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Reference Distributions for the Positive Acute Phase Serum Proteins, α_1-Acid Glycoprotein (Orosomucoid), α_1-Antitrypsin, and Haptoglobin: A Practical, Simple, and Clinically Relevant Approach in a Large Cohort

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Most clinical conditions are accompanied by corresponding changes in serum levels of some, if not all, of the acute phase proteins. While conditions that affect the acute phase proteins are usually inflammatory in nature, non-inflammatory conditions also can cause changes (e.g., malnutrition, some malignancies without secondary inflammation, and genetic polymorphism). Only after the confounding effects of non-inflammatory conditions are taken into account can these measurements be used to detect and stage the inflammatory process and to evaluate the impact of treatment. In this third article in a series, reference ranges for serum levels for three of the acute phase proteins that increase during inflammation are examined: α_1-acid glycoprotein (orosomucoid), α_1-antitrypsin, and haptoglobin. The study is based on a cohort of 55,199 Caucasian individuals from northern New England, tested in our laboratory between 1994 and 1999. Measurements were standardized against CRM 470 (RPPHS) and analyzed using a previously described statistical approach. Individuals with unequivocal laboratory evidence of inflammation (C-reactive protein of 10 mg/l or higher) were excluded. Levels of α_1-acid glycoprotein changed little during life and between the sexes. Levels of α_1-antitrypsin varied somewhat by age, rising slightly beyond age 55; males followed a pattern similar to that for females. For this protein, it was necessary to apply two equations to describe the lower levels associated with certain phenotypes. Haptoglobin levels fell significantly during the first decade of life for both males and females and climbed thereafter. Males and females displayed a similar pattern. When values were expressed as multiples of the age- and gender-specific median levels, the resulting distributions fitted a log–Gaussian distribution well over a broad range. When patient data are normalized in this manner, the distribution parameters can be used to assign a centile corresponding to an individual's measurement, thus simplifying interpretation. J. Clin. Lab. Anal. 14:284–292, 2000.

Key words: acute phase proteins; reference range; reference material; Caucasian; α_1-acid glycoprotein; α_1-antitrypsin; haptoglobin; orosomucoid

The traditional acute phase response is a physiological reaction to injury, foreign bodies, or endotoxin. It is a regulatory response critical to the body's effort to maintain or re-establish homeostasis. These effects are modulated by the production of multifunctional cytokines, which are responsible for the up- or down-regulation of synthesis of this hepatocyte-derived family of proteins.

The primary cytokines (interleukin-1, interleukin-6, and, to a lesser extent, tumor necrosis factor) control the hepatic synthesis of the acute phase proteins. Levels of α_1-acid glycoprotein (estrogen transport), α_1-antitrypsin (proteinase inhibition), and haptoglobin (hemoglobin binding) increase within hours of insult. Conversely, haptoglobin levels can fall to zero within a few minutes of the release of hemoglobin from damaged red cells into the circulation (e.g., extracorporeal oxygenation, crush injury and impact sports, or administration of outdated blood). Precise measurement of these proteins in serum plays an increasingly important role in the clinical evaluation of the pathophysiologic process.

Wide use of high-quality analytical instruments together with the release of a reliable reference material (CRM470/RPPHS (1)) now provides clinicians with accurate labora-

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tory measurements. Hampering the diagnostic and prognostic utility of serum protein measurements is the lack of reliable age- and gender-specific reference data. Although frequently addressed in the literature, the issue of satisfactory reference ranges for the acute phase proteins has not been accomplished. Most studies do not meet the criteria of a large number of cases, appropriate laboratory methods and standardization, and health assessment of relevant study subjects.

The work described here complements our previous studies on the negative acute phase proteins (2) and the immunoglobulins (3). The population is primarily composed of Caucasian individuals who have entered the health care system. A companion paper (see this issue p 265–270) presents a meta-analysis of these same three proteins covering the time period from 1962 to 1999 and places the current findings in that context.

MATERIALS AND METHODS

Serum Protein Reagents and Instrumentation

Antisera were obtained from several sources (Atlantic Antibodies, Scarborough, ME; INCSTAR, Stillwater, MN; and Midland Bioproducts, Scarborough, ME), after assurance that the materials were sufficiently specific, potent, and stable (2). Each new batch of antiserum was compared to previous batches for value recovery and proportionality in actual assays (4). A clarified, delipidated, commercially available serum calibrant was used during the study (INCSTAR, Stillwater, MN). Values for this pool were obtained from the International Reference Preparation for Proteins in Human Serum (RPPHS) (Certified Reference Material 470) (1). The basic reference material is obtainable from either the Bureau of Community Reference in Brussels or the College of American Pathologists (Chicago, IL). Buffers, other reagents, and the analytic instruments were identical.

Selection of Patient Results

Coding of Diagnosis/Symptom Strings

Overall, the great majority of samples had an associated diagnosis or symptom provided by the referring physician. Because these alphanumeric strings varied greatly (over 28,000 unique alphabetic strings), they were classified into 93 groups by a clinician experienced in the interpretation of serum protein measurements (RFR). Diagnostic groups with codes between 101 and 165 contained individuals with diagnoses that were expected to have a large impact on serum protein levels (e.g., multiple myeloma, cirrhosis, hepatitis, infection, lung disease, leukemia, renal failure, immunodeficiency, or thyroid disease), while diagnostic groups 166 through 195 contained individuals with conditions expected to have minimal or no effect on serum protein levels (e.g., allergies, hypertension, headache, neck pain, neuropathy, syncope, seizures, fatigue, or depression). Code 100 indicated that no diagnosis was provided. If more than one diagnosis code was present, the lowest (most severe) was used. The diagnostic groups 166 and above (with the exception of 170, pregnancy) were designated as the preliminary reference population.

Refinement of the Reference Population

The methodologies for selecting referent individuals and computing the reference ranges have been described in detail elsewhere and above (2,3,5). However, in brief, determining which of the diagnostic groups would contribute to the final reference population required the use of a published method that employs symmetric trimming of values prior to calculating means and standard deviations (5). In this way outliers were identified among the diagnostic group parameters associated with codes of 166 and higher. A logarithmic transformation of the variance corrected for the non-gaussian right skewness of the observed data. The resulting trimmed mean value $\pm$ 1.96 standard deviations (for both the median and the log variance) defined limits of acceptability. Measurements from any diagnostic group falling within the limits were considered reference values, and those falling outside were not.

The current data set is similar to the previous one (2) with the exception that the current cohort was for a shorter period, namely, September 1994 through September 1999. Because commercially available calibrants for α_1-antitrypsin fluctuated unacceptably prior to the introduction of CRM 470, data collected before 1994 were omitted and a new later subset was added. After exclusion of patient records due to duplication (and other reasons (2)), 55,199 were available for study. In addition to the statistical selection process used previously (5), we used a statistical model (6) to describe the phenotypic subpopulation of α_1-antitrypsin as evaluated by electrophoretic Pi typing.

α_1-Antitrypsin is affected by two commonplace circumstances: increased estrogenic drive (7–13) and the genetic make-up of the individual. The circulating protein can be found in at least 75 different isoforms, four of which can depress circulating levels significantly (9,10). In order to model the expected distribution of α_1-antitrypsin values in a population with varying rates of phenotypic expression, the following four groups were computer generated using parameters consistent with our local population. The first represents that genotype with a synthetic rate that is considered normal (PI*MM, 100%), making up 93.3% of our population. The second group, occurring in 3.5% of the population, has moderately reduced rates (averaging 80% of normal for PI*MS, 60% for PI*MZ, and several other phenotypes of synthesis of α_1-antitrypsin). The third group comprising 3.1% of the population produces only about 40% of normal (PI*SZ). The last group is rare, about 0.1% or less, and produces very low levels of the protein (20% or less, for PI*ZZ and other very rare genotypes, PI*Snull, PI*Znull, and PI*null/null). Under the assumption that population variances for these groups are

similar, random measurements from these four groups were taken proportionately to simulate the expected α_1-antitrypsin population distribution and used as a comparison to our observed distribution (in multiples of the median (MoM)).

RESULTS

Selection of Diagnostic Groups to Form the Reference Population

After preliminary adjustment for age and gender, the median α_1-acid glycoprotein levels were plotted versus the variance for each of the 85 diagnostic code groups (2) with at least 20 observations (Fig. 1). The circles indicate the preliminary reference population (codes 166 and above) comprising relatively benign diagnoses that are not expected to have much impact of serum protein levels. The squares indicate the diagnostic groups with codes 165 and below, where major protein effects are expected. The rectangle is drawn at the trimmed mean ± 1.96 standard deviations for both the median levels (horizontal axis) and log variances (vertical axis). Overall, the median and log variances for 64 of the 85 groups lie within the rectangle. Results from individuals within these 64 groups compose the referent population for α_1-acid glycoprotein. Individuals from the 21 diagnostic groups represented by filled circles or squares were not included as part of the α_1-acid glycoprotein reference population. This analysis was repeated for α_1-antitrypsin and haptoglobin, resulting in the inclusion of 60 and 51 diagnostic groups, respectively (figures available upon request).

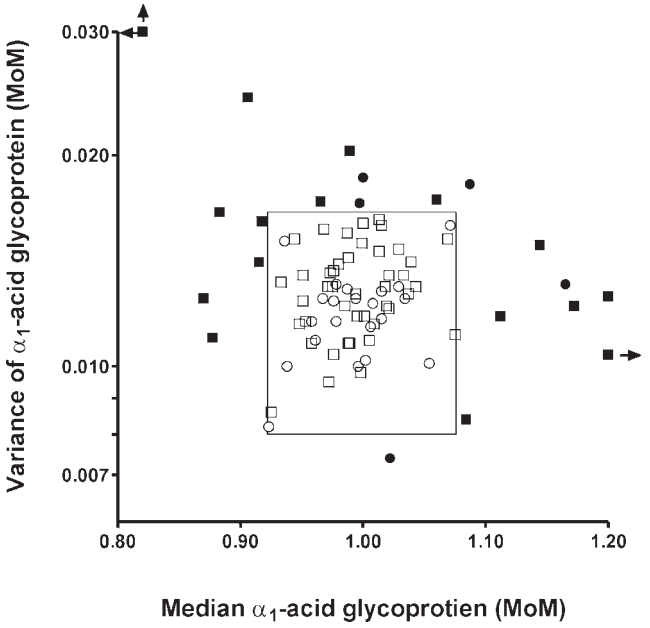

Fig. 1. Scatterplot of α_1-acid glycoprotein median levels versus variance for the diagnostic categories after preliminary adjustment for age and gender. Diagnostic codes 100 through 165 are displayed as squares (□, ■), while codes 166 and above are circles (○, ●). The lower the diagnostic group number, the more severe the illness or complaint. The rectangle represents the 95% confidence intervals (after trimming) of the median (horizontal axis) and variance (logarithmic vertical axis) for the diagnostic categories 166 and above. Open symbols (○, □) represent those categories whose values comprise the reference population. Closed symbols (●, ■) represent categories not included in reference ranges.

Computation of Age- and Gender-Specific Median Values and Selected Centiles

There were 29,673, 30,596, and 27,382 referent individuals available after the above selection process to calculate reference ranges for α_1-acid glycoprotein, α_1-antitrypsin, and haptoglobin, respectively. Figure 2 shows selected α_1-acid glycoprotein centiles for males (Fig. 2A) and females (Fig. 2B). The logarithmic horizontal axis shows the mean age for each interval, while the logarithmic vertical axis shows the median α_1-acid glycoprotein value (filled circles) along with the 5th and 95th centiles (lower and upper open circles, respectively). The observed centiles for individuals under age 10,

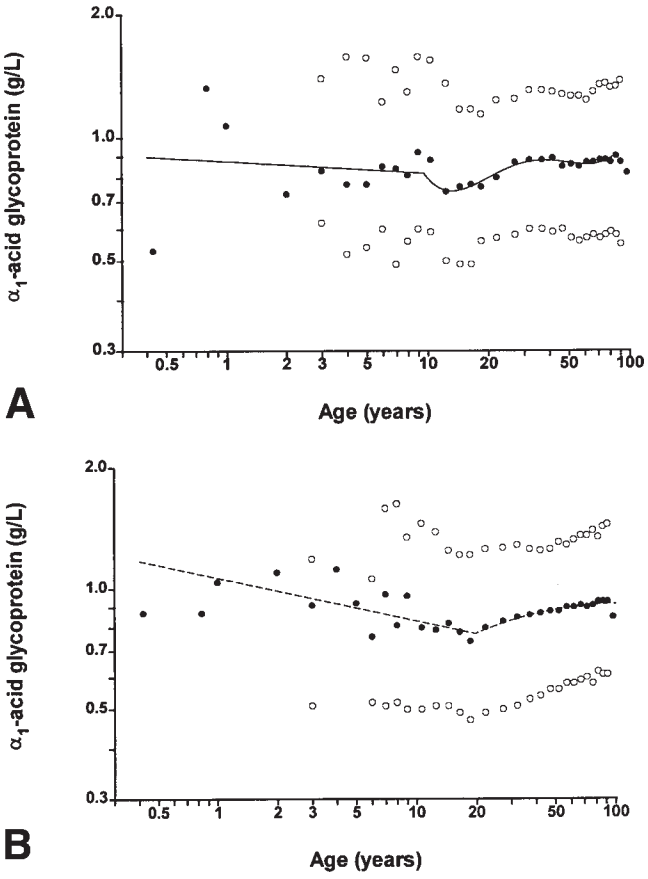

Fig. 2. α_1-Acid glycoprotein centiles and medians versus age. The individual's age is displayed on the horizontal logarithmic axis versus the α_1-acid glycoprotein level on the vertical logarithmic axis. The closed circles (●) represent the observed median level; the lower and upper open circles (○) represent the observed 5th and 95th centiles respectively. The lines represent the predicted median values. Panels **A** (upper) and **B** (lower) show the α_1-acid glycoprotein results for males and females, respectively.

10–30, 30–80, and over 80 years old are based on about 25, 70–500, 300–1000, and less than 300 observations for males, and about 25, 100–1500, 800–2,400, and less than 400 observations for females, respectively. The solid line indicates the fitted median levels for α_1-acid glycoprotein in males, and the dashed line indicates the fitted medians for females. Results from patients younger than 1 month or older than 85 years were not included in the regression analysis. Figures 3 and 4 display similar data for α_1-antitrypsin and haptoglobin, respectively. For all three analytes, a straight line fits the median levels in both younger males and females. The pattern becomes more complicated in older individuals, reflecting physiologic maturation and aging, similar to data for other parameters, such as anthropomorphic features and more familiar biochemical measurements.

There is less than a 10% difference in the α_1-acid glycoprotein median levels between males and females through life, and those levels vary only slightly by age. The α_1-antitrypsin values change little during the first decade of life. Haptoglobin levels for both males and females drop by a factor of 2 during the first decade of life and nearly double by age 80. Table 1 contains the gender-specific regression equations for α_1-acid glycoprotein, α_1-antitrypsin, and haptoglobin median values along with the age range over which each equation is valid. A sample calculation can be found as a footnote to Table 1.

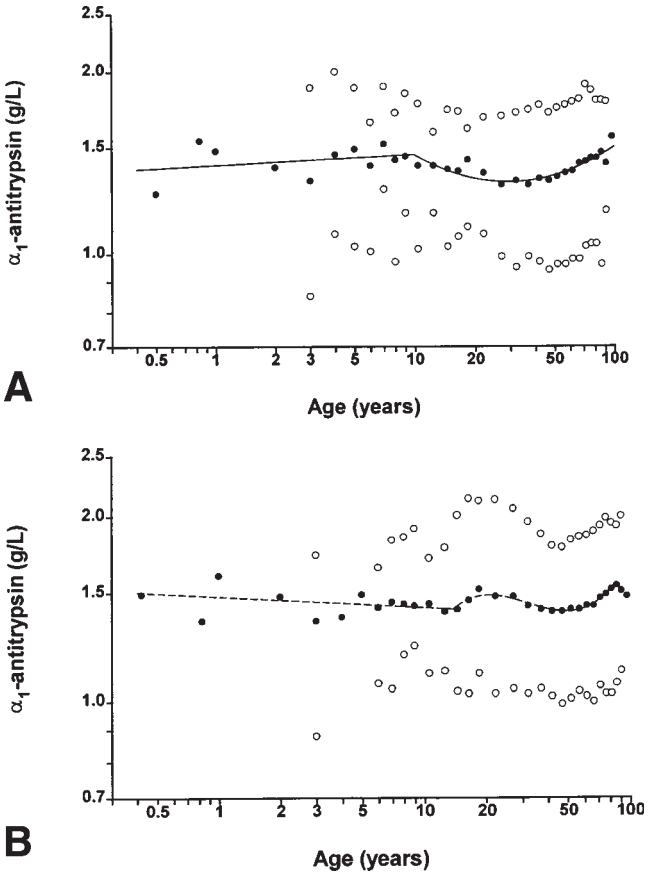

Fig. 3. α_1-Antitrypsin centiles and medians versus age. The data are presented in the same symbol format as Fig. 2A (upper) and 2B (lower) and show α_1-antitrypsin results for males and females, respectively.

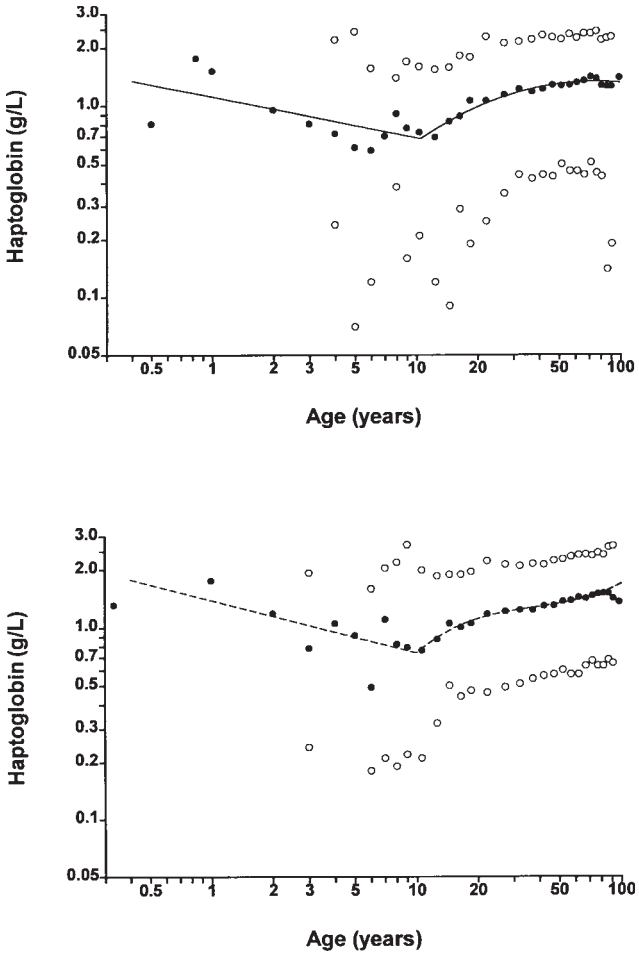

Fig. 4. Haptoglobin centiles and medians versus age. The data are presented in the same symbol format as Fig. 2A (upper) and 2B (lower) and show haptoglobin results for males and females, respectively.

For α_1-acid glycoprotein, the distance from the median to the 95th centile and that from the median to the 5th centile are approximately equal across the entire age range (Fig. 2). This indicates that the distribution of α_1-acid glycoprotein is symmetric after a logarithmic transformation and that the variance of the distribution does not change appreciably by age. For α_1-antitrypsin (Fig. 3) and haptoglobin (Fig. 4) measurements, the 5th centile is farther below the median than the 95th centile is above, indicating asymmetry. This asymmetry may be due to genetic variations for α_1-antitrypsin and undeclared bruising or physical hemolysis in a relatively large fraction of the population, leading to lower than haptoglobin expected levels. In addition, the interval width between the 5th and 95th centiles appears to be broader in young children compared to teenagers and adults for both α_1-antitrypsin and haptoglobin.

TABLE 1. Regression models and coefficients for media α_1-acid glycoprotein (AGP), α_1-antitrypsin (A1AT), and haptoglobin (HPT) measures by age and gender

		Age range (years)		Constant	Coefficients[1]			
Analyte	Sex	From	To	A0	A1	A2	A3	A4
AGP	Males	0.5	9	–0.057731	–0.029216	0	0	0
		10	85	11.4526	–32.8168	34.1405	–15.4503	2.57609
	Females	0.5	19	0.027757	–0.108020	0	0	0
		20	85	–0.630965	0.592427	–0.148026	0	0
A1AT[2]	Males	0.5	9	0.146821	0.017497	0	0	0
		10	85	0.559442	–0.600308	0.204274	0	0
	Females	0.5	13	0.169880	–0.0178556	0	0	0
		14	85	–3.09815	6.70620	–4.52758	1.00304	0
HPT	Males	0.5	10	0.047023	–0.211539	0	0	0
		11	85	–1.29116	1.50695	–0.400771	0	0
	Females	0.5	9	0.140607	–0.272984	0	0	0
		10	85	–3.09463	5.77852	–3.56934	0.755173	0

[1]The general form of the equation is Median = $10^{(A0 + A1\ \log(age) + A2\ \log(age)\hat{}2 + A3\ \log(age)\hat{}3 + 4A\ \log(age)\hat{}4)}$

[2]The medium α_1-antitrypsin level for a 20-year-old female is $10^{(-3.09815 + 6.7062\ \log(20) - 4.52758\ \log(20)\ \log(20) + 1.00304\ \log(20)\ \log(20)\ \log(20))}$ or 1.49.

Fitting the Reference Measurements Expressed in Multiples of the Median (MoM) to a Population Distribution

Each individual reference measurement (in mass units) was then converted to a multiple of its age- and gender-specific median value. Figure 5A–C shows probability plots composed of approximately 2,000 normalized referent measurements for α_1-acid glycoprotein, α_1-antitrypsin, and haptoglobin (expressed in MoM), respectively. The samples were randomly selected to equally represent each year of age. α_1-Acid glycoprotein measurements (Fig. 5A) fitted the distribution well between the 1st and 99th centiles (logarithmic mean and standard deviation of 0.0000 and 0.1155, respectively).

Measurements of α_1-antitrypsin (Fig. 5B) fitted well between the 10th and 99th centiles (logarithmic mean and standard deviation of 0.0000 and 0.0689, respectively). The poor fit at lower centiles is likely due to the effect of genetic variation in the amount of protein released by otherwise normal hepatocytes. The synthetic capacity of the various alleles has been well described (9). A data transformation, in the form of log(MoM + C), was attempted to straighten the distribution but was unsuccessful. In order to better fit the data below the 10th centile, a second line was fitted below a MoM level of 0.80 (logarithmic mean and standard deviation of 0.1248 and 0.1689, respectively). The shape of this probability plot (Fig. 5B) is nearly identical to that of a simulated distribution composed of weighted genotypic subpopulations for α_1-antitrypsin described in the methods. Simplifying the interpretation of this data is the fact that few conditions other than genetic depression of synthesis result in depressed α_1-antitrypsin levels, especially in the apparently healthy. The exceptions in pathology are associated with severe protein-losing states such as the nephrotic syndrome and protein-losing enteropathy.

Haptoglobin measurements (Fig. 5C) fitted well between the 1st and 99th centiles (logarithmic mean and standard deviation of 0.3010 and 0.0904, respectively) after the addition of a constant value of 1.00. The addition of a constant was necessary because of a poor fit below the 30th centile that was still evident even after a simple logarithmic transformation (data not shown). The logarithmic mean of MoM distributions in apparently healthy individuals is usually 0.0000 (log of 1.00). Since a constant value of 1.00 is added to each haptoglobin MoM value, the logarithmic mean for this distribution is 0.3010 (i.e., the logarithm of 2.00). The following is a sample calculation for the 5th centile of haptoglobin. The 5th centile is 1.645 standard deviations below the mean value. Given the population mean and standard deviation values provided above, the 5th centile for haptoglobin is 0.42 MoM ($10^{[(-1.645 \times 0.0904) + 0.3010]} - 1$). This value can then be multiplied by the age- and gender-specific median value to express the 5th centile in mass units for any given individual.

When this analysis was restricted to individuals less than 10 years of age or over 85 years of age, the logarithmic means were similar to the overall values (within 0.032) for all three proteins. The log standard deviations of the older groups were reasonably consistent with the consensus values (all three within 7%). For the youngest age group, however, all three protein distributions were broader with constant increases in the standard deviations of 16%, 32%, and 56%, respectively. The increase in standard deviation was so large for haptoglobin measurements that a second standard deviation of 0.1409 was used to estimate centiles for children 12 and under rather than the consensus standard deviation presented above. Therefore, the 5th centile of haptoglobin for children age 12 and younger would be 0.17 MoM ($10^{[-1.645 \times 0.1409 + 0.3010]} - 1$) rather than the 0.42 MoM for used for adults.

Predicting Age- and Gender-Specific Centiles

Within this range of centiles fitted above, patient values can be assigned an age and gender-specific centile. Table 2

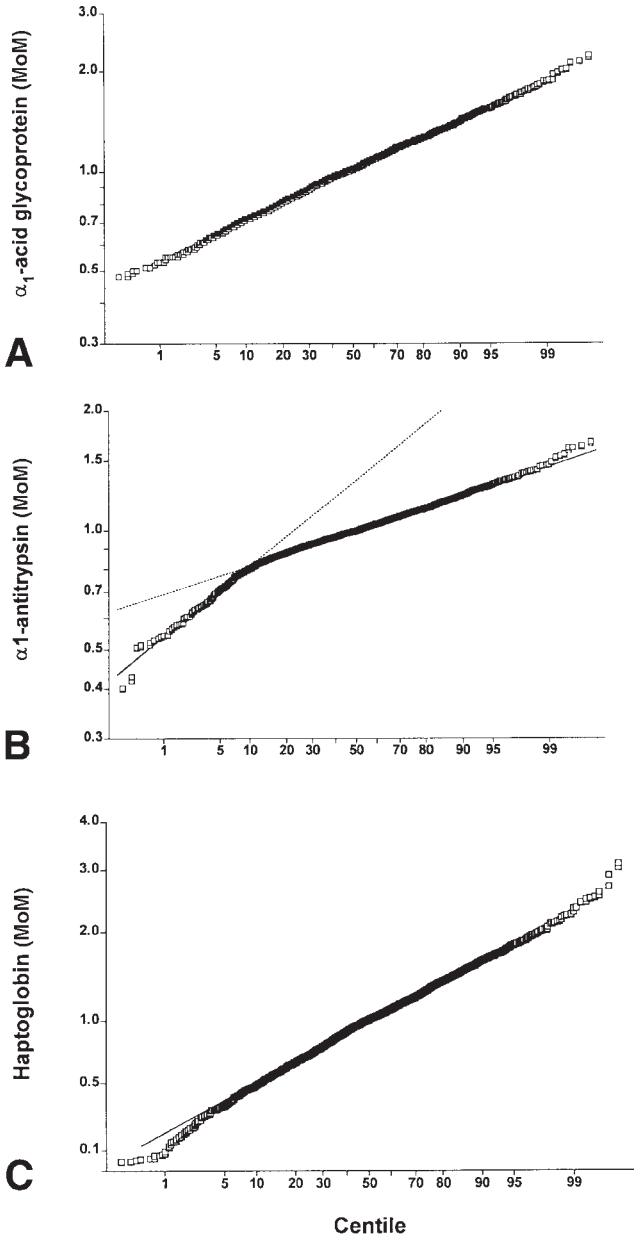

Fig. 5. Probability plots of positive acute phase protein measurements expressed as multiples of the median (MoM). Approximately 2,000 observed MoM values are plotted vertically on a logarithmic axis and horizontally on the Gaussian centile scale. The latter is based on the rank of the observation. If the points fit a straight line, then the distribution is log–Gaussian. Panels A (top), B (middle), and C (lower) show results for α_1-acid glycoprotein, α_1-antitrypsin, and haptoglobin, respectively. The thin line represents the fitted Gaussian means and standard deviations contained in the text. The transformation used for haptoglobin (C) includes the addition of a constant value of 1.0 prior to transformation (see text).

contains the predicted 2.5th, 50th, and 97.5th centiles for selected age and gender categories using the log–Gaussian parameters described above. As a further example, consider an α_1-acid glycoprotein measurement of 1.30 g/l. If this value were to be reported for a 40-year-old male, it would be considered relatively normal (multiple of the median of 0.98 [1.30/1.32] and would be assigned the 45th centile [*z* score of –0.13 ((log(0.98) – log(1.00))/0.0689)]. However, if the value were from a 20-year-old female, the interpretation would be quite different (0.87 multiples of the median [1.30/1.49] and would be assigned the 19th centile [*z* score of –0.88 ((log(0.87) – log(1.00))/0.0689)].

Verification of Results Over the Course of the Study

In order to check the consistency of results during this study, a median MoM was computed for each of the 20 calendar quarters of the study period. The median MoM level was reasonably consistent over time for α_1-acid glycoprotein (1.00 ± 0.08), α_1-antitrypsin (1.00 ± 0.03), and haptoglobin (1.00 ± 0.08). With about 2,000 observations available for each quarter, this variability is highly significant but likely represents the best control over pre- and intra-analytical assay conditions that can be achieved in routine practice. The median age of patients remained relatively constant at 49 years of age while the percentage of males remained unchanged at 31%.

DISCUSSION

The importance of assessing the acute phase reaction in a wide range of conditions has been emphasized in recent publications (14–17). With serum protein technology measurement at a high level of precision and accuracy (18), a reliable reference material in wide use (1), and the availability of age- and gender-specific reference ranges, conditions are right for more effective use of these protein measurements in diagnostic medicine.

This study is a sequel to the development of age- and gender-specific reference ranges for the three major immunoglobulins and the negative acute phase proteins from a single laboratory (2,3) in a large cohort. As in the previous study of the negative acute phase proteins (2), all referent individuals with a C-reactive protein value of 10 mg/l or higher were removed from analysis, thereby excluding results from individuals with the most overt evidence for inflammation. The acute phase response is often an unrecognized, ubiquitous condition, representing a normal, curative process for the repair of tissue damage due to injury, necrosis, or microbial invasion. Commonplace conditions are likely to stimulate the acute phase response, but the response may not reach levels clinically evident at the time of physician visit. However, in the laboratory more sensitive indicators can be measured inexpensively. Recent publications also have shown that even relatively minor changes in levels of the acute phase proteins are harbingers of significant disease (14–17). However, other conditions also may alter the serum levels of the acute phase proteins; for example, the confounding effects of nonsteroi-

TABLE 2. Predicted acid glycoprotein (AGP), α_1-antitrypsin (A1AT), and haptoglobin (HPT) medians and selected centiles stratified by age and gender

Decimal age (years)	AGP (g/l)			A1AT (g/l)			HPT (g/l)		
	2.5th	50th	97.5th	2.5th	50th	97.5th	2.5th	50th	97.5th
Males									
1.0	0.52	0.88	1.47	0.87	1.40	1.91	0.07	1.11	3.10
4.0	0.50	0.84	1.42	0.89	1.44	1.96	0.05	0.83	2.31
7.0	0.49	0.83	1.39	0.90	1.45	1.98	0.04	0.74	2.05
10.0	0.47	0.80	1.34	0.91	1.46	1.99	0.04	0.68	1.90
1.40	0.44	0.74	1.25	0.86	1.38	1.88	0.27	0.81	1.63
18.0	0.46	0.78	1.31	0.83	1.34	1.83	0.31	0.93	1.87
20.0	0.47	0.80	1.34	0.83	1.33	1.82	0.32	0.98	1.97
30.0	0.51	0.87	1.46	0.82	1.31	1.79	0.38	1.15	2.31
40.0	0.52	0.88	1.48	0.82	1.32	1.81	0.41	1.24	2.50
50.0	0.52	0.87	1.46	0.84	1.35	1.84	0.43	1.30	2.60
60.0	0.51	0.86	1.45	0.85	1.37	1.87	0.44	1.32	2.65
70.0	0.51	0.86	1.45	0.87	1.40	1.92	0.44	1.33	2.68
80.0	0.53	0.89	1.49	0.89	1.43	1.96	0.44	1.33	2.68
Females									
1.0	0.63	1.07	1.80	0.92	1.48	2.02	0.08	1.38	3.84
4.0	0.54	0.92	1.55	0.90	1.44	1.97	0.06	0.95	2.63
7.0	0.51	0.86	1.45	0.89	1.43	1.95	0.05	0.81	2.26
10.0	0.49	0.83	1.40	0.88	1.42	1.94	0.04	0.74	2.05
14.0	0.48	0.80	1.35	0.88	1.41	1.93	0.31	0.95	1.90
18.0	0.46	0.78	1.31	0.92	1.48	2.02	0.35	1.07	2.14
20.0	0.46	0.77	1.31	0.92	1.49	2.03	0.37	1.11	2.23
30.0	0.50	0.83	1.40	0.90	1.45	1.98	0.40	1.22	2.45
40.0	0.52	0.87	1.46	0.88	1.41	1.93	0.42	1.28	2.57
50.0	0.53	0.89	1.50	0.87	1.40	1.91	0.44	1.33	2.67
60.0	0.53	0.90	1.52	0.88	1.41	1.93	0.46	1.38	2.77
70.0	0.54	0.91	1.53	0.90	1.45	1.98	0.48	1.44	2.90
80.0	0.54	0.91	1.54	0.94	1.51	2.07	0.50	1.51	3.04

dal anti-inflammatory medications on serum protein levels have not been examined extensively.

α_1-Acid glycoprotein is reportedly affected by hormonal changes, which again are part of everyday life for females (19). It has been shown to change little over time and between the sexes, with the exception of those conditions that are accompanied by an inflammatory drive. It is similar to C-reactive protein in this regard and the effect of anti-inflammatory modulation of serum α_1-acid glycoprotein levels will need to be studied. Despite the fact that C-reactive protein ranges over 1000-fold range while α_1-acid glycoprotein varies only 4-fold, α_1-acid glycoprotein can provide an extremely valuable back-up to C-reactive protein analysis because of its constancy over age and between the sexes.

Haptoglobin levels are extremely sensitive to the release of hemoglobin into the circulation. To place this in perspective, the hemoglobin content of less than 5 ml of whole blood will remove all circulating haptoglobin from the intravascular space within minutes. The release of smaller amounts can be the result of minor ecchymoses from trauma, common in the young, and from bruises that are so frequent even in healthy elderly persons, hence reduced levels are an everyday occurrence. Further, impact sports such as jogging, boxing, handball, and even playing certain musical instruments can be responsible for red cell damage (20). With a population that is increasingly physically active, it is not surprising that degrees of clinically insignificant hemolysis from mechanical red blood cell damage will manifest as lower than expected levels of the protein. Investigators in the past have presented concern that the three phenotypes of haptoglobin, being somewhat different in constituent units, may actually yield different values dependent on phenotype (21). A detailed study of the effect of phenotype on haptoglobin measurement (22) as well as additional characteristics of the assay concluded that when compared to the more common phenotype 2-1, phenotype 1-1 will be overestimated approximately 30%. While there may be measurable differences as the result of constituent make-up, the final result has little or no impact on reference ranges for each phenotype and, as a result, has little effect on the clinical implications (23).

The positive acute phase proteins α_1-acid glycoprotein, α_1-antitrypsin, and haptoglobin, while more dramatic in their response to acute inflammation than the negative acute phase proteins (albumin, transferrin, and transthyretin), are nevertheless less responsive than C-reactive protein. C-reactive protein and serum amyloid A protein have clinically measurable ranges that span more than 3 orders of magnitude while the other acute phase proteins vary less than 1 order of magnitude. Specifically, measurement of α_1-antitrypsin, whose

values rarely increase more than 4-fold, can be used as an indicator of risk from pulmonary and neonatal liver disease, if they are discordantly very low in comparison to other acute phase proteins. This can be done without the knowledge of the inflammatory status of the subject. In addition to up-regulation by inflammatory drive, increases in estrogen levels after puberty, during pregnancy, or in conjunction with contraceptive medication use can significantly increase circulating levels (7–13). (See Fig. 3B for regulatory impact at about the time of puberty through the reproductive years.) α_1-Acid glycoprotein, although less responsive than C-reactive protein, appears to increase soon after the onset of inflammation and can be a cost-effective means of confirming and monitoring the progress of an acute phase response. Haptoglobin levels represent a dynamic balance between normal synthetic rate, inflammatory drive, and removal from the circulation as the result of the release of and combination with free hemoglobin. Synthetic levels of haptoglobin may be increased over normal, whereas because of minor hemoglobin release from bruising or effete red blood cells, serum levels may actually be near zero. Levels of these three acute phase proteins have been shown not to be affected by the administration of anti-inflammatory medications, including over-the-counter products (24,25), whereas C-reactive protein is suspected of doing so. To ensure sensitive detection of this confounding factor, all individuals found to have an acute phase response (as indicated by C-reactive protein levels over 10 mg/l) have been eliminated from consideration in this study.

The deviation from expected seen for α_1-antitrypsin in Fig. 5B could be viewed as indication that pathology exists in these subjects. However, in this situation, while the data may deviate from an expected set, it represents an everyday circumstance that cannot be corroborated without additional expense. For α_1-antitrypsin, clinical concern is for the small number of very low values (less than 0.4 MoM). Because these are both positive acute phase proteins, the principle clinical focus is on those cases that deviate at the higher centiles. Finding the small number of cases that deviate in the lower centiles, while important, constitutes unexpected additional information often not relevant to the reason for seeking medical help. The more detailed calculation for those cases below the 10^{th} centile further reduces the number of cases requiring additional study. In the case of haptoglobin, it is not the intermediately low values that are of clinical concern but rather the very low levels found in the absence of clinical history of sports activity that could explain the finding without additional expense.

Our proposed method of converting laboratory results to multiple of the age- and gender-specific medians (MoM) has several advantages. Conversion permits each analyte to fit a logarithmic Gaussian distribution reasonably well, allowing each MoM level to be assigned a centile. Thus, a laboratory measurement can be reported not only in mass units but also, through conversion to MoM, as the associated centile based on that individual's age and sex. This process greatly simplifies the interpretation of these serum protein values.

This study is of sufficient size to reliably estimate the effects of age and gender in a Caucasian population and to describe values harmonized with an accepted reference material and measured by a single protocol. Because the current study is derived from a relatively homogeneous Caucasian population, the findings cannot be applied to other ethnic groups with certainty. Individual usage of tobacco products, over-the-counter anti-inflammatory medications, contraceptives, or hormone supplementation was not taken into account. Since the half-life of these drugs is short, only carefully designed studies could take their usage into account. As shown in our companion paper (see this issue p 265–270) and in previous papers, our data compare favorably with previous small studies, which employed guidelines defining health status (26,27).

CONCLUSION

A large cohort of Caucasian individuals with routine serum measurements performed at a single laboratory has been examined to determine the reference distributions for α_1-acid glycoprotein, α_1-antitrypsin, and haptoglobin in persons who have no apparent active inflammation. The values are consistent with a review of much smaller studies in the world's literature, which often verify the health status of each participant (26,27). For the first time, reference ranges for these proteins have been described over life and between the sexes. For two of these analytes, both age and gender are important covariates to the normal ranges and necessitate separate reference ranges; α_1-acid glycoprotein is the exception showing only small variation. Converting the analyte measurements to multiples of the age- and gender-specific medians simplifies interpretation and enhances the clinical utility of their measurements. After verification of the appropriateness of medians presented in the current study, the predicted values should be considered for use by laboratorians or clinicians making interpretations of α_1-acid glycoprotein, α_1-antitrypsin, and haptoglobin.

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