Secondary monoclonal gammopathy of undetermined significance after allogeneic stem cell transplantation in multiple myeloma

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ABSTRACT

In the course of multiple myeloma, patients may develop a M-protein band different from the original: secondary monoclonal gammopathy of undetermined significance. In this retrospective single center analysis, we describe the occurrence and clinical relevance of secondary monoclonal gammopathy of undetermined significance after allogeneic stem cell transplantation (post-transplant monoclonal gammopathy of undetermined significance). A total of 138 patients who had undergone 139 allogeneic stem cell transplantations (39.6% in the upfront setting and 60.4% for relapsed multiple myeloma) were included in the study. Sixty-seven (48.2%) patients developed secondary monoclonal gammopathy of undetermined significance, after a median latency of 6.9 months. Secondary monoclonal gammopathy of undetermined significance occurred more often in patients who achieved at least very good partial response after allogeneic stem cell transplantation, compared to partial response or less (54.8% *vs.* 26.5%; P=0.005). The incidence was also higher in the upfront setting as compared to relapsed disease, or with a sibling donor compared to matched unrelated donor, but less often after T-cell depletion. Importantly, development of post-transplant monoclonal gammopathy of undetermined significance as a time-dependent variable independently predicted for superior progression-free and overall survival (median progression-free survival 37.5 *vs.* 6.3 months, P<0.001; median overall survival 115.3 *vs.* 31.0 months, P=0.004). Clinicians should be aware of the benign nature of this phenomenon, and secondary monoclonal gammopathy of undetermined significance should not be confused with relapse or progression of disease. (Trial registered with *trialregister.nl*; HOVON 108: NTR 2958.)

Introduction

Multiple myeloma (MM) is a hematologic malignancy originating from plasma cells that typically produce a monoclonal immunoglobulin (M-protein). The types of heavy and light chain of this M-protein are specific to the myeloma clone. Therefore, it is used as a marker for diagnosis and monitoring of disease and response in MM.

In the course of MM, patients may develop monoclonal bands of different isotypes to the original myeloma M-protein.[1-17] Several terms have been used to describe this phenomenon, including abnormal protein band, oligoclonal protein bands, transient mono- or oligoclonal gammopathy, apparent isotype switch, oligoclonal humoral response, atypical serum immunofixation pattern, and in myeloma patients, as we will use in this study, secondary monoclonal gammopathy of undetermined significance MGUS (sMGUS).[18] We consider post-transplant MGUS as a subtype of sMGUS.

Secondary MGUS occurs more frequently after treatment with autologous stem cell transplantation (auto-SCT) (10%-73%)[1-6,8-14,17] than in patients who have not undergone auto-SCT (1.6%-3.1%).[4,9] Several studies also showed a higher frequency of sMGUS in patients treated with novel agents when compared to conventional chemotherapy.[7,16,17] Importantly, sMGUS is not a sign of relapse or progression of MM.[3,17,19,20] In fact, some studies found that patients who develop sMGUS have a superior prognosis in terms of progression-free survival (PFS) and overall survival (OS) than those without sMGUS.[1,2,4,6,9,10,12-14,17] However, other studies, often with a small sample size, concluded that the presence of sMGUS does not relate to the prognosis of MM, or that it is only associated with a better response and not with a benefit in terms of PFS or OS.[5,7,8,11]

In MM, allogeneic stem cell transplantation (allo-SCT) results in a high rate of molecular remissions and lower risk of relapse by virtue of the graft-versus-myeloma (GvM) effect.[21,22] However, because of high treatment-related mortality (TRM), especially after myeloablative conditioning, and the emergence of the novel agents which are effective and have relatively mild toxicity, the value of this approach is disputed and still has to be determined in clinical trials.

After allo-SCT for various conditions, a high frequency of abnormal protein bands has been reported.[1,5,19,20,23-25] However, the total number of patients undergoing allo-SCT included in previous studies was very low, and allo-SCT was not always analyzed separately from other treatment regimens. Furthermore, most studies evaluated a heterogeneous population of patients with a variety of malignant and benign hematologic disorders, and patients with MM were often not included. Therefore, there are currently no data available regarding the frequency of sMGUS and its prognostic significance in MM patients who had undergone allo-SCT. Here,

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we describe the occurrence of post-transplant MGUS and its association with response, PFS and OS in this group of patients.

Methods

Patients, definitions, and diagnostic criteria

Between 1st January 2001 and 31st December 2012, 149 myeloma or primary plasma cell leukemia (pPCL) patients underwent a total of 154 allo-SCTs in the University Medical Center Utrecht (UMCU), Utrecht, the Netherlands. Patients were treated according to clinical protocols that were approved by the local ethics committee, and all patients gave their informed consent. When a patient received more than one allo-SCT in this period (n=4 patients), only successfully engrafted transplants were included in the analysis. Two patients experienced graft failure and 2 patients received two allo-SCTs several years apart during the inclusion period for this study. Cases were excluded from the analysis if sufficient data about protein bands were not available (n=12 allo-SCTs in 11 patients). Altogether, 139 allo-SCTs in 138 patients were included in the analysis.

Diagnosis of MM or pPCL was based on standard clinical assessment. Secondary MGUS was defined as appearance of a protein band on immunofixation or electrophoresis that is different from the original myeloma M-protein in heavy-chain or light-chain isotype, or in its migration pattern. M-protein analysis was performed using serum protein electrophoresis and immunofixation using the Sebia Hydragel system (Norcross, GA, USA), according to the manufacturer's instructions. M-proteins were detected by screening using amidoblack staining and a pentavalent antiserum directed against human IgG, IgA, IgM, kappa, and lambda, and typed using monovalent antisera. Serum M-protein levels were determined by densitometric scanning at 570 nm (HYRYS densitometer, Sebia), which has a lower limit of M-protein quantification of around 2.0 g/L.

Acute graft-*versus*-host-disease (GvHD) was grade I-IV according to Seattle criteria[26] and chronic GvHD was defined as limited or extensive according to Shulman *et al.*[27] Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) reactivation were determined by quantitative PCR. Invasive aspergillosis was diagnosed if the patient fulfilled criteria for possible, probable, or proven aspergillosis.[28] Response to treatment (before and after allo-SCT) and progression were determined according to the criteria formulated by the International Myeloma Working Group.[29]

Overall survival was measured in months and defined from the date of allo-SCT to the date of death or last follow up. PFS was defined as the time from SCT to date of progression or death from any cause or last follow up. TRM was defined as death owing to any cause other than disease progression or relapse occurring at any time after transplant.

Data and follow up

Data on treatment, response, progression, and clinical events were extracted from patient files. The clinical events that were collected included acute and chronic GvHD, reactivation of EBV/CMV, and invasive aspergillosis. Information on the original M-protein and sMGUS was collected from the administration of the laboratory of medical immunology. We registered the number of protein bands, the isotype and quantity of immunoglobulin, the time to appearance calculated from date of allo-SCT, as well as the duration of the sMGUS.

Follow up with laboratory analysis including serum protein electrophoresis and immunofixation was usually performed every 1-2 months until death or, in case of survival, on 31st July 2013. Clinical events, including sMGUS, were registered until progression occurred, or were censored at the date of death or last follow up.

Statistical analysis

Overall survival and PFS were estimated by the Kaplan-Meier method. Univariate Cox regression was used to determine the prognostic value for OS and PFS. The factors that showed a significance of $P \leq 0.10$ were included in a multivariate Cox regression model (backward analysis) to identify independent predictive factors. sMGUS was analyzed as a time-dependent covariate.

Differences in categorical variables were determined with the Fisher's exact test for 2x2 tables and otherwise with the Pearson's χ^2 test. All P-values were two-sided; $P<0.05$ was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics v.20.0 and 21.0 (SPSS Inc., IL, USA).

Results

Patients' and transplant characteristics

We included a total of 138 patients who underwent 139 allo-SCTs. A hundred and thirty-four patients (96.4%) were diagnosed with MM while 5 patients (3.6%) had pPCL (Table 1). Fifty-five allo-SCTs were performed as part of first-line treatment (39.6%) and 84 (60.4%) for relapsed MM. Induction therapy included novel agents in 47.3% of newly diagnosed patients (no novel agent 52.7%; bortezomib 12.7%; lenalidomide 0.0%; thalidomide 34.5%) and 83.3% of patients who received allo-SCT in the relapse setting (no novel agent 16.7%; bortezomib 17.9%; lenalidomide 35.7%; thalidomide 17.9%; bortezomib + IMiD 11.9%) ($P<0.001$). Transplantation was performed in a non-myeloablative setting in most patients (76.3%). Thirty patients (21.6%) received a semi-ablative conditioning regimen, consisting of alemtuzumab and melphalan plus fludarabine (HOVON 108), or fludarabine plus busilfex. Only 3 patients (2.2%) received a myeloablative conditioning regimen. T-cell depletion was performed with anti-thymocyte globulin (ATG; *in vivo*) in case of an unrelated donor or HLA-mismatch between donor and recipient in 54 transplantations (38.9%), or with alemtuzumab (*in vivo* as well as "in the bag") as part of the HOVON 108 trial in 30 (21.6%) transplantations. All other patients' and transplant characteristics are shown in Table 1.

Transplant outcome and survival

After allo-SCT, 56 patients (40.3%) achieved complete remission (CR) and at least a very good partial response (VGPR) was achieved in 104 patients (74.8%) (Table 2). Fifty-five patients (39.6%) developed acute GvHD of at least grade 2 and chronic GvHD occurred in 60 patients (43.2%); it was classified as extensive in 43 patients and limited in 17 patients. Reactivation of CMV and EBV occurred in 41 (29.5%) and 41 (29.5%) patients, respectively. Twenty patients (14.4%) met criteria for invasive aspergillosis. TRM at six and 12 months after allo-SCT was 8.7 and 11.1%, respectively. The median PFS and OS for all patients, as well as for the subgroups of patients who received allo-SCT as part of first-line treatment or in the relapse setting, is shown in Table 2.

Secondary MGUS

Sixty-seven (48.2%) of the patients developed at least one sMGUS after allo-SCT. A total of 217 new bands were

Table 1. Patients' characteristics.

	N (%)
Age at the time of allo-SCT (years)	
Median	55.7
Range	32.0-66.4
Sex	
Male	90 (64.7%)
Female	49 (35.3%)
Disease	
Multiple myeloma	134 (96.4%)
Primary plasma cell leukemia	5 (3.6%)
Original M-protein	
IgG-kappa	61 (43.9%)
IgG-lambda	29 (20.9%)
IgA-kappa	23 (16.5%)
IgA-lambda	5 (3.6%)
IgM-kappa†	1 (0.7%)
IgM-lambda†	2 (1.4%)
Light-chain kappa	8 (5.8%)
Light-chain lambda	6 (4.3%)
Non-secretory	4 (2.9%)
Remission status before allo-SCT	
CR	14 (10.1%)
VGPR	60 (43.2%)
PR	52 (37.4%)
Less than PR	13 (9.4%)
Conditioning regimen	
Non-myeloablative	106 (76.3%)
Semi-ablative	30 (21.6%)
Myeloablative	3 (2.2%)
T cell depletion with ATG or alemtuzumab	
Yes	84 (60.4%)
No	55 (39.6%)
Prior treatment with novel agents	
Yes	96 (69.1%)
Bortezomib	22 (15.8%)
Lenalidomide	30 (21.6%)
Thalidomide	34 (24.5%)
Combination	10 (7.2%)
No	43 (30.9%)
Extent of prior therapy	
Part of first-line treatment	55 (39.6%)
Relapse setting	84 (60.4%)
Donor type	
Sib	79 (56.8%)
MUD	60 (43.2%)
HLA mismatch	
Yes	23 (16.5%)
No	116 (83.5%)
Patient/donor: male/female	
Yes	31 (22.3%)
No	108 (77.7%)

SCT: stem cell transplantation; CR: complete remission; VGPR: very good partial response; PR: partial response; ATG: anti-thymocyte globulin; Sib: sibling donor; MUD: matched unrelated donor; MM: multiple myeloma. †All 3 patients with IgM MM had extensive osteolytic bone lesions; cytogenetic analysis was performed in 2 patients with cytogenetic abnormalities typical of MM in both [one with t(11;14) and one with ampl(1q)]; all 3 patients were characterized by an aberrant plasma cell phenotype.

Table 2. Outcome after allogeneic stem cell transplantation.

Remission status after allo-SCT	
CR	56 (40.3%)
VGPR	48 (34.5%)
PR	22 (15.8%)
Less than PR	11 (7.9%)
Too early to evaluate	1 (0.7%)
Acute GVHD grade 2-4	55 (39.6%)
Chronic GVHD	60 (43.2%)
Limited	17 (12.2%)
Extensive	43 (30.9%)
EBV reactivation	41 (29.5%)
CMV reactivation	41 (29.5%)
Invasive aspergillosis	20 (14.4%)
Median PFS	
Whole group	13.4
First line treatment	35.4
Relapse treatment	8.3
Median OS	
Whole group	78.1
First-line treatment	118.3
Relapse treatment	37.7

SCT: stem cell transplantation; CR: complete remission; VGPR: very good partial response; PR: partial response; GvHD: graft-versus-host disease; EBV: Epstein-Barr virus; CMV: cytomegalovirus; PFS: progression-free survival; OS: overall survival.

identified. Twenty-five patients had only one new protein band (18.0%), 9 (6.5%) had 2 bands, 8 (5.8%) had 3 bands, and 25 (18.0%) had 4 or more. The most common types of secondary MGUS were IgG-kappa (31.3%), IgG lambda (25.3%), IgM lambda (10.6%), and IgM kappa (7.8%). In most cases it was not possible to quantify the level of sMGUS. Abnormal protein bands could be quantified in only 4 patients, with a maximum level of 11 g/L.

Secondary MGUS first appeared after a median latency of 6.9 months (range 1.1-111.3 months) after allo-SCT. In 27 patients (40.3% of all sMGUS patients) sMGUS appeared within six months from allo-SCT, in 50 patients (74.6%) within 12 months, and in 58 patients (86.6%) within 18 months.

The median duration of all sMGUS cases was 4.47 months (range 0.0-74.5 months). Median duration of the longest sMGUS case per patient was 7.59 months (range 0.0-74.5 months). In 45 patients (67.2%), the longest sMGUS case persisted for six months or more, while in 25 patients (37.3%) the longest sMGUS persisted for 12 months or more. There was no progression of sMGUS to MM or other lymphoproliferative diseases.

Remission status after transplantation was associated with occurrence of sMGUS. Patients who achieved CR or VGPR more frequently developed a new protein band compared to patients who achieved PR or less (54.8 *vs.* 26.5%; P=0.005). Secondary MGUS also occurred more often in newly diagnosed MM patients compared to relapsed patients (60.0% *vs.* 40.5%; P=0.037). Patients with a sibling (sib) donor had a higher incidence of sMGUS, compared to matched unrelated donor (57.0% *vs.* 36.7%; P=0.026). Patients treated with novel agent-based induction therapy had a lower incidence of sMGUS compared to patients treated with conventional chemotherapy alone (39.6% *vs.* 67.4%; P=0.003). There was no difference in the frequency of sMGUS in patients treated with bortezomib, thalidomide, or lenalidomide. Furthermore, sMGUS was less often observed after T-cell depletion with ATG or alemtuzumab (39.3% *vs.* 61.8%; P=0.025). There was a trend towards a higher frequency of sMGUS in patients who developed chronic GvHD (41.8% *vs.* 56.7%; P=0.090). Gender, conditioning regimen, HLA-mismatch, remission status before allo-SCT, EBV reactiva-

tion, CMV reactivation, invasive aspergillosis, acute GvHD and type of original M-protein were not associated with development of sMGUS. One out of 3 patients (33.3%) with IgM MM and 4 out of 5 pPCL (80.0%) patients developed sMGUS.

Predictive factors for PFS and OS

Univariate Cox regression analysis with sMGUS as a time-dependent variable showed that patients who developed sMGUS had a significantly longer PFS and OS when compared to patients who did not develop sMGUS (median PFS: 37.5 *vs.* 6.3 months, *P*<0.001; and median OS: 115.3 *vs.* 31.0 months, *P*=0.004) (Figure 1). There was no significant difference for both PFS and OS between patients who developed sMGUS during the first six, 12, or 18 months after allo-SCT or later. In addition, PFS and OS were not associated with the duration of the longest sMGUS (both for duration ≥6 months and ≥12 months). However, the total number of sMGUS cases after allo-SCT was associated with PFS (*P*<0.001) and OS (*P*<0.001). Patients who developed 3 or more sMGUS cases had a better PFS and OS than patients who developed 1 or 2 protein bands or no sMGUS (median PFS: 78.5 *vs.* 21.1 *vs.* 6.3 months; median OS: not reached *vs.* 74.5 *vs.* 31.0 months). Similar results were obtained when patients with IgM MM and pPCL (rare plasma cell dyscrasias), were excluded from the analysis. Since most TRM occurred in the first six months after allo-SCT (12 out of 15 TRM cases; 80%), we also performed a landmark analysis at this time point. PFS and OS remained significantly superior in patients with sMGUS (n=100 patients; median PFS: 31.5 *vs.* 4.9 months, *P*<0.001; median OS: 109.3 *vs.* 57.3 months, *P*=0.015). Although similar survival advantages were observed in a landmark analysis at 12 months after allo-SCT (n=69 patients; median PFS: 46.0 *vs.* 18.2 months; median OS not reached *vs.* 119.7 months), these differences were no longer statistically significant.

In a subgroup analysis for patients who received allo-SCT as part of first-line treatment, development of sMGUS (time-dependent covariate) also predicted for improved PFS (median: 49.2 *vs.* 10.9 months, *P*=0.010). In this group, OS was longer in patients who developed sMGUS, but this did not reach statistical significance (median: not reached *vs.* 99.5 months; *P*=0.154) (Figure 2). In addition, although median PFS and OS following allo-SCT were shorter in the relapse setting, development of sMGUS predicted for both better PFS (median: 22.5 *vs.* 5.1 months; *P*<0.001) and OS (median: 78.9 *vs.* 24.1; P=0.028).

Another subgroup analysis showed that median PFS was 41.2 months in patients who achieved at least VGPR and developed sMGUS after allo-SCT, while it was only 7.5 months in CR/VGPR patients who did not develop sMGUS after allo-SCT (*P*=0.002) (Figure 3). Also for patients who achieved less than VGPR after allo-SCT, the development of sMGUS was associated with better PFS (14.3 *vs.* 3.8 months; *P*<0.001). Median OS in patients who achieved at least VGPR and developed sMGUS was 115.3 months, compared to 37.1 months in these patients without sMGUS (*P*=0.042). However, in patients who achieved less than VGPR, OS was not significantly better in patients who developed sMGUS after allo-SCT (median OS: 50.0 *vs.* 27.2 months; *P*=0.325).

Other factors associated with both PFS and OS after allo-SCT are age at the time of allo-SCT, extent of prior

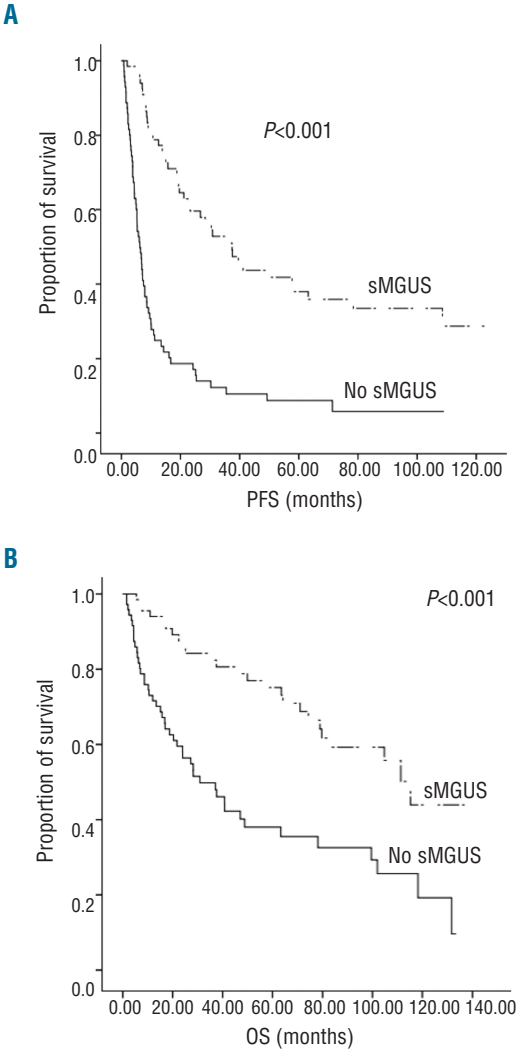

Figure 1. Progression-free survival (PFS) (A) and overall survival (OS) (B) according to the presence of secondary monoclonal gammopathy of undetermined significance (sMGUS). Kaplan-Meier analysis was used to test the statistical significance of differences between the survival curves.

treatment, chronic GvHD, use of novel agents, stem cell source, T-cell depletion, and remission status after allo-SCT. In addition, development of acute GvHD and a non-myeloablative conditioning regimen predicted for improved PFS, and presence of HLA mismatch for inferior OS (Table 3).

Multivariate Cox regression analysis

Multivariate Cox regression analysis showed that development of sMGUS as a time-dependent variable (PFS: HR 0.032, *P*<0.001; OS: HR 0.11, *P*=0.002), age at allo-SCT (PFS: HR 2.45, *P*<0.001; OS: HR 2.42, *P*=0.002), and use of novel agents (PFS: HR 1.90, *P*=0.005; OS: HR 1.97, *P*=0.019) were independent predictive factors for PFS and OS. Conditioning regimen (HR 0.33, *P*<0.001), stem cell source (HR 0.46, *P*<0.001), acute GvHD (HR 0.66, *P*=0.049), and remission status after allo-SCT (HR 0.28, *P*<0.001) also independently predicted for PFS, and T-cell depletion (HR 3.25, *P*<0.001) for OS.

Since type of conditioning regimen and stem cell source,

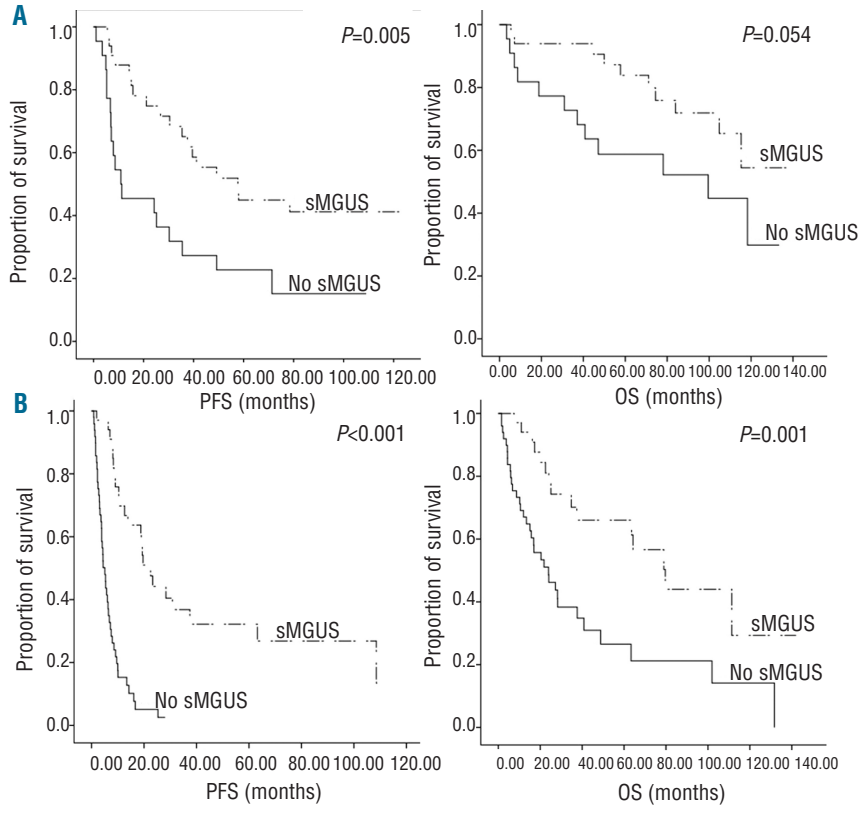

Figure 2. Progression-free survival (PFS) and overall survival (OS) for patients who received allogeneic stem cell transplantation (allo-SCT) as part of first-line treatment (A) or after relapse (B) according to the development of secondary monoclonal gammopathy of undetermined significance (sMGUS). Kaplan-Meier analysis was used to test the statistical significance of differences between the survival curves.

Table 3. Univariate analysis of prognostic indicators of progression-free survival and overall survival.

	PFS; P-value	HR	95% CI	OS; P-value	HR	95% CI
Secondary MGUS						
(Present - time dependent)	<0.001	0.062	0.020-0.195	0.004	0.127	0.0.031-0.524
(Number of sMGUS[1])	<0.001	*	*	<0.001	*	*
Duration longest sMGUS						
(≥6 months)	0.144	0.615	0.321-1.181	0.722	0.852	0.353-2.058
(≥12 months)	0.894	1.043	0.562-1.938	0.970	1.015	0.457-2.257
Sex (Male)	0.189	0.752	0.492-1.151	0.986	1.005	0.609-1.656
Age at time of SCT (> 50 years)	0.035	1.623	1.036-2.542	0.046	1.750	1.010-3.032
Extent of prior therapy (Part of first line treatment)	<0.001	0.438	0.289-0.662	<0.001	0.343	0.203-0.581
Remission status before allo-SCT						
(CR)	0.906	0.962	0.500-1.848	0.507	1.360	0.548-3.390
(CR and VGPR)	0.527	0.758	0.601-1.299	0.769	1.073	0.672-1.712
Remission status after allo-SCT						
(CR)	<0.001	0.232	0.148-0.363	<0.001	0.241	0.133-0.437
(CR and VGPR)	<0.001	0.305	0.199-0.469	0.011	0.524	0.319-0.860
EBV reactivation	0.830	1.047	0.690-1.590	0.385	1.252	0.755-2.075
CMV reactivation	0.448	1.178	0.772-1.795	0.144	1.479	0.875-2.500
Aspergillosis	0.850	1.054	0.610-1.824	0.122	1.637	0.876-3.058
Acute GVHD (grade 2-4)	0.009	0.581	0.386-0.876	0.308	0.774	0.473-1.266
Chronic GVHD (limited and extensive)	0.002	0.536	0.361-0.796	0.008	0.519	0.318-0.845
Prior treatment with novel agents	0.004	1.876	1.224-2.875	0.003	2.299	1.336-3.957
Conditioning regimen (non-myeloablative)	0.002	0.497	0.317-0.778	0.097	0.615	0.346-1.092
Donor type (sib)	0.005	0.569	0.383-0.843	<0.001	0.363	0.222-0.593
HLA mismatch (yes)	0.290	1.311	0.794-2.160	0.007	2.088	1.218-3.584
Patient/donor (male/female)	0.490	0.855	0.548-1.334	0.896	0.965	0.563-1.654
T-cell depletion with ATG or alemtuzumab	<0.001	2.252	1.497-3.390	<0.001	3.534	2.058-6.061

*[1]Groups compared: no sMGUS vs. 1-2 sMGUS vs. ≥3 sMGUS. *Not applicable (multiple categories). PFS: progression-free survival; OS: overall survival; HR: hazard ratio; CI: confidence interval; MGUS: monoclonal gammopathy of undetermined significance; sMGUS: secondary monoclonal gammopathy of undetermined significance; SCT: stem cell transplantation; CR: complete remission; VGPR: very good partial response; PR: partial response; GvHD: graft-versus-host disease; EBV: Epstein-Barr virus; CMV: cytomegalovirus; sib: sibling donor; ATG: anti-thymocyte globulin.*

presence of HLA mismatch, as well as use of novel agents and T-cell depletion (ATG and/or alemtuzumab) are associated with treatment line (upfront *vs.* relapse setting), we also performed a separate multivariate analysis without these variables. This analysis showed that development of sMGUS as a time-dependent variable (PFS: HR 0.047, *P*<0.001; OS: HR 0.13, *P*=0.004), age at allo-SCT (PFS: HR 1.96, *P*=0.004; OS: HR 2.19, *P*=0.006), chronic GvHD (PFS: HR 0.62, *P*=0.02; OS: HR 0.58, *P*=0.037), extent of prior treatment (PFS: HR 0.46, P<0.001; OS: HR 0.46, *P*=0.004), and remission status after allo-SCT (≥VGPR *vs.* <VGPR; PFS HR 0.27, *P*<0.001; OS HR 0.60, *P*=0.05) were independent prognostic factors for both PFS and OS.

Discussion

This is the first study that evaluates the significance of sMGUS in MM patients treated with allo-SCT. In our population, almost half of the MM and pPCL patients developed sMGUS after allo-SCT, which was associated with an improved outcome. In at least 27.6% of the patients, the isotype did not differ from the original M-protein, but had a different migration pattern. The high frequency of post-transplant MGUS clearly demonstrates the importance for clinicians to recognize this benign phenomenon, so that it will not be confused with relapse or progression of disease.

Importantly, development of sMGUS after allo-SCT was associated with a better quality of response, as well as significantly improved PFS and OS, both in patients transplanted in the upfront setting and at the time of relapse. Since development of sMGUS is a post-allo-SCT event, we included sMGUS as a time-dependent variable in both univariate and multivariate Cox regression analysis, and we complemented these studies with a landmark analysis. The association of development of sMGUS with an improved outcome after allo-SCT, as demonstrated in this study, is consistent with several other reports that showed a better survival in MM patients with emergence of sMGUS after autologous SCT or chemotherapy.[1,2,4,6,9,10,12-14,17] However, some studies did not observe a favorable outcome in patients with sMGUS. This may be related to differences in sample size, duration of follow up, technical and procedural variability, use of novel agents, and the percentage of patients that underwent autologous transplantation, or, as shown here, allo-SCT. Importantly, we show that emergence of sMGUS is an independent predictor of PFS and OS after allo-SCT, with improved PFS and OS also in those patients achieving CR or VGPR after allo-SCT.

The oligoclonal bands appeared after a median of 6.9 months after allo-SCT. After auto-SCT the median time to development of sMGUS varied between 1.4 and 10 months after transplantation.[1,3-6,8,11,12,14] We frequently observed an alternating pattern of different oligoclonal bands, which is consistent of what has been described after autologous SCT.[17] The median duration of sMGUS after allo-SCT was 4.47 months (range 0.0-74.5 months). After autologous SCT the median duration of the abnormal protein bands varied between 3 and 17 months.[5,6,11-14,17] In our series of patients, we could not investigate the emergence of secondary light-chain MGUS[30] after allo-SCT, since the free-light chain measurement was performed infrequently, mainly to determine stringent CR, to exclude light-chain escape, or in the follow up of light-chain MM.

The emergence of sMGUS reflects a strong humoral immune response and is a sign of immune reconstitution

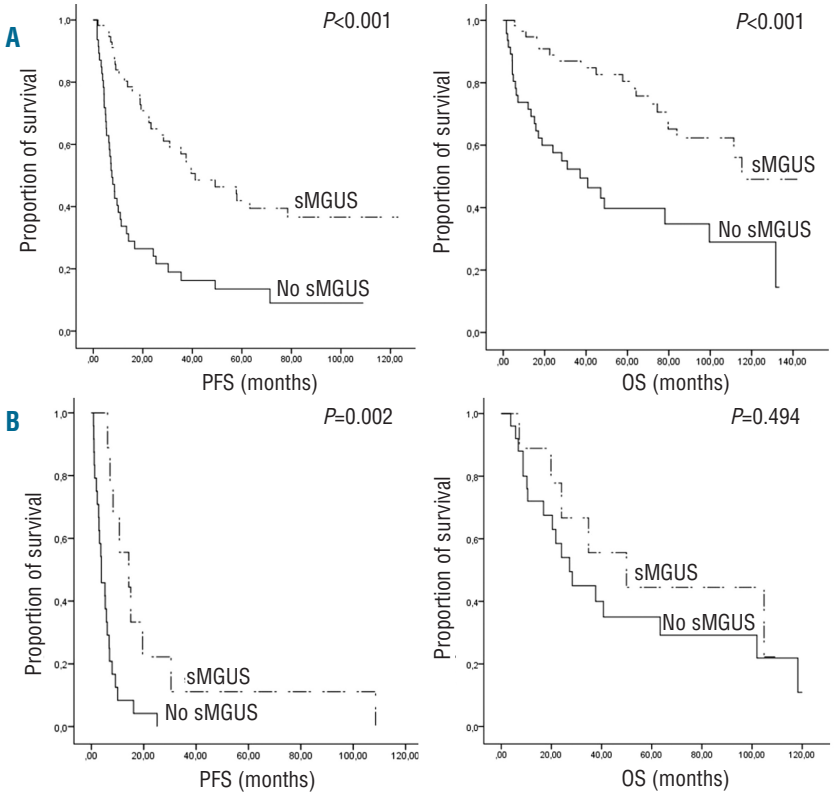

Figure 3. Progression-free survival (PFS) and overall survival (OS) for patients who achieved at least a very good partial response (VGPR) after allogeneic stem cell transplantation (allo-SCT) (A) or less than a very good partial response (VGPR) (B) after allo-SCT according to the development of secondary monoclonal gammopathy of undetermined significance (sMGUS). Kaplan-Meier analysis was used to test the statistical significance of differences between the survival curves.

after allo-SCT, autologous SCT, or novel agent-containing regimens. We and others observed a higher frequency of sMGUS in patients with high-quality responses, which suggests that major tumor reduction contributes to strong immune reconstitution and development of oligoclonal bands. Furthermore, one study demonstrated that patients receiving myeloablative conditioning prior to allo-SCT had a higher incidence of post-transplant MGUS.[19] In our series, conditioning had no effect on the incidence of post-transplant MGUS, which may be explained by the infrequent use of myeloablative conditioning regimens in our patients. In contrast to other studies,[7,16,17] we observed a lower incidence of sMGUS in patients who received novel agent-based induction therapy, which is probably explained by the less frequent use of novel agents in patients who received allo-SCT as part of first-line treatment compared to patients who were transplanted in the relapse setting. Interestingly, lenalidomide was used more frequently than bortezomib in patients with relapsed disease. This preference for lenalidomide may be related to the presence of polyneuropathy due to prior bortezomib and thalidomide therapy, and its convenience of oral administration. Lenalidomide was not part of first-line treatment since in the Netherlands it is only approved for use in the relapse setting. There was no difference in the frequency of sMGUS between bortezomib, thalidomide, or lenalidomide-treated patients.

After stem cell transplantation, impaired T-cell regulation of B-cell proliferation in the bone marrow may also be implicated in the pathogenesis of post-transplant MGUS.[4-8] The role of T cells in sMGUS development is also demonstrated by our finding that T-cell depletion with ATG or alemtuzumab resulted in a lower frequency of post-transplant MGUS.

There is no evidence that these abnormal protein bands are related to the myeloma clone. Guikema et al. used ASO-PCR and DNA sequencing to demonstrate that new serum M-components after auto-SCT are not produced by myeloma cells but rather by the regenerating B-cell compartment.[31] Furthermore, sMGUS not only occurs in MM patients but also after treatment for other hematologic malignancies, and even solid organ transplantations.[19,20,23,25]

It is currently not clear which antigens trigger the oligoclonal humoral immune response leading to sMGUS. Some studies found associations between occurrence of sMGUS and CMV or EBV reactivation or development of (acute/chronic) GvHD.[19,23,30-35] This suggests that oligoclonal bands may represent the generation of an immune response to infectious agents or that the oligoclonal bands mediate alloimmune reactions after allo-SCT. In our study, we demonstrated a trend towards a higher incidence of post-transplant MGUS in the presence of chronic GvHD, but no association with CMV, EBV, or aspergillus infections. In addition, the favorable prognosis conferred by sMGUS suggests that the oligoclonal bands may also be involved in an anti-myeloma immune response. Interestingly, a recent study showed that oligoclonal bands also target recurrent myeloma antigens, including MAGEA4 and heat-shock proteins.[36]

Limitations to this study are in a great part related to the retrospective design. There were no fixed moments of follow up. In addition, the patients received different induction and conditioning regimens prior to allo-SCT, and also treatment of relapse after transplantation was heterogeneous among the patients. Furthermore, this study encompassed a 12-year time period during which treatment and supportive care rapidly evolved.

We conclude that development of sMGUS after allo-SCT is a favorable prognostic factor for PFS and OS, independent of the response achieved after allo-SCT. Importantly, in order to avoid unnecessary treatment clinicians should be aware that sMGUS does not represent disease recurrence or development of a new malignancy.

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References

1. Maisnar V, Tichy M, Smolej L, Zak P, Radocha J, Palicka V, et al. Isotype class switching after transplantation in multiple myeloma. Neoplasma. 2007; 54(3):225-8.
2. Alejandre ME, Madalena LB, Pavlovsky MA, Facio ML, Corrado C, Milone G, et al. Oligoclonal bands and immunoglobulin isotype switch during monitoring of patients with multiple myeloma and autologous hematopoietic cell transplantation: a 16-year experience. Clin Chem Lab Med. 2010; 48(5):727-31.
3. Hovenga S, de Wolf JT, Guikema JE, Klip H, Smit JW, Smit Sibinga CT, et al. Autologous stem cell transplantation in multiple myeloma after VAD and EDAP courses: a high incidence of oligoclonal serum Igs post transplantation. Bone Marrow Transplant. 2000; 25(7):723-8.
4. Wadhera RK, Kyle RA, Larson DR, Dispenzieri A, Kumar S, Lazarus HM, et al. Incidence, clinical course, and prognosis of secondary monoclonal gammopathy of undetermined significance in patients with multiple myeloma. Blood. 2011; 118(11):2985-7.
5. Sucak G, Suyani E, Ozkurt ZN, Yegin ZA, Aki Z, Yagci M. Abnormal protein bands in patients with multiple myeloma after haematopoietic stem cell transplantation: does it have a prognostic significance? Hematol Oncol. 2010; 28(4):180-4.
6. Zent CS, Wilson CS, Tricot G, Jagannath S, Siegel D, Desikan KR, et al. Oligoclonal protein bands and Ig isotype switching in multiple myeloma treated with high-dose therapy and hematopoietic cell transplantation. Blood. 1998; 91(9):3518-23.
7. Mark T, Jayabalan D, Coleman M, Pearse RN, Wang YL, Lent R, et al. Atypical serum immunofixation patterns frequently emerge in immunomodulatory therapy and are associated with a high degree of response in multiple myeloma. Br J Haematol. 2008; 143(5):654-60.
8. Manson GV, Campagnaro E, Balog A, Kaplan D, Sommers SR, Fu P, et al. Secondary MGUS after autologous hematopoietic progenitor cell transplantation in plasma cell myeloma: a matter of undetermined significance. Bone Marrow Transplant. 2012; 47(9):1212-6.
9. Jo JC, Yoon DH, Kim S, Lee K, Kang EH, Jang S, et al. Clinical significance of the appearance of abnormal protein band in patients with multiple myeloma. Ann Hematol. 2014; 93(3):463-9.
10. Guimaraes C, Bergantim R, Ramalho R, Couto N, Guimaraes JT, Trigo F. Prognostic value of unrelated atypical serum immunofixation patterns during multiple myeloma therapy. J Hematol Oncol. 2012; 5:33. doi: 10.1186/1756-8722-5-33.:33-5.

11. Byrne E, Giles C, Andrews J, Rahemtulla A, Naresh KN. Lack of correlation between emergence of an abnormal protein band or of oligoclonal bands and survival in patients with multiple myeloma achieving complete remission following autologous stem cell transplantation. Haematologica. 2011;96 (4):e29.
12. Liu AJ, Zong H, Yang GZ, Zhai YH, Li LH. Significance of oligoclonal bands after stem cell transplantation in multiple myeloma cases. Asian Pac J Cancer Prev. 2012; 13(4):1483-6.
13. Jimenez-Zepeda VH, Reece DE, Trudel S, Franke N, Winter A, Chen C, et al. Oligoclonal and monoclonal bands after single autologous stem cell transplant in patients with multiple myeloma: impact on overall survival and progression-free survival. Leuk Lymphoma. 2014;55(10): 2284-9.
14. Hall SL, Tate J, Gill D, Mollee P. Significance of abnormal protein bands in patients with multiple myeloma following autologous stem cell transplantation. Clin Biochem Rev. 2009;30(3):113-8.
15. de Larrea CF, Cibeira MT, Elena M, Arostegui JI, Rosinol L, Rovira M, et al. Abnormal serum free light chain ratio in patients with multiple myeloma in complete remission has strong association with the presence of oligoclonal bands: implications for stringent complete remission definition. Blood. 2009;114(24):4954-6.
16. Fernandez de LC, Tovar N, Cibeira MT, Arostegui JI, Rosinol L, Elena M, et al. Emergence of oligoclonal bands in patients with multiple myeloma in complete remission after induction chemotherapy: association with the use of novel agents. Haematologica. 2011;96(1):171-3.
17. Tovar N, de Larrea CF, Arostegui JI, Cibeira MT, Rosinol L, Rovira M, et al. Natural history and prognostic impact of oligoclonal humoral response in patients with multiple myeloma after autologous stem cell transplantation: long-term results from a single institution. Haematologica. 2013;98(7): 1142-6.
18. van de Donk NW, Palumbo A, Johnsen HE, Engelhardt M, Gay F, Gregersen H, et al. The clinical relevance and management of monoclonal gammopathy of undetermined significance and related disorders: recommendations from the European Myeloma Network. Haematologica. 2014; 99(6):984-96.
19. Mitus AJ, Stein R, Rappeport JM, Antin JH, Weinstein HJ, Alper CA, et al. Monoclonal and oligoclonal gammopathy after bone marrow transplantation. Blood. 1989;74(8): 2764-8.
20. Nagashima T, Muroi K, Kawano-Yamamoto C, Komatsu N, Ozawa K. Paraproteinemia after hematopoietic stem cell transplantation. Leuk Lymphoma. 2004;45(1):135-7.
21. Lokhorst H, Einsele H, Vesole D, Bruno B, San MJ, Perez-Simon JA, et al. International Myeloma Working Group consensus statement regarding the current status of allogeneic stem-cell transplantation for multiple myeloma. J Clin Oncol. 2010;28(29): 4521-30.
22. Corradini P, Cavo M, Lokhorst H, Martinelli G, Terragna C, Majolino I, et al. Molecular remission after myeloablative allogeneic stem cell transplantation predicts a better relapse-free survival in patients with multiple myeloma. Blood. 2003; 102(5):1927-9.
23. Hebart H, Einsele H, Klein R, Fischer I, Buhler S, Dietz K, et al. CMV infection after allogeneic bone marrow transplantation is associated with the occurrence of various autoantibodies and monoclonal gammopathies. Br J Haematol. 1996; 95(1):138-44.
24. Fischer AM, Simon F, Le DF, Blanche S, Griscelli C, Fischer A. Prospective study of the occurrence of monoclonal gammapathies following bone marrow transplantation in young children. Transplantation. 1990;49(4):731-5.
25. Gerritsen EJ, van Tol MJ, Lankester AC, van der Weijden-Ragas CP, Jol-van der Zijde CM, Oudeman-Gruber NJ, et al. Immunoglobulin levels and monoclonal gammopathies in children after bone marrow transplantation. Blood 1993; 82(11):3493-02.
26. Thomas ED, Storb R, Clift RA, Fefer A, Johnson L, Neiman PE, et al. Bone-marrow transplantation (second of two parts). N Engl J Med. 1975;292(17):895-902.
27. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med. 1980; 69(2):204-17.
28. Segal BH. Aspergillosis. N Engl J Med 2009; 360(18):1870-84.
29. Durie BG, Harousseau JL, Miguel JS, Blade J, Barlogie B, Anderson K, et al. International uniform response criteria for multiple myeloma. Leukemia 2006; 20(9):1467-73.
30. Dispenzieri A, Katzmann JA, Kyle RA, Larson DR, Melton LJ, III, Colby CL, et al. Prevalence and risk of progression of light-chain monoclonal gammopathy of undetermined significance: a retrospective population-based cohort study. Lancet. 2010; 375(9727):1721-8.
31. Guikema JE, Vellenga E, Veeneman JM, Hovenga S, Bakkus MH, Klip H, et al. Multiple myeloma related cells in patients undergoing autologous peripheral blood stem cell transplantation. Br J Haematol. 1999;104(4):748-54.
32. Regamey N, Hess V, Passweg J, Hess C, Steiger J, Erb P, et al. Infection with human herpesvirus 8 and transplant-associated gammopathy. Transplantation 2004; 77(10):1551-4.
33. Badley AD, Portela DF, Patel R, Kyle RA, Habermann TM, Strickler JG, et al. Development of monoclonal gammopathy precedes the development of Epstein-Barr virus-induced posttransplant lymphoproliferative disorder. Liver Transpl Surg. 1996; 2(5):375-82.
34. Babel N, Schwarzmann F, Pruss A, Volk HD, Reinke P. Monoclonal gammopathy of undetermined significance (MGUS) is associated with an increased frequency of Epstein-Barr Virus (EBV) latently infected B lymphocytes in long-term renal transplant patients. Transplant Proc. 2004;36 (9):2679-82.
35. Drouet E, Chapuis-Cellier C, Bosshard S, Verniol C, Niveleau A, Touraine JL, et al. Oligo-monoclonal immunoglobulins frequently develop during concurrent cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections in patients after renal transplantation. Clin Exp Immunol. 1999; 118(3):465-72.
36. Rahlff J, Trusch M, Haag F, Bacher U, Horst A, Schluter H, et al. Antigen-specificity of oligoclonal abnormal protein bands in multiple myeloma after allogeneic stem cell transplantation. Cancer Immunol Immunother. 2012;61(10):1639-51.