Reference Distributions for Immunoglobulins A, G, and M: A Comparison of a Large Cohort to the World's Literature

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Clinical interpretation of immunoglobulin measurements requires accurate and precise assays and widely accepted reference preparations, as well as reliable age- and gender-specific reference ranges. This last requirement, the topic of numerous publications, has not been addressed adequately. By a combination of computerized and hand searching of the literature from 1961 to 1997, we identified 109 publications presenting IgA, IgG, and/or IgM reference data in healthy individuals. After eliminating studies that lacked appropriate clinical, statistical, or reference material information, data from the 17 acceptable studies were converted to a common reference material, CRM 470/RPPHS. When median levels from our recently published

large cohort study are superimposed on these published medians, they fall within the ranges of reported medians. The widths of published 95 percentile reference ranges (where each individual's health was verified) were also found to agree closely with the reference range widths found in our data (where inclusion was based on the reported diagnosis). The current combined study of narrowly applied reference ranges validates our recently published age- and gender-specific reference data for immunoglobulins A, G, and M. Those new data can now be considered as a source of reliable reference ranges to be used by laboratories when interpreting immunoglobulin measurements. *J. Clin. Lab. Anal.* 12:371–377, 1998. © 1998 Wiley-Liss, Inc.

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INTRODUCTION

Since the introduction of serum immunoglobulin measurements into the laboratory in the 1960s, enthusiasm for their clinical use has been dampened by uncertainty of the diagnostic meaning of patient values. Some of this uncertainty has been resolved by the introduction of accurate and precise assays, and a widely accepted reference material. As a consequence of these improvements, however, the lack of reliable age- and gender-specific reference ranges has been highlighted. Many reports of reference ranges have appeared, but few meet the specified criteria of sufficient sample size, description of health status, analytic description, authoritative reference materials, and adequate statistical methods. The current study aims to identify reports from 1961 to 1997 that satisfy these criteria and report immunoglobulins A, G, and M measurements after normalization to a new, widely accepted reference material (CRM 470/RPPHS). That published experience is then compared with the reference values and ranges recently presented in our large cohort study (1) as a means of validation.

MATERIALS AND METHODS

Identification of Published Reference Data

The literature was searched by direct review of the Cumulative Index Medicus published compendia and the comput-

erized MEDLINE CD-ROM (OVID Technologies, New York, NY). A manual search of the period from 1961 to 1966 was performed. The CD-ROM set covered the years 1966–1997. The CD-ROM search strategy included the name of the protein followed by MeSH headings of “reference values,” “or “case controls.” Searching the reference listing of retrieved publications identified additional sources. Articles that were exclusively methodological or reported measurements only in individuals with known disease were removed. Articles were acceptable if they: 1) identified the assay method and reference material, 2) provided details of the study population such as age (an average or closed interval) and numbers of individuals tested, and 3) provided the information required for statistical analysis. Historically, IgA and IgG reference values do not differ greatly between males and females and, for that reason, publications that did not provide information on gender were acceptable. However, IgM measurements do differ between males and females and publications not providing information on gender were excluded from analysis.

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Estimating the Center and Range of Published Reference Distributions

For each study, the center of the distribution was estimated by either the median or geometric mean, because each of the immunoglobulins are known to be right-skewed. If neither of these estimates was provided, the center was estimated to be halfway between the log of provided symmetric centiles (e.g., 2.5th and 97.5th). If that also was unavailable, the study was excluded. The majority of studies provided either an observed or predicted reference range (2.5th–97.5th centile). In order to compare the width of the reference ranges, independent of the actual analyte level, the ranges were also converted to multiples of the median value (or other estimate of the center as described above). When multiple estimates for the reference range were provided (e.g., for various age groups) an average was computed and weighted by the number of observations in each group. Data were collected, edited, and verified by two team members (ON and LMN). When reviewers disagreed, inclusion or exclusion from the analysis was by group consensus.

Conversion of Reported Results to a Single Reference Material

Results were standardized using the Certified Reference Material 470 (CRM 470) / Reference Preparation for Proteins in Human Serum (RPPHS) (2). This material was released by the International Federation of Clinical Chemists (IFCC), the Community Bureau of References (BCR) in Brussels, and the College of American Pathologists (CAP) in the United States. Appropriate conversion factors to CRM 470/RPPHS were calculated (3–7), based on either reported reference materials or the package insert from the manufactured kits in use at the time. Briefly, the conversion factors listed by the manufacturers in their package inserts were used in a multiplicative fashion. Factors ranged from 0.87 to 1.00 with the majority using a factor of about 0.90. That these factors are reasonably accurate is supported by the observation that those groups with usable data fell within a narrow range after conversion (8).

RESULTS

Over 500 papers were identified that were exclusively methodological or reported immunoglobulin results only in individuals with a specific disorder. These were not eligible for analysis. A total of 109 publications were identified as containing immunoglobulin measurements on healthy individuals (9–117). Thirty-two studies reported only an arithmetic mean and insufficient data to estimate a reliable central estimate (13,18,21–23,25,27,29,33,37,39,40,44,45,49,54,55,57,58,62,63,65,67,70,80,86,87,92,93,108,115,117). Twenty-two studies did not state a reference material and/or an assay method and were also excluded (11,12,14,16,

19,20,24,28,32,41,48,50,52,66,77,78,84,89,96,100,101,104). Nine studies did not report age (26,31,34,42,53,82,102,103,106) and 29 others (9,10,30,35,36,38,43,47,59,64,69,71,73–75,79,81,88,95,97–99,105,109,110,112–114,116) had multiple reasons for exclusion. The remaining 17 studies (Fig. 1A) were considered acceptable for IgA measurements (15,17,46,51,56,60,61,68,72,76,83,85,90,91,94,107,111). All but one of these studies (72) also was acceptable for IgG (Fig. 1B). Less than a third of these papers stratified their results by gender. Only nine (15,60,61,68,72,76,83,107,111) were included in the IgM analysis (Fig. 1C). In a separate analysis of reference range width (Figures 2A, 2B, 2C), 14 studies were available for comparison (15,17,46,56,60,68,76,83,85,90,91,94,107,111).

Comparison of Median Values

Many reports contained multiple estimates of the median value because of stratification by age and/or gender. Overall, 119 IgA, 109 IgG, and 59 IgM median estimates were available for analysis. All were standardized to CRM 470/RPPHS. Figure 1 displays the reported mean (or central) age on the logarithmic horizontal axis versus the reported median (or geometric mean) analyte level on the logarithmic vertical axis covering two orders of magnitude. Each observation is shown as a circle with the largest circles representing estimates based on between 10 and 49 subjects and the smallest circles representing estimates based on more than 100. In one highly stratified study (15) many categories contained less than 10 observations and were collapsed to form just six groups. For comparison, the solid and dashed lines represent the median levels found for males and females, respectively, in our large study (1). The estimates of the median IgA and IgG levels (Figures 1A and 1B) are remarkably consistent between published studies including single age- and gender-specific consensus values generated soon after CRM 470 was released (118,119). These narrow estimates are in excellent agreement with those found in our earlier study (1). Published reference data for IgM levels (Figure 1C) are, however, more variable. Although the lower levels in males (squares) versus females (circles) generally holds true, it is not possible to determine a consensus estimate based on the literature. Our earlier, age- and gender-specific median values (1) are within the range of a compilation of narrower published central estimates.

Comparing Published Reference Ranges

The published studies included in these analyses were generally small (all had less than 1,000 study subjects) and for this reason, it was possible to verify the health status of each individual. This was not possible for our study of 115,017 individuals (1). Although the medians derived in our study are in agreement with published median values, it is possible that the reference ranges may be wider than the other pub-

Fig. 1. A summary of published immunoglobulin median reference values. The published median (or geometric mean) IgA (A), IgG (B), and IgM (C) levels are displayed on the logarithmic vertical axis (spanning two orders of magnitude) versus the mean (or median) age on the logarithmic horizontal axis. The three symbol sizes represent the number of observations in each group (large, medium and small circles representing 10–49, 50–99, and 100 or more observations, respectively). The solid (males) and dashed (females) lines are the regressed median levels from our large cohort study (1).

lished reference ranges because we did not verify health status and instead relied on the diagnosis provided on the laboratory slip. In order to determine whether this was the case, we reanalyzed the published reference ranges using our earlier finding that the widths of the ranges are nearly completely independent of age and gender (1). Figure 2 shows the 14 studies for which it was possible to calculate a 2.5th to 97.5th centile range (in multiples of the median). The reference numbers are shown on the horizontal axis, sorted by decreasing 95% reference range width. For example, Figure 2A shows that one study of IgA measurements, based on 252 observations reports the 2.5th centile to be nearly one-quarter the median level, (0.27) and the 97.5th centile to be nearly four times the median (3.76). The 95% reference ranges for our study are represented as open bars. One is the observed reference range and the other is based on parametric estimates fit to a log-Gaussian model. Figures 2B and 2C show a similar analysis for IgG and IgM reference ranges, respectively. For IgA and IgG measurements the observed and predicted values from our large cohort study are within the weighted average of the ranges reported in smaller studies (dotted lines) where health status was verified on each individual. For IgM measurements, our two estimates are slightly wider than the weighted average.

Ethnic Group-Specific Reference Ranges

Several studies included in the above analyses reported immunoglobulin measurements for specific ethnic or racial groups. Ten reported measurements in Caucasian or mostly Caucasian populations (17,46,51,56,60,65,83,85,91,106). Five reported results from both Caucasian and other racial groups separately (11,68,70,72,116). Two studied only Asians (90,103), and two studied only black Africans (107,117). The remaining four did not provide the racial composition of study subjects (15,71,73,76). Five within-study comparisons of immunoglobulin measurements in healthy Caucasian and Black individuals were made (11,68,70,72,116). The findings were not entirely consistent, but on average, the Black individuals tended to have slightly higher values. No further attempt was made to estimate racial differences between studies.

DISCUSSION

During the last decade, analytical instruments used to measure serum protein levels have improved greatly with the result that the need for an accepted, authoritative, and stable reference material became extremely important. This matter was resolved by the release of CRM 470/RPPHS (2). Our publication of a new set of age and gender specific reference

Fig. 2. Reference intervals from published studies. The bars represent the interval between the 2.5th and 97.5th centile for each study with the number of subjects above the bar. The horizontal axis shows the reference number for each study. The selected centiles have been presented as multiples of the median (MoM), compensating for age, gender and reference materials. **A**, **B**, and **C**, respectively, show the analyses for IgA, IgG, and IgM on identical axes. The dotted lines display the weighted consensus value at the 2.5th centile and the 97.5th centile for all studies excluding our large cohort (1). The hollow bars represent the observed and predicted ranges from our study (1).

ranges derived from a large, ethnically homogeneous cohort (1) demonstrates how important consideration of these two variables in the interpretation of immunoglobulin values has become. Laboratories have historically relied on reference ranges derived from published studies, but there may still be reluctance to convert to newly published reference ranges. This literature review provides reassurance that our proposed reference values stratified by age and gender are consistent, overall, with compiled median values in use today. It must be noted however, that many published reference ranges, while correct for a limited segment of the population, are incorrect for the broad scope of the population experience. In addition, these studies are, on average, in agreement, but individual studies may be much more discrepant.

When each study's reference limits are divided by their own population medians (i.e., converted to multiples of the median), they are, except for a few outlying estimates, consistent. This is extraordinary given the diverse ethnic/racial makeup represented in Figure 2: Hong Kong Chinese (90), Somalies (107), and Turkish subjects (85). Furthermore, the reference range widths for children (91), when compared to an elderly group (94), also are remarkably similar even though the median values vary widely. The establishment of a com-

mon reliable set of reference data for males and females throughout life would facilitate future review comparing immunoglobulin levels in similar populations. These reliable reference data for healthy individuals also would allow comparison between healthy and diseased individuals and would set the stage for reliable screening or diagnostic performance to be computed for specific disease status. In the long run, this would result in improved clinical interpretation of immunoglobulin test results.

It has been suggested, that reference ranges may differ in similar populations from different geographic areas, and for different ethnic groups within a single geographic area. However, published data has not provided satisfactory documentation of this phenomenon. Now that peripheral issues such as assay performance (8) and reference materials (2) have been dealt with, and more reliable age- and gender-specific reference data is verified, such studies can be undertaken without the need to match the populations under study for age and gender. Rather, the group's results can be expressed as multiples of the age- and gender-specific medians and tested for differences.

The guidelines published by The National Committee for Clinical Laboratory Standards to assist laboratories to design

and establish local reference ranges (120) and more recently the International Federation for Clinical Chemistry in developing a protocol to guide laboratories for constructing local cohorts for serum protein reference range analysis (121) has been made easier with the completion of this review and our large cohort study (1).

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