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Serum levels of alpha-1 microglobulin and beta-2 microglobulin in bone marrow transplant recipients treated with cyclosporin A

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Abstract. The levels of alpha-1 microglobulin (α_1m) and beta-2 microglobulin (β_2m) in serum were estimated in 77 bone marrow transplant recipients. In comparison to pre-transplant levels, the highest levels of α_1m and β_2m were found during impairment of renal function, i.e., during cyclosporin-induced nephrotoxicity and during treatment with other nephrotoxic drugs ($P < 0.001$). The α_1m levels were less elevated during infections and acute graft-versus-host disease ($P < 0.01$), while β_2m levels were markedly elevated during the same conditions ($P < 0.001$). The linear correlations between serum creatinine and α_1m and creatinine and β_2m were $r = 0.7$ and 0.8, respectively ($P < 0.001$). The overall correlation between α_1m and β_2m was 0.4 ($P < 0.001$). It is concluded that α_1m might be a complement to serum creatinine levels in monitoring renal function after bone marrow transplantation.

Key words: Alpha-1 microglobulin, in bone marrow transplantation – Beta-2 microglobulin, in bone marrow transplantation – Bone marrow transplantation, microglobulins

Alpha-1 microglobulin (α_1m) and beta-2 microglobulin (β_2m) are two low-molecular weight proteins. Serum levels of β_2m have been claimed to be useful as indicators of renal function since this molecule is eliminated by glomerular filtration in the kidney [11, 12]. β_2m is almost completely reabsorbed from primary urine and catabolized in the proximal tubular epithelium. The same mechanisms of renal handling have also been suggested for α_1m; however, these have been investigated less often [5, 16].

Cyclosporin A (CyA) is currently used to prevent acute graft-versus-host disease (GVHD) alone or in combination with methotrexate (MTX) in patients undergoing bone marrow transplantation (BMT) [10, 15]. The major side effect of CyA treatment is nephrotoxicity, reported to occur in more than 80% of BMT patients [8]. BMT recipients are also given other nephrotoxic drugs, such as amphotericin B, aminoglycoside antibiotics, and co-trimoxazole. These patients may also be in a catabolic state due to treatment with cytostatic drugs and irradiation, infections, and/or GVHD. Serum creatinine (s-crea) is, therefore, not an ideal indicator of the glomerular filtration at such times since the levels are increased during catabolic conditions.

The aim of this study was to evaluate the usefulness of monitoring serum α_1m and β_2m levels after allogeneic BMT as indicators of renal function.

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Patients and methods

Patients

A total of 77 BMT recipients (28 females and 49 males) with a median age of 32 years (range 11–50 years) were included. Seventy-five patients underwent BMT because of hematological malignancy, one because of Fanconi anemia, and one because of amyotrophic lateral sclerosis. All patients received grafts from phenotypically HLA-identical donors. Mixed lymphocyte cultures were mutually non-reactive.

Treatment

Treatment has previously been described in detail [9]. Patients with hematological malignancies were conditioned with cyclophosphamide and total body irradiation. The patient with amyotrophic lateral sclerosis was treated with busulphan and cyclophosphamide. GVHD prophylaxis consisted of CyA alone ($n = 20$) or in combination with methotrexate ($n = 57$) [10]. CyA alone was initially given i.v. at a dose ranging from 2.5–7.5 mg/kg per day divided into two doses and then, if tolerated, orally at a dose of 12.5 mg/kg per day for 6 months. Thereafter, the dose was tapered by 2 mg/kg per day every other month and discontinued after 1 year. MTX + CyA were combined according to a protocol from Seattle [15]. MTX was given i.v. in four doses on days + 1, 3, 6, and 11. CyA was given i.v. on days − 1 and 0 at a dose of 7.5 mg/kg per day divided into three doses, and then in the same dosage as when CyA was given alone.

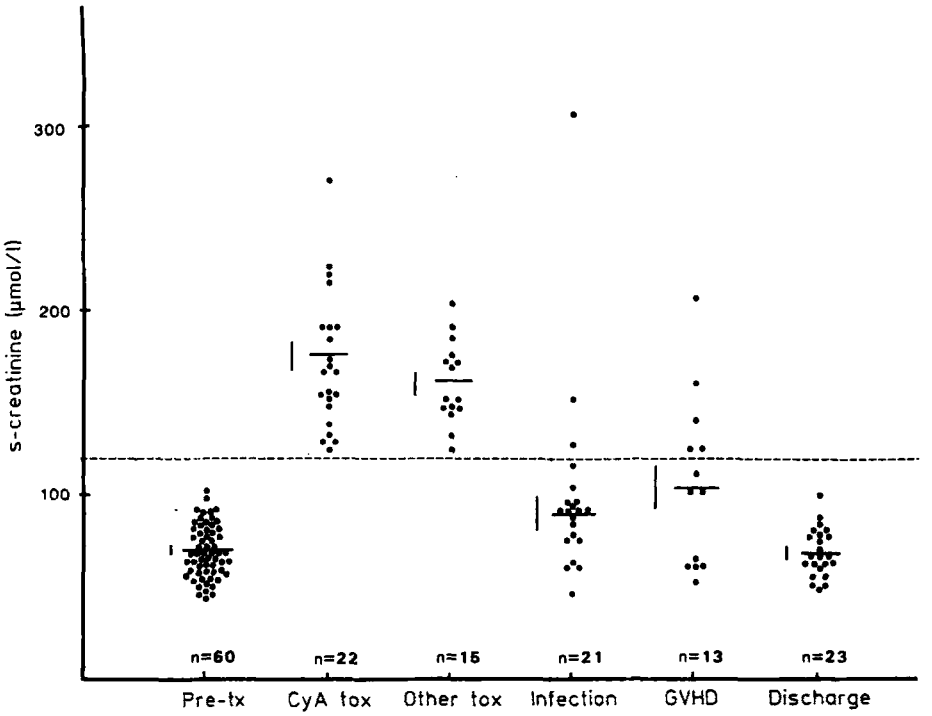

Fig. 1. Individual serum creatinine levels in bone marrow transplant recipients. *Horizontal lines* indicate means, *vertical bars* SEM, and the *dotted line* the upper normal limit. *PRE-Tx* Pretransplant; *CyA tox* cyclosporin-induced nephrotoxicity; *Other tox* nephrotoxicity induced by other drugs; *GVHD* acute graft-versus-host disease; *Discharge* discharge from the hospital

Diagnoses of nephrotoxicity, infections, and GVHD

The CyA dose was reduced when signs of nephrotoxicity were seen and not in response to the plasma levels. CyA nephrotoxicity (CyA tox, $n = 22$) was defined as more than 100% increase in s-crea in comparison to the pretransplant levels. An improvement in renal function after reduction of the CyA dose was taken as evidence for CyA-induced nephrotoxicity. Nephrotoxicity due to other drugs (other tox, $n = 15$) was defined as more than 100% increase in s-crea during treatment with aminoglycosides (netilmicin), amphotericin B, or co-trimoxazole compared to pretransplant s-crea levels. The dosage of nephrotoxic drugs was reduced or treatment stopped, and subsequent improvement in renal function was taken as evidence for nephrotoxicity induced by these drugs.

GVHD ($n = 13$) was diagnosed clinically or by biopsies from skin or oral mucosa and was graded from I to IV [13]. Acute GVHD was treated with prednisolone (2 mg/kg per day) and, in severe cases, methylprednisolone (0.25–0.5 g/day) was added. Grade I GVHD was observed in six patients, grade II in four, and grades III–IV in three patients.

Infections ($n = 21$) consisted of bacterial ($n = 16$), diagnosed by positive blood cultures, and fungal ($n = 5$) infections, diagnosed by positive cultures and serological tests [14]. One patient with invasive candidiasis had free-circulating *Candida* mannan antigen and positive blood cultures for *Candida albicans*. Four additional patients had colonization at several anatomic sites or had persistent colonization of the oropharynx or gut, but free-circulating *Candida* mannan antigen was not detected.

Analyses of α_1m, β_2m, and creatinine

Serum samples were frozen at −20 °C and subsequently analyzed. Serum samples were obtained before transplantation ($n = 60$), on specific occasions ($n = 71$), and on the day of discharge from the transplant unit ($n = 23$). α_1m levels were measured by single radial immunodiffusion (Behringwerke, Marburg, FRG) with a normal range of 20–42 mg/l, and β_2m by an enzyme-linked immunosorbent assay (Behringwerke, Marburg, FRG), normal range 1.1–2.4 mg/l. S-crea, normal value less than 115 µmol/l, was analyzed using the kinetic Jaffé method. The accuracy of this method has been evaluated with a reference method based on isotope dilution-mass spectrometry [3].

Statistics

Statistical analyses were made using the Mann-Whitney U-ranking test, linear correlation, and chi-square analysis. Values from day of diagnosis of the different conditions were used for comparisons. Values are given as mean ± SEM.

Results

Pretransplant and post-transplant levels and nephrotoxicity

The pretransplant and discharge levels of s-crea, α_1m, and β_2m were, in general, within the normal ranges (Figs. 1–3). The pretransplant levels of α_1m and β_2m were 33.8 ± 0.6 mg/l (mean ± SEM) and 1.6 ± 0.1 mg/l, respectively, and were in the same range as the discharge levels. The mean α_1m levels during CyA- and other tox were 59.1 ± 2.2 mg/l (± SEM) and 61.4 ± 2.9 mg/l, respectively, and were significantly elevated in comparison to the pretransplant and discharge levels ($P < 0.001$). The β_2m was also significantly $P < 0.001$) elevated during the same conditions: 6.0 ± 0.6 mg/l (mean ± SEM) and 5.0 ± 0.3 mg/l, respectively, as were the s-crea levels ($P < 0.001$, Fig. 1).

Infections and GVHD

The levels of α_1m and β_2m during infections were 44.0 ± 3.3 mg/l (mean ± SEM) and 3.4 ± 0.3 mg/l, respectively, and were significantly elevated in comparison to

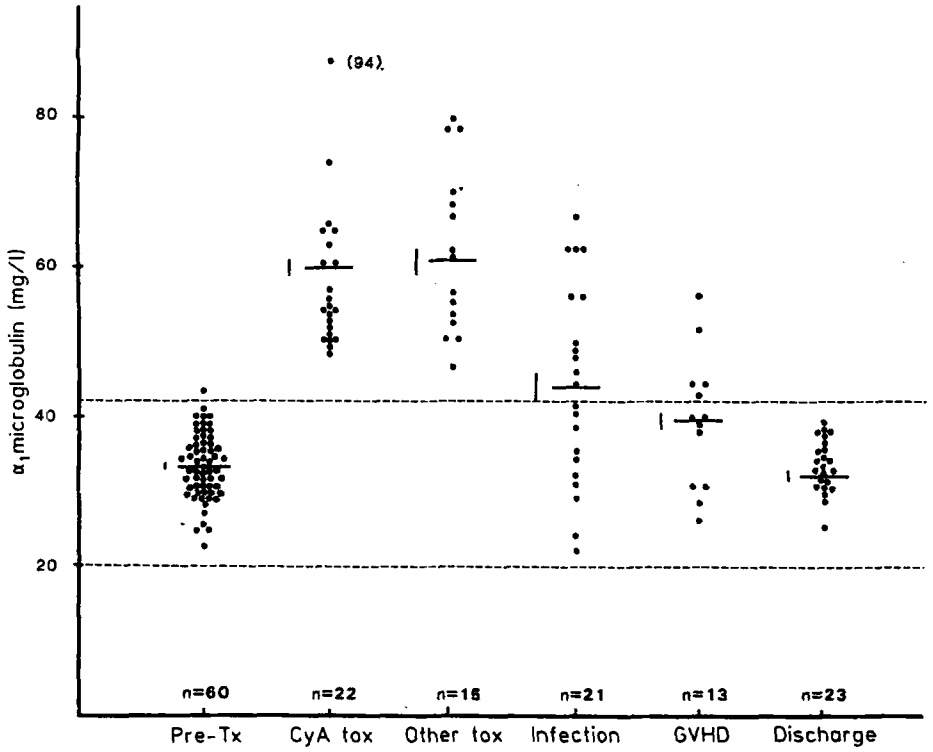

Fig. 2. Individual serum alpha-1 microglobulin levels after bone marrow transplantation. *Horizontal lines* indicate means, *vertical bars* SEM, and the *dotted lines* the limits of the normal range. Abbreviations as in Fig. 1

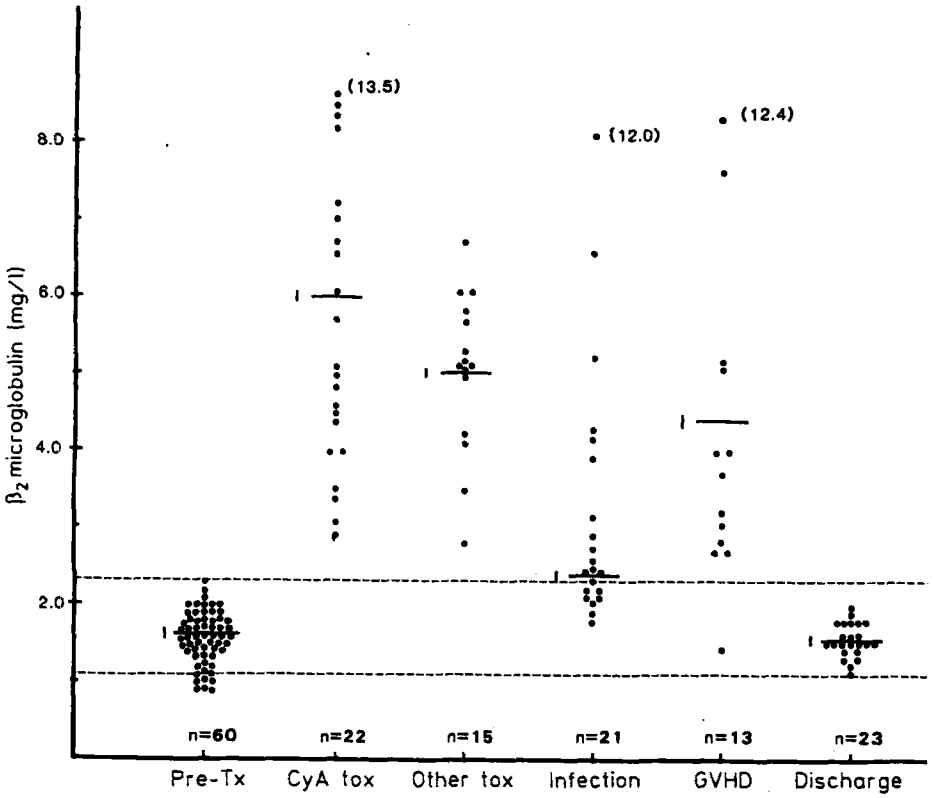

Fig. 3. Individual serum β_2 microglobulin levels after bone marrow transplantation. *Horizontal lines* indicate means, *vertical bars* SEM, and the *dotted lines* the limits of the normal range. Abbreviations as in Fig. 1

the pretransplant and discharge levels ($P < 0.01$ and $P < 0.001$, respectively; Figs. 2, 3). S-crea was also elevated (90 ± 9 µmol/l, $P < 0.001$) but was still within the normal range (Fig. 1). The α_1m and s-crea levels displayed 1.3-fold increases, while the mean β_2m increase was more than twofold. There were no differences in α_1m or β_2m levels when comparing patients with fungal and bacterial infections: 44.0 ± 3.3 mg/l (mean ± SEM) and 3.4 ± 0.3 mg/l, respectively, (NS). During GVHD (grades I–IV), both mean α_1m and β_2m were elevated ($P < 0.05$ and $P < 0.01$, respectively; Figs. 1–3). However, 8/13 of the patients had α_1m and s-crea levels within the normal range, while only 1/13 of the β_2 levels were within the normal range ($P < 0.001$). When comparing different grades of

Table 1. Serum creatinine, α_1m, and β_2m levels in patients with and without different grades of acute GVHD (means ± SEM)

GVHD (grade)	Serum creatinine (μmol/l)	α_1m (mg/l)	β_2m (mg/l)
0 (discharge, $n = 23$)	70 ± 3	32.7 ± 0.9	1.6 ± 0.1
		*	**
I ($n = 6$)	101 ± 16	45 ± 3.0	4.0 ± 0.4
		NS	NS
II–IV ($n = 7$)	109 ± 20	35 ± 3.0	4.8 ± 1.5

* $P < 0.05$; ** $P < 0.01$

Table 2. Linear correlation between s-crea, α_1m, and β_2m during different conditions after bone marrow transplantation

		S-crea vs α_1m	S-crea vs β_2m	α_1m vs β_2m
All patients	($n = 77$)	$r = 0.7$**	$r = 0.8$**	$r = 0.4$*
Pretransplant	($n = 60$)	$r = 0.1$ NS	$r = 0.1$ NS	$r = 0.2$ NS
CyA tox	($n = 22$)	$r = 0.6$**	$r = 0.7$**	$r = 0.4$*
Other tox	($n = 15$)	$r = 0.5$**	$r = 0.6$**	$r = 0.3$*
Infections	($n = 21$)	$r = 0.4$*	$r = 0.8$**	$r = 0.4$*
GVHD	($n = 13$)	$r = 0.4$*	$r = 0.8$**	$r = 0.4$*
Discharge	($n = 23$)	$r = 0.1$ NS	$r = 0.2$ NS	$r = 0.1$ NS

*$P < 0.01$; **$P < 0.001$

GVHD, there were no significant differences in s-crea, α_1m, or β_2m levels (Table 1). The highest β_2m and s-crea levels were obtained in patients with grades II–IV GVHD (NS), while the α_1m level during the same condition was the same as in the discharge group (NS).

Correlations between α_1m, β_2m, and s-crea levels

The linear correlations (r) between α_1m, β_2m, and s-crea during the conditions investigated are shown in Table 2. The highest degrees of linear correlation were found between s-crea and β_2m during infections and GVHD ($r = 0.8$, $P < 0.001$). The overall correlations between α_1m, β_2m, and s-crea were significant ($r = 0.4$–0.8). The correlations were generally positive and significant, except during pretransplant and at discharge.

Discussion

The highest levels of α_1m and β_2m were found during episodes of nephrotoxicity. This is not surprising since it is known that the levels of α_1m and β_2m increase during deteriorations in renal function [1, 2, 4, 12]. Interestingly, the levels of β_2m displayed a more pronounced increase than those of α_1m during nephrotoxicity, indicating different ways of renal metabolism of these two proteins.

β_2m has been suggested to be a more sensitive indicator of the glomerular filtration rate than s-crea since s-crea might be normal despite slight to moderate deteriorations in the glomerular filtration rate. It was previously shown that α_1m and β_2m increased before elevations of s-crea were observed [1, 3, 4, 12]. It is, therefore, interesting that both the pretransplant and discharge levels of α_1m and β_2m were within the normal ranges in bone marrow transplant recipients. This suggests that BMT recipients, in general, have a relatively normal renal function despite treatment with irradiation and cytostatic and nephrotoxic drugs.

α_1m and β_2m were also elevated during infections and GVHD. It was previously shown in renal transplant recipients that β_2m was markedly elevated during infections and inflammatory events, and that α_1m levels were less influenced during the same conditions [2]. Serum levels of α_1m were less affected by GVHD than those of β_2m, and a significantly larger number of the individual α_1m levels were within the normal range in comparison to β_2m levels. This further indicates different ways of production and metabolism of these two molecules. β_2m is the small invariate chain of the HLA antigens [7], but the site of production of α_1m is unknown. Acute GVHD may be activated by differences in HLA antigens between recipient and donor or by minor histocompatibility antigens in the HLA-identical situation. After the effector phase of GVHD, the class I HLA antigens may be targets for cytotoxic T cells. It is possible that this cytotoxic reaction results in an increased release of β_2m from the cell surface because of the linkage between β_2m and the HLA-antigenic determinants. Such a mechanism may explain the higher serum levels of β_2m during acute GVHD. There was no difference in patients with grade I or more severe GVHD. The reason for this may be that in more severe forms of GVHD, nonspecific cytotoxic cells responsible for necrosis and cell damage are recruited. During CMV infections in transplant recipients, the levels of serum β_2m are highly increased [1, 6]. This release may also be due to cytotoxic T cells reacting with HLA antigens in target cells.

In general, the linear correlations between the parameters studied were positive and significant with two important exceptions, i.e., the pretransplant and discharge levels. It was shown in renal transplant recipients that patients with a stable renal function displayed a linear correlation between α_1m, β_2m, and s-crea during stable renal function. There are, however, important differences between recipients of bone marrow and recipients of renal allografts. Renal transplant recipients have one kidney that is denervated and are known to have deteriorations in renal function despite stable s-crea levels within the normal range. The different degrees of linear correlation between α_1m, β_2m, and s-crea during the different conditions again stress the probability of different modes of renal handling for α_1m and β_2m. However, further studies need to be done in order to elucidate the exact manner of renal handling and metabolism of α_1m.

In conclusion, serum levels of α_1m and β_2m were elevated during drug-induced nephrotoxicity and inflammatory events. The levels of α_1m were, however, less influenced by acute GVHD. Determination of α_1m and β_2m in serum might be a complement to s-crea as an indicator of renal function.

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