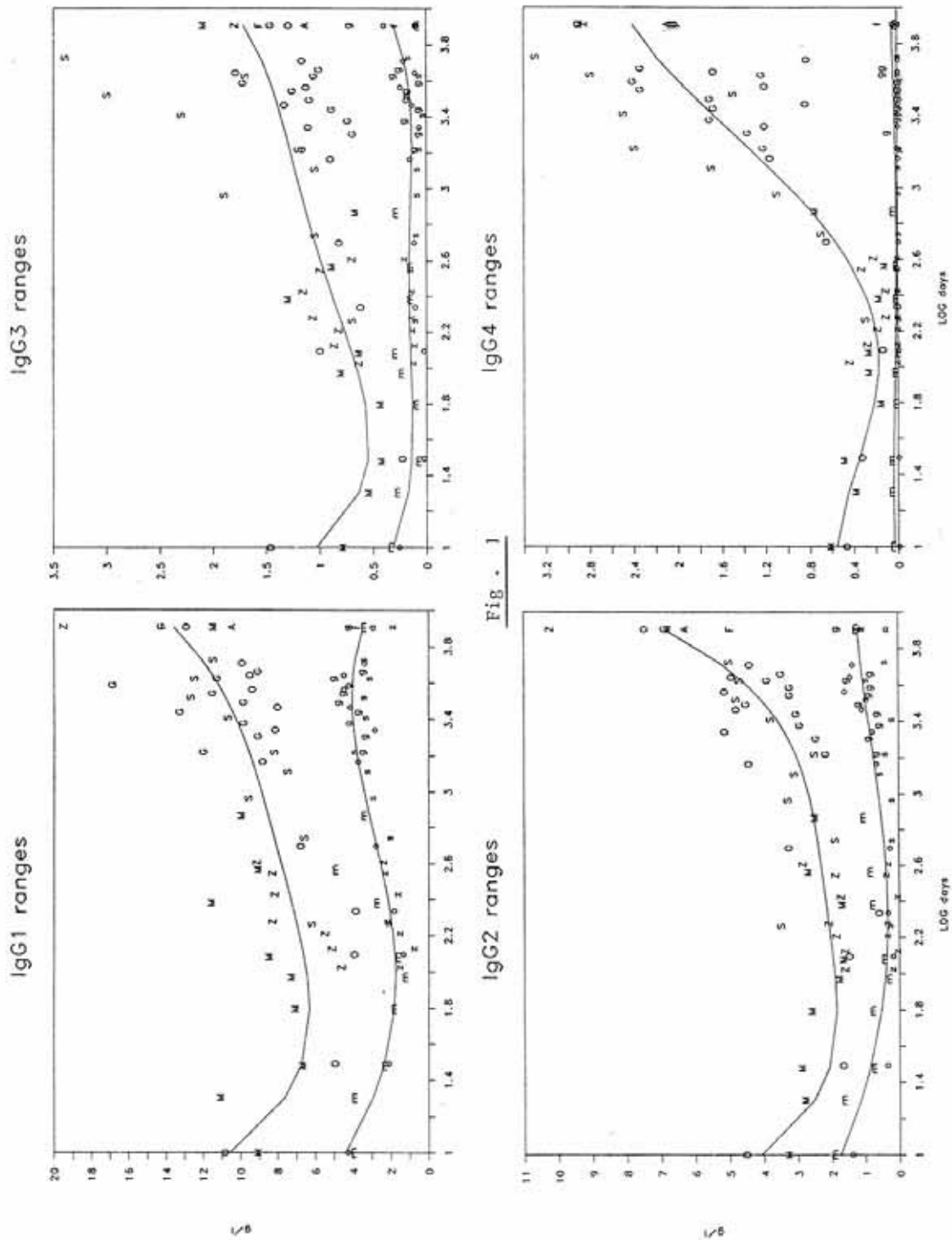|	IgG1		IgG2		IgG3		IgG4	
	low	high	low	high	low	high	low	high
A	-0.1691	0.8842	-0.03441	0.7294	0.07716	0.1423	-0.00547	-0.1677
B	0.6368	-9.362	0.1623	-7.026	-0.7623	-1.555	0.06241	1.596
C	2.568	37.31	0.6514	25.4	2.731	6.225	-0.236	-4.784
D	-12.28	-63.76	-3.909	-40.13	-4.189	-10.3	0.3394	5.315
E	13.66	45.5	4.873	25.12	2.459	6.521	-0.1218	-1.399

Fig . 1

TABLE II

Coefficients of correlation for the 4th degree polynomial regression line calculated for the ranges of the concentrations at different ages.

	IgG1	IgG2	IgG3	IgG4
lower range	0.95	0.87	0.74	0.41
upper range	0.94	0.94	0.83	0.91

In addition to the regression analysis we calculated the IgG subclass concentrations corresponding to the 97.5 percentile and the 2.5 percentile. To calculate these values the data from corresponding age groups in different studies were pooled. The following age groups were made: up to three months(containing altogether 82 children), three months to one year(207), one to three years(140), three to ten(321) and ten to eighteen(144) years of age. The values for adults are from our own studies on 90 sera. The values for percentiles were taken according to Elveback and Taylor (1969). The total number of individual observations in each age group was taken as the basis for the number of observations to be skipped from the lowest and highest concentrations measured. Curves drawn through the concentrations indicating the 2.5 and 97.5 percentiles in the different age groups are shown in Fig 2a, 2b, 2c and 2d for IgG1, IgG2, IgG3 and IgG4, respectively, together with the 4th degree polynomial regression line calculated before. The results of the two methods agree very well, especially for the lower ranges.

The calculated concentrations of the upper or lower ranges of the four subclasses in a particular age group were added and the total amount of IgG thus obtained was compared with the value for IgG ranges reported by Stiehm and Fudenberg(1966) and by Zegers et al(1975). The sum of subclasses corresponded well with total IgG as can be seen in Fig 3, except for the values at birth and for the values for the adults.

To evaluate the possibility of using the calculated upper and lower ranges for the evaluation of future studies on IgG subclasses we have measured IgG subclasses in sera from 85 healthy children and compared the results with the calculated reference ranges of the 4th degree polynomial curve. For IgG1 no

Fig 2

Concentrations of IgG subclasses in different age groups corresponding to 2.5 and 97.5 percentiles. The continuous curves are the 4th degree polynomial regression lines from Fig 1.
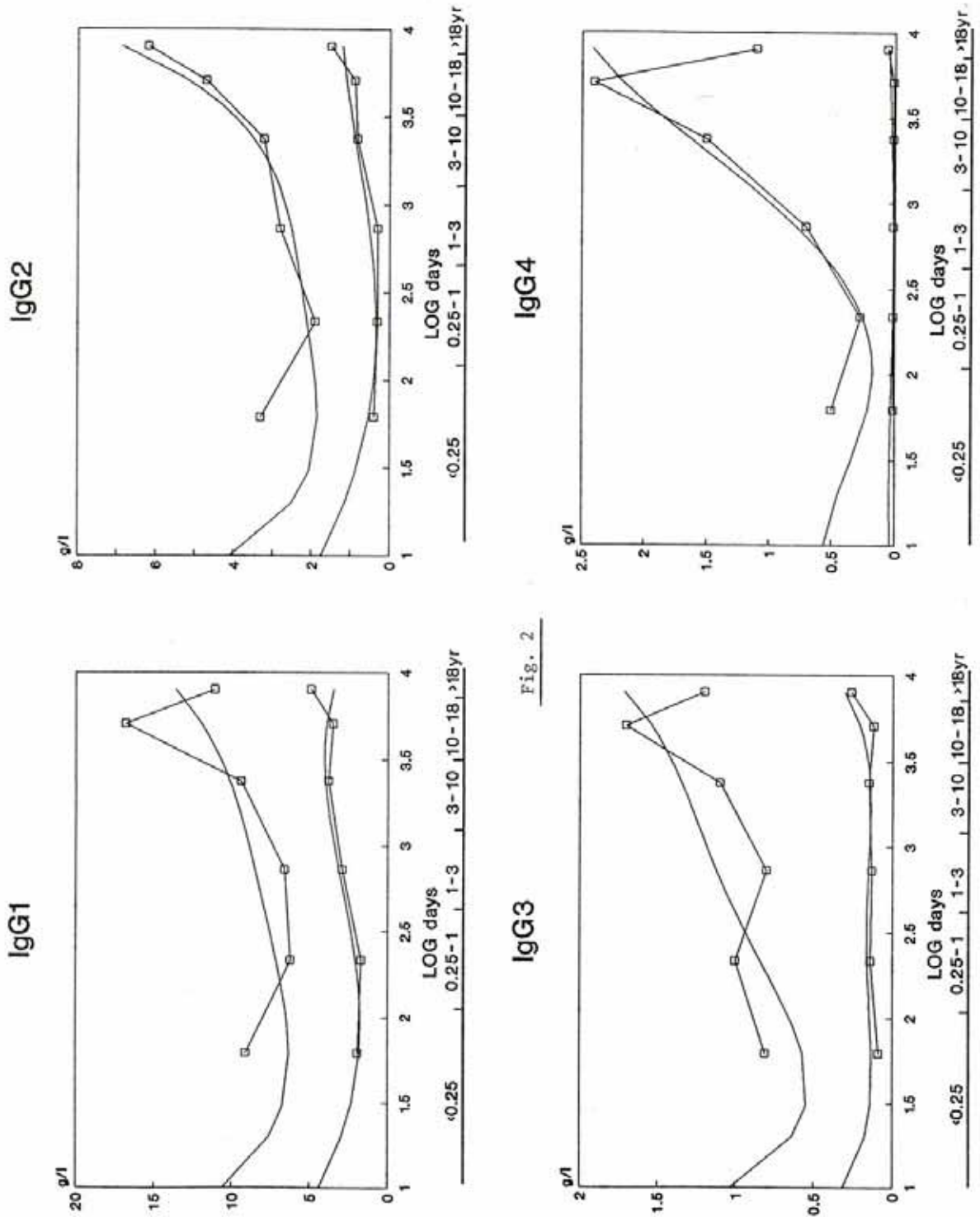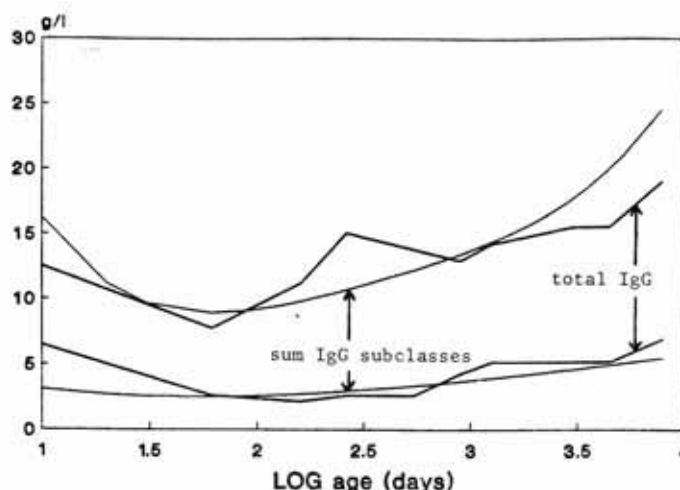
Fig. 2

Fig 3.

Comparison of upper and
lower ranges for the sum
of IgG subclasses with
those for total IgG.

concentrations below the values for the lower range were found; seven sera
had IgG2 below the values for the lower range. None of the sera showed
decreased IgG3. Results for IgG4 could only be evaluated after the
application of an ELISA for the determination of IgG4(not shown here).

Also results from 1245 sera (68% of which were from children below 11
years of age) that were submitted for determination of IgG subclasses were
compared with the calculated reference ranges. In 7% of these sera decreased
IgG1 concentrations were found; 21 % had decreased IgG2 and 11% had decreased
IgG3.

DISCUSSION

The determination of subclasses of IgG in serum is relevant for the esta-
blishment of possible deficiencies (Bentwich et al, 1988). An important
problem in the interpretation of the results is the lack of sufficient
reference data in the various age groups of children. We have therefore
compared the values of IgG subclasses from different papers and we have
explored the possibility of using reference values calculated on the basis of
the pooled data.

The selection of which degree of polynomial equation had to be used was
done as follows. On all sets of data a polynomial from the second till the
eight degree was fitted. The higher the degree of a polynomial the more bends
occurred in the curves. The fourth degree polynomial was chosen since a
higher degree gave no really different form of regression line. A higher
degree of polynomial always fitted better (gave a higher correlation), as
more outliers were fitted to the curve by making extra bends in the curve.

The calculation of the 4th degree polynomial through the values measured
for the upper and lower range in the individual reports results in an average
upper and lower range for those studies. The significance of such ranges in

relation to the whole population of observations from all papers is dependent on the number of observations in each group in every study. When for example that number is 50 it can be calculated that averaging the lower and upper limits results in new lower and upper limits indicating the 1 to 3 percentile and the 97 to 99 percentile, respectively (Rumke and Bezemer, 1972). It is not surprising therefore that the two procedures that we have applied in the analysis of the data yield corresponding results.

When the calculated curves were plotted together with the original data(Fig 1) especially the values for the lower ranges of IgG1 and IgG2 were close to the calculated ranges. The calculated correlations were high except for the lower ranges of IgG3 and IgG4. For IgG4 this can be explained by the fact that the radial immunodiffusion assay does not always allow to measure the low concentrations of IgG4 present and that the lower limit of detection of IgG4 differed in one study from the other.

The ranges of summed values of the subclasses are in a good agreement with data for total IgG.

The comparison of results of IgG subclass determinations in sera from controls with the calculated reference ranges indicate that the calculated ranges may be very useful for research purposes. No concentrations below the lower limits were found for IgG1 and IgG3. For IgG2 8% of the sera were below the lower range, which may be related to the finding (Massadery et al, 1988) that in the lower range the IgG2 concentrations did not follow the curve of the log normal distribution. This phenomenon may make it difficult to discriminate between normal and pathological low concentrations of IgG2.

The combinations of results from different studies as done here implies that data may have been derived from different ethnic groups and that the results have been obtained with different methods at different places and different times. This makes the calculated range probably wider than to be expected from one local study. On the other hand the comparison of data from different laboratories may stimulate the discussion on the significance of IgG subclass deficiencies. A further improvement of the reliability of the ranges can be obtained when data from all individual determinations are taken into account.

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