Reference Distributions for Complement Proteins C3 and C4: A Practical, Simple and Clinically Relevant Approach in a Large Cohort

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The two serum proteins of the complement cascade in the highest concentrations, C3 and C4, respond to various conditions in much the same manner as do other positive acute-phase proteins. A major difference is that they are relatively sluggish in response to cytokine drive, requiring several days rather than hours to be detectably elevated by serial measurements. As with other acute-phase proteins, there are many processes that up- or down-regulate synthesis, including infection or inflammation, hepatic failure, and immune-complex formation. Clinicians may find it difficult to distinguish among these processes, because they often occur simultaneously. The situation is further complicated by genetic polymorphism, with rare instances of markedly reduced synthesis and circulating levels, and consequent vulnerability to infection. C3 and C4 are measured for clinical purposes to help define certain rheumatic and immunologically mediated renal diseases. Interpreting the measured blood levels of these two components requires one to consider the intensity of the inflammatory drive, the timing of the suspected clinical process, the production of complement-consuming immune complexes, and the possible existence of benign circumstances. In this fifth

article in a series, reference ranges for serum levels of two complement proteins (C3 and C4) are examined. The study is based on a cohort of over 55,000 Caucasian individuals from northern New England, who were tested in our laboratory in 1994–1999. Measurements were standardized against certified reference material (CRM) 470/reference preparation for proteins in human serum (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. Our results show that the levels of C3 and C4 change little during life and between the sexes, except that they increase slightly and then fall after age 20 in males and at about age 45 in females. 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.* 18:1–8, 2004.

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INTRODUCTION

In vivo immunological events are frequently accompanied by reactions involving complement proteins. As with other positive acute-phase proteins, inflammation or infection stimulates cytokine production, thus up-regulating complement synthesis. However, circulating levels decrease as immunoglobulins form complexes with antigen (autologous or foreign) and complement. These complexes are subsequently removed from the

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circulation. Immune-complex deposition in tissues initiates variable degrees of inflammation, which in turn stimulates increased hepatocyte synthesis. The net result may be apparently normal circulating levels of C3 and C4 in the face of unequivocal immune-complex-mediated disease. This push-pull reaction can be viewed as the body's effort to provide the necessary elements for immunological homeostasis.

The primary cytokines (interleukin-1, interleukin-6, and, to a lesser extent, tumor necrosis factor) control the hepatic synthesis of the acute-phase proteins. Synthesis of C3 and C4 increases within days of the insult, in contrast to more traditional acute-phase proteins, such as C-reactive protein, orosomucoid, and α_1 -antitrypsin, which respond in less than 24 hr. As already noted, complement levels can fall to very low levels within a few hours due to the development of immune complexes. Apart from cytokine stimulation, the serum concentration of C3 is affected by three commonplace circumstances: increased estrogenic drive (1–6), immune-complex deposition, and the genetic make-up of the individual. The circulating protein can be found in at least five different isoforms, only one of which depresses circulating levels significantly (7). Circulating levels of C4 are measured by immunoturbidometric methods. C4 levels can drop significantly during activation of the alternative complement pathway. Genetic deficiencies are known where no C4 is measurable.

High-quality analytical instruments and a reliable reference material (certified reference material (CRM) 470/reference preparation for proteins in human serum (RPPHS) (8)) now provide clinicians with accurate laboratory measurements. Providing reliable age- and gender-specific reference data will set the stage for increased clinical utilization of serum proteins in diagnostic medicine.

In this study we present an analysis of C3 and C4 levels from a large, allegedly healthy, Caucasian cohort, measured in one laboratory by the same method and measured against the principle reference material currently available. The work described here is a continuation of our previous studies on immunoglobulins (9), negative acute-phase proteins (10), positive acute-phase proteins (11), and serum iron and transferrin saturation (12). A companion paper (13) presents a meta-analysis of these same two analytes covering the time period of 1962–2002, and places the current findings in that context.

MATERIALS AND METHODS

Serum Protein Reagents and Instrumentation

Values were transferred from CRM 470 (obtainable from the Bureau of Community Reference in Brussels)/

RPPHS (8). The buffers, other reagents, and analytic instruments used were the same as previously described (9). Antisera was obtained from the same source as in our previous studies and verified in an identical fashion (9). A clarified and delipidated commercial serum calibrant was used as previously described (9).

SELECTION OF PATIENT RESULTS

Coding of Diagnosis/Symptom Strings

Most samples sent for study were accompanied by a suspected diagnosis or description of symptoms. Computer processing this large number of unique strings (over 28,000) required that they be classified into 93 categories representing related conditions. For example, the diagnoses of "RA," "R A," "R.A.," "Rheu Arth.," and over 300 similar entries were clustered into a single category (code 130 representing rheumatoid arthritis, ICD-9 code 714.0) by a clinician experienced in the interpretation of serum protein measurements (R.F.R.).

The diagnostic codes for computer processing ranged from 100 (indicating that no diagnosis was provided) to 195 (indicating that the listing was either trivial or could not be understood).

Code numbers 101–165 were assigned to diseases expected to have a significant impact on serum protein levels (e.g., multiple myeloma, cirrhosis, hepatitis, infection, lung disease, leukemia, renal failure, rheumatic disease, and immunodeficiency). Diagnostic codes 166–194 indicated conditions expected to have minimal or no effect on serum protein levels (e.g., allergies, hypertension, headache, neck pain, neuropathy, syncope, seizures, fatigue, and depression). When several diagnoses were supplied, the lowest code number was given precedence ensuring that the most significant diagnosis was used. Diagnostic groups coded as 166 and above (with the exception of 170 (pregnancy)) were designated as the preliminary reference population.

Refinement of the Reference Population

The methodologies we used to select referent individuals and compute the reference ranges are described in detail above, as well as elsewhere (9–12). Briefly, to determine which of the diagnostic groups would constitute the final reference population, we used a published method that employs symmetric trimming of values prior to calculating means and standard deviations (SDs) (14). In this way, outliers among the diagnostic group parameters associated with codes of 166 and higher (except 170) were identified and trimmed. A logarithmic transformation of the variance corrected for the non-random right skewness of the observed data. The resulting trimmed mean value

± 1.96 SD (for both the multiples of the median (MoM) and the log variance) defined limits of acceptability. All measurements from any diagnostic group falling within the limits were considered reference values, whereas those falling outside were not.

RESULTS

Selection of Diagnostic Groups to Form the Reference Population

The median C3 and C4 serum levels adjusted for age and gender were plotted vs. the variance for each of the 85 diagnostic code groups (9), with at least 20 observations made in the same fashion as described in detail previously (Fig. 1). Circles represent diagnostic groups (codes 166 and above) comprising relatively benign physiologic diagnoses that are not expected to have a significant impact on serum protein levels. The squares indicate the diagnostic groups with codes 165 and below, where major protein effects are expected. The rectangle encompasses the trimmed mean ± 1.96 SD for both the median levels (horizontal axis) and log variances (vertical axis). Overall, the median and log variances for 57 of the 85 groups lie within the rectangle. Results from individuals within these 57 groups comprise the referent population for C3. Individuals from the 28 diagnostic groups represented by filled circles or squares were not included. This analysis was repeated for C4, resulting in the inclusion of 54 diagnostic groups and the exclusion of 31 groups.

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

The selection process described above yielded 28,277 and 26,651 referent individuals in which to calculate reference ranges for C3 and C4, respectively. Figure 2 shows selected C3 centiles for males (A) and females (B). The logarithmic horizontal axis shows the mean age for each interval, while the logarithmic vertical axis shows the median C3 value (filled circles) along with the 5th and 95th centiles (lower and upper open circles, respectively). The observed centiles for individuals <10, 10–30, 30–80, and >80 years old are based on about 25, 70–500, 300–1,000, and <300 observations for males, and about 25, 100–1,500, 800–2,400, and <400 observations for females, respectively. The solid line indicates the fitted median levels for C3 in males, and the dashed line indicates the fitted medians for females. Results from patients less than 1 month or more than 85 years old were not included in the regression analysis. Figure 3 displays similar data for C4. For both analytes, a straight line fits the median levels in both younger males and females. The pattern

Fig. 1. Scatterplot of C3 and C4 median levels vs. variance for the diagnostic categories after preliminary adjustment for age and gender. **A:** Levels of C3. Diagnostic codes 100–165 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. **B:** The same analysis for C4.

for both C3 and C4 becomes more complicated in older individuals, reflecting physiologic maturation, hormonal effects, and aging. This is similar to data derived from other parameters, such as anthropomorphic features and more familiar biochemical measurements.

There is a <10% difference in C3 median levels between males and females throughout life. Levels vary somewhat at two periods: around ages 25 ($\pm 8\%$) and 70 ($\pm 5\%$). At other periods, the values are virtually identical. C4 values change little from age 1–24 in males, and increase slightly for females during the same interval. From age 24–55, values increase for both males and females (more steeply for females). After age 55, values for males and females drop to the values seen in

Fig. 2. C3 centiles and medians vs. age. The individual's age is displayed on the horizontal logarithmic axis vs. the C3 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. The lines represent the predicted median values. **A** (upper) and **B** (lower) show the C3 results for males and females, respectively.

their third decade of life. Table 1 contains the gender-specific regression equations for C3 and C4 median values, along with the age range over which each equation is valid. A sample equation is shown as a footnote to Table 1.

For C3 and C4 the distances from the median to the 95th centile, and from the median to the 5th centile are approximately equal across the entire age range (Figs. 2 and 3). This indicates that the distribution of C3 is symmetric after a logarithmic transformation, and that the variance of the distribution does not change appreciably by age.

Fitting the Reference Measurements Expressed in MoM to a Population Distribution

Each individual reference measurement (in mass units) was then converted to a multiple of its age and

Fig. 3. C4 centiles and medians vs. age. The data are presented in the same symbol format as in Fig. 2. **A** (upper) and **B** (lower) show C4 results for males and females, respectively.

gender-specific median value. Figure 4 shows probability plots composed of approximately 2,000 randomly selected normalized referent measurements for C3 and C4 (expressed in MoM), respectively. The samples were selected to equally represent each year of age. C3 measurements (Fig. 4A) fitted the distribution well between the 2nd and 99th centiles (logarithmic mean and SD = 0.0000 and 0.0816, respectively). C4 measurements (Fig. 4B) fitted well between the 5th and 99th centiles (logarithmic mean and SD = 0.0000 and 0.1241, respectively).

When this analysis was restricted to individuals < 10 or > 85 years of age, the logarithmic means were similar to the overall values (within 0.04) for both proteins (data not shown). The log SDs of the older groups were reasonably consistent with the consensus values (both within 8%). For the younger age group, the log SD for C3 was 7% lower and the log SD for C4 was 25% higher.

TABLE 1. Regression models and coefficients for median C3 and C4 measurements by age and gender

Analyte	Sex	Age Range (years)		Constant A0	Coefficients ^a	
		From	To		A1	A2
C3	Males	0.5	23	0.062472	0.0076962	0
		24	85	−1.2274	1.6155	−0.48886
	Females	0.5	39	0.07349	0.0045844	0
C4	Males	40	85	−4.2676	4.9456	−1.39199
		0.5	20	−0.616435	0.018581	0
	Females	21	85	−1.92547	1.6408	−0.4786
		0.5	35	−0.63025	0.044539	0
		36	85	−5.03957	5.10559	−1.4326

^aThe general form of the equation is $\text{Median} = 10^{(A0 + A1 \cdot \log(\text{age}) + A2 \cdot \log(\text{age})^2 + A3 \cdot \log(\text{age})^3 + A4 \cdot \log(\text{age})^4)}$.
The median C3 level for a 40-year-old female is $10^{(-5.03957 + 5.10559 \cdot \log(40) - 1.4326 \cdot \log(40) \cdot \log(40))}$ or 0.29.

Fig. 4. Probability plots of complement C3 and C4 measurements expressed as 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, the distribution is log-Gaussian. **A** and **B** show results for C3 and C4, respectively. The thin line represents the fitted Gaussian means and SDs contained in the text.

Predicting Age- and Gender-Specific Centiles

Within this range of centiles fitted above, patient values can be assigned age- and gender-specific centiles. Table 2 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 a C3 measurement of 1.30 g/L. If this value was reported for a 40-year-old male, it would be considered relatively normal (multiple of the median of 1.02 [1.30/1.28]) and would be assigned to the 54th centile [z-score = 0.11 ((log(1.02) – log(1.00))/0.0816)]. If the value was from a 20-year-old female, the interpretation would be 1.08 MoM [1.30/1.20], and it would be assigned to the 66th centile [z-score = 0.41 ((log(1.08) – log(1.00))/0.0816)].

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 C3 (1.00 ± 0.02) and C4 (1.00 ± 0.06). With about 2,000 observations available for each quarter, this variability is statistically 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

It is important to measure complement levels when several life-threatening conditions are present (15–18). With serum protein measurement technology at a high

TABLE 2. Predicted C3 and C4 medians and selected centiles stratified by age and gender

Decimal Age (yrs)	C3 (g/L)			C4 (g/L)		
	2.5th	50th	97.5th	2.5th	50th	97.5th
Males						
1.0	0.80	1.15	1.67	0.14	0.24	0.42
4.0	0.81	1.17	1.69	0.14	0.25	0.43
7.0	0.81	1.17	1.69	0.14	0.25	0.44
10.0	0.81	1.18	1.70	0.14	0.25	0.44
14.0	0.82	1.18	1.70	0.15	0.25	0.44
18.0	0.82	1.18	1.71	0.15	0.26	0.45
20.0	0.82	1.18	1.71	0.15	0.26	0.45
30.0	0.86	1.24	1.79	0.16	0.28	0.50
40.0	0.88	1.28	1.84	0.17	0.30	0.52
50.0	0.88	1.28	1.85	0.17	0.30	0.53
60.0	0.87	1.26	1.82	0.17	0.30	0.53
70.0	0.85	1.23	1.77	0.17	0.30	0.52
80.0	0.83	1.19	1.72	0.17	0.29	0.51
Females						
1.0	0.82	1.18	1.71	0.13	0.23	0.41
4.0	0.82	1.19	1.72	0.14	0.25	0.44
7.0	0.83	1.19	1.73	0.15	0.26	0.45
10.0	0.83	1.20	1.73	0.15	0.26	0.45
14.0	0.83	1.20	1.73	0.15	0.26	0.46
18.0	0.83	1.20	1.73	0.15	0.27	0.47
20.0	0.83	1.20	1.74	0.15	0.27	0.47
30.0	0.83	1.20	1.74	0.16	0.27	0.48
40.0	0.83	1.20	1.74	0.17	0.29	0.51
50.0	0.91	1.31	1.89	0.18	0.32	0.55
60.0	0.92	1.33	1.93	0.18	0.32	0.57
70.0	0.91	1.31	1.90	0.18	0.32	0.56
80.0	0.88	1.27	1.83	0.18	0.31	0.54

level of precision and accuracy (19), and a reliable reference material in wide use (8), the availability of age- and gender-specific reference ranges is more critical than ever. This study is part of the fifth set presenting age- and gender-specific reference ranges for the two major complement components from a single laboratory (9–12) in a large, relatively homogeneous cohort. As in the two previous studies addressing acute-phase proteins, 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 overt laboratory evidence of inflammation. Complement proteins C3 and C4 are sluggish in their response to acute inflammation; nevertheless, increases up to two- to threefold can occasionally be seen if a patient is monitored for 1–2 weeks following an inflammatory event. The acute-phase response is common, and often goes unrecognized in the general patient population. It must be considered whenever complement-consuming processes are being considered. Inexpensive laboratory techniques to measure sensitive indicators of inflamma-

tion are available, and are necessary to avoid misinterpretation of complement component concentrations. Minor changes in the levels of acute-phase proteins have demonstrated how important this consideration can be (15–18).

Other conditions may alter the serum levels of complement proteins as well. The confounding effects of sex hormones on serum protein levels have not been examined extensively, and since many women use such medications, as well as experience increased endogenous hormones during pregnancy, these effects should be investigated to further document previous findings (1–6). In addition to up-regulation by inflammatory drive, increases in estrogen levels due to puberty, pregnancy, or contraceptive medications can significantly increase circulating levels (20). A comparison of both panels of Fig. 2 shows that the disparity between males and females is most marked during times of estrogenic drive in the child-bearing years, and later when estrogen replacement regimens may be in use.

In addition, C3 levels have been shown to rise significantly with cholestasis secondary to mechanical biliary obstruction and/or drug reaction (21–23). Here again, during the progressive development of certain forms of chronic liver disease, including protracted toxic hepatitis (23), the failure of hepatic synthesis can obscure the increase in C3 as a result of cholestasis (21,24). The mechanism is not well understood, but it may reflect immune complex activity to acetaldehyde adducts (25). In immunologically mediated progressive liver disease, C4 appears to be particularly sensitive. C3 levels increase early in progressive biliary cirrhosis, possibly as a result of intrahepatic cholestasis (20). As the disease progresses, both C3 and C4 decrease (21,24).

Conversely, C3 levels are commonly diminished in the face of immune complex formation and deposition as part of certain rheumatic and renal diseases, as well as cryoglobulinemia from many types (26). The critical role played by the complement proteins in many pathological processes again emphasizes the importance of precisely measuring these proteins in serum and placing the values in proper perspective for age and sex.

Our proposed method of converting laboratory results to age- and gender-specific MoMs 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, by the associated centile based on that individual's age and sex. This process greatly simplifies the interpretation of these serum protein values.

The large size of the current cohort enabled us to reliably estimate the effects of age and gender in

a Caucasian population based on measurements standardized with an accepted reference material and measured by a single protocol. Because these findings are derived from a relatively homogeneous Caucasian population, they may be applicable to other ethnic groups as well. Individual usage of tobacco products, over-the-counter anti-inflammatory medications, contraceptives, or hormone supplements were not taken into account. Since the half-life of these drugs is short, only carefully designed studies would be able to take such usage into account. As shown in our companion paper (13), these data compare favorably with previous, smaller studies in which health status was defined on an individual basis (27,28).

CONCLUSIONS

Routine serum measurements for C3 and C4 performed at a single laboratory in a large, homogeneous cohort of Caucasians were examined to determine the reference distributions in persons who had no apparent active inflammation or complement-consuming process.

For these two analytes, both age and gender are important covariates to the normal experience, and they necessitate the use of separate reference ranges. C3 values clearly differ between the sexes to a considerable degree during the period when sex hormone levels change (around ages 15–50). C4 levels change little throughout life, although values in females are higher than in males, and reach a maximum at age 55. Converting the analyte measurements to age- and gender-specific MoMs simplifies interpretation and enhances the clinical utility of these measurements. After the appropriateness of the reference ranges presented in the current study is verified, the predicted values should be considered for use by laboratorians and clinicians when interpreting C3 and C4 measurements in individuals who have no apparent inflammation.

REFERENCES

1. Haram K, Augensen K, Elsayed S. Serum protein in normal pregnancy with special reference to acute-phase reactants. *Br J Obstet Gynaecol* 1983;90:139–145.
2. Giacoia GP. Concentration of serum prealbumin and retinol-binding proteins during pregnancy. *South Med J* 1984;77:1261–1263.
3. Whicher J. Complement component C3. In: Ritchie RF, Navolotskaia O, editors. *Serum proteins in clinical medicine*. Vol. I. Laboratory section, Foundation for Blood Research, Scarborough; 1996. Chapter 10.01, p 1–7.
4. Song CS, Merkatz IR, Rifkin AB, Gillette PN, Kappas A. The influence of pregnancy and oral contraceptive steroids on the concentration of plasma proteins. *Am J Obstet Gynecol* 1970;108:227–231.
5. Dotchev D, Liappis N, Hingerland H. Sex-specific differences of serum proteins in adults and influence of oral hormonal contraceptives on serum protein compositions. *Clin Chim Acta* 1973;44:431–435.
6. Navolotskaia O. Pregnancy/hormone effect. In: Ritchie RF, Navolotskaia O, editors. *Serum proteins in clinical medicine*. Vol. II. Clinical section, Foundation for Blood Research; 1999. p 106.0:1–11.
7. Alper CA, Colten HR, Gear JS, Rabson AR, Rosen FS. Homozygous human C3 deficiency. The role of C3 in antibody production, C-1s-induced vasopermeability, and cobra venom-induced passive hemolysis. *J Clin Invest* 1976;57:222–229.
8. Whicher JT, Ritchie RF, Johnson AM, et al. New international reference preparation for proteins in human serum (RPPHS). *Clin Chem* 1994;40:934–938.
9. Ritchie RF, Palomaki GE, Neveux LM, Navolotskaia O, Ledue TB, Craig WY. Reference distributions for immunoglobulins A, G and M: a practical, simple and clinically relevant approach in a large cohort. *J Clin Lab Anal* 1998;12:363–370.
10. Ritchie RF, Palomaki GE, Neveux LM, Navolotskaia O, Ledue TB, Craig WY. Reference distributions for the negative acute phase proteins, albumin, transferrin and transthyretin: a practical, simple and clinically relevant approach in a large cohort. *J Lab Clin Anal* 1999;13:273–279.
11. Ritchie RF, Palomaki GE, Neveux LM, Navolotskaia O. Reference distributions for the positive acute phase proteins, α_1 -acid glycoprotein (orosomucoid), α_1 -antitrypsin, and haptoglobin: a practical, simple, and clinically relevant approach to a large cohort. *J Clin Lab Anal* 2000;14:284–292.
12. Ritchie RF, Palomaki GE, Navolotskaia O, Neveux LM, Ledue TB, Craig WY. Reference distributions for serum iron and transferrin saturation: a practical, simple, and clinically relevant approach in a large cohort. *J Clin Lab Anal* 2002;16:237–245.
13. Ritchie RF, Palomaki GE, Navolotskaia O, Neveux LM, Ledue TB, Craig WY. Reference distributions for complement proteins C3 and C4: a comparison of a large cohort to the world's literature. *J Lab Clin Anal* 2004;18:9–13.
14. Healy MJR. Outliers in clinical chemistry quality-control schemes. *Clin Chem* 1979;25:675–677.
15. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;336:973–979.
16. Joyce PR, Hawes CR, Mulder RT, Sellmen JD, Wilson DA, Boswell DR. Elevated levels of acute phase proteins in major depression. *Biol Psychiatry* 1992;32:1035–1041.
17. Thompson DK, Haddow JE, Smith DE, Ritchie RF. Serum protein changes in women with early breast cancer. *Cancer* 1981;48:793–798.
18. Maes M, Wauters A, Neels H, et al. Total serum protein and serum protein fractions in depression: relationship to depressive symptoms and glucocorticoid activity. *J Affect Disord* 1995;34:61–69.
19. Ledue TB, Johnson MJ, Cohen LA, Ritchie RF. Evaluation of proficiency survey results for serum immunoglobulins following the introduction of a new international reference material for human serum proteins. *Clin Chem* 1998;44:878–879.
20. Ramcharan S, Sponzilli EE, Wingard JC. Serum protein fractions. Effects of oral contraceptives and pregnancy. *Obstet Gynecol* 1976;48:211–215.
21. Gardinali M, Conciato L, Cafaro C, et al. Complement system is not activated in primary biliary cirrhosis. *Clin Immunol Immunopathol* 1998;87:297–303.

22. Ohshio G, Furakawa F, Manabe T, Tobe T, Hamashima Y. Relationship between secretory IgA, IgA-containing (C3-fixing) circulating immune complexes, and complement components (C3, C4) in patients with obstructive jaundice. *Scand J Gastroenterol* 1986;21:151–157.
23. Sopena B, Martinez-Vazquez C, de la Fuente J, et al. Serum levels of immunoglobulins and complement in alcoholic liver disease. *Rev Clin Esp* 1993;193:419–423.
24. Schlesinger M, Benbassat C, Schoefeld Y. Complement profile in primary biliary cirrhosis. *Immunol Res* 1992;11:98–103.
25. Munoz LE, De Villiers D, Markham D, Whaley K, Thomas HC. Complement activation in chronic liver disease. *Clin Exp Immunol* 1982;47:548–554.
26. Lamprecht P, Moosig F, Gause A, et al. Immunological and clinical follow up of hepatitis C virus associated cryoglobulinemic vasculitis. *Ann Rheum Dis* 2001;60:385–390.
27. National Committee for Clinical Laboratory Standards. How to define and determine reference intervals in the clinical laboratory. Approved guideline. NCCLS document C28-A. Villanova, PA: NCCLS; 1995.
28. Gräsbeck R, Siest G, Wilding P, Williams GZ, Whitehead TP. Provisional recommendation on the theory of reference values. Part 1. The concept of reference values. *Clin Chim Acta* 1978;87:461F–465F.