Reference Intervals for Serum IgG, IgA, IgM, C3, and C4 as Determined by Rate Nephelometry

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We report reference intervals for IgG, IgA, IgM, C3, and C4 for a population of 750 well children and 120 healthy adults. Ranges were established by rate nephelometry (previous studies have been based on immunodiffusion). Our results generally agree with previously established immunoglobulin ranges, except for some disagreement as to ages when adult values are attained.

Additional Keyphrases: pediatric chemistry • immunoglobulins • age-related values • proteins • complement

With the introduction of nephelometric procedures for the quantitation of specific proteins (1) discrepancies between values reported by radial immunodiffusion and nephelometry for immunoglobulins and other proteins on the same specimen have been noted (2, 3). Such discrepancies are ascribed to several factors: differences in molecular configurations, methodology, antisera, and standards or calibrators. The purpose of our study is not to document these factors, but rather to establish reference intervals for a pediatric and adult population for the more commonly assayed specific proteins: IgG, IgA, IgM, C3, and C4. To date, no such intervals have been reported for these populations as determined by rate nephelometry.

Materials and Methods

Population. Participants in this study were screened by their pediatricians or internists for any disease that might increase or decrease concentrations of immunoglobulins or complement.

All pediatric participants (25 boys and 25 girls) were accepted in a random fashion as they presented themselves to two pediatric clinics and a pediatric hospital. Cord-blood was sampled and blood from these subjects at one, two, three, four, five, six, and seven to nine months, 10 to 12 months, one year, two years, three years, four to five years, six to eight years, and nine to 10 years.

The same procedure was utilized for selection of the adult patients. The adult population consisted of 60 men and 60 women, ranging in age from 16 to 62 years.

All values represent individual patients. Pediatric specimens were obtained by heel puncture and venipuncture. No specimens were accepted if difficulty was experienced in obtaining adequate blood flow by heel puncture. Cord blood was obtained by the obstetrician during delivery and was delivered to the laboratory within 1 h. None of the newborns from whom cord blood was sampled showed clinical evidence of intrauterine infection; follow-up studies confirmed this. Specimens from adults were all by venipuncture.

Procedure. IgG, IgA, IgM, C3, and C4 were determined by rate nephelometry with the Beckman Immunochemistry System (ICS; Beckman Instruments, Inc., Brea, CA 92621). Each determination was performed as specified in the manufacturer's kit insert for the specific protein being analyzed, with antisera, calibrators, buffer solutions, and diluents provided by the manufacturer.

The only change from the recommended procedure was to refilter the diluent and buffer solutions through a polycarbonate membrane (0.4-μm pore size, 47-mm, stock no. 111107; Nucleapore Corp., Pleasanton, CA 94566) held by a Nucleapore 47-mm holder attached to a 50-mL plastic syringe. (We later found this step to be unnecessary with the newer formulation of the solutions provided.)

Each determination was performed in duplicate, and only results agreeing within the ±5% range were accepted. The two results were averaged and recorded.

Statistical tools. Distribution of values within the reference intervals for most age groups were skewed, with more being below the mean than above. In such instances the upper and lower limits of the reference interval (95%) were calculated after logarithmic transformation of the values (4, 5). IgM values for the seven- to nine-month group and C3 values for the 10- to 12-month and one-year groups had a gaussian distribution; reference intervals for these groups were determined by ±2 SD from the mean. Log transformation was not necessary for the adult population.

Results

Table 1 lists ranges for each protein as well as the mean for each age group studied. Age-related changes are evident in all proteins examined. IgG in the newborn is the same as for adults, owing to placentation transfer of this immunoglobulin. At three months the concentration declines to a minimum, then gradually increases, reaching adult concentrations at six to eight years of age as the immune system matures.

IgA concentration is lowest at birth, increasing to low adult values at four to five years. Mean adult concentrations are not reached until puberty. Due to numerous and different antigenic challenges in individuals, the heterogeneous production of immunoglobulins varies greatly throughout life. IgG and IgA values are essentially in agreement with values previously reported by investigators who used radial immunodiffusion (6–15). There is some discrepancy from previous studies as to when adult concentrations are reached for these two immunoglobulins.

IgM values reported by various investigators show considerable variation (9, 13). In our study, IgM concentrations in cord blood are markedly low and gradually increase to the mean adult value by four to five years of age.

Reports of reference intervals for C3 and C4 are quite limited (11, 16). Our data show that C3 concentrations are 65% of the adult value at birth and attain adult values by one year. C4 concentration is half the adult value at birth, but by six months reaches adult concentrations. These values are in agreement with studies by Gitlin and Gitlin (11).

Discussion

In general the results obtained by rate nephelometry for

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| Table 1. Mean and Range of IgG, IgA, IgM, C3, and C4 in Relation to Age |
|------------------|-----------------|-----------------|-------------------|-----------------|
|                  | IgG             | IgA             | IgM               | C3              |
|                  | Mean g/L        | 95% Range g/L   | Mean g/L          | 95% Range g/L   |
| Cord Blood       | 11.21 6.36-16.06| 0.023 0.014-0.036| 0.13 0.063-0.25  | 0.83 0.57-1.16  |
| 1 month          | 5.03 2.51-9.06  | 0.13 0.013-0.53  | 0.45 0.20-0.87    | 0.83 0.53-1.24  |
| 2 months         | 3.65 2.06-6.01  | 0.15 0.028-0.47  | 0.46 0.17-1.05    | 0.96 0.59-1.49  |
| 3 months         | 3.34 1.76-5.81  | 0.17 0.046-0.46  | 0.49 0.24-0.89    | 0.94 0.64-1.31  |
| 4 months         | 3.43 1.96-5.58  | 0.23 0.044-0.73  | 0.55 0.27-1.01    | 1.07 0.62-1.75  |
| 5 months         | 4.03 1.72-8.14  | 0.31 0.001-0.04  | 0.62 0.33-1.08    | 1.07 0.64-1.67  |
| 6 months         | 4.07 2.15-7.04  | 0.25 0.081-0.68  | 0.62 0.35-1.02    | 1.15 0.74-1.71  |
| 7-9 months       | 4.75 2.17-9.04  | 0.36 0.11-0.90   | 0.80 0.34-1.26*   | 1.13 0.75-1.66  |
| 10-12 months     | 5.94 2.94-10.69 | 0.40 0.16-0.84   | 0.82 0.41-1.49    | 1.26 0.73-1.80* |
| 1 year           | 6.79 3.45-12.13 | 0.44 0.14-1.06   | 0.93 0.43-1.73    | 1.29 0.84-1.74* |
| 2 years          | 6.85 4.24-10.51 | 0.47 0.14-1.23   | 0.95 0.48-1.68    | 1.20 0.81-1.70  |
| 3 years          | 7.28 4.41-11.35 | 0.66 0.22-1.59   | 1.04 0.47-2.00    | 1.17 0.77-1.71  |
| 4-5 years        | 7.80 4.63-12.36 | 0.68 0.25-1.54   | 0.99 0.43-1.96    | 1.21 0.86-1.66  |
| 6-8 years        | 9.15 6.33-12.80 | 0.90 0.33-2.02   | 1.07 0.48-2.07    | 1.18 0.88-1.55  |
| 9-10 years       | 10.07 6.08-15.72| 1.13 0.45-2.36   | 1.21 0.52-2.42    | 1.34 0.89-1.95  |
| Adult            | 9.94 6.39-13.49 | 1.71 0.70-3.12   | 1.56 0.56-3.52    | 1.25 0.63-1.77  |

* All ranges based on a log transformation of the data to eliminate skewness except ranges indicated, which were distributed in a gaussian fashion.
IgG, IgA, C3, and C4 approximate those obtained in other age-related studies established by radial immunodiffusion. Values obtained for IgM with the ICS vary from those previously reported. These differences appear to be related to differences in calibrators supplied by the various manufacturers of radial immunodiffusion kits and to assignments of standard values based on results obtained from various reference laboratories utilizing differing radial immunodiffusion techniques (1–3, 18–20).

Wide ranges of values within the various age groups leads one to be aware of the futility of basing a clinical decision on measurement of a single specific protein. As with determinations of other blood constituents, serial measurements may well be useful in some disease states.

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References