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Serum albumin level during intestinal exfoliative rejection: a potential predictor of graft recovery and patient outcome

Zambernardi A, Gondolesi G, Cabanne A, Martinez MI, Solar H, Rumbo M, Rumbo C. Serum albumin level during intestinal exfoliative rejection: a potential predictor of graft recovery and patient outcome.

Abstract: Exfoliative rejection is a severe complication after intestinal transplant. The assessment of mucosa histology is restricted to the area reached by endoscopy. We aim to evaluate the serum albumin (SA) value as a parameter of graft damage and clinical prognosis in intestinal exfoliative rejection (ExR). The present study is a retrospective analysis of 11 episodes of ExR occurred in a cohort of 26 patients. SA levels were measured 24 h after diagnosis and twice a week thereafter and then correlated with parameters of clinical and graft histological recovery (HR). During ExR, all patients had very low SA levels, reaching a minimum average of 1.9 ± 0.3 g/dL. According to the value of albumin levels at ExR diagnosis, the patients were grouped finding a correlation with their clinical evolution. Six ExR episodes presented with severe hipoalbuminemia (<2.2 g/dL; $p < 0.05$) that correlated with worse patient and graft outcome, ranging from graft loss and need for re-transplantation to delayed clinical and HR. SA at ExR diagnosis may be an indicator of the severity of the ExR process, and it could also be used as an early predictor of patient and graft outcome.

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Intestinal transplant (ITx) is indicated in patients with irreversible intestinal failure that have developed serious complications related to parenteral nutrition support. Acute cellular rejection is one of the most common complications in the post-transplant period. Early diagnosis and appropriate treatment are the bases to achieve a successful outcome. In some cases, a late diagnosis or insufficient pharmacological treatments lead to the occurrence of intestinal exfoliative rejection (ExR) (1–3). This implies loss of the gut mucosa integrity and the occurrence of a systemic inflammatory response syndrome that could evolve into sepsis and eventually turn into multiple organ failure and death (4–6). This condition is highly correlated with graft loss and reduced patient survival.

At present, the gold standard method for diagnosis of intestinal rejection is the endoscopic evaluation and histological assessment of the graft (1). Although the intestinal mucosa is easily available through the ileostomy after transplant, this procedure has the disadvantage that the endoscope can only reach a small area of the total intestinal length and mucosal surface; one has to assume that the screened area reflects the condition of the whole graft. If the process is patchy or if it is localized in a specific area of the graft, it could be missed (7–9). Furthermore, during ExR episodes, the risk of having complications associated with the endoscopy procedure such as bacterial translocation or intestinal perforation is increased; consequently, it would be advisable to keep endoscopies to a minimum number during the intestinal mucosa recovery (8). So far, the clinical follow-up of intestinal transplantation lacks of established biochemical markers for rejection diagnosis, assessment of graft damage extension or to evaluate treatment efficiency (10).

Acute graft versus host disease (GVHD) with gastrointestinal involvement shares many features with ExR after ITx, because there is an immunological aggression to the intestinal mucosa that

leads to enteropathy and barrier dysfunction (11, 12), which leads to protein-losing enteropathy syndrome. This situation presents many of the diagnosis challenges described above for ExR after ITx, such as the evaluation of the extension of the damage and the lack of markers for mucosa damage. Recent studies have proposed serum albumin (SA) levels as a parameter of severity during GVHD (13–15). Due to the similarities in the physiopathology and the diagnosis challenges, we aimed to evaluate the value of SA as a parameter of graft damage and recovery during ExR.

Patients and methods

This is a retrospective analysis of the ExR episodes that occurred in a cohort of ITx patients at a single center. From June 2006 to January 2012, 11 episodes of ExR (seven occurred in children) were diagnosed in 26 isolated ITx recipients. The study was conducted according to the Helsinski Declaration of 1975. The main features of each case are shown in Table 1.

The diagnosis of ExR was based on the histology criteria established in the "*Pathology Workshop, at the VIIIth International Small Bowel Transplant Symposium, Miami, Florida*" (16). SA values were considered normal when in the range of 3.5–5.2 g/dL (Colorimetric Albumin BCG). Additional laboratory data and clinical variables were considered to rule out SA variations due to causes other than epithelial damage. Liver function was followed by determination of liver enzymes and bilirubin; prothrombin time determination was used to assess liver synthetic function. Kidney function was estimated by plasma and urine creatinine concentration (creatinine clearance calculated by Cockcroft-Gault formula); daily urine test strips (Siemens multistix 10SG, Siemens, Munich, Germany) were carried out to rule out albumin loss through the kidney. One case among the 11 episodes analyzed was excluded due to proteinuria (Table 1). In the same way, to avoid factitious increases in SA level, amino acids administrated were standardized by parenteral nutrition regimen on 2–2.5 and 1–2 g/kg/d for pediatric and adult patients, respectively. Hydroelectrolytic balance, daily patient weight and infectious intercurrences were also considered. Intravenous albumin infusion was not used in this group of patients during the treatment of rejection. SA levels were collected at least twice a week starting 24 h after the diagnosis of ExR to avoid inaccurate values due to shock or dehydration.

The indicators used for functional graft recovery were clinical recovery (CR) and histological recovery (HR). CR was defined as the point when enteral nutrition was successfully restarted (achieving at least 50% of the total caloric daily requirements) together with the decrease in stool output to normal range (<40 mL/kg/d in children and <1500 mL/d in adults). HR was considered after obtaining normal histology in two consecutive intestinal biopsies.

Statistics

Comparisons between groups of data were performed with the Student's *t*-test or binomial proportion test. All the statistical analyses were performed using MacAnova 5.05 free shareware from University of Minnesota.

Results

The SA level progression was analyzed weekly; at least two samples per week were obtained. According to the initial level of SA, the patients were divided into two groups: Group A (GA) intermediate hypoalbuminemia, consisting of four ExR episodes with initial SA value >2.2 g/dL and Group B (GB), severe hypoalbuminemia, consisting of six ExR episodes with initial SA value <2.1 g/dL (Fig. 1). Considering the complete series of SA values from diagnoses to the time of recovery, all patients had low SA levels, showing a minimum average of 1.9 $\pm$ 0.3 g/dL. However, the progression of SA levels during the evolution of the rejection process was different between groups.

GA initially showed decreasing values of SA, reaching the lowest point during the third week after diagnosis (Fig. 2). This was followed by a steady recovery and normalization of SA. The SA curve for GB showed the lowest SA value at the time of diagnosis; after that, the SA curve showed a rising trend and until recovery.

As shown in Fig. 3, all patients in GA normalized SA values within eight wk at median (range 5–11) of treatment, whereas in GB, SA normalization occurred on week 15 at median (range 4–39). Two patients in GB were explanted with no recovery of SA levels. During the pre-rejection period, all the patients had documented SA levels higher than the ones obtained during the rejection episode. During this period, only one patient had SA levels below 3 g/dL. This patient was suffering from an intra-abdominal collection and underwent laparotomy right before the onset of rejection.

CR, as defined in the previous section, occurred in an average of five wk in GA. Only one patient in GB had CR in a short time (three wk), similar to GA (Fig. 3). The other patients in GB reached

Table 1. Patients' description

Patient no.	Age[a]	Pre-Tx immune risk[b]	Time from Tx to ExR (d)	Time from symptoms to diagnosis (d)	Initial SA (g/dL)	ACR[c] before ExR	Outcome[d] SAR/CR/HR (wk)
1	A	L	13	1	4.0	NO	Median-term recovery 11/08/08
2	P	H	20	1	3.0	NO	Dead due to CMV sepsis 70 d after ExR 09/04/05
3	P	H	26	1	2.8	NO	Median-term recovery 05/04/06
4	P	L	22	1	2.3	NO	Median-term recovery 08/03/03
5	P	H	1157	>7	2.1	1 mild ACR	Explanted NR/NR/NR[f]
6	P	L	640	7	2.0	1 mild ACR	Long-term recovery 08/03/08
7	P	L	347	3	2.0	NO	Long-term recovery 04/14/13
8	A	L	971	10	1.8	2 mild ACR	Long-term recovery 39/08/50
9	P	L	914	>7	1.5	2 mild ACR	Listed for re-transplantation 15/NR/NR
10[e]	A	L	1226	>20	1.6	1 mild ACR	Dead due to refractory rejection/sepsis NR/NR/NR
11	A	L	235	>7	2.05	NO	Explanted NR/NR/NR

[a]Age: P, pediatric; A, adult. [b]Pre-Transplant Immunological Risk (see text for details): L, Low; H, High. [c]ACR: acute cellular rejection; ExR: exfoliative rejection. [d]SAR: serum albumin recovery to normal range; CR: clinical recovery; HR: histological recovery. [e]Patient excluded from the study due to proteinuria during ExR. [f]NR= no recovery.

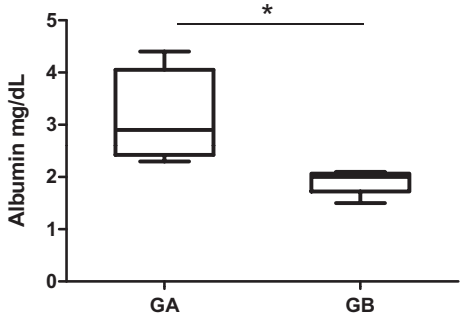

Fig. 1. Albumin level at the time of ExR diagnosis. GA (group A): patients with mild hypoalbuminemia. GB (group B): patients with severe hypoalbuminemia. *$p < 0.01$.

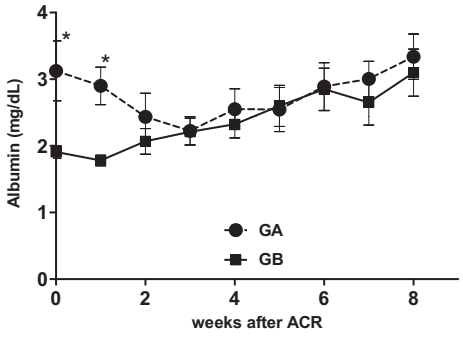

Fig. 2. Evolution of serum albumin levels during the first 8 weeks after ExR diagnosis. GA = Group A, patients with mild initial hypoalbuminemia level. GB = Group B, patients with severe initial hypoalbuminemia. *Significant differences between groups, $p < 0.01$.

CR in longer terms (8 and 14 wk) or never recovered graft function; two were explanted and a third patient developed chronic rejection, remaining on parenteral nutrition until re-transplantation.

HR is achieved when the graft restores its normal architecture and cellular distribution. To avoid sampling errors, we defined HR when it was documented with two consecutive biopsies. In GA, HR was reached in a median of five wk. In GB, it was reached in a median of 13 wk post-ExR diagnosis (Fig. 3). As previously described, two patients in GB were explanted at the end of week 2 and another evolved into chronic rejection; these patients are not included in the graphs of Fig. 3. Considering all cases analyzed, HR was consistently different in both groups when analyzed by the binomial exact test of difference of proportions ($p < 0.01$).

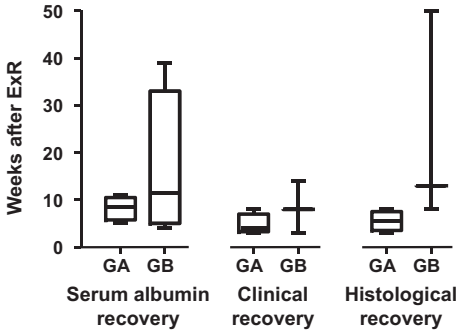

Fig. 3. Comparison of recovery times using different criteria. GA: Group A, patients with initial mild hypoalbuminemia. GB: Group B, patients with initial severe hypoalbuminemia. Three patients of this group never achieved complete histological and clinical recovery and two patients of this group did not achieve serum albumin recovery, due to chronic rejection or graft removal, consequently they are not included in the plot.

All ExR episodes were initially treated using the same immunosuppressive protocol: anti-thymocyte globulin 1.5 mg/kg/dose until T-cell depletion was achieved and extended up to a maximum of 14 d; three intravenous boluses of corticosteroids in consecutive days followed by taper; and tacrolimus levels were brought to the range of 12–15 ng/mL. One patient in GB was additionally treated with three doses of infliximab (3 mg/kg/dose, every two wk) due persistent rejection after thymoglobulin treatment. We did not observe any particular changes in SA evolution during this particular treatment.

Discussion

SA measurement is a standardized test, inexpensive, quick, and widely available in biochemistry laboratories. Due to its widespread use and availability, SA levels have been proposed as a prognostic factor in several clinical conditions, such as critically ill patients (17, 18) or chronic renal patients (19). In all these situations, low SA levels correlate with a poor patient outcome. This simple biomarker could be applied to aid real-time clinical decision making in the setting of intestinal ExR. In the present work, we showed that SA levels at the diagnosis of ExR correlate with the time for recovery and patient outcome.

SA levels are influenced by several physiological and physiopathological situations, such as hepatic and renal dysfunction, inflammatory status, and intestinal barrier alterations, among others (20). For example, a decrease in hepatic albumin synthesis due to an inflammatory response or due to hepatic dysfunction would affect SA levels. Fluid and electrolyte imbalance might affect SA levels; therefore, all these points need to be considered when using SA as a marker in intestinal mucosa damage.

Severe damage of the intestinal barrier or renal damage causing significant proteinuria could cause massive loss of albumin and consequently lead to hypoalbuminemia (21). In the present study, we have documented renal and hepatic function of all the patients during the ExR process to rule out these factors as another cause of SA variations. One patient with ExR diagnosis was excluded of this study due to renal damage. All the SA values included in the analysis were measured once the fluid and electrolyte balance was re-established as primary intervention in the dehydrated patients at hospital admission, to avoid variations due to hemoconcentration.

Intestinal protein loss due to barrier dysfunction leads to protein-losing enteropathy (22). Different clinical situations, such as bacterial or viral infections, inflammatory bowel disease, or acute GVHD have been identified as causes of this entity (23–25). Moreover, an increase in protein loss in ileostomy content during cases of ITx acute, rejection was observed by Goulet et al. (26). Severe cases of protein-losing enteropathy are associated with hypoalbuminemia. Most of these situations involve at least two important components that contribute to the hypoalbuminemia: mucosal histological damage and either a local or systemic inflammatory condition. It has been shown that inflammatory mediators produced in different situations have an impact on the intestinal barrier permeability, and this may lead to protein loss without major structural damage (4, 27). This situation may worsen when tissular damage is present. In the case of ITx ExR, these features are also present, so we reasoned that SA levels could be an indicator of the degree of intestinal damage caused by the rejection process.

Acute GVHD with gastrointestinal involvement shares many features with ExR after ITx, because there is an immunological aggression to the intestinal mucosa leading to enteropathy and barrier dysfunction. In the clinical practice, acute GVHD with mucosal involvement has been associated with protein-losing enteropathy from several decades ago (28, 29). Endoscopic studies to assess the intestinal barrier integrity are not a standard practice in this situation; consequently, the SA levels emerged as an indicator of the clinical course and prognosis of acute GVHD (13). This has recently been confirmed in a cohort of patients that underwent bone marrow transplant conditioned with reduced-intensity regimes that produce minimal regime-related gut toxicity; thus, it excludes protein loss

secondary to the ablation therapy (14, 15). The authors concluded that SA levels at the onset of acute GVHD are a predictor of the severity of the episode.

In the case of ITx, the monitoring of graft status is available through ileostomy with histopathological analysis (30) or after ileostomy closure through colonoscopy. However, the rejection process may have dissimilar impact on different portions of the graft (7). The evaluation of the graft damage extension is usually a difficult task, relying in different clinical signs, and extrapolations of endoscopic observations. So far, other tests such as fecal calprotectin or plasma citrulline have been investigated as possible markers to diagnose and follow up the acute intestinal rejection process in the post-transplant setting in a non-invasive manner. Plasma citrulline determinations have not gained a role in the rejection diagnosis due to lack of specificity and the need of an extensive damage in order to present a significant reduction in plasma citrulline level; furthermore, no study has confirmed its use to assess mucosa recovery (31). In the same line, fecal calprotectin dosage has been suggested as an indicator to be used only as first-line detection test by different groups either due to great variability within patients (32) or due to low specificity for the diagnosis of intestinal rejection (33). As mentioned before, SA can be regarded as a marker of severity in different illnesses, provided that different factors that influence its levels are critically evaluated. However, no work until the present has studied SA variation during ExR. The results shown here demonstrate that SA levels are modified by the occurrence of ExR. SA levels at the time of diagnosis allowed grouping of patients that correlated with the impact of the ExR episode, showing the potential utility of SA levels to prospectively evaluate the effect of the ExR episode on the graft. Different clinical parameters, such as white blood cells count, blood loss, bacterial translocation, need of intensive care unit admission, could be used to evaluate CR. For the purpose of the present analysis, decrease in ostomy/stool output to normal range together with the restitution of enteral nutrition was chosen, considering them as clinically relevant for specific intestinal recovery. Once the ExR episode was controlled with changes in the immunosuppressive therapy, SA levels tended to normalize in both groups showing, however, different timing for clinical and HR. Noteworthy, the patients in our cohort that had the worse outcome in the ExR episodes (such as graft loss or chronic graft disfunction) were among the patients with the lower SA levels at ExR diagnosis. Due to technical limitations, the evaluation of the total intestinal surface affected by the rejection process is difficult; consequently, we could not establish a correlation between the extension of the mucosal lesion and SA levels in our cohort of patients.

In conclusion, SA levels can be a readily available parameter from the onset of the ExR process that can be used as indicator of severity at the diagnosis of ExR episodes, and potentially, they could be used to follow up the graft recovery during the ExR treatment.

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