Levels of α₁ acid glycoprotein and ceruloplasmin predict future albumin levels in hemodialysis patients

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Background. Serum albumin concentration predicts mortality in hemodialysis (HD) patients. While serum albumin concentration correlates with serum concentration of C-reactive protein (CRP) and is dependent upon CRP in multiple regression models in cross-sectional studies, CRP does not predict future albumin levels, possibly because CRP changes rapidly, yielding large month-to-month variability in CRP. If inflammation causes rather than is simply associated with hypoalbuminemia, then changes in the levels of acute phase proteins should precede changes in serum albumin concentration.

Methods. The levels of long-lived positive and negative acute-phase proteins (APPs) (C-reactive protein, ceruloplasmin, α₁ acid glycoprotein, transferrin and albumin) were measured longitudinally in 64 HD patients and a regression model was constructed to predict future albumin levels. Normalized protein catabolic rate (nPCR) was measured monthly. The number of repeated measurements ranged from 9 to 39 in each patient (median 22 and a mean of 23 measurements). To construct a model that would predict serum albumin concentration at any time j, values of all longitudinally measured APPs, positive and negative at any time j − 1, approximately 30 days prior to time j, were used. Other demographic factors (such as, race, access type, and cause of renal failure) also were incorporated into the model.

Results. The model with the best fit for predicting serum albumin at time j included albumin, ceruloplasmin, and α₁ acid glycoprotein measured at time j − 1. The only demographic variable with subsequent predictive value was diabetes.

Conclusions. The finding that changes in the concentration of the long lived APPs measured one month earlier are associated with predictable changes in the future concentration of serum albumin suggest that changes in inflammation are likely to be causal in determining serum albumin concentration in hemodialysis patients.

Key words: inflammation, hypoalbuminemia, hemodialysis, C reactive protein, acute phase proteins.

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A low serum albumin is closely associated with the excessive mortality of hemodialysis (HD) [1, 2]. The association has been attributed to consequences of malnutrition [3] and/or inflammation [4, 5]. However, the principal cause of death in hemodialysis patients is cardiovascular [6]. Interestingly, inflammation also has been identified as an epidemiologically important risk factor for cardiovascular disease in the general population [7–11].

In multiple regression analysis, inclusion of C-reactive protein (CRP) [5, 12] or interleukin-6 (IL-6) [5] reduced or eliminated the predictive value of albumin on survival, suggesting that inflammation was responsible for the predictive effect of albumin on mortality, including cardiovascular mortality. It must be emphasized, however, that associations cannot define a cause-effect relationship. In fact, a longitudinal study observed that while CRP correlated inversely with the current albumin level, it did not predict future serum albumin levels, possibly because CRP changed so rapidly (half-life 19 hours [13]) that a time relationship between serum CRP and albumin was obscured [14, 15]. It also is possible that very acute inflammatory events might have time to resolve prior to the subsequent measurement of albumin, approximately a month later.

The goal of this study was to establish whether changes in the levels of acute phase proteins having longer half-lives than CRP would predict future serum albumin levels. Alpha-1 acid glycoprotein (α₁ AG) has a half-life of approximately 65 hours [15] and ceruloplasmin has a fractional catabolic rate of 16.6 ± 2.3 percent of the plasma pool per day corresponding to a half-life of approximately three days [16]. It was reasoned that if inflammation increased in production of acute phase proteins and caused lower values of serum albumin, markers of inflammation would precede and hence predict future albumin levels. It was postulated that changes in the levels of more long lived acute-phase proteins (APPs)
might have a greater predictive value on future serum albumin levels compared to more rapidly changing proteins (such as CRP) or cytokines. A linear prediction model with variable selection to assess the actual predictive value of both short- and long-lived APPs was developed.

METHODS

Patient selection

The individual Institutional Review Boards at each institution approved the protocol and informed consent was obtained from each patient. Sixty-four patients enrolled in the National Institutes of Health HEMO Study at the University of California Davis in Sacramento, California, at Beth Israel Medical Center in New York, New York, and at Emory University in Atlanta, Georgia were recruited to participate in this longitudinal study. During the first six weeks serum concentrations of CRP, albumin, transferrin, α1 AG and ceruloplasmin were measured weekly. Subsequently, each protein was measured monthly on serum obtained from pre-dialysis samples. The 64 patients had been on dialysis for a mean time of 6.44 ± 3.45 years (1.32 to 21.56 years; median 6.40 years; 25th percentile, 3.73 years and 75th percentile, 7.68 years).

The times of measurements were labeled as 1, 2, . . . , j − 1, j, j + 1, . . . and the measurement at time j − 1 was one month prior to that at time j.

The possible predictors included demographic variables (sex, age, race), patient characteristics (time on dialysis, and the cause of renal failure as diabetic or non-diabetic) and the most recent measurements of all longitudinally measured acute phase proteins (CRP, ceruloplasmin, α1 AG and transferrin) obtained at the previous measurement time j − 1, approximately 30 days earlier. The time interval used for prediction was approximately 30 days prior to the time of the predicted albumin concentration at current time j. The variable selection method used was to find the set of most pertinent predictors among these variables. This model was constructed by using a forward variable selection procedure with Akaike’s information criterion (AIC) criterion [19].

Laboratory methods

Albumin was measured in duplicate using bromocresol green. All other proteins were measured with rate nephelometry using a Beckman Array automated nephelometer (Beckman Instruments, Fullerton, CA, USA) [17]. All nephelometric measurements were made in duplicate in each of two optical systems. The average of these values was used for calculations. The intra-assay coefficient of variation for albumin was 0.22%, with an inter-assay coefficient of variation ranging from 2.42% to 3.16%. The intra-assay coefficient of variation for α1 AG was 0.14%, with an interassay coefficient of variation ranging from 0.07% to 0.50%. The intra-assay coefficient of variation for ceruloplasmin was 0.27. The intra-assay coefficient of variation for transferrin ranged from 0.026% to 0.07%, with an interassay coefficient of variation ranging from 2.56% to 4.38%.

Samples were frozen initially on dry ice and then stored at −20°C until assay. Biochemical assays were performed once a week on samples received during that week in a central laboratory (Davis, CA, USA).

The means for each value were calculated by first calculating the means within subjects, then taking the means of these means. The standard deviations were calculated by generating the standard deviations within subjects, and then taking the mean of these standard deviations.

Statistical analysis for predicting albumin concentration

The goal was to obtain the best fitting model for predicting serum albumin concentration at any arbitrary time j by using a longitudinal, mixed-effects linear model [18]. The times of measurements were labeled as 1, 2, . . . , j − 1, j, j + 1, . . . and the measurement at time j − 1 was one month prior to that at time j.

The possible predictors included demographic variables (sex, age, race), patient characteristics (time on dialysis, and the cause of renal failure as diabetic or non-diabetic) and the most recent measurements of all longitudinally measured acute phase proteins, (CRP, ceruloplasmin, α1 AG and transferrin) obtained at the previous measurement time j − 1, approximately 30 days earlier. The time interval used for prediction was approximately 30 days prior to the time of the predicted albumin concentration at current time j. The variable selection method used was to find the set of most pertinent predictors among these variables. This model was constructed by using a forward variable selection procedure with Akaike’s information criterion (AIC) criterion [19].
The number of repeated measurements ranged from 9 to 39 in each patient (median 22 measurements).

Statistical analysis was performed using the statistical software package S-Plus, version 4.5 using the linear mixed effects model function.

Since CRP predicts contemporaneous albumin values in cross sectional studies, but not future albumin levels [14], we reasoned that whatever physiologic factors establish CRP levels may interact with serum albumin concentration in a different way than do those factors that exert their effects in the future. In order to test this hypothesis, we performed additional analysis in which current levels of CRP were introduced (at time $j$) to establish (1) whether the effect of CRP on albumin concentration at time $j$ was still evident) and (2) to establish whether the addition of CRP changed the relationships between terms that predicted future albumin levels (that is, $\alpha$1 AG at time $j - 1$, ceruloplasmin at time $j - 1$ and albumin at time $j - 1$).

**RESULTS**

Serum albumin values in individual patients varied over time as previously observed [14]. The mean and standard deviation of the mean value for each protein during this time interval is provided in Table 1.

By far the best single predictor for the current serum albumin was its previous concentration. This result is not surprising because the albumin pool is large (~350 g/1.73 m²) and turns over slowly (half-life from 14 to 21 days) [20–24].

Consequently, changes in either the synthesis or catabolism of albumin are reflected only slowly in the serum albumin concentration (that is, the changes are well buffered). Besides the prior albumin value, the current albumin concentration at any time can be predicted by the concentration of both $\alpha$1 AG and by ceruloplasmin measured at approximately one month prior to the predicted value. The levels of other positive or negative acute phase proteins did not improve the association between serum ceruloplasmin or $\alpha$1 AG and serum albumin. Specifically, levels of CRP or transferrin had no value in predicting future serum albumin concentration even though the two proteins correlated with contemporaneous serum albumin levels (Table 3). It is noteworthy that both $\alpha$1 AG and ceruloplasmin were found to be additional significant predictors in a model, which already contained the previous albumin concentration as a predictor (Table 2).

Finally, the signs of the parameter estimates for ceruloplasmin at $j - 1$, $\alpha$1 AG at $j - 1$ and disorder (presence of diabetes) are all negative, while the sign of the parameter estimate for albumin at $j - 1$ is positive. Hence, the best model to predict albumin at time $j$ included albumin concentration at time $j - 1$, ceruloplasmin concentration at time $j - 1$, and $\alpha$1 acid glycoprotein concentration $j - 1$, as well as the presence of diabetes.

The results in Figure 1 show the longitudinal relationships between prior measurements of serum ceruloplasmin, $\alpha$1 AG and the current value of serum albumin in four randomly selected patients; the predicted serum albumin concentration also is presented. To simplify the presentation, earlier values of serum albumin were not included (even though they are included in the final
Fig. 1. Temporal relationship between observed (▲) and predicted (○) serum albumin concentration (g/dL) measured or predicted at time \( j \) and the concentration of ceruloplasmin (▼) (mg/dL) and \( \alpha 1 \) acid glycoprotein (○) (mg/dL) measured at time \( j - 1 \), approximately a month before time \( j \) in 4 patients on chronic hemodialysis, chosen from a total of 64 such patients.
prediction model). Each panel includes an insert showing the relationship between serum protein concentrations at times $j$ and $j - 1$. It is apparent that there is an inverse or reciprocal relationship between the serum concentrations of the positive acute-phase proteins at time $j - 1$, and that of the predicted and observed albumin level at the at subsequent time, $j$. Secondly, the predicted serum albumin concentration closely parallels the observed serum albumin concentration in individual series and when all of the data are combined (Fig. 2), the predicted and observed values agree closely. The combination of all three longitudinal predictors, plus the diabetes status, produces the least scatter around the regression relating observed and predicted values. It should be noted that the observations in this scatter-plot are not independent observations, (they include all repeated measurements) so no regression line has been plotted for these data.

When CRP at time $j$ was added to the model it had an additive and highly significant effect (Table 3) on describing current albumin (at time $j$). More importantly, the coefficients and the significance of the other variables at time $j - 1$ were unchanged when CRP at time $j$ was added. This suggests that CRP controls albumin concentration by a separate mechanism than do events controlling serum albumin, ceruloplasmin, $\alpha_1$ AG (all at time $j - 1$) and the presence of diabetes.

Adding nPCR at $j - 1$ to the final model that already includes albumin at $j - 1$, ceruloplasmin at $j - 1$, $\alpha_1$ AG $j - 1$ and disorder (presence of diabetes) does not improve the model. The individual variable nPCR at $j - 1$ had a positive coefficient (as expected) but was not significant, with a $P$ value of 0.1878. Including nPCR at $j - 1$ in the final model does not affect the significance of the other variables.

Table 3. Model that most closely predicts serum albumin concentration at time $j$ using measurements of serum proteins at prior time $j - 1$ and initial demographic and disease-specific variables with the addition of CRP at time $j$

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>SEM</th>
<th>z ratio</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>2.998301</td>
<td>0.173285</td>
<td>17.3027</td>
<td>—</td>
</tr>
<tr>
<td>Albumin at $j - 1$</td>
<td>0.34821</td>
<td>0.038013</td>
<td>9.1602</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Ceruloplasmin at $j - 1$</td>
<td>$-0.0034$</td>
<td>0.001092</td>
<td>$-3.1133$</td>
<td>0.0019</td>
</tr>
<tr>
<td>$\alpha_1$ AG at $j - 1$</td>
<td>$-0.00073$</td>
<td>0.000282</td>
<td>2.576</td>
<td>0.01</td>
</tr>
<tr>
<td>Diabetes</td>
<td>$-0.09001$</td>
<td>0.038487</td>
<td>$-2.3387$</td>
<td>0.0194</td>
</tr>
<tr>
<td>CRP at $j$</td>
<td>$-0.04148$</td>
<td>0.005686</td>
<td>$-7.2958$</td>
<td>$&lt;0.0001$</td>
</tr>
</tbody>
</table>

The effect of addition of current CRP values (at time $j$) on the ability of prediction of current serum albumin concentration (at time $j$) by the concentrations of both albumin and long-lived acute phase proteins occurring approximately a month earlier (at time $j - 1$). Note that while CRP at time $j - 1$ had no predictive effect on future serum albumin concentration, CRP at time $j$ had a powerful effect on controlling current serum albumin concentration while not in any way disrupting the effects of earlier events.

**DISCUSSION**

Serum albumin level is controlled by nutritional factors [25–28], external loss [29], inflammation [30, 31], and by changes in its distribution volume. In earlier reports, we found that a short lived marker of inflammation (CRP level) was the factor most closely correlated with current serum albumin in both cross sectional [32] and longitudinal studies [14] of maintenance hemodialysis patients. Both albumin and CRP decreased and increased continuously with one another during the longitudinal study [14], so the level of CRP was not useful in predicting serum albumin at the time of the next measurement approximately a month later. But correlations between variables in a cross-sectional analysis cannot alone infer causality. Partial data from the first 37 of these patients were analyzed and reported previously [14]. The time of observation on these same patients has been extended here and combined with data from the additional patients. Despite the increase in time of observation and the number of patients the observations that we reported previously persist. CRP continues to correlate with contemporaneous values of serum albumin but cannot predict future albumin values. Additionally, the model used for analysis in the current study was different than that done from that used previously, allowing us to focus on prediction of future albumin.

None of the lagged predictors were of additional value in the previous study [14] because current predictors were included at all time points. The lagged predictors then did not add significant prediction value. In the present study, we only allow lagged predictors, assuming none of the current values were available. The aim therefore was a real prediction of future measurements from the values. Our present findings offer more compelling evidence for a cause and effect relationship between inflammation and hypoalbuminemia since changes in both ceruloplasmin and $\alpha_1$ AG clearly precede the change in albumin concentration. As expected, serum albumin at
any given time exerted a powerful effect on future values because the pool of albumin is large [33, 34] and its rate of catabolism low.

Albumin is a negative acute phase protein, meaning that its serum concentration will decrease during periods when that of positive acute phase proteins, such as ceruloplasmin [35] and α1 acid glycoprotein increase [36]. While changes in serum albumin synthetic rate occur quickly [16, 17], there is no rapid change in serum albumin concentration because of the large mass of total body albumin (~5 g/kg body weight). Furthermore, changes in serum albumin concentration during inflammation also may be a consequence of increased fractional catabolic rate [37]. Changes in serum albumin concentration resulting from an altered rate of albumin catabolism would require time to be apparent.

The most common laboratory markers of inflammation used in clinical studies have been either CRP or interleukin 6 (IL-6), yet their levels in plasma do not predict future changes in serum albumin concentration. Even when CRP measured simultaneously with albumin is included in the relationship α1 AG and ceruloplasmin still have a predictive effect on future albumin levels. This suggests that the processes reflected by serum α1 AG and ceruloplasmin levels may be different than processes measured by CRP levels. It suggests that these processes interact with albumin concentration in different ways. It is also possible that these longer-lived proteins may provide an integrated measurement of inflammation over an extended period.

The usefulness of the short-lived proteins may be limited because these proteins turn over very rapidly and serum levels are highly variable over time. It is possible that the short-lived markers of inflammation, such as CRP and IL-6 interact with serum albumin concentration in a different way than do markers with longer half-lives, such as α1 acid glycoprotein. If indeed changes in albumin concentration mediated by processes that control α1 AG and ceruloplasmin remain biologically relevant after a month, then why are changes in albumin concentration mediated by processes that control CRP immediately? One possibility is that processes that control albumin concentration and occur within short time frames, such as redistribution of albumin pools may reflect processes that also regulate CRP levels, and that these may be different than processes that take more time to be expressed, such as changes in albumin synthesis or fractional catabolic rate. Since these latter changes require more time to be seen, they may be those that correlate with longer-lived acute-phase proteins.

We conclude that the persistence of a chronic inflammatory condition leads to hypoalbuminemia in hemodialysis patients. The severity of future hypoalbuminemia (and by inference, the risk of an adverse outcome) can be predicted when high levels of longer-lived acute-phase proteins, such as ceruloplasmin and α1 AG are found. Inflammation precedes and likely causes changes in albumin concentration in dialysis patients.

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REFERENCES


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