Reference Distributions for the Positive Acute Phase Proteins, α_1 -Acid Glycoprotein (Orosomucoid), α_1 -Antitrypsin, and Haptoglobin:

A Comparison of a Large Cohort to the World's Literature

Robert F. Ritchie,* Glenn E. Palomaki, Louis M. Neveux, and Olga Navolotskaia

Foundation for Blood Research, Scarborough, Maine

Limiting bedside use of positive acute phase protein measurements (α_1 -acid glycoprotein (orosomucoid), α_1 -antitrypsin, and haptoglobin) has been the lack of satisfactory methods for quantifying serum levels and a credible reference material. Great strides have been made in the last few years. The remaining barrier to 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. Sixty publi-

cations reporting reference intervals have been identified which meet the criteria used in our prior two studies, and these have been analyzed statistically. Previous small studies of these individual proteins agree on average, over their constrained age ranges, with our life-long reference ranges. This meta-analysis provides support for our reference ranges and places them in the perspective of previous publications. *J. Clin. Lab. Anal.* 14:265–270, 2000. ©2000 Wiley-Liss, Inc.

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Inflammation, infection, tissue necrosis, and malignancy can trigger the acute phase response. Diagnostic and prognostic application of these measurements has previously been limited by the uncertainty of the appropriate reference ranges for a given patient. Difficulties are especially evident when attempting to interpret studies for very young or elderly individuals. Significant inflammation may manifest with seemingly normal levels of the acute phase proteins that would be considered high were reference ranges to be available for the appropriate age group. The three proteins examined in this study, α_1 -acid glycoprotein, α_1 -antitrypsin, and haptoglobin, represent the major positive acute phase proteins whose synthesis is up-regulated by the cytokines IL-1 and IL-6, which drive the acute phase response (APR). This study, like its predecessors (1,2), aims to identify publications that provided reference data for these proteins and satisfied minimal acceptance criteria. Published results were normalized against CRM 470/RPPHS (3) before comparison. Sixty publications from the period between 1961 and 1999 were identified and reviewed (4–63).

MATERIALS AND METHODS

Identification of Published Reference Data

Briefly, the methods used to search for relevant publications were the same as for the previous studies (1,2) with the

addition of on-line searching PubMed (<http://www.ncbi.nlm.nih.gov/PubMed>) using the same key word headings as were used for MEDLINE CD-ROM searches (OVID Technologies, New York, NY). The Cumulative Index Medicus published compendia were also manually searched. Methodological papers or those that only examined patients with specific diseases were excluded. Articles were considered acceptable if they (i) identified the assay method and reference material, (ii) provided minimum details of the study population, such as age, and (iii) provided sufficient numerical information for statistical analysis.

Estimating the Central Estimate and Reference Range From Published Studies

The mean, median, or geometric mean was used as the estimate of the center of the distribution as in the previous studies. If they were not directly available, they were computed using observed or smoothed centiles (e.g., between the 2.5th and 97.5th centile). If none of these were available, the study

*Correspondence to: Robert F. Ritchie, M.D., Foundation for Blood Research, U.S. Route 1, P.O. Box 190, Scarborough, Maine 04074.
E-mail: ritchie@fbr.org or palomaki@fbr.org

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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 (1,2). This material (3) 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 labeling the material Reference Preparation for Proteins in Human Serum (RPPHS).

RESULTS

Number of Studies Available for Analysis

A total of 34 publications were identified that contained information on α_1 -acid glycoprotein in apparently healthy individuals (4–6,13–19,21,23,24,27–36,43,44,47,48,51,55,57,59–62). Eleven studies did not include adequate data on either reference material or assay methodology (18,28,34,35,44,47,55,59–62). Five studies did not include the age of the study subjects (16,17,27,29,43), and two had multiple reasons for exclusion (15,33). The remaining 16 studies (4–6,13,14,19,21,23,24,30–32,36,48,51,57) provided acceptable central estimates. Of these, 12 also provided acceptable reference ranges (4–6,19,21,24,30–32,36,48,51).

A total of 27 publications were identified that contained information on α_1 -antitrypsin in apparently healthy individuals (4–6,8,11,13,17,18,20–26,37–41,45,49,51–53,56,63). Four studies did not include adequate data on a reference material or an assay methodology (8,18,39,49). One study did not include information on the age of the study subjects (17), and one had multiple reasons for exclusion (38). The remaining 21 studies (4–6,11,13,20–26,37,40,41,45,51–53,56,63) provided acceptable central estimates. Of these, 17 provided acceptable reference ranges (5,6,11,20–22,24–26,37,40,41,45,51,52,56,63).

A total of 19 publications were identified that contained information on haptoglobin in apparently healthy individuals (4–6,13,16,18,21,23,24,27,37,41,42,44,46,48,51,54,58). Four studies did not include data on a reference material or an assay methodology (18,44,46,54). Two studies did not include age (16,27), and one study had multiple reasons for exclusion (42). The remaining 12 studies (4–6,13,21,23,24,37,41,48,51,58) provided acceptable central estimates. Of these, eight studies provided acceptable reference ranges (5,6,21,24,37,41,51,58).

Fig. 1. Summary of published acute phase protein median reference values. The published central estimates for α_1 -acid glycoprotein (A), α_1 -antitrypsin (B), and haptoglobin (C) levels are displayed on the logarithmic vertical axis (spanning a factor of 10) versus 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 representing >100, 50–99, and 10–49 observations, respectively). The solid (males) and dashed (females) lines are the regressed median levels from our large cohort study (see this issue, p 284–292).

Comparison of the Central Estimates

Figure 1 shows the central estimates of α_1 -acid glycoprotein (A), α_1 -antitrypsin (B), and haptoglobin (C). The reported mean (or median) age of the study population is shown on the logarithmic horizontal axis versus the central estimates on the logarithmic vertical axis, over a several-fold range in values. Each observation is shown as a circle with the smallest and darkest circles representing the more reliable estimates (over 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 (see this issue, p 284–292).

Figure 1A shows the reported α_1 -acid glycoprotein median values from the 16 published studies considered acceptable. Most data are from persons in narrow age ranges. Our median levels for α_1 -acid glycoprotein are in the mid-range of values from a number of published estimates for subjects over age 1 year of age. Our estimates are slightly higher than the published estimates for children under 1 year of age. The estimates of our median α_1 -antitrypsin levels (Fig. 1B) are in the mid-range of published studies for individuals over 20 years of age (64,65). The few published estimates in populations under age 20 tend to be somewhat lower. The estimates of our haptoglobin medians (Fig. 1C) generally agree with the few published observations, especially in individuals less than 3 years of age.

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 eight of 25 studies included more than 100 observations. Thus, verification of the health status of each individual was usually possible, whereas our observed reference ranges were each based on over 27,000 observations where verification was not possible for each individual, although each had been seen by a physician. It is possible that our reference ranges may be wider than other smaller studies because we relied on the diagnosis provided on the laboratory slip (66,67). For that reason, we compared our 95% reference limits with the published limits (see this issue, p 284–292). Figure 2 shows the previous studies after conversion to multiples of the median (e.g., each study's reference limits divided by their own population medians). The publication numbers are shown on the horizontal axis, sorted by decreasing the 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. Figures 2A–C show the analysis for α_1 -acid glycoprotein, α_1 -antitrypsin, and haptoglobin reference ranges, respectively. For

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 material differences between studies. Panels A, B, and C show the analyses for α_1 -acid glycoprotein, α_1 -antitrypsin, and haptoglobin measurements, respectively, on identical logarithmic vertical axes. The horizontal dotted lines display the weighted consensus value at the 2.5th centile and the 97.5th centile for previously published studies. The broken bars represent the observed and predicted reference ranges from our large cohort study (see this issue, p 284–292).

all three analytes, our ranges are similar to the combined consensus estimate from the literature.

Ethnic Group-Specific Reference Ranges

Of the 30 studies included in these analyses, 11 (11,13,22, 26,37,41,45,52,53,56,63) did not provide racial or ethnic information, 16 were mainly Caucasian (4–6,14,21,23–25,30–32,36,40,51,57,58), one consisted mainly of black Africans (48), and two consisted of Asians (19,20). There was insufficient data to determine whether racial or ethnic differences exist.

DISCUSSION

This is the third in a series of meta-analyses for serum proteins reference ranges. Our practical approach applied to large populations produces data comparable to previous smaller studies and can be used to derive reliable reference ranges throughout life, separately for males and females.

Our companion study (see this issue, p 284–292) reports reference data for α_1 -acid glycoprotein, α_1 -antitrypsin, and haptoglobin based on a large data set (over 27,000 observations) with less reliance on the documentation of each individual's health status. The current meta-analysis, like the previous two, shows our reference data to be consistent with these more limited studies indicating that local laboratory ranges may already be close to optimal if the ranges are already calibrated to CRM 470/RPPHS and are only applied to their constrained population as to age and sex.

The availability of a reliable set of reference data for both sexes and throughout life facilitates more reliable incorporation of serum protein values into diagnostic and prognostic patient evaluation. These ranges also set the stage for studying possible differences in population subgroups (e.g., racial or geographic). By employing recommended protocols (68,69) 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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