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Reference Distributions for α_2-Macroglobulin: A Comparison of a Large Cohort to the World's Literature

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The lack of satisfactory methods for quantifying serum levels and a credible reference material has limited bedside use of serum alpha$_2$-macroglobulin (α2M) measurements. 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. A total of 40 publications reporting reference intervals have been identified that meet the criteria used in our prior five studies, and these have been analyzed statistically. On average, previous small studies of these individual proteins agree with our lifelong reference ranges over their constrained age ranges. This meta-analysis provides support for our reference ranges and places them in the perspective of previous publications. J. Clin. Lab. Anal. 18:148–152, 2004.

Key words: serum proteins, alpha$_2$-macroglobulin, meta-analysis, CRM 470/RPPHS

INTRODUCTION

A wide variety of physiological and pathophysiological conditions can change synthetic rates of serum proteins. However, whatever the process, it is usually accompanied by processes that destroy, lose, or sequester an individual species in ways that remove them from the circulation. In other words, increased synthesis can be masked by increased catabolism with the resultant appearance of normal levels. Diagnostic and prognostic application of alpha$_2$-macroglobulin (α2M) measurements has previously been largely limited to assessing the extent of glomerular damage and protein loss in the classical nephrotic syndrome. There has been suggestive evidence during the last two decades that α2M has the potential of predicting early onset diabetic nephropathy, an increasingly prevalent disorder in America, and the hepatorenal syndrome.

The protein examined in this study represents the anti-protease in the highest mass concentration. This study, like its predecessors (1–5), aims to identify publications that provided reference data for this analyte and that satisfy minimal acceptance criteria. Published results were normalized against Certified Reference Material (CRM 470)/Reference Preparation for Proteins in Human Serum (RPPHS) (6) before comparison. A total of 40 publications from the period between 1961 and 2000 were identified and reviewed (7–46).

MATERIAL AND METHODS

Identification of Published Reference Data

The methods used to search for relevant publications were the same as for the previous studies (1–5), with the same key-word headings as were used previously 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 without control groups, were excluded. Articles were considered acceptable if they: 1) identified the assay method and reference material; 2) provided minimum details of the study population such as age and gender; and 3)

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provided sufficient numerical information for statistical analysis.

Estimating the Central Estimate and Reference Range From Published Studies

As in the previous studies, the mean, median, or geometric mean was used to estimate the center of the distribution. 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 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 (1–5). This material (6) was released by the International Federation of Clinical Chemists (IFCC; Milan, Italy), the Community Bureau of Reference (BCR) in Geel, Belgium, and the College of American Pathologists (CAP; Glenview, IL) 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 40 publications were identified that contained information on α2M in apparently healthy individuals (7–46). Five studies did not include adequate data on either reference material or assay methodology. Five studies did not include the age of the study subjects and five additional studies had multiple reasons for exclusion. Twenty-five provided acceptable central estimates. Of these, 23 studies also provided acceptable reference ranges. A total of 19 studies provided acceptable data for both figures.

Comparison of the Central Estimates

Figure 1 shows the reported α2M median values from the 25 published studies considered acceptable, although the estimates vary widely. Most data are from persons in narrow age ranges. Each observation is shown as a circle with the smallest and darkest circles representing the more reliable estimates (over 100 individuals). If a published observation was based on less than 10 cases, it was combined with an adjacent age group from the same publication, if possible. For purposes of comparison, the solid and dashed lines represent the median levels found for males and females, respectively, in our companion study (47). Our median levels for α2M are in the low-range of values from a number of published estimates for subjects 10 year of age and older. The one published estimate in a population under age 10 are very similar to ours (16).

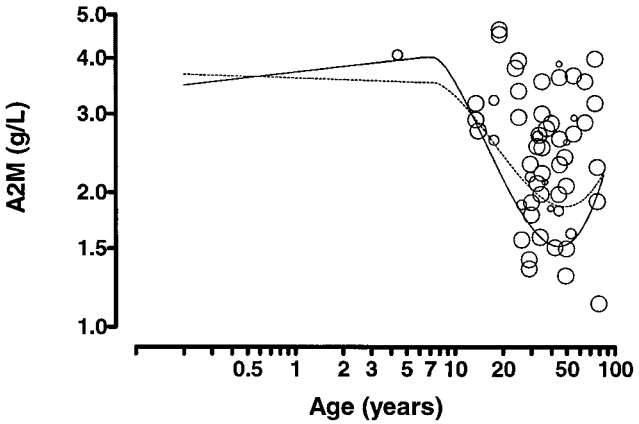

Fig. 1. A summary of published acute phase protein median reference values. The published central estimates for α2M levels are displayed on the logarithmic vertical axis versus the mean (or median) age on the logarithmic horizontal axis. The three symbol sizes represent the number of observations in each group (small, medium, and large circles representing over 100, 50 to 99, and 10 to 49), respectively. The solid (males) and dashed (females) lines are the regressed median levels from our large cohort study (47).

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 five 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 40,420 observations. Although each had been seen by a physician, verification was not possible for each individual. 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. For that reason, we compared our 95% reference limits with those published. Figure 2 shows the previous 23 studies after conversion to multiples of the median (i.e., each study's reference limits divided by their own population medians). The publication numbers are shown on the horizontal axis; sorted by decreasing 95% reference range width. Except for four studies representing a very small range (12,29,36,43), and one with a very broad one (21), the reference intervals are

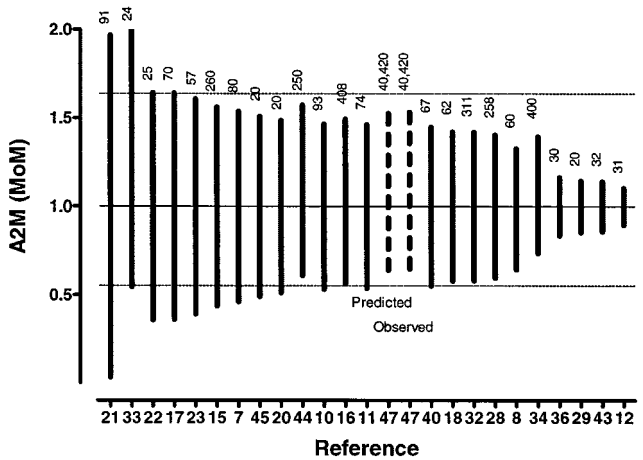

Fig. 2. Reference intervals from published studies. The bars represent the interval between the 2.5th and 97.5th centile for each study with the number of subjects above the bar. The horizontal axis shows the reference number for each study. The selected centiles have been presented as multiples of the median (MoM), compensating for age, gender, and reference material differences between studies. 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 (47).

reasonably consistent. 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. For α2M, our ranges are slightly tighter than the combined consensus estimate from the literature.

Ethnic Group-Specific Reference Ranges

Of the 40 studies included in these analyses, 23 publications provided values on age, sex, and racial or ethnic groups (7,8,10–12,15–18,20–23,28,29,32–34,36, 40,43–45). A total of 16 were in Caucasian, or mainly Caucasians, cohorts One reported separate results in Caucasian and black Africans (21). Two reported on Asians (33,45) and two reported on black Africans (22,36). The remaining 17 did not provide information on their study subjects. There was insufficient data to determine whether racial or ethnic differences existed.

DISCUSSION

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

Our companion study (47) reports reference data for α2M based on a large dataset (40,420 observations) with less reliance on the documentation of each individual's health status. The current meta-analysis, like the previous five, shows our reference interval to be consistent with these more limited studies indicating that local laboratory ranges may already be close to optimal if the ranges are 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 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 (48,49), 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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