makes its measurement in blood useful to ascertain that dosage is adequate. The long half-lives are an advantage once therapeutic doses are achieved, but the time required to achieve steady state may be unduly long if there is no monitoring. In addition, only patients on no additional psychoactive drug have as yet been treated. Use of fluoxetine in other groups of patients with more variable drug history makes drug monitoring useful to ensure adequacy of the drug trial in these patients.

This work was supported in part by an NIMH Center Grant (MH 41115) to the Department of Psychiatry, University of Texas Southwestern Medical Center, and a grant from Eli Lilly Laboratories. Support was also provided by the Waters Division of Millipore.

References

Reference Interval for Prealbumin for Children Two to 36 Months Old
BettyLou Sherry,1,2 Rhona M. Jack,3 Allan Weber,1 and Arnold L. Smith1

To define a reference value for serum prealbumin (transthyretin) concentration, we used a rate immunonephelometric microassay to quantify it in 76 healthy children, ranging in age from two to 36 months. Age-specific ranges (±2 SD from the mean) are: 2–5.9 months, 142–330 mg/L; 6–11.9 months, 120–274 mg/L; 12–17.9 months, 115–259 mg/L; 18–23.9 months, 143–243 mg/L; 24–36 months, 108–258 mg/L. When the data were grouped into those for subjects younger and older than 12 months of age, the mean for the 2–11.9 month age group (210 mg/L) significantly (P < 0.01) exceeded that of the 12–36 month age group (187 mg/L). We propose that in spite of a decrease in prealbumin concentration with increasing age, it is acceptable to use the reference interval 116–281 mg/L (±2 SD from the mean) for children from two to 36 months old. We also compared concentrations of prealbumin in serum and plasma of 41 individuals, finding the mean difference to be +11.7 mg/L; concentrations in plasma averaged 6.7% greater than those in serum.

Additional Keyphrases: pediatric chemistry • transthyretin • newborns

Concentrations of prealbumin (transthyretin) in serum are used as a short-term indicator of nutritional status (1) or as one component of an inflammatory and nutritional index for predicting outcome of certain diseases (2). A specific rate immunonephelometric microassay is used in clinical laboratories (3), is rapid and specific, and is usually applied to serum. This assay provides results within a few hours of sample collection. However, no comparisons of concentrations in serum and plasma have been published, and relatively small data sets have been used to define the pediatric normal reference interval (3, 4). We sought to compare prealbumin concentrations in serum and plasma, and to define reference values for children between two and 36 months old.

Materials and Methods

Samples
Whole blood, 250 µL, obtained by heel- or finger-stick, was placed in a standard or heparinized microtainer, centrifuged, and the plasma or serum removed with a Pasteur pipette and placed in an Eppendorf tube, which was sealed with Parafilm and stored at –70 °C until assay. For comparison of concentrations in plasma and serum we used blood samples obtained from the same individuals within a 3-h period. We used blood leftover from physician-requested laboratory tests. Sixteen samples from healthy adult volunteers were used for preliminary comparison of serum and plasma values. Twenty-five paired samples were obtained from infants and children six days to 12 years old. Most of these were hospitalized for cardiac surgery or cancer treatment. These two sources gave us a wide range of values for prealbumin concentrations (30–340 mg/L) for the serum/plasma comparison.

Blood samples used to defined the reference interval were collected from 76 healthy, afebrile children ranging in age from two to 36 months. Informed consent was obtained from the parents. These children were in good health at the time
of venesection. One to two months before this study, all subjects had been hospitalized an average of 10 days for *H. influenzae* type meningitis, but were all in good health at the time of venesection. We found that these children had lost an average of less than 1% of their pre-illness body weight during hospitalization, but had an appropriate rate of weight gain one month after discharge. We thus had no reason to believe that these children—who were afebrile, eating normally, and gaining weight—would have plasma prealbumin concentrations not representative of those for normal children.

**Equipment and Reagents**

We measured prealbumin by rate immunonephelometry, using the Immunochemistry system (ICS), the Accu-Prep system 222A pipetor dilutor, prealbumin antisera, and ICS calibrator number 3, all supplied by Beckman Instruments, Brea, CA 92621. Immunochemistry system diluent and buffer were from Kaltestad Laboratories, Austin, TX 78701. Control sera were "Level I" (Gilford Instruments, Irvine, CA 92713) or "Calibrator 1" (Atlantic Antibodies, Scarborough, ME 04074).

Control, calibrator, and patients' samples were diluted 36-fold with ICS diluent (phosphate-buffered saline) before assay. Samples with values <70 mg/L or >500 mg/L were assayed after six- or 216-fold dilution, respectively.

**Method**

To perform the assay, one places 600 μL of buffer (polyethylene glycol in phosphate-buffered saline) in a reaction cell with a stir bar. After an initial light-scatter measurement of buffer, the rate of increasing light scatter is measured after 42 μL of calibrator, control, or specimen, and 42 μL of prealbumin antisera are added. We found the between-run CV to be 2.95% at 227 mg/L (n = 15).

**Results and Discussion**

Figure 1 shows a comparison of prealbumin concentrations in serum and plasma. The mean difference is 11.7 mg/L (by paired t-test; \( t = -4.04, P <0.01 \); values for plasma averaged 6.7% higher. This may be ascribed to trapping of prealbumin in the clot or to the relative insolubility of fibrinogen under these assay conditions.

Figure 2 shows the age-specific mean (± 2 SD) concentrations of prealbumin for our 76 subjects. The number of children in each age group are: 0–5.9 months, 13; 6–11.9 months, 24; 12–17.9 months, 20; 18–23.9 months, eight; and

![Fig. 1. Differences between concentrations of prealbumin in serum and plasma](image)

Mean difference of plasma from serum is +11.7 mg/L; standard error of the difference = 18.6 mg/L; n = 41

![Fig. 2. Mean ± 2 SD for prealbumin from age two to 36 months (n = 76)](image)

24–35.9 months, 11. This does not differ significantly from a normal distribution as defined by the chi-square goodness-of-fit test; therefore, we used values for the mean and standard deviation to define the reference interval.

Younger children evidently have higher values. However, because of the small sample size in each age group, this data set does not have the statistical power to define six-month age-range reference intervals. When stratified into age groupings of less than and greater than 12 months of age (n = 37 and 39), the children from two to 11.9 months of age showed statistically significantly higher mean prealbumin concentrations in plasma as compared with the older children (mean = 210 mg/L vs mean = 187 mg/L, \( P <0.01 \)). These differences are statistically significant, but we have no evidence to indicate that they are clinically significant. We suggest that, for children from two to 36 months of age, the overall mean ± 2 SD would be an appropriate reference value. Our overall mean was 198 mg/L; ± 2 SD yields a normal range of 116 to 282 mg/L. This reference value is similar to that of Goldsmith and Munson (3): 109–273 mg/L for children ranging in age from one day to 18 years. Our data, derived from 76 children between two and 36 months old, provides additional validation of their results. Their wider age range was derived from 93 children. Our overall mean is 3.6% higher than their (3) found: 198 mg/L vs 191 mg/L. Although their results might be expected to be higher than ours because of higher values observed in older children, our values were slightly higher. This difference may be attributable to our use of plasma, not serum, as samples.

Our sample size was not large enough to evaluate potential age-dependent gender-related differences. Valquist et al. (4) reported no sex-related difference in serum prealbumin concentration until puberty. They also showed that children nine weeks to 13 months old had higher concentrations of prealbumin in serum than did older children. Our results confirm this finding, and indicate that the higher concentrations seen in our younger children are not simply ascribable to the small sample size. Our reference interval cannot be used to evaluate preterm or term newborns. Such subjects reportedly (5, 6) have lower serum concentrations of prealbumin in serum.

We conclude that the normal reference interval for concentrations of prealbumin for children between the ages of two and 36 months, as determined with a rate-immunonephelometric microassay, is 116 to 282 mg/L.
This research was supported in part by Beckman Instruments; The National Institutes of Child Health and Human Development (HD 17080); Maternal and Child Health Training Grant (MCJ-009043); Mead Johnson Nutritional Group, a Division of Bristol-Myers U.S.; and the Chicago Bridge and Iron Foundation.

References

A Fluorescence Polarization Immunoassay Evaluated for Quantifying Astromicin, a New Aminoglycoside Antibiotic

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We evaluated a fluorescence polarization immunoassay (FPFA) procedure for measuring astromicin, a new aminoglycoside antibiotic. The calibration curve can be stored for at least 32 days without loss of accuracy. There were no interferences from hemoglobin (up to 4000 mg/L) or bilirubin (up to 200 mg/L), nor were there any cross-reactivities with amikacin, gentamicin, or tobramycin. Within-run precision (CV) for 20 replicates, each at concentrations of 5, 15, and 25 mg/L, was 1.25–2.11%. Between-run precision for these same concentrations, measured on five occasions, was 2.13–2.74%. Linear-regression analyses of data by FPFA (y) and HPLC (x) for plasma obtained from six healthy male volunteers who were given 200 mg of astromicin by 30-min intravenous infusion and for sera prepared by adding arbitrary amounts of astromicin showed highly significant correlations: r = 0.996 (n = 66), y = 0.986x – 0.48 mg/L and r = 0.981 (n = 59), y = 0.968x – 0.25 mg/L, respectively. This assay offers a rapid, efficient, and accurate system for therapeutic monitoring of astromicin in blood.

Additional Keyphrases: TDx system, HPLC compared · pharmacokinetics

Astromicin, a new aminoglycoside antibiotic developed in Japan, is expected to have a similar or better clinical efficacy for treating infections with Gram-negative bacteria, less nephrotoxicity than amikacin (1, 2), and less otoxicity than ribostamicin (3). The "therapeutic window" for astromicin concentration in plasma is reportedly almost the same as for amikacin, i.e., peak concentration 15–25 mg/L, trough concentration <5 mg/L (4).

Here we report our evaluation of the precision and accuracy of a fluorescence polarization immunoassay (FPFA) for astromicin in blood and compare results with those obtained by HPLC.

Materials and Methods

Assays. FPFA, a homogeneous immunoassay technique for drug measurement in serum or plasma, is based on the competitive binding between the drug in the patient’s sample and the tracer (drug labeled with fluorescent) for the antibody. For the present FPFA, we used the "TDx" system (Dainabot, Tokyo, Japan), an automated fluorescence polarization analyzer. The Dainabot reagent kits contained three vials: rabbit polyclonal antibody to astromicin, fluorescein bound to astromicin, and a pretreatment solution that is used to release the drug from protein. Six calibrators (0, 4, 10, 18, 30, and 50 mg/L) were used to construct the standard curve. Low (5 mg/L), medium (15 mg/L), and high (25 mg/L) controls were included in the daily assay runs. Emission and detection wavelengths were 485 nm and 525 nm, respectively. HPLC assay was performed according to the methods reported elsewhere (5). In brief, plasma samples or their dilutions were introduced into a CM-Sephadex C-25 miniature column and washed twice with 2-mL portions of sodium acetate buffer (pH 7, 0.4 mol/L). Drug adsorbed onto the column was eluted three times with 0.5 mL of 0.4 mol/L sodium acetate containing 10 mmol of sodium hydroxide (pH 11); 0.5 mL of micromic sulfate solution (1 mg/L) was added as internal standard, and the mixture was diluted to 5 mL with the solution used for the mobile phase. We used a 150 × 6 mm (i.d.) column packed with YMC-PACK AM-312 (octadecylsilane; Gasukurokogyo Inc., Tokyo, Japan). The mobile phase consisted of 28.5 g of anhydrous sodium sulfate, 1 mL of glacial acetic acid, 20 mL of paired-ion chromatography reagent (PIC-B7; Waters Associates, Milford, MA) and 50 mL of mercaptoethanol in 929 mL of distilled water (flow rate: 1 mL/min). For fluorometric detection after post-column reaction with o-phthalaldehyde.

CLIN. CHEM. 34/9, 1880–1882 (1988)

1880 CLINICAL CHEMISTRY, Vol. 34, No. 9, 1988