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ORIGINAL PAPER

Density of microvessels positive for CD105 (endoglin) is related to prognosis in meningiomas

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Abstract Microvessel density (MVD) is considered to be a prognostic marker in many tumours. Nevertheless, conflicting results were achieved regarding its prognostic role in meningiomas when it was quantified through pan-endothelial markers such as CD34, CD31 or Factor VIII. In the present study, MVD was assessed in meningiomas through the specific marker for neo-angiogenesis CD105. Fifty-four formalin fixed, paraffin embedded, surgical cases of meningiomas (WHO 28 grade I and 26 grade II) as well as ten normal leptomeningeal samples were submitted to immunohistochemical analysis for CD105. CD34 immuno-expression was also evaluated on consecutive parallel sections. For each case, MVD was estimated in terms of number of vessels/mm^2. CD105 was not evidenced in normal samples, whereas it was demonstrated in the vessels within 14/28 WHO grade I cases and within 24/26 WHO grade II meningiomas. On the contrary, CD34 antibody stained blood vessels in both normal and neoplastic samples; moreover, in each case, it stained more microvessels than CD105 antibody (25.33 ± 21.16 vs. 50.72 ± 26.75). Higher CD105 counts were significantly correlated with higher histological grade and Ki-67 LI $> 4\%$. No statistical significant correlations were encountered between MVD measured by either CD105 and CD34 and sex, age, site of tumour or extent of surgical resection. CD105-MVD, but not CD34-MVD, showed an inverse significant correlation with overall survival and recurrence-free survival. In conclusion, our study suggests the higher specificity of CD105 in comparison to pan-endothelial markers in the evaluation of meningioma neo-angiogenesis, and its higher prognostic significance. CD105 might serve as a target for therapeutic approaches blocking blood supply in meningiomas.

Key words CD105 · Endoglin · Meningioma · CD34 · Survival

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Introduction

Microvessels density (MVD) provides a quantitative measure of angiogenesis in a variety of pathologic processes, including neoplasia. It has been extensively analyzed in a number of malignancies and it is considered to be a useful prognostic marker in many types of cancer [16]. Nonetheless, the markers utilized in these studies are pan-endothelial markers, such as FVIII, CD31 and CD34, which also react with pre-existing normal host vessels and not only with the newly formed tumour vessels. As a consequence, they do not seem to be the most adequate markers to precisely quantify MVD in tumours. By contrast, endoglin (CD105), a 180 kDa homodimeric transmembrane glycoprotein, which is a component of the TGF-ß receptor complex [7], is predominantly expressed on cycling vascular endothelial cells in regenerating or inflamed tissues, or in tumours, all of which undergo active angiogenesis [6, 23, 38]. It is well known that its expression is up-regulated by hypoxia and by TGF-ß1 [33, 45] and, in solid tumours, the antibody against CD105 binds preferentially the activated endothelial cells of peri- and intra-tumour vessels that are

actually involved in tumour neo-angiogenesis, whereas a negative/weak reaction is evidenced in vascular endothelium of normal tissues [6, 39, 40].

Meningiomas account for approximately 25% of all primary intracranial neoplasms [17]. Although they are often histologically benign and at times undergo an indolent clinical course, meningiomas still display a poor outcome in some cases. Even if the most powerful prognosticators for these tumours include histologic grading and extent of surgical resection, their adverse clinical course in terms of mortality and morbidity has been associated in some instances with the degree of tumour vascularity and with the extent of peri-tumoral vasogenic oedema [27]. Several studies have been performed in order to quantify MVD in meningiomas by using endothelial markers such as FVIII, CD31 and CD34 [2, 19–21, 28, 29, 35, 44]. Nevertheless, the correlations with clinico-pathological parameters showed conflicting results [19–21, 28, 35]. In fact, some authors found a correlation between MVD and tumour grade [20, 28, 35], while no significant relationship was reported with respect to histological grade and recurrences of meningiomas in other investigations [19, 21]; finally, even an inverse correlation between vascularity and tumour grade was observed by Yoo and colleagues [44].

Up to now, MVD has never been evaluated by the use of anti-CD105 antibody in human meningiomas. Therefore, taking into consideration its specificity in the identification of newly formed vessels, we have performed the immunohistochemical evaluation of CD105 expression in a series of human meningiomas. Moreover, we have considered of interest the analysis of possible correlations between CD105 pattern and clinico-pathologic parameters, such as age and gender of the patient or histological grade, histotype, Simpson's grade of surgical resection and growth fraction revealed by Ki-67 LI of the tumour. Finally, a comparison with the CD34 immunohistochemical expression in the same cases of meningiomas has also been performed.

Materials and methods

Fifty-four cases of surgically resected meningiomas, obtained from 32 female (59%) and 22 male (41%) patients (age range 21–84 years; mean age 61.7 years) and occurring between 1996 and 1998, were taken from the files of the Department of Human Pathology, University of Messina and of the Unit of Pathology, M. Bufalini Hospital, Cesena, Italy. More precisely, 23 cases, diagnosed as atypical meningiomas, were randomly selected. Subsequently, a comparable number of cases comprising meningothelial, transitional and fibrous histotypes were considered. Moreover, cases of meningiomas displaying a more unusual histotype and occurring in the same years were also recruited. All cases were histologically re-evaluated according to WHO 2000 [17]. Finally the cohort of the study comprised: 12 meningothelial (22%), 5 transitional (9%), 4 fibrous (7%), 4 microcystic (7%), 3 secretory (6%), 1 clear cell (2%), 2 chordoid (4%) and 23 (43%) atypical meningiomas. Thus, according to the WHO 2000 classification system, 28 cases displayed a histological grade I and 26 a histological grade II [17]; 5/23 atypical meningiomas displayed brain infiltration at the haematoxylin and eosin staining. The tumour localization was subdivided into three sites: convexity (32%), parasagittal (35%), and basal (33%). For each case, Simpson's grade of surgical resection [36] as well as immunohistochemical assessment of growth fraction determined by Ki-67 labelling index (LI) were available. On the basis of Simpson's grade, two main groups were considered: the first one (59%) representing grade 1 tumours (complete excision, including dura and bone), the second group (41%) comprising both grade 2 (complete excision plus apparently reliable coagulation of dural attachments) and grade 3 (complete excision of the solid tumour, but with insufficient dural coagulation or bone excision) meningiomas. Follow-up data, including patients survival and recurrences, were available for 39/54 (72%) of the patients. Recurrence was defined as detection of recurrent tumour by neuroradiological investigations in those patients with a previous complete surgical excision.

Ten samples of human leptomeninges, obtained at autopsy from adult and neonate patients without any brain disease, were utilized as normal tissue controls.

Immunohistochemistry

All meningeal specimens were fixed in 10% neutral formalin for 24 h at room temperature, embedded in paraffin at 55°C and cut into parallel consecutive 4 μm thick sections for the subsequent immunohistochemical study. Briefly, the endogenous peroxidase activity was blocked with 0.1% H_2O_2 in methanol for 20 min; then, normal sheep serum was applied for 30 min to prevent unspecific adherence of serum proteins. For the CD105 epitope retrieval, specimens were pre-treated with proteinase K (S3020, DAKO Cytomation) at room temperature for 15 min, whereas CD34 antigen was unmasked by microwave oven pre-treatment in 10 mM, pH 6.0 sodium citrate buffer for 3 cycles × 5 min. Sections were successively incubated at 4°C overnight with the primary monoclonal antibodies against CD105 (DAKO Corporation, Denmark, clone SN6 h, w.d. 1:50) and CD34 (DAKO Corporation, Denmark, clone QBEnd10, w.d. 1:50); a sheep anti-rabbit immunoglobulin antiserum (Behring Institute; w.d. 1:25) was applied and the bound primary antibody was visualized by avidin–biotin–peroxidase detection using the Vectastain Rabbit/Mouse Elite Kit, according to the manufacturer's instructions. To reveal the

immunostaining, the sections were incubated in darkness [42] for 10 min with 3–3′ diaminobenzidine tetra hydrochloride (Sigma Chemical Co., St. Louis, MO, USA), in the amount of 100 mg in 200 ml 0.03% hydrogen peroxide in phosphate-buffered saline solution (PBS). Nuclear counterstaining was performed by Mayer's haemalum. Specificity of the binding was assessed by omitting the primary antiserum or replacing it with normal rabbit serum or phosphate buffered saline solution (PBS pH 7.4). Moreover, the syncytiotrophoblast present in specimens of human term placenta was tested as a positive control for CD105 immunoreaction [14]. Sections of renal cell carcinoma known to express CD105 and CD34 were used as additional positive controls [34]. In parallel sections obtained from the same tissue blocks, Ki-67 antigen was unmasked by retrieval procedures (10 mM, pH 6.0 sodium citrate buffer heated in a microwave oven for 3 cycles × min) and then Ki-67 antiserum (clone MIB-1, DAKO, Glostrup, Denmark; w.d. 1:50) was applied for 30 min at room temperature.

Quantification and statistics

The quantification of microvessels was performed according to the procedure described by Weidner et al. [41]. The three most vascularized areas detected by CD105 were initially selected (so-called hot spots) under 40× field. Microvessels were then counted in each of these areas under a 400× field. Single endothelial cells or cluster of endothelial cells, with or without a lumen, were considered to be individual vessels. The mean value of three ×400 field (0.30 mm^2) counts was recorded as the microvessel density (MVD) of the section. Then the MVD value was converted into the mean number of microvessels/mm^2 for the statistical analyses. The vessels were counted using a Zeiss microscope by two independent observers blinded to the clinico-pathological data. The same procedure was carried out on corresponding human meningioma slides stained by CD34.

The Ki-67 LI was calculated as mean percentage by counting the stained nuclei of tumour cells for 1,000 cells in three representative neoplastic fields; all degrees of nuclear staining intensity were taken into consideration. A Ki-67 value of 4% was utilized as a cut-off point to determine low and high Ki-67 expression, as suggested by Perry et al. [26].

The Mann–Whitney and Kruskal–Wallis tests were used to analyse the correlations between CD105-MVD and the clinico-pathological variables of meningiomas, whereas the Spearman correlation test was applied to verify the correlation between the CD105 and the CD34 in the identification of MVD.

Overall survival and recurrence-free survival were assessed by the Kaplan–Meier method, with the date of primary surgery as the entry data. The same criteria used in our previous study on meningiomas were applied for defining the end point for overall survival [3]. It was characterized as the length of survival to death for meningioma or for intercurrent diseases strictly related with it, such as status epilepticus, diabetes insipidus with electrolytic imbalance, metachronous meningiomas and intra- or post-surgical complications related to the high vascularity of the meningioma. Patients died of diseases independent from the meningioma (myocardial infarction, other malignant neoplasias not involving the CNS) were censored. The end point for the recurrence-free survival analysis was the length of survival to the detection of a recurrent tumour. The Mantel–Cox log-rank test was applied to assess the strength of association between survival time or recurrence-free interval and each of the parameters (age and gender of the patient, site, Simpson's grade, histologic grade, CD105-MVD and Ki-67 LI of the tumour) as a single variable. Successively, a multivariate analysis (Cox regression model) was utilised to determine the independent effect of each variable on survival.

For CD105-MVD overall and recurrence-free survival analyses, cases were subdivided into two groups by using the median MVD value as the cut-off value (CD105-MVD median value 20).

Moreover, overall and recurrence-free survival analyses were performed for CD34-MVD, utilizing the median MVD value as the cut-off value (CD34-MVD median value 48.85).

A probability (P) value less than 0.05 was considered statistically significant. Data were analysed using the SPSS package version 6.1.3 (SPSS Inc., Chicago, IL, USA).

Results

The clinico-pathological characteristics of the analysed meningiomas and the corresponding CD105-MVD and CD34-MVD are shown in Table 1. In the ten normal leptomeningeal samples, blood vessels were only stained by the CD34 antibody, whereas no staining was evidenced by using the CD105 antibody (Fig. 1).

With reference to the neoplastic samples, CD105 positive vessels were evidenced in 38/54 (70%) meningiomas; among CD105 negative cases, 14/16 were grade I meningiomas, 1/16 was a clear cell meningioma and 1/16 was an atypical meningioma. In positive cases, CD105 immunoreaction was observed in the endothelial cells of stained vessels (Fig. 2). A positive immunoreaction was also noted in vascular smooth muscle cells of hyalinized vessels within meningiomas displaying a microcystic histotype and in the neoplastic cells of fibroblastic meningiomas (Fig. 2e). Moreover, when the adjacent brain tissue was invaded, vessels present in these areas were also stained by the CD105 antibody.

Table 1 Clinicopathological characteristics and CD105-MVD and CD34-MVD data of 54 analyzed meningiomas

Case	Sex	Age	Site	Histotype	Grade	Ki-67 (%)	Simpson	CD105-MVD (v/mm^2)	CD34-MVD (v/m m^2)	Status	FU (months)	Recurrences	DFI (months)
1	F	31	Basal	Atypical	2	8	1	20.00	46.6	NED	93	Yes	48
2	M	60	Convexity	Secretory	1	1	1	0.00	22.2	NED	118	Not	118
3	M	38	Parasagittal	Atypical	2	10	3	23.33	47.7	DOD	80	–	–
4	M	57	Parasagittal	Atypical	2	5	2	30.00	63.3	NED	104	–	–
5	M	60	Basal	Meningothelial	1	0.5	2	0.00	23.3	NED	112	–	–
6	F	59	Basal	Meningothelial	1	4	1	43.33	76.6	–	–	–	–
7	M	75	Basal	Secretory	1	0.50	2	30.00	66.6	DOD	2	–	–
8	F	71	Convexity	Atypical	2	6	1	66.66	83.3	NED	89	Not	89
9	F	77	Basal	Meningothelial	1	0.5	2	46.66	77.7	DID	12	–	–
10	M	67	Convexity	Atypical	2	10	1	0.00	26.6	NED	120	Not	120
11	F	76	Convexity	Microcystic	1	1	2	83.33	100	DID	76	–	–
12	M	55	Basal	Microcystic	1	3	3	33.33	76.6	–	–	–	–
13	M	54	Basal	Meningothelial	1	0.5	1	5.33	23.3	NED	114	Not	114
14	F	63	Parasagittal	Atypical	2	1	1	15.33	30	–	–	–	–
15	F	73	Parasagittal	Atypical	2	5	2	20.00	50	NED	114	–	–
16	M	65	Parasagittal	Transitional	1	1	1	13.33	26.6	–	–	–	–
17	M	64	Convexity	Atypical	2	5	1	93.33	133.3	NED	94	Not	94
18	F	70	Basal	Meningothelial	1	4	2	12.00	26.6	–	–	–	–
19	M	61	Parasagittal	Atypical	2	5	3	20.00	50	–	–	–	–
20	M	56	Parasagittal	Atypical	2	5	1	51.00	80	DOD	91	Yes	16
21	M	66	Parasagittal	Atypical	2	6	1	26.66	58.8	NED	108	Not	108
22	F	54	Convexity	Transitional	1	0.5	1	0.00	23.3	NED	100	Not	100
23	M	63	Convexity	Transitional	1	2	1	4.33	22.2	NED	118	Not	118
24	M	63	Convexity	Meningothelial	1	0.5	1	0.00	16.6	NED	115	Not	115
25	M	63	Convexity	Meningothelial	1	1	1	0.00	23.3	–	–	–	–
26	M	65	Parasagittal	Atypical	2	8	3	35.33	80	–	–	–	–
27	F	70	Basal	Transitional	1	0.5	2	0.00	22.2	NED	119	–	–
28	F	63	Parasagittal	Atypical	2	20	1	63.33	80	–	–	–	–
29	F	59	Basal	Fibroblastic	1	0.5	1	0.00	16.6	NED	106	Not	106
30	F	21	Convexity	Clear cell	2	0.5	1	0.00	22.2	NED	98	Not	98
31	F	70	Convexity	Atypical	2	10	1	27.66	60	AWD	101	Yes	41
32	M	73	Parasagittal	Atypical	2	30	2	55.33	78.8	DID	111	–	–
33	F	47	Basal	Chordoid	2	5	1	23.33	56.6	–	–	–	–
34	F	47	Basal	Secretory	1	1	3	5.33	26.6	NED	96	–	–
35	F	48	Parasagittal	Transitional	1	1	1	0.00	23.3	NED	118	Not	118
36	F	72	Basal	Atypical	2	10	2	3.33	23.3	–	–	–	–
37	M	53	Parasagittal	Microcystic	1	4	1	106.66	173.3	NED	110	Not	110
38	F	60	Convexity	Atypical	2	10	1	23.33	53.3	DOD	7	–	–
39	F	63	Basal	Meningothelial	1	1	1	30.00	66.6	–	–	–	–
40	F	70	Basal	Fibroblastic	1	1	1	0.00	16.6	NED	96	Not	96
41	F	64	Parasagittal	Fibroblastic	1	3	1	0.00	18.8	NED	108	Not	108
42	F	65	Convexity	Fibroblastic	1	1	1	0.00	16.6	–	–	–	–
43	M	40	Parasagittal	Atypical	2	3	1	20.00	51.1	NED	106	Not	106
44	F	84	Parasagittal	Atypical	2	30	2	23.33	53.3	DOD	10	–	–
45	F	61	Basal	Meningothelial	1	1	3	0.00	16.6	NED	116	–	–
46	F	74	Convexity	Atypical	2	15	1	85.33	100	NED	91	Not	91

Table 1 continued

Case	Sex	Age	Site	Histotype	Grade	Ki-67 (%)	Simpson	CD105-MVD (v/mm^2)	CD34-MVD (v/m m^2)	Status	FU (months)	Recurrences	DFI (months)
47	F	53	Parasagittal	Atypical	2	8	3	55.33	80	NED	91	-	-
48	F	73	Basal	Meningothelial	1	1	2	0.00	16.6	–	–	–	–
49	M	77	Parasagittal	Atypical	2	10	3	18.66	31.1	DOD	17	–	–
50	M	75	Parasagittal	Atypical	2	20	2	40.00	78.3	DOD	41	–	–
51	M	65	Convexity	Meningothelial	1	0.5	1	0.00	18.8	NED	105	Not	105
52	F	63	Basal	Meningothelial	1	1	2	13.33	26.6	–	–	–	–
53	M	75	Convexity	Chordoid	2	5	1	63.33	81.1	DOD	1	–	–
54	M	43	Convexity	Microcystic	1	1	1	36.66	76.6	NED	97	Not	97
												–	–

MVD Microvessel density, *NED* no evidence of disease, *DOD* dead of disease, *DID* dead of independent disease, *AWD* alive with disease, *FU* follow-up

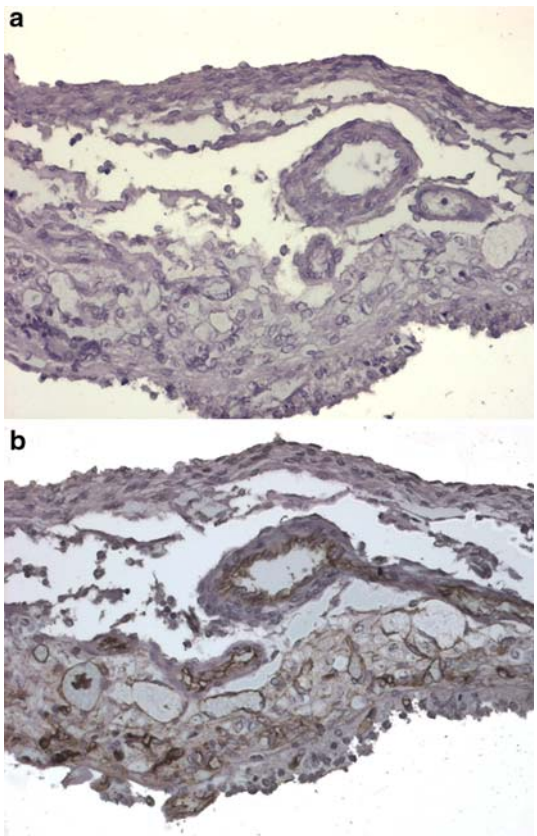

Fig. 1 **a** No staining was evident in the human normal leptomeninges with CD105 antibody (CD105 stain; original magnification ×100) **b** By contrast, an intense positive immunoreaction was evidenced in the vessels of normal leptomeninges by using CD34 antibody (CD 34 stain; original magnification ×100)

When CD105 immunoreactivity was considered in the different meningioma histotypes, no CD105 positive vessels were seen in the only analysed clear cell meningioma, whereas all the microcystic meningiomas displayed a CD105-MVD value higher than the cohort MVD median value. Nonetheless, given the small number of chordoid, secretory, microcystic and clear cell variants, it was not possible to study the statistical correlations between CD105MVD counts and these meningioma histotypes.

CD34 immunoreaction stained all kinds of vessels present in all meningioma cases. In the case-by-case analysis, the number of microvessels stained by CD105 was lower than the one revealed by CD34 staining (25.33 ± 21.16 vs. 50.72 ± 26.75) (Fig. 3); by Spearman's test, there was a highly significant correlation between CD105-MVD and CD34-MVD ($r = 0.960$, $P = 0.0001$).

Concerning the statistical correlations between CD105-MVD and the clinico-pathological variables, when meningioma cases were stratified by histological grade, a significant difference ($P = 0.0014$) in CD105-MVD was found between grade I and grade II tumours, the latter ones displaying higher MVD values (Table 2).

A variously represented Ki-67 nuclear reactivity was found in meningiomas, with a rate of stained cells ranging from 0.5 to 30% (mean value 5.3%). Despite the high (20 or 30%) Ki-67 LI value encountered, some cases were classified as atypical and not as malignant meningiomas since the criterion of 20 or more mitoses per 10 high power fields in order to classify them as grade III tumours was not fulfilled [27]. A significantly higher CD105-MVD value ($P = 0.0002$) was evidenced in cases displaying Ki-67 LI levels greater than 4% (Table 2).

No statistically significant differences in CD105-MVD were recorded with reference to the gender and age of the patient, or to the site and Simpson's grade of the tumour (Table 2).

Regarding the clinical course, univariate analysis identified histological grade, Ki-67 LI and CD105-MVD, but not

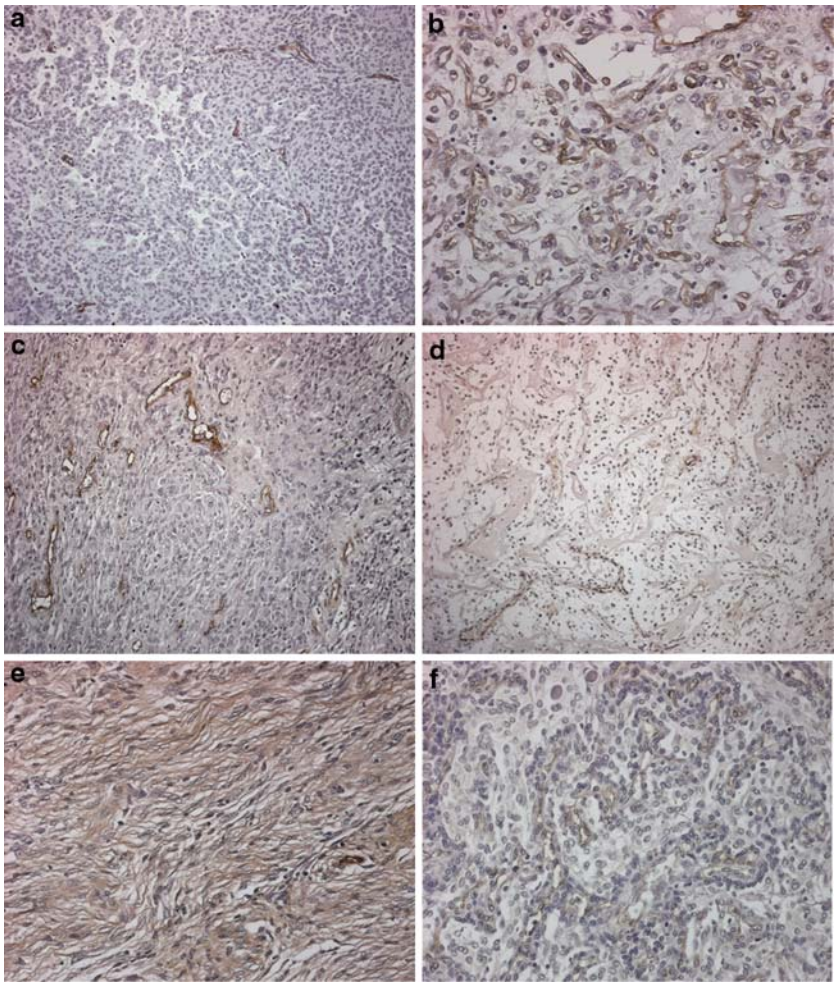

Fig. 2 **a** CD105 antibody stained only rare vessels in meningothelial meningioma (CD105 stain; original magnification ×100). **b** A tumour high MVD was evidenced by CD 105 staining in a microcystic meningioma (CD105 stain; original magnification ×200). **c** An intense positive immuno-reaction for CD 105 was present in numerous vessels within atypical meningioma (CD105 stain; original magnification ×100). **d** CD105 staining in the vessels of a chordoid meningioma (CD105 stain; original magnification ×100). **e** CD105 positive immunoreaction was evident in rare vessels as and in the neoplastic cells within fibrous meningioma (CD105 stain; original magnification ×200). **f** A slight weakly positive CD105 immunoreaction was present in few vessels in secretory meningioma

CD34-MVD, as significant prognostic factors for meningiomas specific survival (Table 3). In particular, the survival of patients with a higher CD105-MVD (≥20 vessels/mm^2) was significantly worse ($P = 0.0419$) than that of patients with lower CD105-MVD (<20 vessels/mm^2). The survival curves of patients with low and high CD105-MVD as well as Ki-67 LI are illustrated in Figs. 4 and 5, respectively. Multivariate analysis indicated that only Ki-67 LI was an independent prognostic factor for patients survival to meningioma (Table 3).

Among the 22 patients with a Simpson's grade 1 meningioma and with an available follow-up, 3 had developed recurrences. Follow-up ranged from 16 to 120 months. All recurrent cases displayed a CD105-MVD count above the cut-off level. Univariate analyses showed that a CD105-MVD ≥ 20 vessels/mm^2, a Ki-67 LI > 4% and a high histologic grade were significant prognostic factors for recurrence (Table 4).

No significant differences in the recurrence-free survival were observed between the patients with a high CD34-MVD and those with a low CD34-MVD (Table 4).

Discussion

CD105 antibody has been largely employed in the assessment of MVD in several kinds of neoplasias and a significant correlation has been demonstrated between a lower CD105-MVD and a better overall survival of patients [4, 9, 10, 17, 30, 31, 37, 43]. In brain tumours, MVD evaluation by CD105 has been immunocytochemically performed in craniopharyngiomas [9], in medulloblastomas [5], in astrocytic tumours [4, 5, 43] as well as in primary central nervous system lymphomas (PCNSL) [37]. In detail, a significant inverse correlation between the MVD revealed by CD105 and the survival emerged for both astrocytic tumours and PCNSL [4, 37, 43].

In the present study we analysed, for the first time, the CD105 immunoexpression in human meningiomas and the corresponding normal leptomeninges in order to evaluate its ability to identify the newly formed neoplastic vessels as well as to establish whether a correlation exists with clinicopathological parameters, so as to verify if MVD documented by CD105 could be utilized for prognostic purposes.

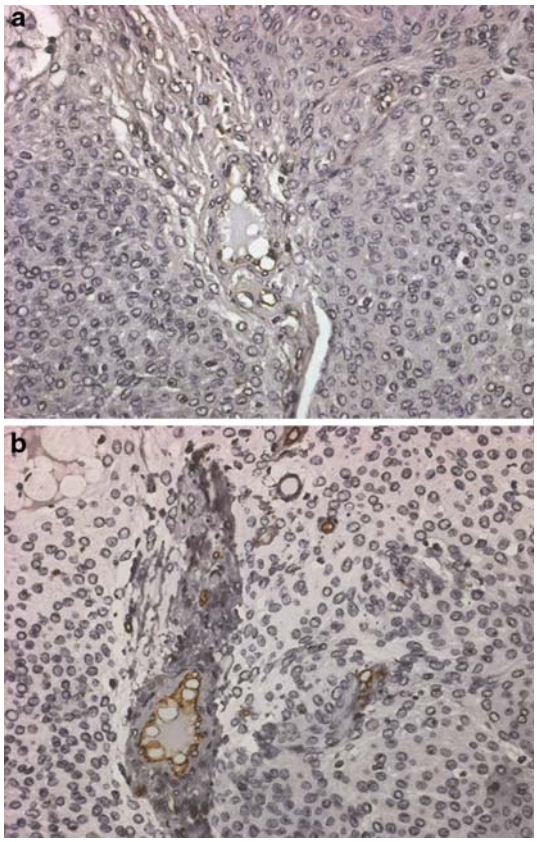

Fig. 3 CD 105 antibody revealed fewer vessels than the CD34 one. Moreover CD105 did not stain the vessels with a muscular wall, in contrast to CD34 (**a** CD105 stain; original magnification ×200; **b** CD34 stain; original magnification ×200)

Table 2 Statistical correlations between CD105MVD and clinicopathological parameters analyzed throughout Mann–Whitney and Kruskal–Wallis tests

Variable	*n*	Mean rank	*P*
Gender			
Male	22	30.39	0.2571
Female	32	25.52	
Age			
≤65 years	34	26.09	
>65 years	20	29.90	0.3834
Site			
Convexity	17	26.35	
Parasagittal	19	33.03	0.1231
Basal	18	22.75	
Grade			
1	28	21	0.0014
2	26	34.50	
Simpson's grade			
Grade 1	32	26.41	0.5322
Grade 2–3	22	29.09	
Ki-67 LI			
≤4%	31	26.41	0.0002
>4%	23	29.09	

Table 3 Univariate and multivariate survival analyses in 39 patients with meningioma

Univariate analysis				
Parameter	X^2	*df*	*P* value	
Sex	1.850	1	0.1737	
Age	0.84	1	0.3602	
Site	2.32	2	0.3141	
Histological grade	4.68	1	0.0305	
Ki-67	6.0458	1	0.012	
CD105-MVD	4.14	1	0.0419	
CD34-MVD	2.672	1	0.102	
Simpson's Grade	2.68	1	0.1014	
Multivariate Analysis	β	SE	Exp (β)	*P* value
Variable				
Ki-67	0.073	0.035	1.076	0.036

In an attempt to test CD105 specificity as a neo-angiogenesis marker, MVD revealed by CD105 staining in the analysed normal and neoplastic leptomeninges was also compared with MVD measured by using the pan-endothelial marker CD34 on parallel tissue sections. The comparison between CD34-MVD and CD105-MVD revealed that CD34 antibody stained more microvessels than the CD105 one. Moreover, vessels in normal human leptomeninges did not express CD105, whereas they were constantly stained by the CD34. This is in line with previous observations [24, 32], in which the CD105 expression is weak or negative in normal tissues. Therefore, we hypothesize that CD34 stained both the host entrapped vessels in meningiomas and the newly formed ones; thus CD34 may be considered as a pan-endothelial marker, while CD105 further appears as a more specific marker for neo-angiogenesis.

On the whole, CD105 positive vessels were evidenced in 70% of meningiomas. Interestingly, CD105 negative cases were all grade I tumours, apart from one clear cell and one atypical meningioma. When cases were stratified by grade, significant differences in CD105MVD counts were achieved, being grade II the parameter characterized by higher CD105 immunoexpression. In grade II tumours, neo-angiogenesis might be stimulated by the secretion of pro-angiogenic factors, such as VEGF, by the neoplastic cells. Indeed, a significantly higher VEGF expression has been demonstrated in atypical meningiomas in comparison to benign meningiomas [19]. As above specified, the clear cell analysed meningioma, which stained completely negative for CD105, represented an exception within grade II meningiomas;

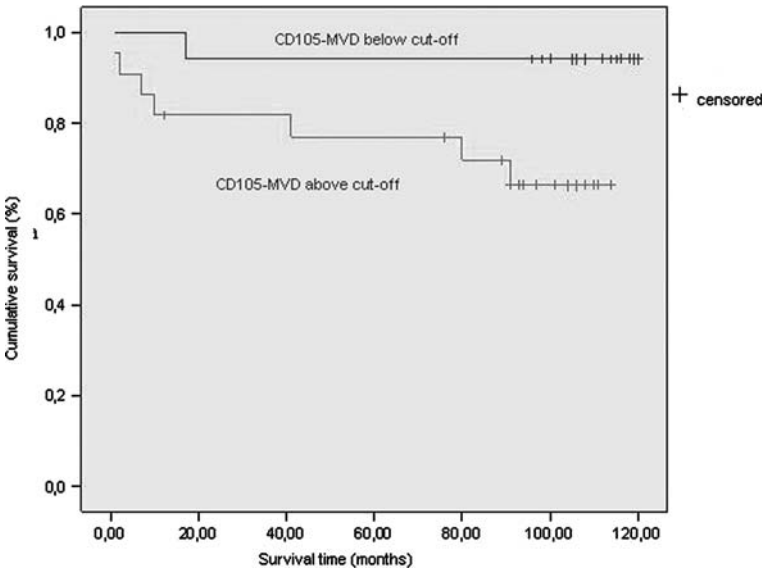

Fig. 4 Kaplan–Meier meningioma-specific survival curve according to CD105-MVD. The survival of the patients with a CD105-MVD above the cut-off value (median value) was significantly worse than that of the patients with a CD105-MVD below the cut-off value

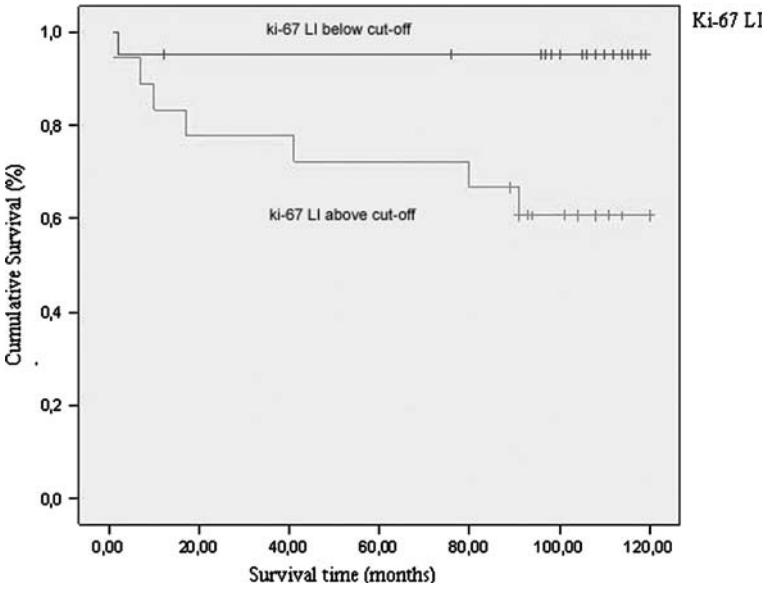

Fig. 5 Kaplan–Meier meningioma-specific survival curve according to Ki-67 LI. The survival of the patients with a Ki-67 LI above the cut-off value (Ki-67 LI $> 4\%$) was significantly worse than that of the patients with a Ki-67 LI below the cut-off

Table 4 Univariate analysis for recurrence-free time in 22 patients with a predetermined Simpson's grade 1 meningioma

Parameter	X^2	*df*	*P* value
Sex	0.5148	1	0.4731
Age	0.0622	1	0.8030
Site	0.0616	2	0.7183
Histological grade	4.93	1	0.0265
Ki-67	6.0458	1	0.0139
CD105-MVD	4.0223	1	0.0449
CD34-MVD	1.0489	1	0.3058

nevertheless, the extreme rarity of this variant did not allow us to obtain statistical results or specific histopathological correlations. Curiously, when the histotype was specifically considered within grade I meningiomas, microcystic tumours revealed to be high vascular tumours, displaying a high CD105 count. Even if microcystic meningiomas were already known for their hypervascularity [8, 25], our study suggests that this feature may be dependent on neoangiogenic processes. Besides, previous studies have already shown a significantly higher expression of VEGF and flt-1 in meningiomas displaying a microcystic histotype in comparison to other grade I meningeal tumours [8]. Finally, in accordance with the previous detection of endoglin m-RNA in human vascular smooth muscle cells [1], we also evidenced CD105 staining in the vascular smooth muscle cells of hyalinized vessels of microcystic meningiomas.

No statistically significant differences in CD105-MVD were encountered with respect to age and gender of the patients or to the neoplastic site and Simpson's grade of the tumour.

In an attempt to investigate the relationship between MVD and growth fraction, CD105 immunoexpression was also analysed in comparison to KI-67 LI, performed in the same series of meningiomas. According to the guidelines proposed by Perry et al. [27], levels of Ki-67 greater than 4% have been associated with an increased rate of recurrence and therefore, we utilized this percentage as a cut-off value to identify tumours characterized by a negative prognostic parameter. Interestingly, in accordance with results already obtained by other authors for glioblastomas [4], cases with a higher Ki-67 LI exhibited also significantly higher CD105 counts. We may speculate that meningiomas with an intrinsically higher capability to proliferate undergo a hypoxic condition which is determined by their increased volume. Hypoxia may be in turn responsible for the endoglin up-regulation, via the hypoxia inducible factor-1 (HIF-1) and consequently for the higher neo-angiogenesis revealed by MVD, as elsewhere suggested [22]. Then, nutrients provided by the newly formed vessels might allow the more rapid growth and progression of the tumour [13, 15]; indeed, it is known that tumour growth is greatly dependent on the formation of new vessels [11, 12].

When the prognostic value of CD105-MVD and CD34-MVD on specific survival to meningioma and on the recurrence-free survival was tested, only the former was significantly associated to the clinical outcome. In fact, a higher CD105-MVD count, but not CD34-MVD count, appeared significantly correlated to a worse survival of patients and to the development of recurrences. Therefore, similarly to what reported for astrocytic brain tumours [4, 43], CD105 shows a more significant predictive value in meningiomas in comparison to other endothelial markers. Nonetheless, multivariate analysis disclosed that CD105-MVD is not an independent prognostic factor for meningioma specific survival, in contrast to Ki-67 LI. The hypervascularity observed also in benign indolent tumours, such as microcystic meningiomas, might account for this finding. Although CD105-MVD cannot be considered as a robust prognostic parameter in predicting the patients survival to meningiomas, however our study gives evidence of the existence of strong correlations between tumour histological grade or Ki-67 LI and the extent of tumour vascularity, when the latter is measured by using a specific marker for tumour neo-angiogenesis, such as CD105. Indeed, meningiomas with a higher histological grade and a higher Ki-67 LI were shown to have a significantly higher density of newly formed vessels, as if they required a high vascular supply to support their growth. In conclusion, the present study suggests that CD105 is a specific marker for neo-angiogenesis in meningiomas and that a high density of vessels expressing this marker is present in aggressive meningiomas; if further studies confirm this issue, then CD105 might be considered as a target for anti-angiogenic selective immuno-therapies able to block tumour blood supply in meningiomas.

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