Selective Use of Transthyretin and Retinol-Binding Protein as Markers in the Postoperative Assessment of Protein Nutritional Status

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We evaluated several markers to judge the postoperative state of protein nutriture in eight patients following surgery for cancer. Seven patients had a good prognosis and had no evidence of infections or other complications. Following surgery, all of the patients showed a shift toward abnormal values for the serum concentrations of albumin, transthyretin (TT), retinol-binding protein (RBP), and the amino acid (AA) ratio of nonessential to essential amino acids. In patients without complications, the AA ratio returned to normal first. When blood specimens were collected at 7-day intervals, concentrations of RBP and TT were revealed to be decreased and recovered at the same time, or TT was

recovered after RBP was normalized. RBP and TT were usually abnormal until the AA ratio became normal. Although albumin moved toward normal concentrations after RBP and TT, the albumin concentrations in some patients were slightly above the lower reference value, whereas RBP and TT were significantly below their lower reference limits. In these patients, assessments over the next 7–14 days showed persistently low values for albumin, TT, and RBP. We recommend the selective use of TT and RBP for the postoperative assessment of protein nutriture in surgical patients. *J. Clin. Lab. Anal.* 17:1–5, 2003. © 2003 Wiley-Liss, Inc.

Key words: albumin; transthyretin; retinol-binding protein; amino acid; nutritional status; Fischer ratio

INTRODUCTION

Postoperative assessment of protein nutriture is important in patients having surgery because of the usual accompanying stress and blood loss. Patients undergoing resection of cancers in the gastrointestinal tract commonly show malabsorption of essential nutrients; i.e., carbohydrates, proteins, fatty acids, vitamins, minerals, and trace elements (1). Many markers have been proposed for the assessment of protein nutriture (2). Plasma concentrations of albumin tend to decline if dietary proteins are inadequate, or if there is cirrhosis or renal loss. Other plasma proteins, such as transthyretin (TT or prealbumin), transferrin, retinol-binding protein (RBP), and somatomedin C have much shorter half-lives than albumin and are better tests for evaluating acute changes in nutritional adequacy. Fibronectin, because it is not synthesized solely by the liver, is a useful test for non-liver-related changes in nutriture. Interleukin-1 is another useful test for as-

sessing acute changes; it has a short half-life of 2–4 hr, much like that of somatomedin C. The adequacy of protein intake can be evaluated by urinary excretion of urea and hydroxyproline, and by a plasma amino acid profile; however, increases in these metabolites in urine may also reflect a catabolic state or kidney disease (1,3). Better tests could reduce both costs and the amount of blood needed for testing. Given the many biochemical markers of nutritional status, our challenge was to identify the most efficient tests for the assessment of protein nutriture for patients undergoing major surgery.

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MATERIALS AND METHODS

Patients

Blood specimens were collected by vein puncture from eight cancer patients before and at 1, 7, 14, 21, and 28 days after surgery. The patients underwent resection of the colon (patients A–D, ages 48–70 years) or stomach (patients E–H, ages 63–75 years). Patients D and F–H were male, and patients A–C and E were female. After surgery, patients A and B received total parenteral nutrition (TPN) containing 80–120 g of AA for 1–9 and 1–3 days, respectively; thereafter they received an oral diet. Six other patients were given an oral diet therapy after surgery. The above diets contained 38–81 g of protein (63 g on average), 18–54 g of lipid (34 g on average), 118–252 g of carbohydrate (220 g on average), and 872–1,797 kcal of energy (1,460 kcal on average) daily. Except for patient B, all patients had lost <2 kg body weight by 28 days postoperatively as compared with their preoperative weight. Patient B had lost 5 kg by day 28. Except for patient B, the patients had no infections or complications.

Written informed consent was obtained from the subjects, and the protocol was approved by the Protection of Human Subjects Committee of Toho University.

Analyses

We used serum albumin, an analyte with a half-life of about 20 days, as an indicator of long-term protein nutriture. Its concentration was measured by a bromocresol blue method in the Hitachi 7250 automated analyzer with a kit method from Dia-Iatron Co. (Tokyo, Japan). We also chose RBP, which has an intermediate half-life of about 12 hr, and TT, which has a half-life of 2 days. The latter two analytes in serum were determined by an immunonephelometric procedure (4). The concentrations of free amino acid (AA) in plasma were measured using a JLC-300V AA analyzer (JEOL, Tokyo, Japan) (5). We also wanted to determine the AA ratio, i.e., the molar ratios of certain nonessential AAs to essential AAs. The AA ratio is calculated as [glycine + serine + glutamine + taurine]/[isoleucine + leucine + valine + methionine] (3). The reference range for the AA ratio in our laboratory is <3.1. The Fischer ratio, a similar tool, was calculated as [isoleucine + leucine + valine]/[phenylalanine + tyrosine]. The AA ratio is typically increased to about 3.5 in kwashiorkor (3). In female patients, the AA ratio was negatively correlated with the Fischer ratio, $r = -0.674$, $P < 0.01$; and $r = -0.346$, $P < 0.20$ in male patients.

Reference ranges in our laboratory for serum albumin are 38–53 g/L; TT, 220–400 mg/L; and RBP,

29–79 mg/L. In addition to these biochemical markers, we determined the serum concentration of C-reactive protein (CRP) by an immunoturbidimetric method in the Hitachi 7250 automated analyzer using the kit from Dia-Iatron. CRP is an acute-phase protein that increases under conditions of tissue injury, inflammation, and infection. Under these conditions, CRP is usually increased, and a negative nitrogen balance occurs owing to the increased catabolism of proteins (2). We determined neutrophil counts using a hematology analyzer (Cell-Dyn 3000; Abbott Diagnostics Division, North Chicago, IL).

RESULTS

As shown in Fig. 1, serum concentrations of albumin, TT, and RBP were markedly decreased in nearly all patients 1 day after surgery; in some patients, these decreased to below the lower reference limit. Although all patients lost blood during surgery, the decline was much more than that calculated from blood loss and the patients' hematocrit values. As shown in Fig. 2, the AA ratio in patient D decreased 1 day after surgery, which looked like good protein nutriture; however, the ratio changed to >3.1, i.e., to a deficiency state at day 7. Ratios >3.1 were also found in patient A at postoperative day 1; in patient B at days 1–14; and in patient H at day 28. The AA ratio of parenteral fluids given to patients A (at days 1–9) and B (at days 1–3) was about 0.10. Except in patient B, in whom a higher AA ratio persisted until the 14th postoperative day, all patients had a good prognosis and did not show any inflammation or infection as judged from their CRP concentrations and neutrophil counts. We found that the AA ratio is a good measure to rule out poor protein nutriture and (or) a catabolic state. The nutritional status of the other nutrients was good in all patients.

Despite the differences in the half-lives, the concentrations of RBP and TT were usually lower than the reference ranges during the same time periods of days 1–7 postsurgery, and both nutritional markers began to elevate thereafter. At days 21–28, nearly all patients' concentrations were within the reference ranges for RBP and TT, although patient B had persistently low values at day 28. We found that the RBP and TT did not return to the reference ranges as long as the AA ratio was abnormal. As illustrated in Fig. 1, the serum albumin in patients A, C, F, and G was in the reference range following the normalization of both RBP and TT. The albumin in patients B, D, E, and H was still below the reference range at 28 days after surgery.

Tables 1 and 2 show the correlation coefficients of the analytes described herein. Except for the AA ratio, all biomarkers were significantly and negatively correlated

Fig. 1. Postoperative changes in serum albumin (left), transthyretin (TT, middle) and retinol-binding protein (RBP, right) in (A-H) eight surgical patients. The shaded areas indicate our reference range.

Fig. 2. Postoperative changes in AA ratio (left), CRP (middle), and white neutrophil counts (right) in (A-H) eight surgical patients. The shaded areas indicate our reference ranges.

with CRP and neutrophil counts in male patients. However, these correlations were poor in female patients. In female patients the AA ratio was negatively correlated with TT and RBP; however, in male patients the Fischer ratio was negatively correlated with TT and RBP. Concentrations of RBP, TT, and albumin were significantly correlated with each other in both male and

female patients. We estimated the concentration of one biomarker with a long halflife from the lower reference value of another biomarker with a shorter halflife and their regression equation (Tables 1 and 2). For example, the concentration of albumin was calculated from 29 mg/L of RBP or 220 mg/L of TT and the linear regression equation of each pair of analytes (regression

TABLE 1. Correlation coefficients between biochemical nutritional markers, CRP, and neutrophils in male patients

Versus	Neutrophils	CRP	Albumin	TT	RBP	Fischer ratio
AA ratio	+0.310	−0.217	−0.341	−0.335	−0.300	−0.346
Fischer ratio	−0.618 ^b	−0.611 ^a	+0.767 (36) ^b	+0.800 (234) ^c	+0.834 (33) ^c	
RBP	−0.421 ^a	−0.664 ^c	+0.652 (36) ^c	+0.845 (206) ^c		
TT	−0.525 ^b	−0.500 ^a	+0.673 (37) ^c			
Albumin	−0.474 ^a	−0.497 ^a				
CRP	+0.538 ^b					

Correlation data for paired variables in four patients having surgery before and 1, 7, 14 and 28 days postoperatively. For example, we obtained the straight-line regression equation from our data to calculate the low-normal value of, e.g., albumin (values in parentheses) from the low-normal value of RBP. In all cases, the calculated low-normal value agreed well with our reference range for albumin. The same is true for other pairs of analytes shown in the table.

^a $P < 0.05$.

^b $P < 0.01$.

^c $P < 0.001$.

TABLE 2. Correlation coefficients between biochemical nutritional markers, CRP, and neutrophils in female patients

Versus	Neutrophils	CRP	Albumin	TT	RBP	Fischer ratio
AA ratio	+0.100	+0.195	−0.170	−0.551 (232) ^a	−0.654 (26) ^a	−0.674 (2.8) ^b
Fischer ratio	−0.541 ^a	−0.624 ^a	+0.257	+0.318	+0.525 (30) ^a	
RBP	−0.260	−0.552 ^a	+0.519 (37) ^a	+0.871 (237) ^c		
TT	−0.045	−0.335	+0.651 (37) ^c			
Albumin	−0.187	−0.330				
CRP	+0.777 ^c					

Correlation data for paired variables in four patients having surgery before and 1, 7, 14 and 28 days postoperatively. For example, we obtained the straight-line regression equation from our data to calculate the low-normal value of, e.g., albumin (values in parentheses) from the low-normal value of RBP. In all cases, the calculated low-normal value agreed well with our reference range for albumin. The same is true for other pairs of analytes shown in the table.

^a $P < 0.05$.

^b $P < 0.01$.

^c $P < 0.001$.

equations not shown). The calculated concentrations from the regression equation agreed well with our lower reference limits. Therefore, we were conservative and considered an albumin of <38 g/L as representing albumin deficiency (a value of <34 g/L is usually used in nutritional assessment (6)).

DISCUSSION

In this study we wanted to identify the most useful biomarker for assessing protein nutriture in cancer patients undergoing surgery of the colon or stomach. We compared the postsurgical concentrations of serum albumin (half-life = 20 days), RBP (half-life = ~ 12 hr), and TT (half-life = 2 days), and the ratio of certain AAs that changed rapidly following intravenous protein infusion or oral feeding. Although the RBP concentrations before surgery in male patients were significantly ($P < 0.05$) higher than those in female patients, no significant differences were found in postoperative

values of all biomarkers for protein nutriture status between male and female patients.

In a previous study (7) it was reported that the AA ratio was affected by fasting: taurine was increased, but serine, isoleucine, leucine, and valine were decreased, and thus the AA ratio was increased from 2.3 to 2.7 during 3 hr of fasting. In the present study, even though blood specimens were collected from patients who had fasted overnight, we found the AA ratio to be a good indicator of protein nutriture. When surgery led to a decrease in TT and RBP, the concentrations of these two proteins did not return to the reference range until the AA ratio was normalized. Because the Fischer ratio is commonly used in studies of nutrition, we investigated whether the AA ratio could be replaced by the Fischer ratio. The Fischer ratio was correlated negatively with the AA ratio and positively with albumin, TT, and RBP. The AA ratio in female patients and the Fischer ratio in male patients showed better correlation with TT and RBP than did the albumin. Therefore, the AA ratio

can be replaced by the Fischer ratio. A barrier to the use of the AA and Fischer ratios is cost. Apparent differences in the statistical results between male and females requires further investigation. Serum CRP concentrations and neutrophil counts in female patients appeared to increase higher and decrease more rapidly than those of male patients.

In the present study, with blood collection every 7 days, we found that TT and RBP increased at the same time or that TT increased after the RBP was normalized. When blood specimens were collected at 7-day intervals, TT and RBP gave the same clinical information despite the differences in their half-lives. Although the usual finding is that albumin returns to normal after TT and RBP, we found patients in whom the albumin was normal but the concentrations of RBP and TT were still below the lower reference limits. This is illustrated by patient B at days 7 and 14 postsurgery (38 and 40 g/L, respectively), patient G at day 14 (39 g/L), and patient H at days 7 and 14 (38 and 39 g/L, respectively). The serum albumin in these individuals was above the lower reference value despite the RBP and TT concentrations, which were still below their lower reference limits. In such patients, protein nutriture assessed over the next 7–14 days by RBP, TT, and albumin were not normal. This may be a consequence of acute changes in nutritional status.

Young and Hill (8) reported the assessment of protein-calorie malnutrition in surgical patients from TT, transferrin, arm muscle circumference (AMC), percentage weight loss, and hemoglobin. Our results are consistent with their observations. The data in our study indicate that fewer tests would suffice to judge nutritional status. Young and Hill (8) concluded that in order to predict postoperative morbidity and mortality, anthropometric data in addition to biochemical markers were required before and after surgery (8). We did not evaluate preoperative data, because all of the tests described herein were within our reference ranges. Postoperative changes in anthropometric data should be studied more thoroughly, because plasma AA concentrations have been correlated with estimates of patients' lean muscle mass (9).

Our results were based on data from only eight patients; nevertheless, we can recommend the selective use of RBP and TT for the postoperative assessment of protein nutriture in patients having surgery. The clinical value of RBP and TT are essentially the same in

estimating protein nutriture, when they were assessed at 7-day intervals. The earlier discharge of patients from the hospital is a major cost-saving measure, and the analysis of RBP and TT at intervals of 7 days or less is useful in making a discharge decision. When normal results are obtained with serial testing, and in the absence of any inflammation or infection (i.e., CRP < 3 mg/L), patients who are able to eat normally can be discharged regardless of hypoalbuminemia.

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