Original paper

Increased angiogenesis seems to correlate with inferior overall survival in myeloid sarcoma patients

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Myeloid sarcomas (MS) are tumors composed by myeloid elements and developing outside bone marrow. The prognosis is overall poor, only stem cell transplantation being consistently reposted as a potentially curative approach.
In this study we explored whether microvessel density, a biomarker of angiogenesis, might be relevant in MS. We studied 60 MS, 24 acute myeloid leukemia, 5 normal bone marrow samples and 2 cases of extramedullary hemopoiesis in patients without evidence of hematological malignancy. We used immunohistochemistry (anti-CD34) to identify and quantify micro-vessel density (MVD) and micro-vessel grading (MVG). We found that MS had significantly higher MVD and MVG than normal bone marrow ($p = 0.0002$ and $p < 0.001$, respectively). We then found that cases with monocytic morphology had significantly higher MVD than myelo-monocytic and blastic ones ($p = 0.005$), while no differences were recorded based on extramedullary site. Finally, we found that higher MVD and higher MVG were associated with inferior outcome in terms of overall survival in multivariate analysis ($p = 0.05$ and $p = 0.02$, respectively), when censoring for stem cell transplantation was undertaken.
In conclusion, we documented for the first time that increased angiogenesis is characteristic of MS and correlates with survival, suggesting that anti-angiogenic approaches might deserve a clinical evaluation in this setting.

Key words: myeloid sarcoma, angiogenesis, micro-vessel density, immunohistochemistry, anti-angiogenic therapy.

Introduction

Myeloid sarcoma (MS), also termed as "granulocytic sarcoma" "extra-medullary myeloid tumor", or "chloroma", is a rare condition that is characterized by the occurrence of one or more tumor masses, consisting of myeloblasts or immature myeloid cells and presenting at an extra-medullary site [1]. The latter more often corresponds to the skin, bone or lymph node, although the process can affect almost every site of the body [2, 1, 3]. It may develop *de novo* or concurrently with acute myeloid leukaemia (AML), myeloproliferative neoplasia (MPN) or myelodysplastic syndrome (MDS) [1, 3]. Interestingly, MS may be the first evidence of AML or precede it by months or years [1, 2]. Finally, it can represent the initial

manifestation of relapse in a previously treated AML in remission [1, 3].

Histologically, MS most commonly consists of myeloblasts (MB), with or without features of promyelocytic or neutrophilic maturation, that partially or totally efface the tissue architecture. In a significant proportion of cases, it displays myelomonocytic (MMo) or pure monoblastic (MoB) morphologic features. However, this morphologic sub-classification has been largely criticized, being no longer included in the WHO classification [1, 4]. In contrast, different studies demonstrated that the immunophenotype is of paramount importance for the lineage definition and differential diagnosis [1, 5, 4]. The latter is mainly based on the differentiation of the process from malignant lymphoma, and specially lymphoblastic lymphoma, Burkitt lymphoma, diffuse large B-cell lymphoma, and anaplastic large cell lymphoma.

The genetic features are quite heterogeneous, monosomy 7, trisomy 8 and MLL rearrangement being the most common alterations in adult patients [3]. Recently, new genetic analyses including next generation sequencing were demonstrated to be able to identify clinically actionable molecular markers in this setting even starting from formalin fixed paraffin embedded samples [6, 7]. The clinical course is more often aggressive and it is likely that intensive treatments including stem cell transplantation can determine improved outcomes [3, 8, 9].

Formation of new blood vessels from pre-existing ones (angiogenesis) is an absolute requirement for the viability and growth of solid tumors [10, 11] This neovascularization is mediated by angiogenic molecules released by tumor cells themselves and by accessory host cells such as macrophages, mast cells, and lymphocytes. In turn, the newly formed endothelial cells of the tumor can stimulate tumor growth in a paracrine fashion [12]. Furthermore, angiogenesis is important for the development of a malignant phenotype [12] and numerous studies [12] have demonstrated that the vascular density of a tumor directly correlates with metastasis and patient outcome. Interestingly, angiogenesis was found to have a significant role in hematological malignancies, such as plasma cell myeloma, acute leukemias, myeloproliferative neoplasms (MPN) and lymphomas [13, 14, 15, 16, 21]. In particular, an increased micro-vascular density (MVD) was demonstrated in the bone marrow of acute myeloid leukemia patients [22]. However, no data have been reported so far concerning angiogenesis in MS.

In the present study, we investigated the extent of angiogenesis in a large series of MS in adult patients, aiming to: 1) assess whether angiogenesis is different in MS, bone marrow of AML patients, and normal bone marrow; 2) assess whether angiogenesis is associated with other clinico-pathological feature in MS; and 3) assess the prognostic impact of angiogenesis related parameters in MS.

Material and methods

Tissue samples and clinical information

Sixty MS cases (median age 60.5, range 16-87 years), 24 AML bone marrow samples, 5 normal bone marrows and 2 cases of extramedullary (ectopic) hemopoiesis encountered in patients without any evidence of hematological malignancy were retrieved from the files of the Hematopathology Unit of Bologna University (collected between 1990 and 2004). These cases were included in a larger panel previously described in details [3], the follow up being updated to January 2015. Complete clinical data were available in all the cases, while conventional cytogenetics and eventually FISH data were available for

Table I. Patients' characteristics

Parameter	Number of patients
Gender (M/F)	30/30
Karyotype*	
Favorable	3
Intermediate	20
Unfavorable	4
Localization	
Lymph node	12
Testis	6
Gastro-intestinal	5
Skin	21
Other**	16
Cytology	
Myeloblastic	28
Mielo-monocytic	15
Monocytic	12
Unclassified	5
Associated hematological malignancy	
AML	27
MDS	6
MPN	6
None	21
CR achievement (Y/N)	14/46
Stem cell transplant (Y/N)	9/51

CR – complete remission; Y – yes; N – no
** According to Grimwade et al. [54]*
*** Other included: biliary tract (n = 3); bone (n = 2); breast (n = 2); CNS (n = 2); serosal (n = 2); gingival mucosal (n = 1); lung (n = 1); spleen (N=1); tonsil (n = 1); and uterus (n = 1).*

31/60 patients. Informed consent was obtained from all patients and the institutional ethical committee approved tissue collection and study design.

Patients' characteristics are detailed in Table I and Supplementary Table I.

Histology and immunohistochemistry

Biopsies (excisional for lymph-nodes and testis; punch for skin and surgical/CT-guided for other sites) were performed in all patients before treatment; all specimens were reviewed by two of the authors to confirm the accuracy of the diagnosis and assess the cytological subtype according to the WHO classification as well as the MVD. The histopathological analysis was conduced as previously reported [3, 23]. The percentage of neoplastic cells positive for immunohistochemical markers (see below) was independently assessed by four experienced pathologists. In case of discrepancy, the slide was collectively discussed at a multi-head microscope until consensus was reached. Each marker was regarded as positive when it was clearly expressed by at least 20% of the neoplastic cells. Bone marrow biopsies have been also evaluated in all the MS cases.

Micro-vascular density analysis

In order to evaluate endothelial cells, anti-CD34 and anti-FVIII antibodies (Ab) were used [17, 18].

Two separate previously described methods were used to estimate MVD [18, 24, 25]. Briefly, in the first method, visual micro-vessel grading, the slides were visually scanned at × 100, × 200 and × 400 magnification and semi-quantitatively graded for the extent of CD34/FVIII staining. To ensure the accuracy of the grading method, each sample was reviewed by two of the authors. Morphologic analysis was performed carefully to ensure vessel specificity of the CD34/FVIII-stained stroma considered for the analysis. Four different micro-vessel grades (MVG) have been considered: MVG 1, normal or slightly increased MVD; MVG 2, micro-vessels easy to detect and definitely increased in respect to normal; MVG 3, abundant microvessels; MVG 4, strongly increased MVD. The second method, visual count, involved counting of micro-vessels according to previously described methods [17, 18, 24, 25]. In performing this visual count, each of the slides was first scanned at × 100 magnification, and three areas with abundant micro-vessels were chosen and defined as 'hot spots'. The number of micro-vessels in each of these hot spots was then determined at × 400 magnification. The final MVD number (micro-vessels for high-power magnification field/× 400) was assigned by taking the average of the three separate visual counts. During the count process large vessels were excluded as well vessels in periosteum or bone as regards bone marrow specimens. Areas of staining with no discrete breaks were counted as single vessels and the presence of a lumen was not required. Five bone marrow samples as well as 2 cases of extramedullary (ectopic) hemopoiesis without tumor evidence were studied as controls.

For MVD, if different scores were proposed by different observers, consensus was reached by simultaneous analysis and discussion.

Statistical analysis

For clinical analysis, all data were evaluated with the Stat view 5.0 software package (SAS Institute Inc, North Carolina, USA). In order to assess possible correlations between angiogenesis parameters (MVD and MVG) and other clinico-pathological features, covariate analysis was performed by the means of Spearman's rank correlation coefficient (rho) for numerical (continuous) variables (i.e. age); conversely, Mann-Whitney and Kruskal-Wallis tests were adopted for nominal (non-continuous) variables (i.e. gender, tissue localization, cytology, cytogenetics, and presence of concomitant hematological malignancy), when two or more groups were evaluated, respectively. Such non parametric tests were chosen to ensure the maximal accuracy also in case of small (<30 cases) subgroups. Survival curves were plotted according to the Kaplan-Meier method [26]. Relapse free survival (RFS) was calculated from the date of complete remission (CR) achievement until relapse, death or date of the last contact; overall survival (OS) was calculated from the date of diagnosis until death or date

Table II. Summary of the descriptive statistics for the analyzed parameters

Parameter	Mean value	Median value	Range	Standard deviation	Mann-Whitney, p-value (vs. MS)
Age (years)	56.3	60.5	16-87	18.14	_
MVD					
MS	21.35	15.65	7.8-124.4	18.30	_
AML	20.73	18	8.2-79	12.78	0.144
Normal samples	4.6	5	4-5	0.55	0.0002

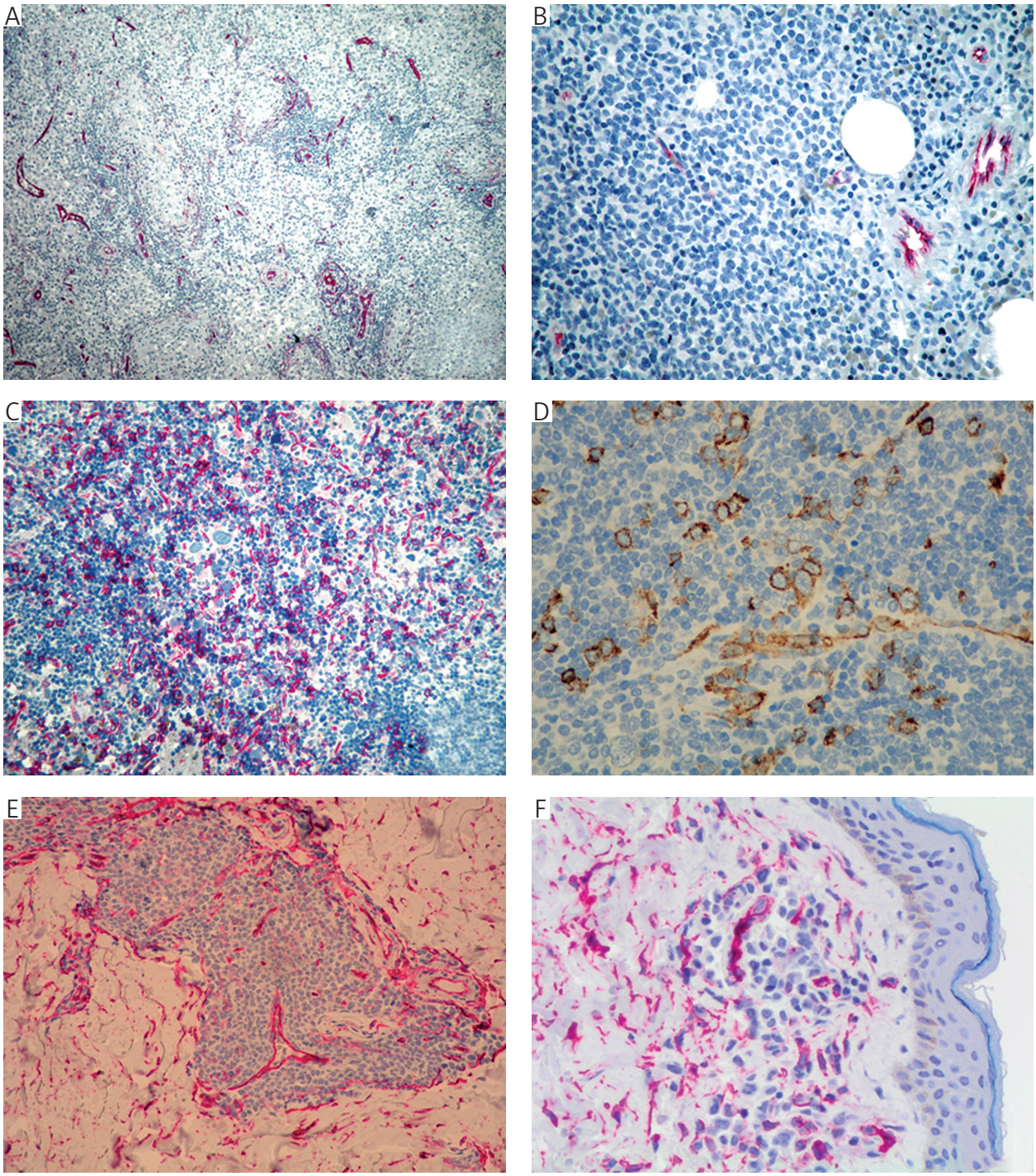

Fig. 1. Microvessel density evaluation in myeloid sarcoma cases. Examples of immunohistochemical evaluation of CD34 in myeloid sarcoma cases. Based on MVG, these cases were graded as following: A) MVG 2 (100 ×); B) MVG 1 (400 ×); C) MVG 3 (100 ×); D) MVG 1 (400 ×); E) MVG 2 (100 ×); F) MVG 2 (400 ×)

of the last contact for living patients. When appropriate, survival data were censored at the time of stem cell transplant (SCT) administration. The univariate association between individual clinical features and RFS/OS was determined with the Cox proportional hazards regression model [27] and, when appropriate, with also the log-rank [28]. A multivariate analysis using the Cox proportional hazards regression model [27] was performed to compare the factors turned out to be significantly associated with survival at univariate analysis [29].

The limit of significance for all analyses was defined as a p value < 0.05; 2-sided tests were used in all calculations. Sample size of groups considered in each analysis as well as the relative descriptive statistics are depicted in Table II.

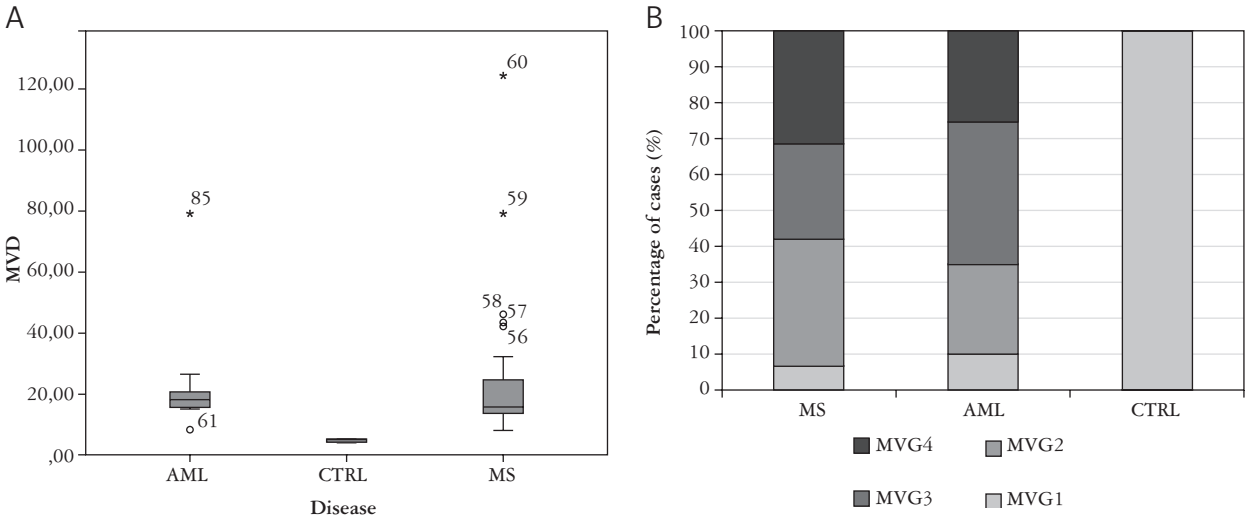

Fig. 2. Angiogenesis in myeloid sarcoma. A) Median MVD in MS, AML and normal samples (Ctrl) is depicted. Bars represent standard errors. B) MS and AML cases, as well as normal samples grouped according to MVG (1-4)

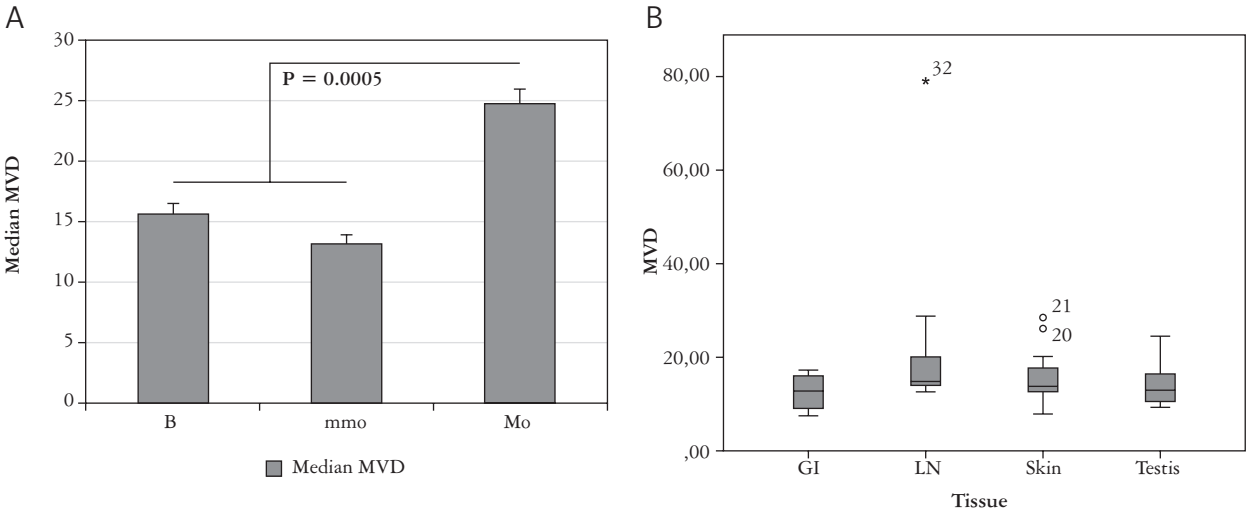

Fig. 3. A) Median MVD in MS with different cytology is shown. B) Median MVD in MS with different localization is shown. Bars represent standard errors

Results

Correlations between angiogenesis and clinicopathological features in myeloid sarcoma

First, we compared MVD in MS, AML and controls. Indeed, although the antibody directed to CD34-stained myeloid progenitors, CD34 has been confirmed to be a useful antigen for assessing intra-tumor angiogenesis, as for other malignancies (Fig. 1) [17, 18]. We found that MS presented with significantly increased MVD in comparison to normal bone marrow. In particular, the median MVD was 15.65 (range, 7.8-124.4) vs. 5 (range, 4-5) in tumors and controls, respectively (Mann-Whitney, $p = 0.0002$). On the other hand, no significant difference was recorded between MS and AML (15.65 vs. 18) (Fig. 2A; Table II). Consistently, according to MV grading, MS were grouped as following: 4 MVG1; 21 MVG2; 16 MVG3; and 19 MVG4. Among AML cases, 2 were classified as MVG1, 5 as MVG2, 8 as MVG3, and 5 as MVG4. Conversely, all the normal bone marrow samples were regarded as MVG1 (χ^2, $p = 0.00000138$) (Fig. 2B). Of note, we observed a remarkable degree of correlation between MVD and MVG (correlation, $r^2 = 0.33$; $p < 0.001$). Similarly, inter-observer concordance was very high concerning MVG after the initial evaluation ($r^2 = 0.8$; $p < 0.001$), while consensus was reached at the end in all instances.

We then tried to assess possible correlations between angiogenesis parameters (MVD and MVG)

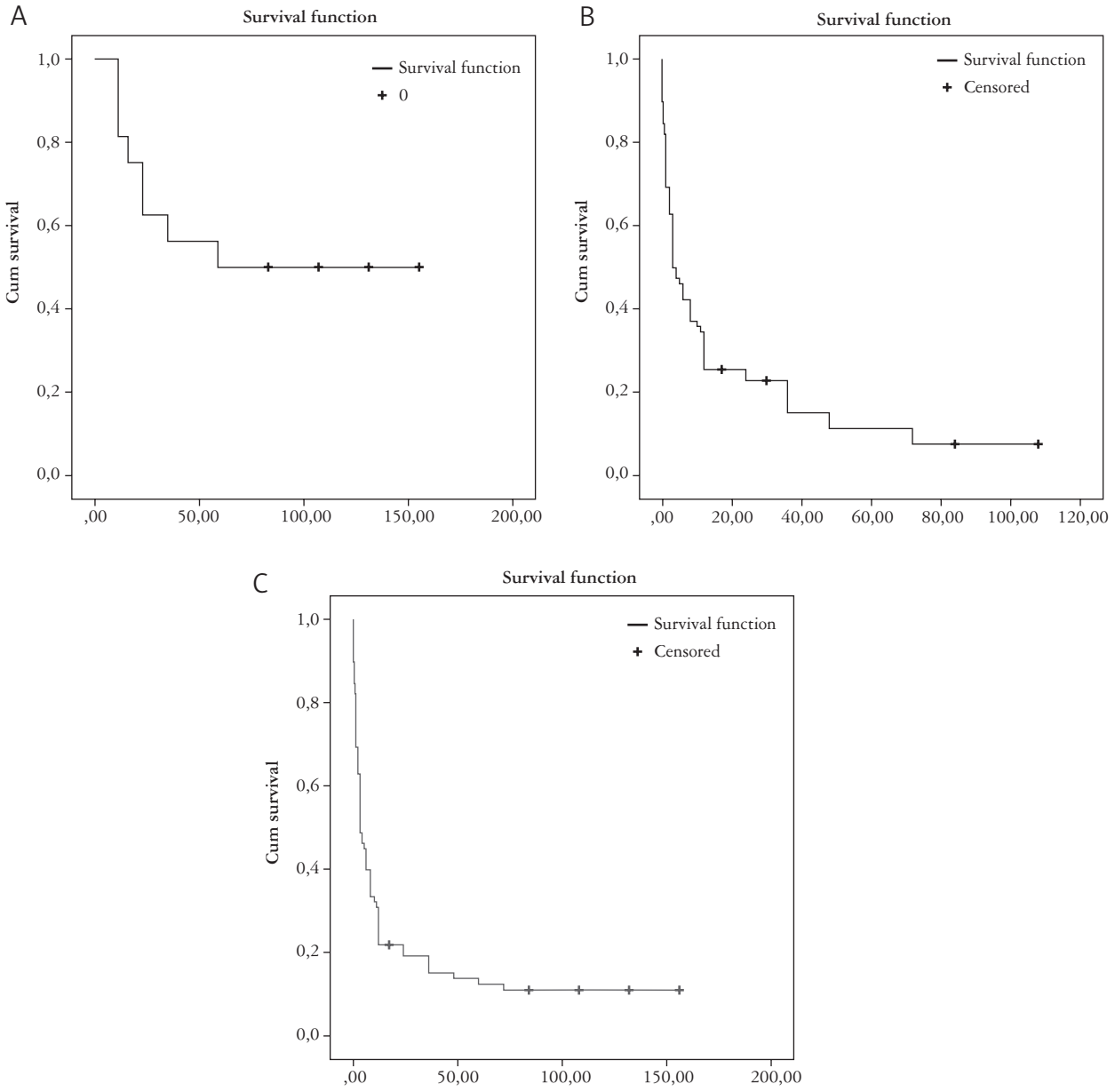

Fig. 4. Relapse free survival (A), OS (B) and OS censored by SCT administration (C) plotted by using the Kaplan-Meier method

and the other clinico-pathological features of MS, including age, gender, cytogenetics, tissue localization, cytological subtype, and presence of a concomitant hematological disease (AML, MDS or MPN). Indeed, we found neither age (Spearman correlation, $p = 0.9$), nor gender (Mann-Whitney, $p = 0.8$), cytogenetics (Kruskal-Wallis, $p = 0.9$), and concomitant disease (Kruskal-Wallis, $p = 0.7$) to be associated with MVD or MVG. Afterward, we evaluated MVD and MVG in MS cytological subtypes (myeloblastic, MB; myelo-monocytic, MMo; and monocytic, Mo). We found that Mo forms had a significantly higher MVD when compared with MB and MMo ones (24.7 vs. 15.65 vs. 13.25, respectively; $p < 0.0005$) (Fig. 3A). Subsequently, we evaluated possible variations in MS cases at different localizations. Skin, gastro-intestinal, testicular and lymph-nodal lesion were considered, as at least 5 cases were available for each group. We found no significant differences in MS cases developing in different tissues (Kruskal-Wallis, $p = 0.7$). On the other hand, when we considered the remaining localizations in one unique group (being the single one too small to be analyzed) (Table I), we found the latter to have a significantly higher MVD (Kruskal-Wallis, $p = 0.007$) (Fig. 3B). In spite of this, due to the heterogeneity of this group, it was not possible to draw definite conclusions, identifying tissues with higher vascular development. However,

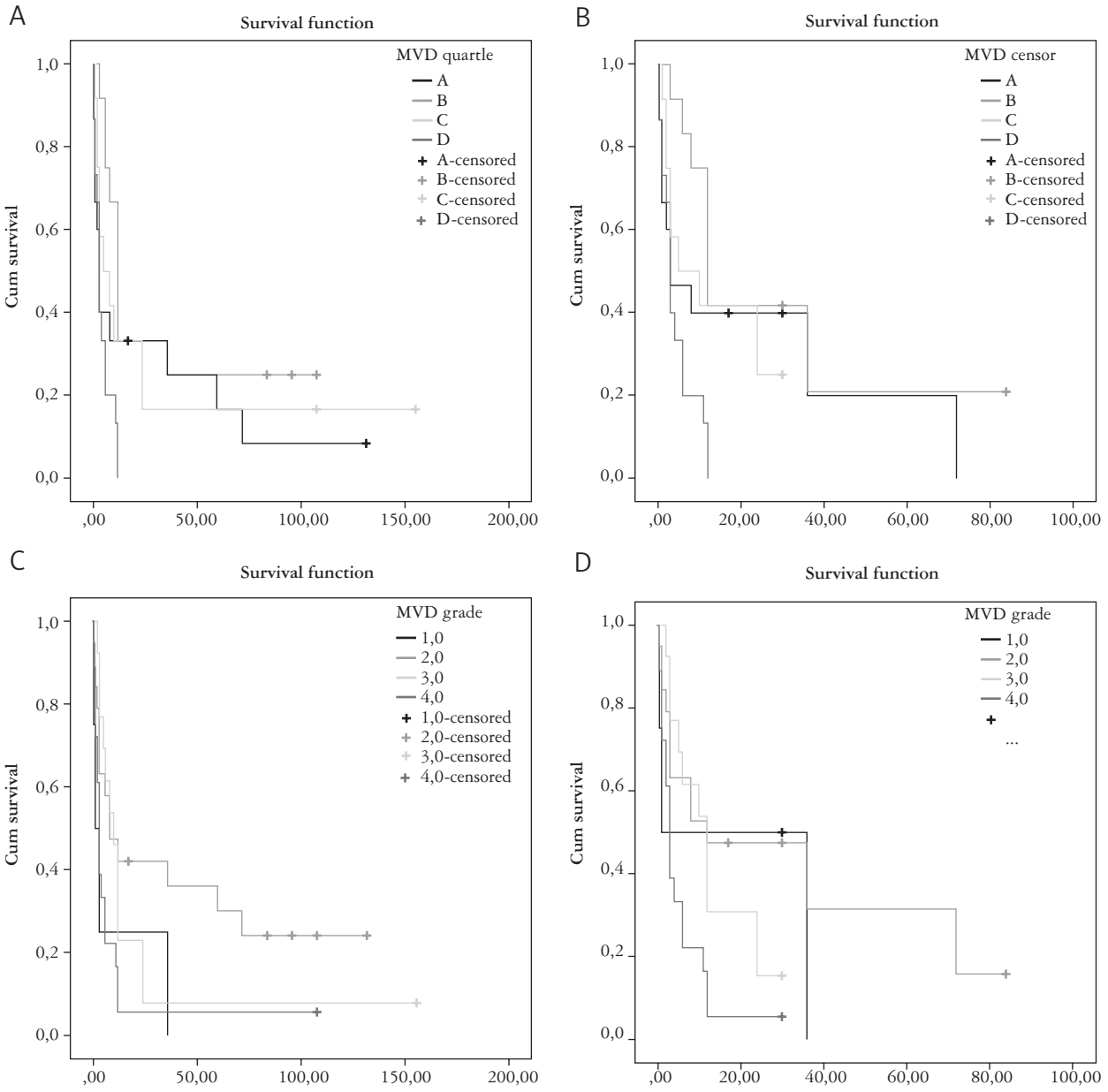

Fig. 5. A) Kaplan-Meier OS curve showing that patients divided into quartiles, according to MVD levels, had a significantly different outcome. B) This was even more evident when OS was censored by SCT administration. C) Kaplan-Meier OS curve showing that patients divided according to MVG had a significantly different outcome. D) This was even more evident when OS was censored by SCT administration

to further explore this phenomenon, and try to definitely exclude the puzzling effects of extra-medullary normal tissues influences, we also evaluated MVD and MVG in two cases of ectopic (extra-medullary) hematopoiesis, not associated with hematological malignancies. Indeed, in such instances, the MVD (4 and 5, respectively) and MVG (grade 1 in both instances) did not differ from those of normal bone marrows. Suggesting that the invaded tissues were not major determinants of micro-vessel development.

Taken together, our results showed that angiogenesis was extremely increased in neoplastic samples in comparison to normal hemopoiesis, without significant differences as far as various clinico-pathological features are concerned, with the exception of the cellular subtype.

Increased angiogenesis correlated with poor survival in myeloid sarcoma patients

Median OS for MS patients was 10 months (range, 0.5-180) (Fig. 4A-B). Seven out of sixty (11.6%) are currently alive, 6 being in continuous CR (10%). The median RFS was 35 (range, 2-179) months (Fig. 4C). The median follow-up for alive patients was 102 (range, 96-180) months.

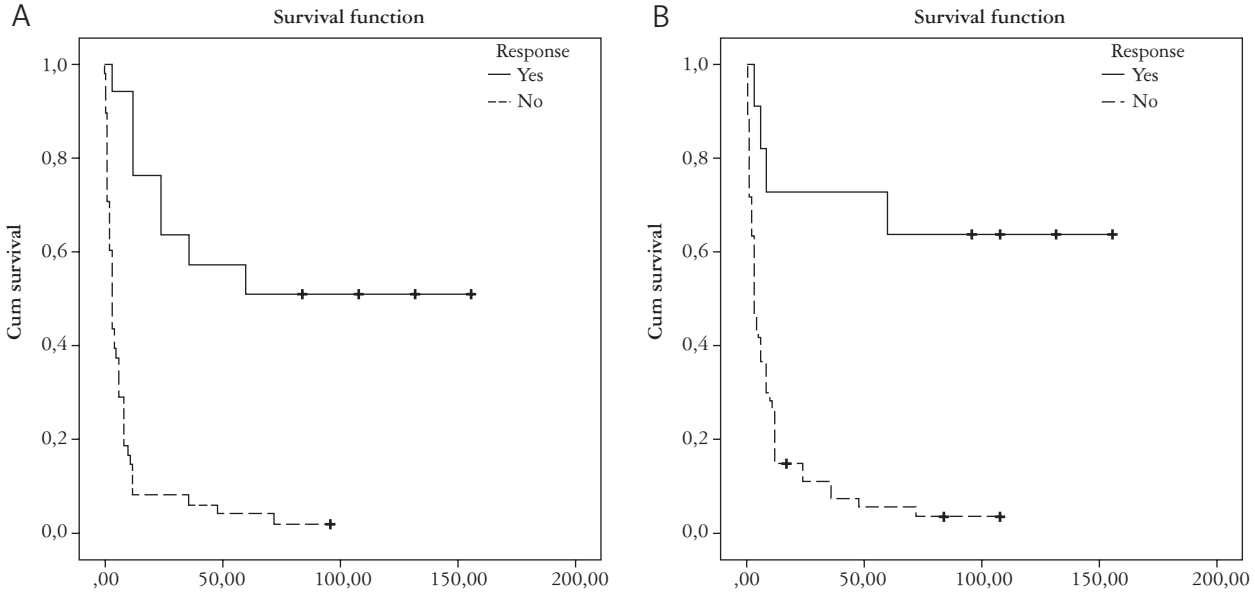

Fig. 6. Overall survival curves according to treatment response (CR vs. no CR) censored by SCT administration (A), and overall survival curves according to treatment type (SCT vs. no SCT) (B) plotted by using the Kaplan-Meier method

To assess whether increased angiogenesis was associated with patients' clinical outcome, we first examined whether MVD correlated with treatment response. Indeed, median MVD was 14.8 (range 10.7-31.4) vs. 16.2 (7.8-124.4) in patients who achieved a CR (responders, n = 15 cases) or not (non-responders, n = 45 cases) (Mann-Whitney, p = 0.3). In addition, we calculated the response rate between patients with higher (n = 30 cases) or lower (n = 30 cases) MVD (50^{th} percentile was taken to divide the two groups); again, we found no significant differences (6/30 vs. 9/30, respectively; Fischer exact test, p = 0.55).

Subsequently, we found that neither MVD (Cox model, p = 0.5) nor MVG (Cox model, p = 0.7; Kaplan-Meier plot, Log-rank, p = 0.3) were significantly correlated to RFS. Consistently, when patients were divided into quartiles according to their MVD levels (high, n = 15; intermediate-high, n = 15; intermediate-low, n = 15; and low, n = 15 cases) still there was no significant difference as far as RFS was concerned (Kaplan-Meier, Log-rank, p = 0.3).

Then, referring to OS, we found that patients with higher MVD tended to have a worse survival than patients with lower values (Cox model, p = 0.07). However, notably, if survival was censored at the time of SCT, MVD turned out to be significantly associated with OS (Cox model, p = 0.03). In addition, by dividing patients into quartiles according to the MVD levels (see above), we found MVD to be significantly associated with OS (Cox model, p = 0.05; Kaplan-Meier, Log-rank, p = 0.03; Fig. 5A). Consistently, when OS was censored for SCT, the association was again significant, and even more evident (Cox model, p = 0.03; Kaplan-Meier, Log-rank, p = 0.01; Fig. 5B). Finally, we found that patients with higher MVG had a slightly though not significantly worse outcome (Cox model, p = 0.09; Kaplan-Meier, Log-rank, p = 0.07; Fig. 5C); however, when OS was censored for SCT, the association turned out to be significant (Cox model, p = 0.04; Kaplan-Meier, Log-rank, p = 0.02; Fig. 5D).

Other clinico-pathological features associated with the clinical outcome

We then evaluated whether other parameters did correlate with survival. We found that age, as expected, was significantly associated with OS (Cox model, p = 0.0006; p = 0.0002 after censoring for SCT). Conversely, it was associated with only an unfavourable trend as far as RFS is concerned (Cox model, p = 0.07). Conversely, neither gender, nor cytogenetics, nor cytological subtype, nor tissue localization, nor the presence of a concomitant haematological malignancy were significantly associated with both RFS and OS (Table III). On the other hand, patients who achieved a CR had a significantly better outcome in terms of both RFS (Cox model, < 0.0001; Kaplan-Meier, Log-rank, p < 0.0001), and OS (Cox model, < 0.0001; Kaplan-Meier, Log-rank, p < 0.0001). Notably, this was true also when OS was censored at the time of transplant (Cox model, < 0.0001; Kaplan-Meier, Log-rank, p < 0.0001; Fig. 6A). In addition, patients who were submitted to SCT (n = 9 cases) presented with a significantly better outcome, in terms of both RFS (Cox

Table III. Summary of univariate analysis, performed according to the Cox proportional hazards regression model [27]

Parameter	p	Confidence interval		p	Confidence interval	
	Uncensored OS	95% Lower	95% Upper	OS censored for SCT	95% Lower	95% Upper
Age	0.0007	1.013	1.049	0.0002	1.016	1.055
CR achievement (Y/N)	< 0.0001	0.073	0.0351	0.0001	0.89	0.453
MVD	0.0695	0.999	1.026	0.0335	1.001	1.027
MVD- by quartiles	0.536	0.266	1.25	0.0268	0.194	1.012
MVG	0.0931	0.273	1.212	0.0419	0.146	1.099
SCT	0.0005	0.056	0.45	NA	NA	NA

CR – complete remission; Y – yes; N – no; MVD – micro-vessel density; MVG – micro-vessel grading; SCT – stem cell transplantation; NA – not applicable

Table IV. Summary of multivariate analysis, performed according to the Cox proportional hazards regression model [27]

Parameter	p-value	Confidence interval		p-value	Confidence interval	
	Uncensored OS	95% Lower	95% Upper	OS censored for SCT	95% Lower	95% Upper
Age	0.1702	0.994	1.033	0.0098	1.007	1.05
CR achievement (Y/N)	0.001	0.112	0.573	0.0052	0.126	0.694
MVD	0.0848	0.997	1.045	0.0157	1.005	1.051
MVG	0.2729	0.087	1.367	0.0494	0.028	0.0762
SCT	0.1124	0.123	1.246	NA	NA	NA

CR= complete remission; Y – yes; N – no; MVD – micro-vessel density; MVG – micro-vessel grading; SCT – stem cell transplantation; NA – not applicable

model, < 0.0001; Kaplan-Meier, Log-rank, p<0.0001), and OS (Cox model, < 0.0001; Kaplan-Meier, Log-rank, p < 0.0001; Fig. 6B). Nonetheless, it should be noted that patients submitted to SCT were mainly those who achieved a CR. In fact, 6/15 responders vs. 3/42 non-responders actually received the procedure (Fischer exact test, p = 0.005).

Multivariate analysis of parameters associated with survival

To definitely assess the prognostic impact of parameters turned out to be associated with OS in univariate analysis, we performed multivariate analysis as previously reported [29]. Notably, when OS was not censored for SCT, only CR achievement significantly correlated with the OS (Cox model, p = 0.001; Table IV). Grippingly, on the other hand, when OS was censored for SCT, either age (p = 0.01), and CR achievement (p = 0.005), and MVD (p = 0.02), and MVG (p = 0.05) turned out to significantly affect the OS (Table IV).

Discussion

In this paper, we evaluated for the first time angiogenesis in MS, providing novel insights as far as both the biology and the clinic of this tumor are concerned. Indeed, we studied a large panel of cases, representative of adult forms [3].

First, we found that MS presented with significantly increased angiogenesis in comparison to normal bone marrow and extra-medullary non-neoplastic hemopoietic tissue. Actually, this was not totally surprising, as other myeloid malignancies, including AML, MDS, and CMN were found to have an increased vascular density [16, 17, 18, 19, 22]. Nevertheless, this is the first demonstration of the phenomenon in MS and may offer relevant issues for further future analyses.

In particular, angiogenesis may represent a relevant phenomenon favoring tissue colonization by neoplastic myeloid elements. To this regard, we found that monocytic forms had a significantly higher MVD in comparison to other cytological subtypes. On the other hand, it is well known that AML with monoblastic/monocytic differentiation do present more often with extra- medullary involvement and MS [30]. Indeed, the higher capability of myeloid blasts with monocytic differentiation to infiltrate and eventually efface different tissues may be related to a higher pro-angiogenic capacity. On the other hand, we failed to demonstrate significant differences among MS as far as the specific tissue localization was concerned. Particularly, though certain localizations

were associated with higher MVD, there was no evidence that in non-neoplastic ectopic hemopoiesis occurring in the same sites was associated with particularly increased micro-vascular development. On the other hand, interestingly, cases developed in tissues which are more frequently involved by MS (skin, lymph nodes, testis, and gastro-intestinal tract) were characterized by lower MVD. Thus, it is conceivable that the spectrum of adhesion molecules would determine the tissue localization [31], while the pro-angiogenic properties would non-specifically favor tissue invasion and MS formation. It should be underlined, however, that we cannot definitely exclude that MS arising at different sites might differently affect the OS. In fact, this study was not powered to answer this issue and certain sites were involved in a very few cases. Similarly, we failed to find any difference between cases with or without an associated (preceding, concomitant or succeeding) bone marrow disease (AML, MDS, CMN). This can appear somehow surprising; however, it should be noted that MS *per se*, independently from any associated malignancy is generally provided with a poor prognosis.

Moreover, we showed that angiogenesis, in terms of both MVD and MVG, did significantly correlate with patients' outcome. In particular, higher degrees of angiogenesis were associated with significantly inferior OS. Remarkably, MVD turned out to be the unique biological feature impacting OS. In fact, cytogenetics, tissue localization, cytology, and presence of concomitant hematological disease did not. To this regard, however, it should be noted that the number of cases for which cytogenetic information were available was relatively limited, probably affecting such result. Conversely, clinical parameters such as age (a major determinant of OS in AML patients as well) and the achievement of CR were significantly related to the OS. Importantly, with an extended, long-term follow up (ranging from 96 to 180 months) the administration of SCT was confirmed to be associated with a better outcome [3]. Nevertheless, it should be underlined that six out of the nine patients who received SCT had previously achieved a CR, while only three did not. Thus, it was not possible to definitely discriminate the beneficial effect of SCT from the obvious advantage represented by a responsive disease. On the other hand, of note, only 2/6 long term survivors did not receive SCT, and one patient, initially refractory, was rescued by the transplant. Further, some cautiousness has to be taken when considering survival data. In fact, treatments were relatively heterogeneous as patients were not included in a specific clinical trial.

Finally, our study offered new evidences for possible targeted treatment of MS patients [32]. In fact, MS is well recognized as an adverse prognostic factor in myeloid tumors [1], and angiogenesis probably contributes to tumor aggressiveness. In particular, new vessels were shown not only to provide metabolic support, but also to directly sustain tumor cells growth and survival. In fact, the interaction between endothelial cells and myeloid blasts have been demonstrated to provide fundamental sustainment through the VEGF/VEGFR2 pathway, being active both autocrine and paracrine loops [33, 34, 35, 36]. Noteworthy, in experimental models, the inhibition of VEGF/VEGFR2 signaling was shown to be essential for inducing durable remissions in AML xenografts [33]. Thus, anti-angiogenic therapy may be proposed also to patients with extra-medullary disease [18, 19, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 17, 50, 51].

The main limitation of the study probably relies in the lack of a complete genomic characterization. Unfortunately, the lack of available material did not allow neither a conventional approach nor a NGS or microarray based one [6, 7, 52, 53] to identify lesion with potential prognostic relevance.

In conclusion, we provided evidence, for the first time to the best of our knowledge, that angiogenesis is increased in MS, assuming a significant prognostic impact in this setting. Thus, we offered the basis for a better comprehension of the biology of the tumor, as well as the rationale basis for novel, targeted therapeutic approaches.

The authors declare no conflict of interest.

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