Reference Distributions for Complement Proteins C3 and C4: A Comparison of a Large Cohort to the World's Literature

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The lack of credible reference materials and satisfactory methods for quantifying serum levels has limited the bedside use of complement protein (C3 and C4) measurements. However, great technological strides have been made in the last few years. The remaining barrier to a more relevant and cost-effective use of serum protein data for diagnosis and prognosis is the availability of reliable reference intervals from birth to old age for both males and females. Fifty-one publications reporting

reference intervals were identified that meet the criteria used in our prior four studies, and these were analyzed statistically. Previous small studies with constrained age ranges agree, on average, with our larger series of life-long reference ranges. This meta-analysis provides support for our reference ranges and places them in the context of previous publications. *J. Clin. Lab. Anal.* 18:9–13, 2004. © 2004 Wiley-Liss, Inc.

Key words: serum proteins; complement; C3; C4; meta-analysis; CRM 470/RPPHS; acute phase proteins; reference range; Caucasian

INTRODUCTION

The acute phase response can induce up-regulation of complement protein synthesis. However, this response (especially when intense) is also accompanied by processes that destroy or sequester complement proteins such that they are removed from the circulation. In other words, increased synthesis can be masked by increased catabolism, with the resultant appearance of normal levels. The diagnostic and prognostic applications of these measurements have for the most part been limited to assessing whether serum levels of C3 and C4 are diminished; the increase in concentrations that accompany the acute phase response or cholestasis and increase from estrogen drive have largely been ignored. Furthermore, the few publications presenting reference ranges for C3 and C4 have not addressed the possibility of coincident inflammation, which can confuse the interpretation of measured values.

The two proteins examined in this study, C3 and C4, represent the major complement proteins whose synthesis is slowly up-regulated by the cytokines IL-1 and IL-6, which drive the acute phase response. This study, like its predecessors (1–4), aims to identify publications that provide reference data for these two analytes and satisfy minimal acceptance criteria. Published results were normalized against CRM 470/RPPHS (5) before com-

parison. Fifty-one publications (6–56) from the period of 1961–2000 were identified and reviewed.

MATERIAL AND METHODS

Identification of Published Reference Data

Briefly, the methods used to search for relevant publications were the same as for the previous studies (1–4). The same key-word headings were used, with the addition of the analyte-specific terms (<http://www.ncbi.nlm.nih.gov/PubMed>) and MEDLINE CD-ROM searches (OVID Technologies, New York, NY). Methodological papers or those that only examined patients with specific diseases were excluded. Articles were considered acceptable if they 1) identified the assay method and reference material; 2) provided minimum details regarding the study population, such as age; and 3) provided sufficient numerical information for statistical analysis.

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Estimating the Central Estimate and Reference Range From Published Studies

The mean, median, and geometric mean were used to estimate the center of distribution, as in the previous studies. If not directly available, they were computed using observed or smoothed centiles (e.g., between the 2.5th and 97.5th centiles). If none of these were available, the study was excluded from all analyses. Observed 95% reference ranges were used for the reference interval analysis. If the range was estimated from parameters or was other than the 95% range, the study was excluded from the reference interval analysis.

Conversion of Reported Results to a Single Reference Material

Using published package inserts, all results from the period of the study were standardized against CRM 470/RPPHS, as previously described (1–4). This material (5) was released by the International Federation of Clinical Chemists (IFCC), the Community Bureau of Reference (BCR) in Brussels, and the College of American Pathologists (CAP) in the United States. The latter labeled the material “Reference Preparation for Proteins in Human Serum” (RPPHS).

RESULTS

Number of Studies Available for Analysis

A total of 50 publications were identified that contained information on C3 in apparently healthy individuals (6–29,31–56). Thirty-six articles had enough data to calculate central estimates (8,9,11–14,16,17,19,20,22–24,26,28,29,32–42,44,46,48,49,52–56). Eighteen studies supplied sufficient information for calculating the reference intervals (8,10,11,13,14,17,21–24,27,37,40,49,50,52–54).

A total of 32 publications were identified that contained information on C4 in apparently healthy individuals (7,9–12,16,17,19,20,22–30,32,34–38,40–47,49–55), and supplied sufficient information for calculating central estimates (9,11,12,16,17,19,20,22–24,26–29,32,34–38,40–42,44,46,47,49,51–55). Thirteen articles supplied sufficient information for calculating the reference intervals (20,22–24,27,28,30,40,49,50,52–54).

Comparison of the Central Estimates

Figure 1 shows the central estimates of C3 (Fig. 1A) and C4 (Fig. 1B). The reported mean (or median) age of the study population is shown on the logarithmic horizontal axis vs. the central estimates on the logarithmic vertical axis. Each observation is shown as a circle, with the smallest and darkest circles representing the

Fig. 1. A summary of published acute phase protein median reference values. The published central estimates for C3 (A) and C4 (B) levels are displayed on the logarithmic vertical axis vs. the mean (or median) age on the logarithmic horizontal axis. The three symbol sizes represent the number of observations in each group (small and dark, medium, and large circles represent >100, 50–99, and 10–49, respectively). The solid (males) and dashed (females) lines are the regressed median levels from our large cohort study (57).

more reliable estimates (more than 100 observations). If a published observation was based on less than 10 observations, it was combined with an adjacent age group from the same publication. For purposes of comparison, the solid and dashed lines represent the median levels found for males and females, respectively, in our companion study (57).

Figure 1A shows the reported C3 median values from the 36 published studies that were considered acceptable. In adults, the range was nearly twofold. Most data are from persons in narrow age ranges. Our median levels for C3 are in the mid-range of values from a number of published estimates in subjects 1 year of age or older. However, our estimates are slightly higher than the published estimates for children under age 1 year. The estimates of our median C4 levels (Fig. 1B) are in the mid-range of published studies for individuals over 20 years of age (58,59). The few published estimates in populations under age 20 tend to be somewhat lower.

Comparison of the Reference Ranges

The reference ranges reported in the studies used in the above analyses were generally based on a small number of observations: only 12 of 36 studies of C3, and eight of 32 studies of C4 included 100 or more observations. Thus, verification of the health status of each individual was usually possible, whereas our observed reference ranges were each based on over 28,277 observations of individuals whose health status could not be verified. It is possible that our reference ranges are wider than other, smaller studies because we

A

B

Fig. 2. Reference intervals from published studies. The bars represent the interval between the 2.5th and 97.5th centiles for each study, with the number of subjects above the bar. The horizontal axis shows the reference number for each study. The selected centiles are presented as multiples of the median (MoM), compensating for age, gender, and reference material differences between studies. Parts A and B show the analyses for C3 and C4 measurements, respectively, on identical logarithmic vertical axes. The horizontal dotted lines display the weighted consensus value at the 2.5th and 97.5th centiles for previously published studies. The broken bars represent the observed and predicted reference ranges from our large cohort study (57).

relied on the diagnosis provided on the laboratory slip (60,61). For that reason, we compared our 95% reference limits with the previously published limits (57). Figure 2 shows other published studies after conversion to multiples of the median (e.g., each study's reference limits divided by their own population medians). The reference numbers are shown on the horizontal axis, sorted by decreasing 95% reference range width. The weighted average of the upper and lower limits of the ranges reported in the literature (excluding our study) is shown by two thin horizontal dashed lines. The 95% reference ranges for our study (both observed and predicted from the population parameters) are represented as thick dashed lines. Figure 2A and B show the analysis for C3 and C4 reference ranges, respectively. For both analytes, our ranges are similar to the combined consensus estimate from the literature, indicating that our method of identifying referent individuals did not lead to underprediction.

Ethnic Group-Specific Reference Ranges

Of the 51 studies included in these analyses, 15 publications provided values on racial or ethnic groups. Only two stated categorically that the subjects were Caucasian (16,23). One reported results in Caucasian and black Africans separately (24). Four described restricted ethnic groups (6,17,53,55). Three reported on Asians (37,45,48) and three examined black Africans (41,52,56). The remaining 40 did not report the racial composition of their study subjects. There was insufficient data to determine whether there are racial or ethnic differences in C3 and C4 levels.

DISCUSSION

This is the fifth in a series of meta-analyses for serum protein reference ranges. Our practical approach applied to large populations produces reference intervals comparable to previous smaller studies, and can be used to derive reliable reference ranges throughout life, separately for males and females. Our companion study (57) reports reference data for C3 and C4 based on a large dataset (over 27,000 observations), with less reliance on the documentation of each individual's health status.

The availability of a reliable set of reference data for both sexes and throughout life facilitates a more reliable incorporation of serum protein values into diagnostic and prognostic patient evaluations. These ranges also set the stage for studying possible differences in population subgroups (e.g., racial or geographic). By employing recommended protocols (62,63), local populations can now be compared more easily and with smaller study groups, since variations by age and sex can be taken into account prior to population comparisons.

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