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Progression of a solitary plasmacytoma to multiple myeloma. A population-based registry of the northern Netherlands

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Plasmacytoma is characterized by a local accumulation of monoclonal plasma cells without criteria for multiple myeloma (MM). The current treatment regimen is local radiotherapy. However, more than 50% of patients develop MM within 2 years after treatment. A population-based registry was consulted for the diagnosis of solitary plasmacytoma between 1988 and 2011. Progression to MM and prognostic features for progression to MM were scored, including hypoxia inducible factors (HIF), vascular endothelial growth factor (VEGF, also termed VEGFA) and micro-vessel density (MVD) expression in biopsy material. A total of 76 patients were included, 34% having extramedullary plasmacytoma (EMP) while 66% had a solitary plasmacytoma of the bone (SBP). Median follow-up was 89 months, (7–293 months). In Seventy per cent of SBP patients developed MM with a median time to progression of 19 months (5–293). Three patients (12%) with EMP developed MM. High expression of VEGF and HIF-2α (also termed EPAS1) was demonstrated in conjunction with an increased MVD in 66% of the patients. No association could be shown between angiogenesis parameters and progression to MM. In conclusion, this population-based study demonstrates that SBP patients have a higher risk of developing MM following local radiotherapy, indicating that this group might benefit from added systemic chemotherapy.

Keywords: solitary plasmacytoma of the bone, extramedullary plasmacytoma, multiple myeloma, angiogenesis, micro vessel density.

A solitary plasmacytoma (SP) is defined as a solitary lesion of clonal malignant plasma cells. About 80% of SPs are located in the bone marrow compartment (SBP) while they are extramedullary in the remaining cases and called extramedullary plasmacytoma (EMP). SP is a rare condition with a median age of onset of 55 years, which is approximately 10 years younger than multiple myeloma (MM) patients (Dimopoulos *et al*, 2000; and Soutar *et al*, 2004). By definition, a plasmacytoma fulfills no criteria for MM except for a small amount of M-protein, which is described in 25–75% of the patients (Dimopoulos *et al*, 2000; Soutar *et al*, 2004; Rajkumar *et al*, 2014).

The current treatment of a plasmacytoma with curative intent is local radiotherapy. However, more than 50% of patients develop MM within 2 years after treatment (Dimopoulos *et al*, 2000; Soutar *et al*, 2004). In view of this unfavourable prognosis, several studies tried to identify predictive markers for progression, including age, lesion size > 5 cm and persistent M-protein 1 year after treatment (Wilder *et al*, 2002; Ozsahin *et al*, 2006). However, none of the features are consistent between the different studies except that SBP has a higher rate of progression to MM than EMP. Approximately two-third of the patients with SBP develop MM while only 10–20% of the EMP progress to MM (Dimopoulos *et al*, 2000; Soutar *et al*, 2004). In addition, tumour-specific markers, such as micro-vessel density (MVD), which is a reflection of increased (neo)-angiogenesis, have been studied. In a study of SBP patients an increase in MVD coincided with an increased risk to progress to MM (Kumar *et al*, 2003).

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In the present population-based study we evaluated the clinical parameters of SP patients and correlated these findings with myeloma progression. In addition, MVD and (neo) angiogenesis-related factors, such as vascular endothelial growth factor (VEGF, also termed VEGFA) and hypoxia inducible factors (HIF) 1α and 2α (also termed HIF1A and EPAS1, respectively), were studied in biopsies of plasmacytoma patients. We correlated these findings with progression to MM to determine whether they can be used as prognostic features.

Materials and methods

Patients

In this retrospective multicentre study, all patients with a SP diagnosed between 1988 and 2011 in the northern area of the Netherlands were included. Inclusion criteria were biopsy-proven plasmacytoma, follow-up of at least 6 months, bone marrow demonstrating less than 10% plasma cells and skeletal survey showing no other lytic lesions than the solitary lesion of the plasmacytoma.

Data were collected at time of diagnosis and included the number of plasma cells in bone marrow aspirate, the serum M-protein level and, when performed, the free light chain level. Results from different imaging techniques [magnetic resonance imaging (MRI), fluorodeoxyglucose positron emission tomography (FDG-PET) and computed tomography (CT)] were included when performed. Furthermore, details of the applied treatment regimens and the M-protein level 1 year after treatment were collected. Progression to MM was defined as the time when the patient started systemic treatment for MM.

Approval for the study was obtained from the local medical ethical committee.

Immunohistochemistry for MVD, VEGFA, HIF 1α and 2α

Biopsies were collected from different pathology laboratories. The expression of angiogenesis- and hypoxia-related features was examined by immunohistochemical staining. After fixation in 10% neutral phosphate buffered formalin (3·6% formaldehyde) for at least 12 h, decalcification in a solution containing 10% (v/v) acetic acid and 10% formalin (v/v; 3·6% formaldehyde) for 1 or 2 days, and paraffin embedding, the plasmacytoma biopsy was cut in sections of 4 μm and stained as follows. Slides were de-paraffinized and rehydrated in graded alcohol solutions. Endogenous peroxidase activity was blocked with hydrogen peroxide. Heat-induced antigen retrieval was performed for all stains, except for VEGF (in which antigen retrieval was done with protease 0·1%). Slides were incubated with the primary antibody for HIF-1α (mouse monoclonal clone 54/HIF-1α, diluted 1:70; BD Biosciences, Franklin Lakes, NJ, USA) HIF-2α (mouse monoclonal ab8365, diluted 1:200; Abcam, Cambridge, UK) and VEGFA (rabbit polyclonal sc-152, diluted 1:50; Santa Cruz Biotechnology Inc., Dallas, TX, USA). Blood vessels were visualized with a CD34 antibody (mouse monoclonal QBEnd 10, Dako, Copenhagen, Denmark). The slides were washed with phosphate-buffered saline and subsequently incubated with a rabbit anti mouse or goat anti-rabbit horseradish peroxidase conjugated as secondary or tertiary antibody. For VEGF staining streptavidine peroxidase was used as a tertiary antibody. The chromogenic reaction was performed with diaminobenzidine for 12 min and after that sections were counterstained with haematoxylin before dehydrating and mounting.

The intensity of staining was analysed semi-quantitatively. The percentage of positive plasma cells was counted

Table I. Patient Characteristics (n = 76).

Characteristic	%
Gender	
Male	60
Female	40
Age (years)	
0–49	20
50–59	30
60–69	24
70+	26
M-protein	
None	43
IgG	40
IgA	5
VLK only	4
IgM	1
Unknown	7
Concentration M-protein (g/l)	
None	47
≤5	10
5·0–9·9	10
10·0–19·9	16
≥20	1
Unknown	16
Bence Jones proteinuria	
Not present	72
Not done	18
>0	10
Site of plasmacytoma	
Bone	66
Extramedullary	34
Radiation dose (Gy)	
20–39	11
40–49	89
Imaging	
X-skeletal	99
CT	47
MRI	42
FDG-PET	11

MRI, magnetic resonance imaging; FDG-PET, fluorodeoxyglucose positron emission tomography; CT, computed tomography.

according the following scoring system: no visibility, 10–30% positive, 30–50% positive, 50–80% positive and more than 80% positive plasma cells (Eckert *et al*, 2011). The vessel count was measured using light microscopy in the areas of the slide that contained the highest numbers of blood vessels per selected area (hotspot). After the hotspots were identified, the total number of vessels per selected image was counted at 400 × magnification. At least five hotspots were counted for each section and the vessel number was expressed as the mean of five counts (Weidenaar *et al*, 2011). The grade of MVD was established as low (MVD lower than 20) and high (MVD 20 or higher) (Kumar *et al*, 2003).

Statistical analysis

Progression-free survival (PFS) was estimated as time from diagnosis of the plasmacytoma to development of MM or the last follow-up using the Kaplan-Meier method. Survival curves were compared using the log rank test. In addition, univariate Cox regression analysis was used to estimate associated risk estimates, i.e., hazard ratio (HR) with 95% confidence interval (95%CI). A P value < 0·05 was used to define statistical significance.

Results

Patient characteristics

Between 1988 and 2011, 76 patients diagnosed with a SP were included in this study. Patient characteristics are shown in Table I. Median follow-up was 89 months (range 7–287 months). Median age was 61 years (range 26–87) and 60% were male. Most of the patients (66%) had an SBP and the plasmacytoma was located in the axial skeletal in 78% of these patients. In the EMP patients the plasmacytoma was most frequently located in the oropharynx or nasopharynx (65%). Forty-six per cent of the patients had an IgG-M-protein, 4% had free light chain disease only and 43% had no M-protein. Median M-protein was 0 g/l (range 0–22·8 g/l).

In view of the time frame of the study, a MRI was only performed in 42% of the cases, mainly for diagnostic purpose of the affected area. FDG-PET scan was performed in 11% of the patients (n = 8). The plasmacytoma size was only documented in 20 cases (25%). M-protein 1 year after treatment was available in 46% of the patients.

Treatment

Treatment consisted of local radiotherapy in 91% of the patients, 7% of the patients received systemic chemotherapy and 11% underwent surgical removal in combination with local radiotherapy. One patient was not treated. The radiotherapy dose was >40 Gy in 89% of the patients. A limited number of patients (n = 8) had a lower dose (20–39 Gy) due to the combined treatment of radiotherapy and chemotherapy.

Progression to MM

Local relapses of the plasmacytoma were not observed. Of patients with a SBP, 70% progressed to MM with a median

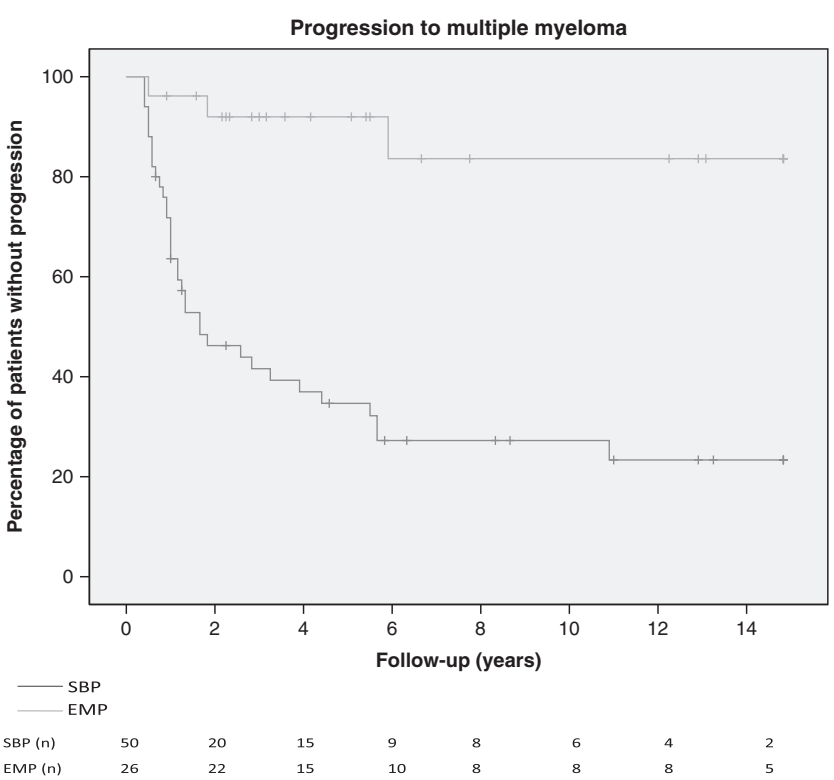

Fig 1. Kaplan–Meier curve of progression to Multiple Myeloma. SBP, solitary plasmacytoma of the bone; EMP, extramedullary plasmacytoma.

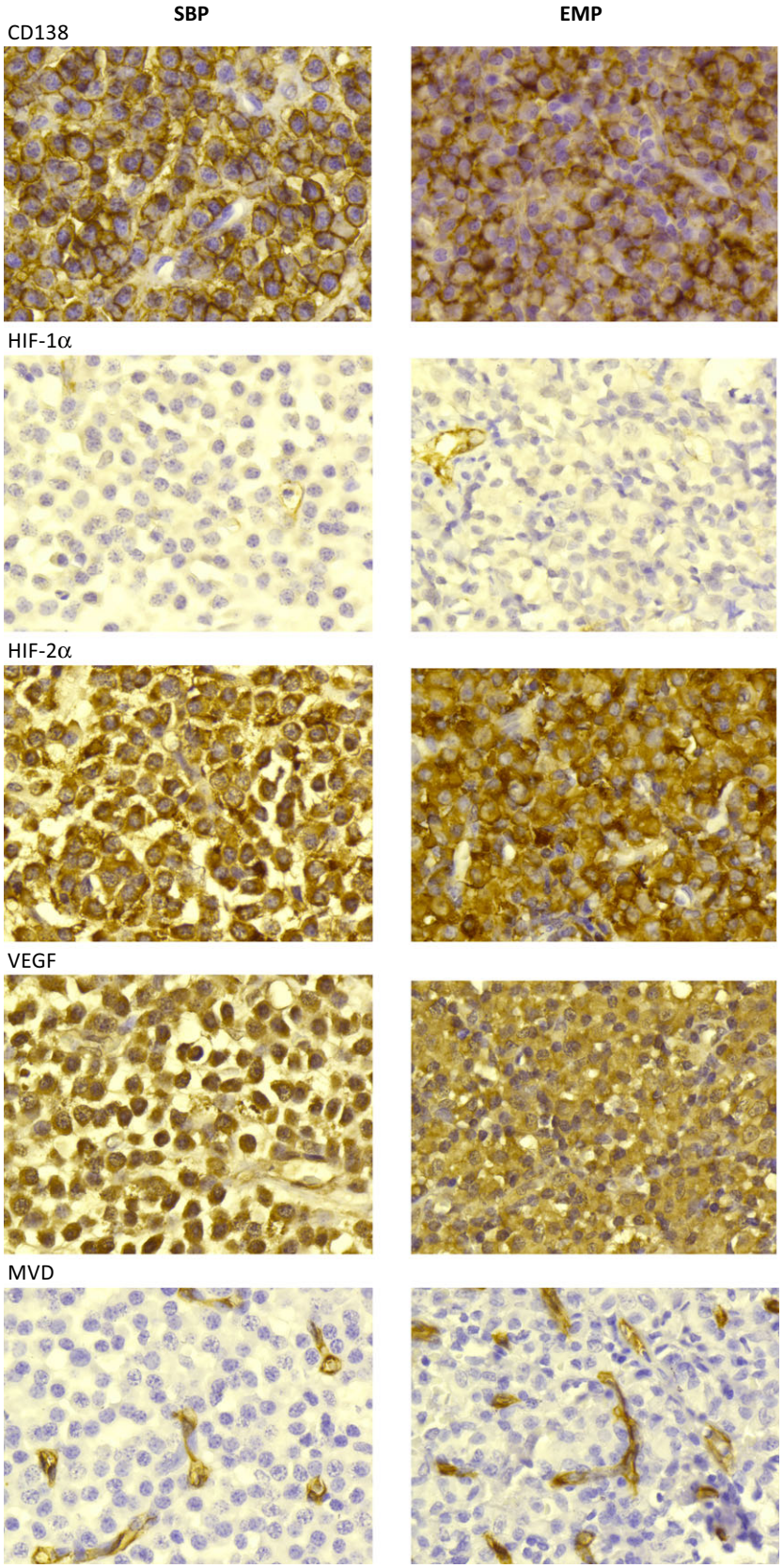

Fig 2. Immunohistochemical staining for HIF-1α, HIF-2α, VEGF and MVD in SBP and EMP biopsies. Immunohistochemical staining on biopsies from a solitary plasmacytoma of the bone (SBP) and extramedullary plasmacytoma (EMP). HIF-1α, HIF-2α, hypoxia inducible factor; VEGF, vascular endothelial growth factor; MVD, micro-vessel density.

time to progression of 19 months (range 5–131, Fig 1). Of the EMP patients, 12% ($n = 3$) progressed to MM after 6, 33 and 71 months. The 5-year PFS was significantly different between SBP and EMP (38% vs. 93%, $P = 0{\cdot}0001$). However, the overall survival (OS) between SBP and EMP was not significantly different ($P = 0{\cdot}294$) with an OS of 70% vs. 81% at 5 years and 64% vs. 77% at 10 years, mainly because 4 EMP patients died within 5 years of diagnosis due to disorders unrelated to MM.

MVD, HIF and VEGF expression by clonal plasma cells

To determine whether tumour-related predictors can be defined for the difference in PFS between SBP and EMP, immunohistochemical staining of plasmacytoma biopsies were performed for SBP ($n = 13$) and EMP ($n = 9$) samples (Fig 2). Six patients progressed to MM (5 SBP and 1 EMP). All biopsies ($n = 22$) demonstrated increased MVD as defined by CD34-positive staining in comparison to normal bone marrow ($3{\cdot}5 \pm 2{\cdot}9$ (mean $\pm$ standard deviation) vessel number per power field (Houwerzijl *et al*, 2013). The MVD between SBP and EMP was not significantly different ($P = 0{\cdot}5$). Plasma cell expression of HIF-1α, HIF-2α and VEGF was studied, as an increase in MVD might be a reflection of locally produced VEGF triggered by hypoxia. A clear distinction was observed for HIF-1α and HIF-2α. HIF-1α was demonstrated in plasma cells in a minority of the cases (36%) although the surrounding endothelial cells demonstrated a distinct positive staining. HIF-2α and VEGFA stains were positive in more than 80% of the plasma cells in all of the studied samples; no difference was observed between SBP and EMP (Fig 3).

Finally, all defined *in vivo* and *in vitro* prognostic parameters were included in a univariate Cox regression analysis. No association with progression to MM was observed except for the location of the plasmacytoma (SBP *versus* EMP; HR = 8·9, 95% CI = 2·7–29·1, $P = <0{\cdot}001$), as depicted in Table II. There was insufficient data to draw any conclusions regarding persistent M-protein at 1 year after treatment and lesion size.

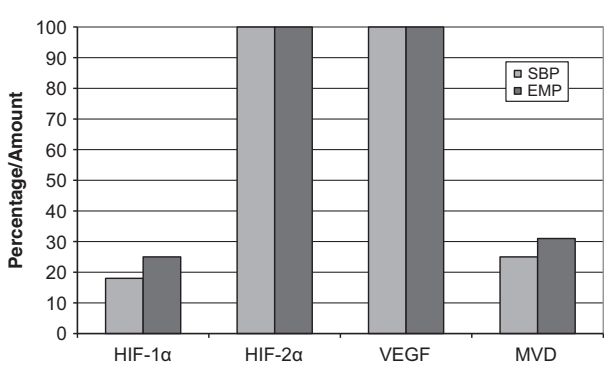

Fig 3. Results of immunohistochemical staining for angiogenic parameters. Percentage of patients with a positive stain for the angiogenic parameters shown in at least of 80% of the plasma cells. SBP, solitary plasmacytoma of the bone; EMP, extramedulary plasmacytoma; HIF, hypoxia inducible factor; VEGF, vasculair endothelial growth factor; MVD, micro vessel density (depicted in absolute numbers).

Discussion

In this large population-based cohort we showed that despite optimal local control with radiotherapy, patients with a SBP are at high risk for progression to MM with a median time to progression of 19 months. No association with progression to MM was observed for other prognostic parameters. However, no consistent results with regard to prognostic parameters have been obtained between different studies except for the difference between SBP *versus* EMP (Wilder *et al*, 2002 and Ozsahin *et al*, 2006).

In addition, tumor-related factors, such as MVD, VEGF and HIF-2α expression, were studied and demonstrated increased expression but without significant difference between SBP and EMP. Also, no association with progression

Table II. Cox regression analysis for progression to MM.

	HR	95% CI	*P*-value
SBP *versus* EMP			<0·001
SBP	8·9	2·7–29·1	
EMP	1		
Age			0·23
≥60 years	1·5	0·78–2·8	
<60 years	1		
M-protein at diagnosis			0·95
0	1		
0–6·9 g/l	1·1	0·49–2·6	
>6·9 g/l	0·95	0·42–2·2	
% plasma cells at diagnosis			0·62
<2%	1		
3%	1·2	0·53–2·6	
>3%	1·5	0·68–3·1	
Bence Jones proteinuria			0·71
Negative	1		
Positive	0·80	0·24–2·6	
HIF-1α			0·53
Negative	1		
Positive	0·58	0·10–3·2	
HIF-2α*			
>80%			
VEGF*			
>80%			
MVD			0·25
Low	2·6	0·51–13·9	
High	1		

SBP, solitary plasmacytoma of the bone; HIF, hypoxia inducible factor; VEGF, vasculair endothelial growth factor; MVD, micro vessel density; HR, hazard ratio; 95% CI, 95% confidence interval.
*All patients had a value >80%, hazard ratio not estimable.

to MM was demonstrated, in contrast to a large cohort reported by Rajkumar *et al* (2002), in which increased MVD on bone marrow samples was associated with progression to MM. The difference might be due to the smaller number of patients studied. In addition, our cohort included a relatively high number of EMP (41%) patients, of which 78% had a high MVD score but a low-relapse rate, suggesting that these factors are not interconnected.

Nowadays more sophisticated techniques can be used for the diagnosis of SP. As advised by the International Myeloma Working Group (IMWG), staging of SBP should include an MRI of the spine and pelvis (Dimopoulos *et al*, 2009). This is based on a small series reported by Lieboss *et al* (1998), in which 15 SBP patients were studied. In our study MRI was performed in 42% of the patients, but this was mainly for diagnostic purposes and did not always include the spine and pelvis. Only limited patient numbers have been reported regarding the relevance of FDG-PET scanning at diagnosis for SBP (Nanni *et al*, 2008; Warsame *et al*, 2012). In the present study, 8 patients (3 EMP, 5 SBP) had an FDG-PET scan at diagnosis. Four of these 8 patients progressed to MM. Remarkably, all 3 of these EMP patients had a negative FDG-PET but still progressed to MM.

Recent studies have demonstrated that more careful examination of the bone marrow of SBP patients identified a clonally related plasma cell population in 68% of the patients. The presence of this occult bone marrow disease (OMD) is of prognostic significance and is highly predictive for progression to MM, with a time to progression of 18–26 months. (Hill *et al*, 2014; Pavia *et al*, 2014). The strong predictive value of OMD and the short PFS supports the potential use of systemic treatment in addition to local radiotherapy in this high-risk group of SBP. Avilés *et al* (1996) compared radiotherapy with and without melphalan/prednisone therapy for 36 months in 58 patients. After a median follow-up of 8·9 years, 21% of the patients in the melphalan/prednisone group progressed *versus* 53% in the radiotherapy group (Avilés *et al*, 1996). That study did not identify high-risk SBP patients and treatment was without the use of the more effective immunomodulatory derivatives (IMIDs) and proteasome inhibitors. In future, early treatment may prevent or delay symptomatic disease in this high risk group, as has been shown in a group of high risk smouldering MM cases, of which 50% progress to MM within 2 years (Mateos *et al*, 2013). Treatment with lenalidomide and dexamethasone resulted in superior PFS and OS compared to no treatment. Moreover, clinical significant symptoms were reduced by the treatment (Mateos *et al*, 2013). A study using a combination of an IMID and a proteasome inhibitor in addition to local radiotherapy in high-risk SBP is now being developed.

In conclusion, based on the population-based cohort in our retrospective study, patients with SBP who are treated with local curative radiotherapy appear to have a high risk of developing MM. Adding systemic treatment with novel agents, such as proteasome inhibitors or IMID might improve the outcome of this patient group.

Author contributions

EGMdW and EV designed the research study, EGMdW and EV analysed the data and wrote the manuscript; NV performed data analysis; ML, JV, FO, WS, SH, MH, MH, and MB collected clinical data and updated the database contributed to results interpretation. AD and EGMdW analysed the immunohistochemistry performed on the biopsies.

Conflict of interest

None.

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