

Dynamic Contrast-Enhanced Magnetic Resonance Imaging Identifies a Subgroup of Patients with Asymptomatic Monoclonal Plasma Cell Disease and Pathologic Microcirculation

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Abstract **Purpose:** The aim of our study was to investigate whether dynamic contrast – enhanced magnetic resonance imaging (DCE-MRI) allows visualization of changes in microcirculation between healthy controls on the one side and early/advanced stages of plasma cell disease on the other. **Experimental Design:** We examined a group of 222 individuals consisting of 60 patients with monoclonal gammopathy of undetermined significance (MGUS), 65 patients with asymptomatic multiple myeloma (aMM), 75 patients with newly diagnosed symptomatic MM (sMM), and 22 healthy controls with DCE-MRI of the lumbar spine. **Results:** A continuous increase in microcirculation parameters amplitude A and exchange rate constant kep reflecting vascular volume and permeability, respectively, was detected from normal controls over MGUS and aMM to sMM. For A and kep , significant differences were found between controls and aMM ($P = 0.03$ and $P = 0.004$, respectively) as well as controls and sMM ($P = 0.001$ and $P < 0.001$, respectively). Although diffuse microcirculation patterns were found in healthy controls as well as MGUS and MM, a pattern with focal hotspots was exclusively detected in 42.6% of sMM and in 3 MGUS and 3 aMM patients. MGUS and aMM patients with increased microcirculation patterns showed significantly higher bone marrow plasmocytosis compared with patients with a low microcirculation pattern. **Conclusions:** Our investigations substantiate the concept of an angiogenic switch from early plasma cell disorders to sMM. Pathologic DCE-MRI findings correlate with adverse prognostic factors and DCE-MRI identifies a distinct group of patients with increased microcirculation parameters in aMM and MGUS patients.

Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic premalignant disorder associated with clonal proliferation of bone marrow plasma cells. It is found in 3% of all individuals beyond the age of 70 years and in 1% of the population older than 50 years (1, 2). A progression into multiple myeloma (MM), amyloidosis, macroglobulinemia, or other lymphoproliferative disorders is seen in 1% of these patients per year. In some patients, an intermediate, also

asymptomatic (no end organ damage), but more advanced stage can be recognized and is called asymptomatic MM (aMM). In contrast to patients with symptomatic MM (sMM) both disorders do not require therapy according to international standards (3). The identification of parameters predictive for the transition of MGUS and aMM into symptomatic disease are crucial to improve the recognition of patients at risk and most important to develop therapeutic strategies to prevent this transition. Kyle et al. (4) did fundamental research in this field and they were able to show prognostic significance of monoclonal protein and bone marrow plasmocytosis. Moreover, distinct cytogenetic abnormalities are associated with transition of MGUS to MM and previous evidence suggests a myeloma-induced progressive activation of bone marrow angiogenesis (5). The generation of new blood vessels from existing ones has been shown to be of great importance for development, growth, and prognosis not only of solid tumors but also of hematologic malignancies as MM (6, 7). It has been postulated by Folkman et al. (8) that the process of malignant transformation requires an “angiogenic switch.” This concept describes that malignant, dormant cancer cells progressively acquire the ability to induce angiogenesis by a change in the balance between proangiogenic and antiangiogenic cytokines.

Detection and quantification of angiogenesis in a clinical setting is a challenge (9). Histologic examination is considered

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Translational Relevance

In multiple myeloma, increased angiogenesis results in changes of microcirculation in bone marrow. These changes can be visualized by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). The imaging technique can be done with standard MRI tomographs, and the contrast agent used is well-tolerated. The present study describes two main findings: Using DCE-MRI (a) a gradually increase of microcirculation from early stages of disease to symptomatic myeloma can be detected and (b) a subgroup of patients with monoclonal gammopathy of undetermined significance and asymptomatic multiple myeloma presenting with increased microcirculation parameters can be distinguished from healthy controls. These patients may benefit from a higher frequency of follow-up. Future clinical studies are required to investigate if DCE-MRI can select asymptomatic multiple myeloma patients that require earlier therapy before symptomatic disease occurs. In addition, the relevance of DCE-MRI for integration of antiangiogenic treatment strategies is an important aspect for clinical evaluation.

to be the gold standard but carries disadvantages as it is an invasive technique and merely represents a limited area of bone marrow. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is a functional imaging technique applied in initial work-up and treatment monitoring of several solid tumors (10, 11). It can be done subsequently to morphologic total body or total spine MRI in MM patients (12). It determines and visualizes changes of contrast enhancement after a defined constant flow-controlled infusion of Gadolinium-diethylenetriaminepentaacetic acid. Pharmacokinetic signal time curves are used to calculate microcirculation parameters amplitude A and exchange rate constant kep , which serve as semiquantitative parameters for microcirculation in the investigated tissue (13). To visualize the distribution of microcirculation parameters in the examined area, a color-coded pharmacokinetic map is superimposed onto the static MR image.

DCE-MRI parameters amplitude A and exchange rate constant kep are significantly increased in patients with active MM compared with healthy controls and correlate with osteolytic bone involvement and prognosis (14). Earlier work of our group shown in 24 myeloma patients the significant correlation of high Amplitude A of DCE-MRI with increased micro vessel density (15). Those investigations were made at the pelvis as bone marrow biopsies are done at this site routinely with very low risk for the patient. The vertebral column is the bone marrow region generally investigated by MRI in patients with MM. Therefore, the primary reason why we did not correlate immunohistochemistry and DCE-MRI again was that we decided to investigate the spine in the present study and biopsies in this region comprise a higher—and in our opinion, not justifiable—risk for the patient. Amplitude A serves as a prognostic factor in patients with relapsed or refractory MM (16) and was found to be predictive for local complications, e.g., vertebral collapse (17). No reports however have been published on DCE-MRI in aMM and MGUS patients.

Therefore, the present study focused on the visual characterization of specific microcirculation patterns and measurement of semiquantitative microcirculation parameters in this group of patients. Effort was made to compare these patients with sMM on the one hand and healthy controls on the other. Clinical prognostic factor was compared in patients with MGUS and aMM with increased diffuse or focal versus low diffuse microcirculation pattern. Furthermore, our aim was to evaluate the potential role of DCE-MRI within routine diagnostics in patients with asymptomatic plasma cell disease. Due to a short follow-up period, no survival data are presented in this study.

Materials and Methods

Patients. From 200 patients included in this study, 125 did not require (systemic) chemotherapy (NRC group; ref. 18). This group consisted of 60 patients with MGUS and 65 patients with aMM. Seventy-five patients had a diagnosis of a sMM.

Age of patients and controls were not significantly different. Diagnostic criteria and staging for MM and MGUS were applied according to the classification of The International Myeloma Working Group (19). Patient characteristics are listed in Table 1. All MM patients requiring therapy were examined before onset of treatment.

Controls. The control group consisted of 22 healthy individuals who participated voluntarily in this study. Characteristics of the healthy controls are also listed in Table 1.

After written informed consent, patients and healthy volunteers were investigated with DCE-MRI of the lumbar spine using a study protocol approved by the institutional ethics committee.

DCE-MRI protocol. The entire spine of all patients and healthy controls was examined on a 1.5-T-Tomograph (Magnetom; Siemens Medical Solutions) from the first cervical vertebra to the sacrum with a sagittal STIR and a sagittal T1-weighted SE. The dynamic MRI protocol used was as follows: Optimized Saturation-recovery-TurboFLASH-Sequence [TR/TE, 79/4.76 ms; FOV, 380 mm; slice thickness, 8 mm; Voxel size, $1.5 \times 1.5 \times 8$ mm; matrix size, 256×380 (FoV read 50%)], in 8 sagittal slices, 22 measurements (total acquisition time, 5.5 min), temporal resolution, 11.25 s per measurement cycle. Contrast media injection was started with the fourth measurement cycle using an automatic power injector (Spectris Solaris EP MR-injection system; Medrad). The i.v. injection of 0.1 mmol/kg Gadolinium-diethylenetriaminepentaacetic acid (Magnevist; Schering) was given over 30 s via a cannula placed in the antecubital vein followed by a saline flush of 30 mL at the same injection rate. Data were analyzed on a conventional PC workstation with the "MeVislab"-Software (MeVis medical solutions AG).

The tissue-specific information contained in DCE-MRI-based signal intensity time curves is described by two relevant model variables: Amplitude A (arbitrary units), which is proportional to the relative signal enhancement and the exchange rate constant kep (min^{-1}) reflecting the contrast agent transit between the extravascular and the intravascular compartment. Using a 2-parametric 16-color map (Fig. 1A) encoding for variables A and kep , each pixel was assigned a color. Each of the different colors represents a discrete interval of A (0.1-0.4, 0.4-0.8, 0.8-1.2, and >1.2) and kep (0-1.6, 1.6-3.2, 3.2-4.8, and >4.8). The resulting color map was overlaid on the morphologic magnetic resonance images (Fig. 1B-F).

Image analysis. The anonymized color-coded pharmacokinetic parameter maps of all 200 patients and 22 healthy controls were categorized into seven distinct patterns of microcirculation jointly by 2 experienced investigators (JH, CMZ) reaching a consensus for each patient. Diagnosis of patients was not known by the investigators during image analysis. Because of the small number of controls and the relatively small number of available investigators, it was not possible to blind the examiners to the volunteers' names. However, efforts were made to abide objectiveness.

Table 1. Characteristics of patients and healthy volunteers

	Control group n = 22 (%)	MGUS n = 60 (%)	Asymptomatic MM n = 65 (%)	Symptomatic MM n = 75 (%)
Female sex	12 (54.6)	30 (50.0)	29 (44.6)	31 (41.3)
Age >60y	8 (36.4)	32 (53.3)	23 (35.4)	47 (62.7)
M-protein type				
Bence Jones		7 (11.7)	2 (3.1)	6 (8.0)
IgG		38 (63.3)	49 (75.4)	47 (62.7)
IgA		9 (15.0)	14 (21.5)	19 (25.3)
IgM		6 (10.0)		
IgG + IgA		0	0	1 (1.3)
Plasma cells in bone marrow > 5%		18 (40.9)	55 (98.2)	59 (86.8%)
		n = 44*	n = 56*	n = 68
Plasma cells in bone marrow > 30%*			7 (56.0)	26 (37.7)
			n = 56*	n = 69*
Serum β 2-microglobulin > 2.5 mg/L (upper level of norm)			7 (14.3)	41 (58.6)
			n = 49*	n = 70*
Myeloma in ISS stage				n = 69*
I				42 (60.9)
II				17 (24.6)
III				10 (14.5)

*Number of patients with available data.

Subsequently, a systematic analysis of regions of interest (ROI) encompassing a complete vertebral body was carried out. For this ROI, the software automatically calculated mean signal intensities on single images, and then plotted the change of mean signal intensity inside each ROI over time. Median estimated volume investigated per vertebral body was 7,910 mm³ (range, 4,672-10,645 mm³). Amplitude *A* and exchange rate constant *kep* were calculated for the signal intensity curve according to the model described by Brix et al. (20). As proven by computer simulations, *kep* can be determined reliably in the range *kep* < 13 min⁻¹ so that all values for *kep* ≥ 13 min⁻¹ were fixed to 13 min⁻¹ and were censored in the statistical analysis (21). The median values of the microcirculation parameters for all examined vertebral bodies of each individual (generally the five vertebrae of the lumbar spine) were calculated separately.

Microcirculation patterns. Changes of signal enhancement and distribution of pharmacokinetic variables as displayed by color-coded DCE-MRI images were classified based on visual aspects as described below, using classifications proposed previously (15, 16, 22, 23). As reported earlier, there were four distinct pattern categories: normal, diffuse, and focal. A normal pattern as it was found in most (59%) of the healthy controls is characterized by mainly dark blue colors representing low values of both microcirculation parameters (*A*, 0.1-0.8; *kep*, 0-3.2) Fig. 1B. If pink (*A*, 0.4-0.8; *kep*, >3.2) spots were detectable in these patients, the pattern was still classified as normal if the (pink) proportion of the investigated area remained below 30%. A diffuse pattern was characterized by a noncircumscribed signal increase resulting in pink areas on a blue background in the color-coded variable maps affecting 30% to 100% of the ROI (Fig. 1C and D). A further pattern was differentiated from the diffuse pattern described above: In some patients, the pattern involving the whole ROI did not only show the intermediate to high *kep* values and low to intermediate *A* values, but extensive levels of both parameters were seen resulting in a confluent white or turquoise (*A*, >0.8; *kep*, >3.2) image named as "diffuse variant" (Fig. 1E).

A focal pattern on normal or diffuse background was characterized by a circumscribed signal increase on different background activity. These "hotspots" were visualized as white, yellow, or turquoise colors in the DCE-MRI maps (Fig. 1F).

Pitfalls in evaluation of pattern and measurement of microcirculation parameters. Revision of the microcirculation patterns revealed five patients and two volunteers with an osteochondrosis in the region of

the lumbar spine. These findings showed circumscribed increased values of both amplitude *A* and exchange rate constant *kep*, and a white color in functional images. However, they were easily differentiated from focal lesions due to their marginal location at the vertebral bodies adjacent to the intervertebral discs. Respective vertebrae were excluded from the subsequent analysis.

Bone marrow plasmacytosis. In recent years, many prognostic markers for sMM were proposed. To investigate the group of patients with MGUS and aMM (pooled as NRC-group), we decided to evaluate plasma cell infiltration in bone marrow, which has been shown to be a valid prognostic marker (4, 24). It was also found to correlate with increased microcirculation parameters in an earlier study examining mainly sMM patients (15). Because some (but not all individuals) of the NRC group showed increased/abnormal microcirculation patterns (diffuse, diffuse variant, or focal patterns) in DCE-MRI, bone marrow plasmacytosis was compared between these two groups.

Statistical analysis. Medians of amplitude *A* of the ROIs of each individual usually consisting of the five vertebral bodies of the lumbar spine were compared between the different patient groups as well as between patients and healthy controls using exact Wilcoxon rank sum tests. As exchange rate constant *kep* can only be calculated reliably in values of <13 min⁻¹, Peto & Peto modification of Wilcoxon-Gehan test for censored data were used for comparison of the different groups (25–27). Because of the multiple tests, a *P* value correction was done using the procedure proposed by Holm (28).

Incidence of the different microcirculation patterns in the patient and control groups were compared and descriptively characterized. Furthermore, Fisher's exact test for Count Data was done to detect differences in distribution of microcirculation patterns between the examined groups.

Comparison of bone marrow infiltration by plasma cells between NRC patients with normal versus abnormal microcirculation patterns was done using an Exact Wilcoxon Rank Sum test.

Because of the limited median time of follow up, the focus of this article was not to collect survival data.

Results

Microcirculation analysis in healthy volunteers. Analysis of microcirculation patterns of 22 healthy volunteers revealed that

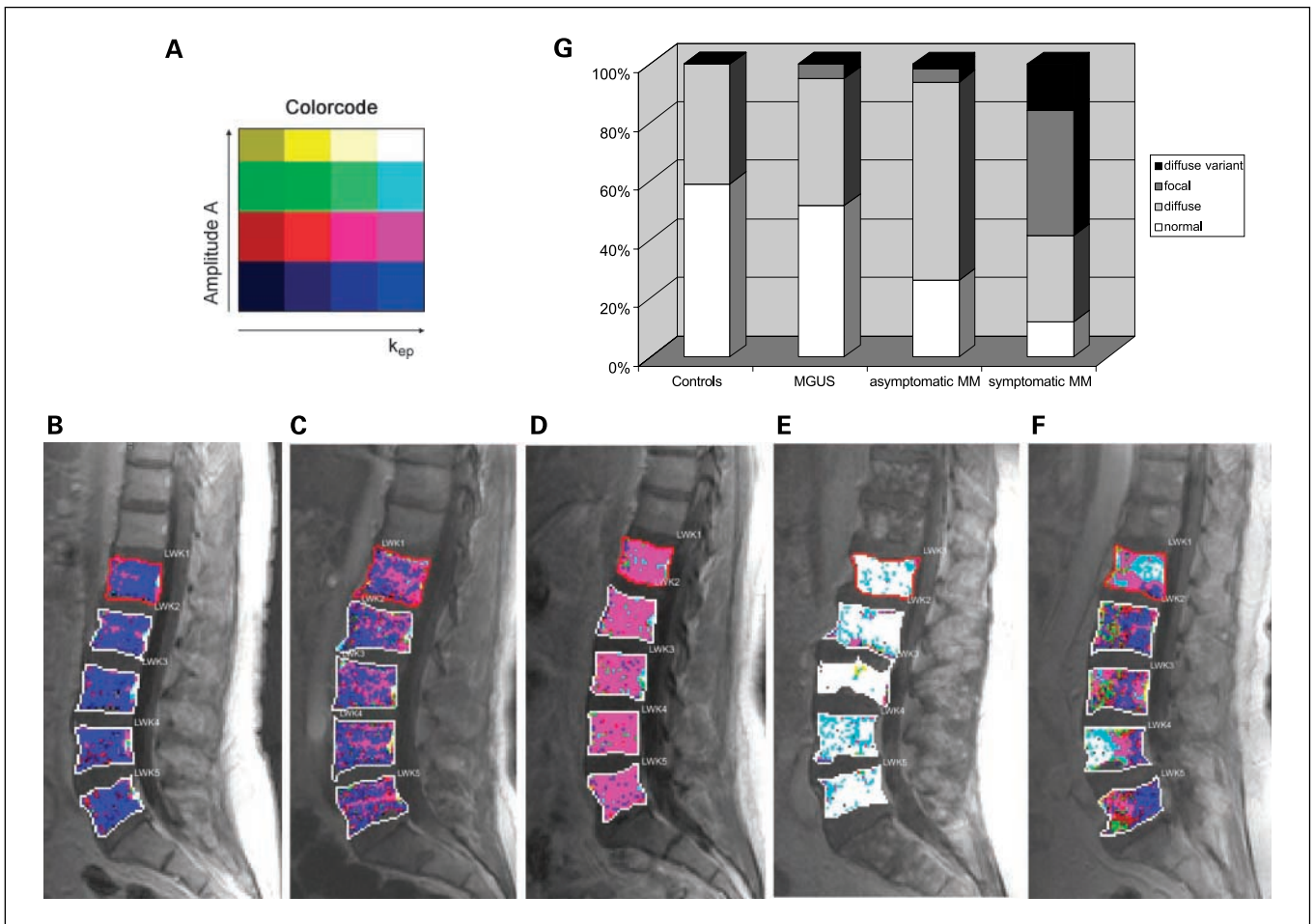


Fig. 1. Color code of DCE-MRI, examples for microcirculation patterns: *A*, color scale used for encoding variables amplitude *A* and exchange rate constant *kep* to create a color coded map that is superimposed on a conventional MRI image. Each of the different colors represents a discrete interval of *A* (0.1-0.4, 0.4-0.8, 0.8-1.2, and >1.2) and *kep* (0-1.6, 1.6-3.2, 3.2-4.8, and >4.8). Examples of microcirculation patterns are given in *B* to *F*. *B*, "normal" pattern (dark blue or gray). Patients with marginal homogeneous signal change affecting <33% of the vertebral body were classified as normal as well. *C* and *D*, "diffuse" pattern: Homogeneous signal increase mostly resulting in a pink color related to high values of *kep* and intermediate values of *A* affecting between 30% and 100% of the vertebral body. *E*, "diffuse variant" (diffuse variant) pattern: Diffuse pattern with the highest values of both parameters affecting the whole ROI characterized (white and turquoise). *F*, "focal" circumscribed signal increase, hotspots, displaying white, yellow or turquoise colors on diffuse background of different intensity. *G*, distribution of microcirculation patterns in the control and patient groups: Focal hotspots on differently intensive diffuse background (focal) prevail in sMM. Controls and the majority of asymptomatic patients show mainly normal or diffuse patterns (normal, diffuse). Except for one patient with aMM, a diffuse variant microcirculation pattern (diffuse variant) is present exclusively in symptomatic disease.

13 of 22 (59%) showed a d_1 pattern according to the criteria mentioned above with low values of *A* and *kep* (Fig. 1G). In healthy controls, median amplitude *A* of all investigated vertebrae was 0.596 arbitrary unit (range, 0.386-0.808 arbitrary

unit), and the median of exchange rate constant *kep* was 3.607 min^{-1} (range, 1.028-12.309 min^{-1}). In the whole patient group, the median of *A* was 0.687 arbitrary unit (range, 0.297-2.481; see below), and the median of *kep* was 6.551 min^{-1} (range,

Table 2. Differences in medians of amplitude *A* and exchange rate constant *kep*

	<i>P</i> value		<i>P</i> value	
	<i>A</i>		<i>kep</i>	
Control group vs. Patients	0.03		<0.001	
Control group vs. MGUS	0.46	n.s.	0.2	n.s.
Control group vs. Asymptomatic MM	0.03		0.004	
Control group vs. Symptomatic MM	0.001		<0.001	
MGUS vs. Asymptomatic MM	0.1	n.s.	0.01	
MGUS vs. All MM	0.002		<0.001	
Asymptomatic MM vs. Symptomatic MM	0.02		0.06	n.s.
NRC vs. Symptomatic	0.001		<0.001	

0.740-13 min⁻¹, according to the chosen cut off). In six subjects, two or more of the five analyzed vertebrae showed a diffuse pattern with one of them even presenting with a focally increased perfusion in the second lumbar vertebra. Review of the conventional MRI revealed that the supposed focal lesion was a blood vessel with an atypical vessel course. The remaining three controls showed a confluent pattern with no focal lesions

(Fig. 1G). Further analysis revealed that two of the latter were the youngest in the control group. Therefore, we analyzed the correlation of age and microcirculation parameters in both the control and the patient group. We found a significant inverse correlation of age and amplitude *A* in healthy controls (*P* = 0.02). As this effect is not detectable in the larger patient group, we assume that it is overlaid by the influences of

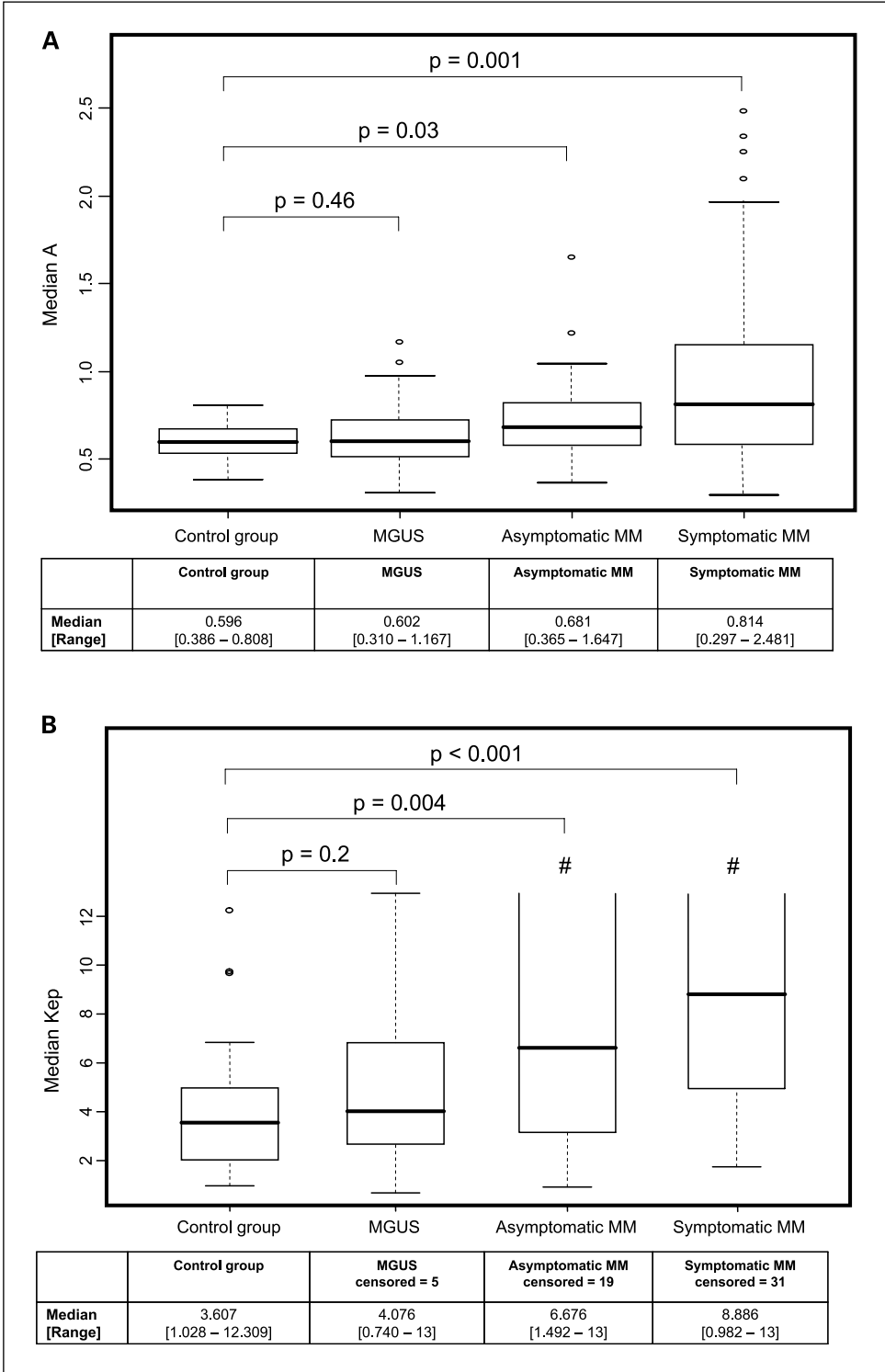


Fig. 2. Values of amplitude *A* and exchange rate constant *kep* *B* in different groups (MGUS, *n* = 60; aMM, *n* = 65; sMM, *n* = 75; controls, *n* = 22): Median and ranges are shown. *Boxes*, the first and third quartile; *whiskers*, the 1.5 times of the interquartile range (of the box). #, exchange rate constant *kep* can be determined reliably in the range *kep* < 13 min⁻¹. Therefore, the box plots are not closed for aMM and sMM.

pathologic changes in microcirculation. In fact, this suggests that there is a continuous decrease of microcirculation in bone marrow with age. No correlation between exchange rate constant kep and age could be shown.

Microcirculation parameters in patients with asymptomatic plasma cell disease and symptomatic myeloma. Comparison of the whole group including individuals with MGUS and MM patients with the healthy controls revealed a significantly higher median of amplitude A and exchange rate constant kep for patients ($P = 0.03$ and $P < 0.001$, respectively). Median of both microcirculation parameters continuously increased from healthy volunteers to sMM. Statistical evaluation of subgroups for the median of A and kep resulted in significant differences of controls and patients with manifest MM (asymptomatic and symptomatic, respectively), between MGUS and MM (aMM plus sMM), and between the NRC-group and the sMM group (Table 2; Fig. 2A and B). For A but not for kep , a significant difference between aMM and sMM ($P = 0.02$ and $P = 0.06$, respectively), and for kep but not for A , a significant difference between MGUS and aMM was found ($P = 0.01$ and $P = 0.1$, respectively).

An amplitude A value higher than the upper limit of normal controls was found in 15% of patients with MGUS ($n = 9$), 21% of patients with NRC ($n = 26$), and 26% of patients with aMM ($n = 17$). Because of the cutoff level of 13 min^{-1} , this evaluation was not done for exchange rate constant kep .

Microcirculation patterns in different patient groups. The distribution of microcirculation patterns within the different groups is shown in Fig. 1G and Table 3. Quantitative and qualitative differences in the DCE-MRI patterns were found when controls were compared with the NRC group and the sMM patients, respectively.

The normal pattern was 59% in controls, 52% in MGUS, 26% in aMM, and 12% in sMM. Those with MGUS and aMM, who had no normal pattern, had mainly diffuse patterns instead. In sMM, there was a predominance of patterns that were not observed in controls at all, i.e., diffuse variant and focal. One case of the asymptomatic group presented with a diffuse variant pattern and subsequently progressed into symptomatic disease, necessitating systemic treatment 6 months later. We did not observe images with focal signal increase in healthy controls. Therefore, the following qualitative changes were considered abnormal: diffuse variant and focal.

For distribution of microcirculation patterns, significant differences were shown between the sMM group and healthy controls ($P < 0.001$) as well as aMM patients and the NRC group, respectively ($P < 0.001$). No significant differences could be found between controls and MGUS, controls, and aMM or MGUS and aMM (Table 4). Differences between the control group and the aMM group as well as between the MGUS and the aMM group nearly reached significance with a P value of 0.08 each.

The NRC group was divided into patients presenting with d₁ pattern as observed in more than half of the controls (59%) and those with higher activity. We found that patients in the NRC group with an increased pattern had a significantly stronger bone marrow infiltration than those NRC patients who presented with a low intensity pattern (12.5% plasma cells versus 7.5%; $P = 0.05$).

Discussion

We describe here in a large group of patients qualitative and semiquantitative DCE-MRI parameters that distinguish sMM from normal controls. We also provide evidence that this technique can identify a distinct patient population in NRC that presents with a pathologic DCE-MRI. Referring to this, pathologic findings according to our analysis were defined by a pattern of strongly increased microcirculation (diffuse variant), focal hotspots in the investigated area (focal pattern), or by an amplitude A above the upper limit of normal controls. It has to be mentioned that a diffuse variant pattern cannot be distinguished from a focal pattern affecting the whole vertebra, which may explain in part why those two patterns seem to seem exclusively in the same, namely the symptomatic patient group.

Different patterns of bone marrow involvement of MM in conventional as well as DCE-MRI have been described concordantly by different authors (14, 23, 29, 30). Diffuse distribution of microcirculation was found in healthy controls as well as in all stages of plasma cell disease. However, the frequency of occurrence of focal "hotspots" with circumscribed and markedly increased blood vessel supply, which were not detectable in controls, increased with stage of disease. These patterns were found in 43% of sMM patients. Comparison of vertebrae with a focal and a diffuse microcirculation pattern revealed significantly higher amplitude A in the focally affected areas. Hypothetically, this can be caused by an actual loss of bone mass in these regions due to an infiltration of the malignant cells into the cancellous bone compared with a diffuse infiltration of bone marrow and simultaneously diffuse increase of angiogenesis and microcirculation. On the other side, the circumscribed areas of increased microcirculation may occur due to the repair of microfractures caused by increased bone disease in patients with advanced stage of MM. The resolution of our conventional MRI images did not enable us to differentiate those two mechanisms.

Although for the whole NRC group, MGUS-group, and aMM-group, no significant differences of the distribution of microcirculation patterns were detectable, we identified 26 NRC patients with an amplitude A higher than the upper limit of normal controls and 6 more patients with a pathologic DCE-MRI pattern [1 patient had an abnormal pattern (diffuse variant) combined with an increase of amplitude A]. At present,

Table 3. Microcirculation patterns in different groups

	normal	%	diffuse	%	diffuse variant	%	focal	%
Controls	13	59	9	41	0	0	0	0
MGUS	31	52	26	43	0	0	3	5
asymptomatic MM	17	26	44	68	1	2	3	4
symptomatic MM	9	12	22	29	12	16	32	43

Table 4. Differences in microcirculation patterns

	<i>P</i>	
Control group vs. Patients	0.09	n.s.
Control group vs. MGUS	0.42	n.s.
Control group vs. Asymptomatic MM	0.08	n.s.
Control group vs. Symptomatic MM	<0.0001	
MGUS vs. Asymptomatic MM	0.08	n.s.
MGUS vs. All MM	<0.0001	
Asymptomatic MM vs. Symptomatic MM	<0.0001	
NRC vs. Symptomatic	<0.0001	

NOTE: Statistical comparison of the distribution of microcirculation patterns within each of the indicated groups (healthy controls and patient groups: MGUS, asymptomatic MM, symptomatic MM, NRC = MGUS + aMM, all MM = aMM + sMM) using the Fisher's exact test (s. Material and Methods).

our clinical follow-up is not long enough to provide substantive results on patient outcome in relation to the DCE-MRI pattern. However, two observations may indicate that pathologic DCE-MRI is a possible adverse prognostic factor:

(a) In the NRC-group, patients with a low intensity pattern of microcirculation (signal increase in <30% of the ROI) showed lower bone marrow plasmacytosis with borderline significance ($P = 0.05$). These findings are limited by the fact that DCE-MRI pattern of the lumbar spine was compared with bone marrow samples obtained from the pelvis. However, this is supported by results of a previous study with mainly sMM patients that showed a significant correlation of bone marrow infiltration and DCE-MRI findings of the same region (15). Increased plasma cell infiltration in bone marrow was found to be an independent risk factor for progression of MGUS into MM (31).

(b) One patient with a pathologic DCE-MRI pattern (diffuse variant) developed a progression to sMM 6 months after the DCE-MRI. The prognostic value of all focal lesions detected in our asymptomatic patients will be evaluated after an adequate time of follow-up. We assume that diffuse and diffuse variant patterns can overlay focal hotspots because response to treatment sometimes leads to a change from increased diffuse to focal microcirculation patterns (data not shown).

A further indication that amplitude *A* is a relevant factor for disease progression is indirectly assisted by the fact that the median amplitude *A* in aMM but not in MGUS was significantly different from the median amplitude *A* in healthy controls. Further follow-up will reveal the clinical significance of these findings.

Early detection of increased risk for progression into myeloma could result in increased follow-up frequency to monitor disease progression. Additionally, prognostic parameters including DCE-MRI for NRC patients with high probability of early development of treatment necessity could lead to clinical studies with novel agents initiating early therapy. As a study investigating the application of thalidomide in indolent or aMM showed a response to treatment and a delay of disease progression (32), a treatment of high-risk patients within the NRC group particularly with agents targeting increased microcirculation and angiogenesis seems promising.

DCE-MRI allows a semiquantitative characterization of microcirculation with amplitude *A* representing vascular

volume and exchange rate constant *kep* giving information about permeability of the blood vessel walls. The limitation of *kep* to values below 13 min^{-1} because of restrictions of the pharmacokinetic model lowers the reliability of this parameter. However, it is interesting that the significance of differences between controls and patients as well as between different patient groups parallels in *kep* and *A*, which has been shown to be a reliable factor (14, 16, 17). Significant differences only of *kep* can be found between MGUS and aMM and only of *A* between aMM and sMM. Furthermore, no difference can be shown between healthy controls and MGUS. This observation led us to the hypothesis that in the process of the augmentation of microcirculation from premalignant to symptomatic disease, the impaired structure of the walls of neoangiogenic blood vessels can be detected (difference of *kep* between MGUS and aMM) before an actual increase in vascular volume appears (difference in amplitude *A* between aMM and sMM).

Our findings fit well in the concept of the angiogenic switch that postulates requirement of angiogenesis for progression beyond a "dormant" and microscopic stage of tumors (8, 33). Analogously to this, immunohistologic assessment of bone marrow biopsies have shown that angiogenesis in bone marrow of individuals with MGUS increases with progression to MM. Works by Kumar et al. (34) and our own group provide experimental evidence that the angiogenic switch from MGUS to sMM may involve a loss of expression of angiogenesis inhibitors by malignant plasma cells. Quite recently, data were published proving that an increased micro vessel density can be shown not only in MM patients with symptomatic disease but also in a subgroup of patients with MGUS and smoldering MM (35). In this study, the degree of angiogenesis increased along with the stage of plasma cell disease. Furthermore, Vacca et al. (36) described an increased proangiogenic potential of bone marrow samples of 76% in myeloma patients and 20% in MGUS patients. The fact that patients with or without evidence of increased microcirculation can be found in each patient group investigated here confirms that angiogenesis is one but not the only factor influencing disease progression.

According to recent work done by others and our own group, we identified three main areas of potential clinical relevance of DCE-MRI in MM: (a) as a prognostic tool for response rate and survival of sMM patients in combination with standard approaches especially if antiangiogenic compounds are used; (b) to predict the probability of local control and local complication rate as pain or nerve compression for lesions within vertebrae; (c) as prognostic marker for the recognition of patients with aMM and MGUS with increased risk for progression to symptomatic disease.

Conclusion: We were able to identify a group of patients with aMM and MGUS with pathologic DCE-MRI. Pathologic DCE-MRI patterns are correlated with increased bone marrow plasmacytosis. This technique might comprise the capability to further characterize patients in the NRC group that require higher frequency of follow-up investigations and that could benefit from early treatment. However, a longer time of follow-up of our patients is needed to determine actual prognostic significance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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