Pathology - Research and Practice 215 (2019) 152648

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Endoglin-based assessment of neoangiogenesis in sporadic VIII cranial nerve schwannoma

Gino Marioni[a,*], Stella Blandamura[b], Lorenzo Nicolè[b], Luca Denaro[c], Diego Cazzador[a,d], Chiara Pavone[a], Luciano Giacomelli[b], Vincenza Guzzardo[b], Ambrogio Fassina[b], Antonio Mazzoni[a], Domenico D'Avella[c], Alessandro Martini[a], Elisabetta Zanoletti[a]

[a] Department of Neuroscience DNS, Otolaryngology Section, Padova University, Padova, Italy
[b] Department of Medicine DIMED, Padova University, Padova, Italy
[c] Department of Neuroscience DNS, Academic Neurosurgery, Padova University, Padova, Italy
[d] Department of Neuroscience DNS, Section of Human Anatomy, Padova University, Padova, Italy

ARTICLE INFO

Keywords:
Vestibular nerve
Schwannoma
Sporadic
Angiogenesis
Endoglin
CD105

ABSTRACT

Although the diagnosis and treatment of sporadic vestibular schwannoma has improved in recent years, no factors capable of predicting its growth have been identified as yet. Endoglin (CD105) is a proliferation-associated protein expressed in angiogenic endothelial cells, and a potential prognostic indicator for several solid tumors. The aim of the present study was primarily to investigate the expression and role of CD105 in a series of sporadic vestibular nerve schwannomas.

In 71 consecutive cases of vestibular schwannoma, vessel cross-sectional area and density were calculated from immunohistochemically assessed CD105 expression using image analysis to correlate them with: (i) tumor dimensions; and (ii) tumor growth rate measured on high-resolution contrast-enhanced MRI (ceMRI).

Based on assessments of CD105 expression, a significant positive correlation was identified between vessel cross-sectional area and tumor size at the time of surgery ($p = 0.0024$), and between vessel density and tumor size ($p = 0.013$). Vessel cross-sectional area ($p = 0.0006$) and vessel density ($p = 0.003$) were significantly greater in tumors measuring ≥ 10 mm in size than in those < 10 mm. Conversely, when tumor growth rate could be calculated from two or more ceMRI (38 cases), there was no significant correlation between tumor growth rate and cross-sectional vessel area or vessel density as assessed with CD105.

Further investigations are needed to ascertain the feasibility of: (i) using circulating endoglin assay to monitor tumor growth; and (ii) targeting neoangiogenesis with anti-endoglin antibodies in sporadic vestibular schwannoma.

1. Introduction

Early diagnosis of vestibular schwannoma not infrequently enables small tumors to be detected promptly before hearing deteriorates, and this raises the question of how this may influence prognosis. The natural history of vestibular schwannoma crucially influences currently-used conservative treatment options [1–3]. The choice between active treatment (surgery or radiotherapy) and observation has to strike a balance between different conditions at diagnosis to offer the best functional outcome in terms of hearing preservation and facial nerve function [3,4]. When a tumor shows growth, active treatment should entail surgical removal (or stopping further growth) without exposing patients to further morbidity. For small tumors diagnosed in patients with good hearing, growth has little or no influence (in principle) on current hearing-preserving options. Predicting the growth of small vestibular schwannomas would nonetheless be crucially important because it would allow a preliminary selection of tumors unlikely to grow that could be followed up successfully with observation alone.

Wait-and-scan protocols currently rely on high-resolution, contrast-enhanced magnetic resonance imaging (ceMRI) to assess tumor growth. The common rule is to measure only the extrameatal portion of the tumor, and growth is assessed disregarding its intrameatal aspect [5]. Radiological monitoring is currently the only available method for the long-term observation of tumor growth. As well as being coarse, it is unable to predict growth, which varies from case to case and over time. Efforts have been made to investigate potential tumor markers capable

* Corresponding author at: Department of Neuroscience DNS, Otolaryngology Section, Padova University Hospital, Via Giustiniani 2, 35128 Padova, Italy.
E-mail address: gino.marioni@unipd.it (G. Marioni).

https://doi.org/10.1016/j.prp.2019.152648
Received 20 July 2019; Received in revised form 16 September 2019; Accepted 16 September 2019

of correlating with schwannoma size and growth rate, with controversial results [6].

The early growth of solid tumors requires no neovascularization as long as tumors are no more than 2–3 mm in diameter, but neoplastic angiogenesis is essential to their further growth [7]. Judah Folkman was the "father of angiogenesis", awarded the Wolf Prize in Medicine in 1992 for his discoveries, from which the concept of angiogenesis originated and the field of research developed. He proposed the first model of tumor angiogenesis according to which tumor cells can sense their increasing distance from existing vasculature and release angiogenic signals in response [8,9]. Tumor angiogenesis is currently considered the outcome of an imbalance between proangiogenic and anti-angiogenic factors produced by tumor and normal cells [10,11]. Endoglin (CD105) is a homodimeric transmembrane glycoprotein, and an auxiliary receptor of transforming growth factor-β (TGF-β), which binds to TGF-β1 and TGF-β3. CD105 modulates TGF-β signaling by interacting with type I and type II TGF-β receptor [12]. It is involved in activating a complex signaling pathway involving the proliferation, migration and adhesion of endothelial cells [13]. Anti-CD105 antibodies have revealed a greater affinity for activated endothelial cells in tissues participating in angiogenesis [14,15]. Unlike pan-endothelial-cell antibodies such as anti-CD31 and anti-CD34, anti-CD105 antibodies may react preferentially with the endothelial cells of all angiogenic tissues, including tumors, but weakly or not at all with those of most normal tissues [16]. Based on the available evidence, CD105 has been suggested as a reliable marker of angiogenesis in clinical studies [17]. Its features have also made CD105 become a prime candidate as a marker for use in prognostics, tumor imaging, and anti-angiogenesis therapy [12], in intracranial tumors too [18].

The present study investigated CD105 expression in a cohort of 71 patients with sporadic vestibular nerve schwannoma. In particular, vessel cross-sectional area and vessel density assessed on CD105 immunohistochemistry were correlated with: (i) tumor size; and (ii) tumor growth rate measured on high-resolution ceMRI.

2. Methods

2.1. Patients

This retrospective study was conducted in accordance with the principles of the Helsinki Declaration. Before undergoing any surgery, all patients signed a detailed informed consent form, and gave their written permission for clinical case publication. Data were examined in agreement with the Italian privacy and sensitive data laws (D. Lgs 196/03), and the internal regulations of Padova University's Otolaryngology Section.

The study was conducted on surgical specimens from 71 patients (37 men and 34 women; mean age 52.8 ± 13.0 years, median 52 years) with primary sporadic VIII cranial nerve (CN) schwannoma, all treated by the same team of surgeons at our institution between 2013 and 2017. Thirty patients underwent surgery within 6 months of their tumor being diagnosed, and 41 after a mean observation period of 31.9 months (range 6–125 months). In 20 cases, hearing-preserving surgery via a retrosigmoid approach was preferred, while the trans-labyrinthine approach was adopted for the other 51 patients. Our institutional indications for managing sporadic acoustic neuroma have been reported elsewhere [6].

Forty-two patients had hearing loss at presentation, which was isolated in 25 cases, and associated with other otological symptoms (tinnitus, fullness) in 17. Thirteen patients presented with isolated tinnitus. Seven had vertigo, and 5 had vertigo associated with other symptoms (hearing loss, fullness or tinnitus). One patient presented with trigeminal neuralgia. Five patients had no symptoms, and their schwannoma was an incidental finding on a ceMRI performed for other reasons.

All patients were diagnosed by high-resolution ceMRI of the brain and cerebello-pontine angle (CPA). The cases managed with hearing-preserving surgery via a retrosigmoid approach and retrolabyrinthine meatotomy also underwent preoperative high-resolution bone CT scan. Complete resection was accomplished in all cases.

The sporadic VIII CN schwannomas were classified as: purely intrameatal (10 cases); small, < 1 cm in the CPA (21 cases); medium-sized, 1–2.5 cm in the CPA (27 cases); or large, > 2.5 cm in the CPA (13 cases) [19].

Final pathological examination confirmed the preoperative diagnosis of schwannoma in all cases.

2.2. Radiological measurements

Tumor size was calculated starting from measurements obtained on the T1w and high-resolution T2w sequences on the latest preoperative ceMRI, selecting the axial image showing the largest part of the lesion [20]. Only the extracanalicular component of the tumor was considered to calculate tumor size, measuring its maximum diameter in the CPA.

In all cases where previous ceMRI findings were available, a tumor growth rate was calculated as follows by comparing the first ceMRI at the time of diagnosis with the last one:

(tumor size at last ceMRI - tumor size at first ceMRI)/time elapsing between the two images. The resulting unit of measurement was mm/month.

2.3. Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded (FFPE) Sections 4–5 μm thick obtained from each tissue sample as described elsewhere [21]. Briefly, staining was done automatically using a fully-automated system (Bond™-maX; Leica, Newcastle Upon Tyne, UK). Sections were pretreated using heat-mediated antigen retrieval with sodium citrate buffer (pH6, Epitope Retrieval Solution 1, Leica) for 30 min at 99 °C. Specimens were then incubated with rabbit polyclonal anti-endoglin (Atlas Antibodies AB, Bromma, Sweden; 1:2500). Staining reactions were then detected using the Bond Polymer Refine Detection Kit (Leica) according to the manufacturer's protocols. Staining was visualized with 3,3'-diaminobenzidine (DAB) and the slides were lightly counterstained with hematoxylin. Sections were then dehydrated, cleared, and mounted. Samples from FFPE human kidney were used as positive controls and serum without the primary antibody as a negative control.

2.4. Image analysis (IA)

For each case, all CD105-stained vessel profiles within 3 randomly-selected hotspots were identified. Each hotspot was selected by a pathologist (LN) in areas showing the greatest vessel density perceivable under the optical microscope. The hotspots were then captured with a Leica 108 DMD digital microscope (Leica Microsystem, Wetzlar, Germany) at a maximum magnification of 20 × . Vessel profiles were automatically quantified using a custom-made algorithm trained to detect stained vessel structures, and developed with Visiopharm™ software, version 4.5.6.5 (Visiopharm, Hoersholm, Denmark) (Fig. 1). The quantitative variables generated were vessel cross-sectional area (AA) and vessel density (VD). AA was obtained with the formula: AA = AV/AH (where: AV = total vessel area; AH = total hotspot area). VD was obtained with the formula: VD = nV/AH (where: nV = number of vessels in the hotspot).

2.5. Statistical analysis

Our data were not normally distributed and the subcohorts considered were often quite small, so the statistical significance of any differences between means was ascertained using a non-parametric measure, the Mann-Whitney *U* test. Spearman's rank correlation test

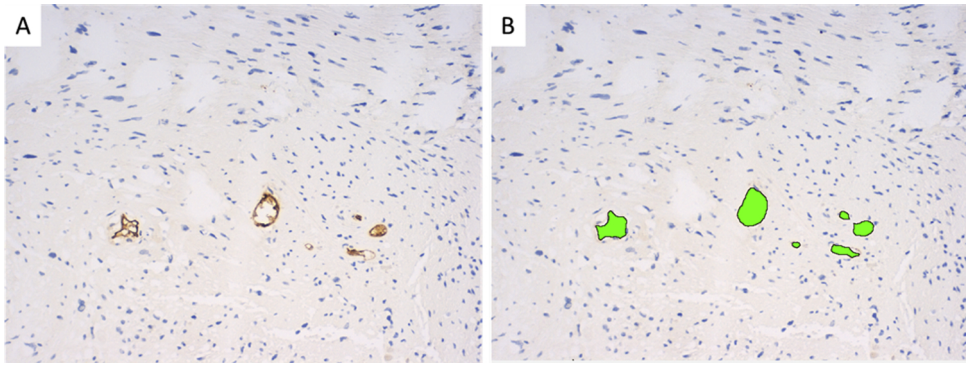

Fig. 1. Representative case of automatic vessel detection and cross-sectional area measurement. Hotspot with higher vessel density captured at maximum magnification of 200X (A). Vessel units labeled in green (B).

was also applied, as appropriate. A p value < 0.05 was considered significant. The STATA 8 statistical package (Stata Corp LP, College Station, TX, USA) was used for all analyses.

3. Results

3.1. Vessel cross-sectional area and density (assessed on CD105 expression) in the whole series

CD105 staining was only evident in the cytoplasm and membrane of endothelial cells (Figs. 2 and 3). Morphologically, no significant differences in vessel architecture were detected between groups with different tumor growth rates, or between groups with different tumor volumes at diagnosis. Interestingly, more sclerotic perivascular stroma was seen in some cases with a rarefied vasculature.

Based on CD105 staining, the mean vessel cross-sectional area was 2.6 ± 2.0 for the series as a whole, and the mean vessel density was 0.25 ± 0.15. The mean tumor size on preoperative ceMRI was 13.2 ± 10.8 mm (median 10 mm). Table 1 summarizes the vessel cross-sectional area and density values stratified by the patients' main demographic and radiological variables.

Spearman's rank correlation test ruled out any significant correlations between age at the time of surgery and vessel cross-sectional area (rho = 0.19, p = 0.10), or vessel density (rho = 0.21, p = 0.07). Among the neoangiogenesis-related variables measured in the whole series of tumors, the following significant positive correlations emerged using Spearman's rank correlation test: (i) between vessel cross-sectional area assessed with CD105 and tumor size on last ceMRI

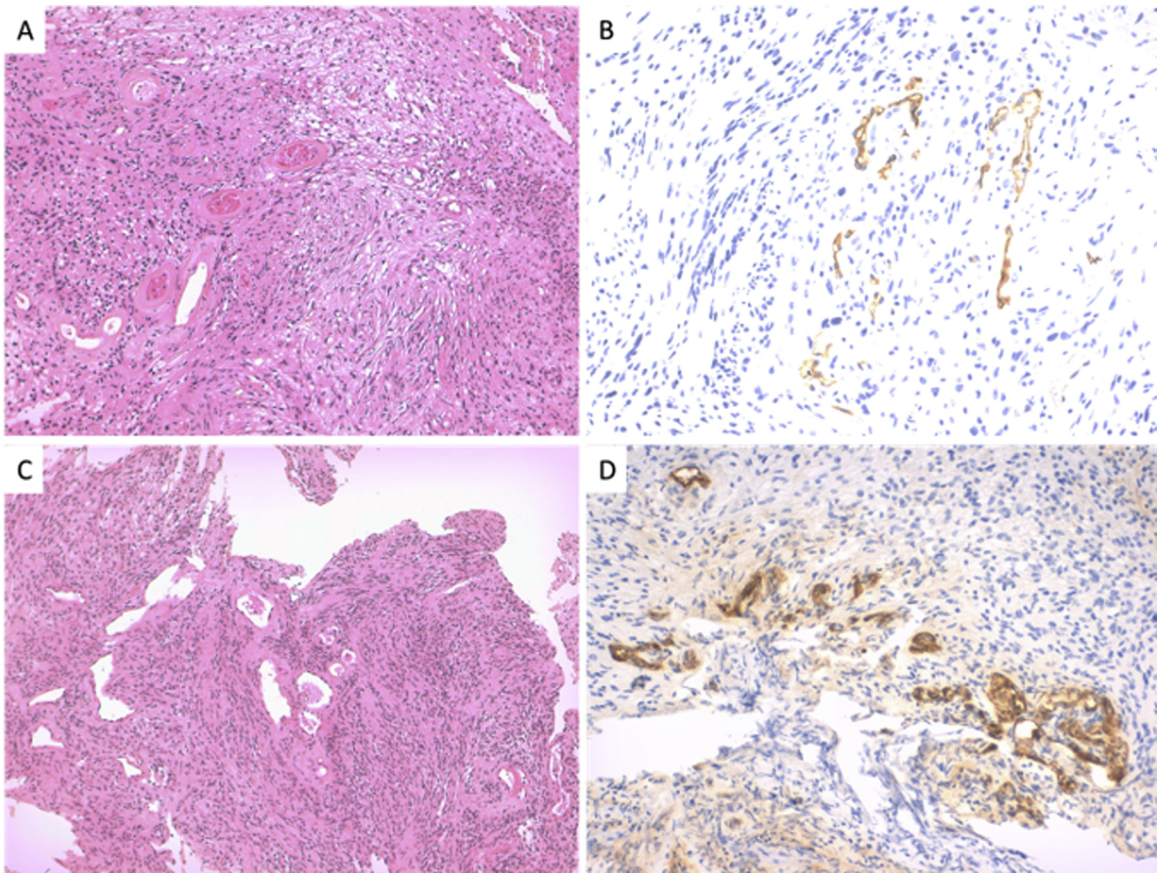

Fig. 2. Representative hotspots of a larger tumor (C and D) versus a smaller tumor (A and B), the larger tumor showing a higher vessel density. Original magnification 100X for (A) and (C), 200X for (B) and (D); hematoxylin and eosin staining for (A) and (C).

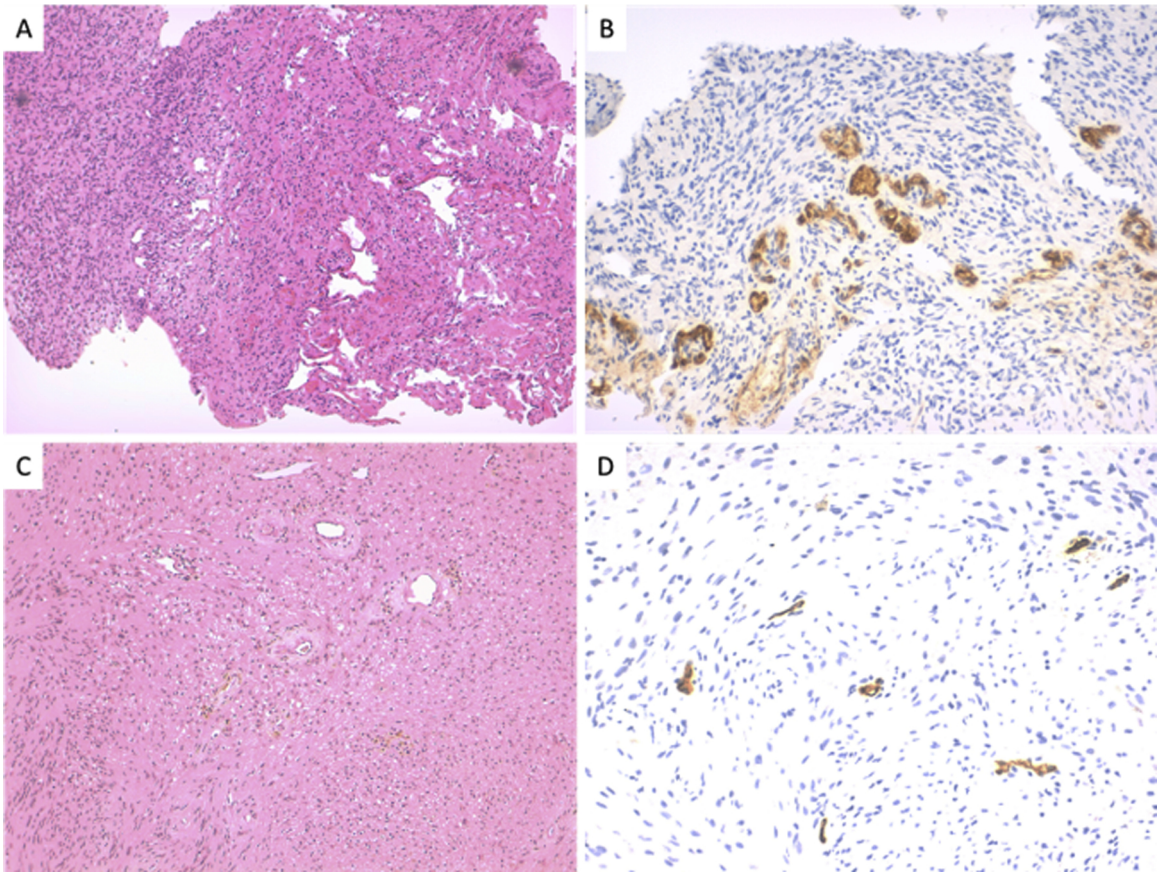

Fig. 3. Representative cases with high (A and B) versus low (C and D) growth rates. Note high cellularity and high vessel density in A and B. Original magnification 100X for (A) and (C), 200X for (B) and (D); hematoxylin and eosin staining for (A) and (C).

Table 1
Whole series of sporadic vestibular schwannomas: CD105-assessed cross-sectional vessel area fraction and vessel density values.

Variable	No. cases	Cross-sectional vessel area fraction (AA) (mean ± SD)	Vessel density (VD) (mean ± SD)	AA p-value*	VD p-value*
Male	37	2.73 ± 1.76	0.27 ± 0.15	0.24	0.07
Female	34	2.56 ± 2.25	0.21 ± 0.14		
Age < 65 years	55	2.67 ± 2.21	0.25 ± 0.15	0.62	0.83
Age ≥ 65 years	16	2.57 ± 1.31	0.25 ± 0.13		
Tumor dimension < 10 mm	30	1.90 ± 1.94	0.19 ± 0.13	0.0006	0.003
Tumor dimension ≥ 10 mm	41	3.20 ± 1.94	0.29 ± 0.15		

* Mann-Whitney *U* test.

(rho = 0.35, p = 0.0024); and (ii) between vessel density and tumor size on last ceMRI (rho = 0.29, p = 0.013). The mean vessel cross-sectional area and mean vessel density assessed with CD105 were significantly higher in tumors measuring ≥10 mm in size (median value of the whole series) than in those < 10 mm (Mann-Whitney *U* test, p = 0.0006, and p = 0.003, respectively) (Fig. 2).

3.2. Subcohort of patients whose tumor growth rate (mm/month) was calculated on serial ceMRI findings

For the 38 patients who underwent ceMRI twice or more preoperatively, their VIII CN schwannoma growth rate calculated as explained in the Methods was a mean 0.42 ± 0.71 mm/month. In 10 cases the schwannoma growth rate was 0 mm.

Spearman's rank correlation test found no significant correlations between age at the time of surgery and schwannoma growth rate (rho = -0.14, p = 0.44).

In this subcohort, the mean vessel cross-sectional area assessed with CD105 was 2.53 ± 2.05 (median value 1.88), and the mean vessel density was 0.25 ± 0.15 (median value 0.21). Statistical analysis ruled out any significant correlation between tumor growth rate and vessel cross-sectional area (Spearman's rank correlation test, rho = 0.13, p = 0.48), or vessel density (Spearman's rank correlation test, rho = 0.17, p = 0.36). (Fig. 3) Considering only the patients in this subcohort whose ceMRI findings revealed tumor growth (28 cases), the Mann-Whitney *U* test found no significant differences in terms of vessel cross-sectional area (3.23 ± 2.49 *vs* 2.08 ± 1.45) or vessel density (0.32 ± 0.17 *vs* 0.22 ± 0.13) between patients whose tumor growth rate was ≥0.22 mm/month (median value) as opposed to < 0.22 mm/month (p = 0.21 and p = 0.15, respectively).

4. Discussion

Although vestibular nerve schwannomas are benign and grow relatively slowly, they need angiogenesis to progress beyond a certain size, and several angiogenesis-related factors in this setting have already been investigated [22]. In 2000, Labit-Bouvier et al. [23] examined vascularization using CD34 in 69 sporadic unilateral vestibular

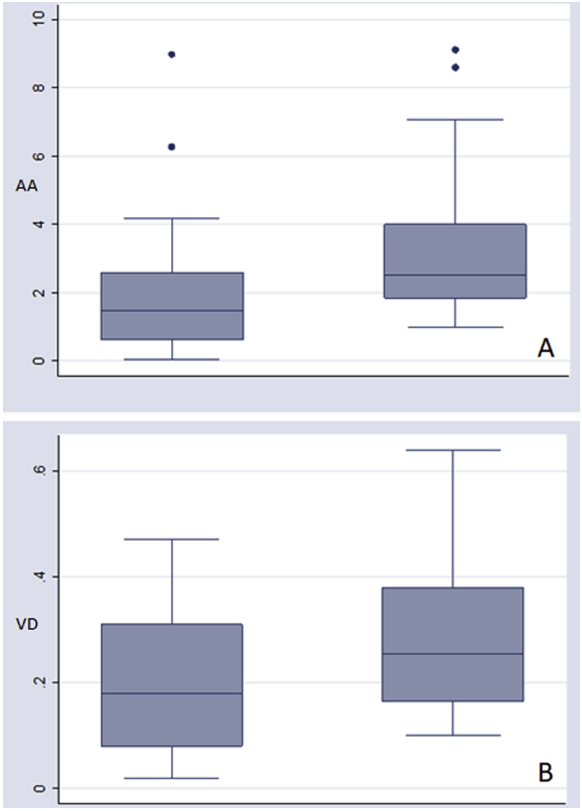

Fig. 4. A. Vessel cross-sectional area (AA) assessed from CD105 staining: box plots of subcohorts of patients with tumor diameters < 10 mm (left) and ≥ 10 mm (right); B. Vessel density (VD) assessed from CD105 staining: box plots of subcohorts of patients with tumor diameters < 10 mm (left) and ≥ 10 mm (right).

schwannomas: their CD34 index did not correlate with a semi-quantitative evaluation of the number of vessels or with symptom duration or the tumor's clinical growth rate. Diensthuber et al. [24] analyzed the surgical samples of 37 sporadic unilateral vestibular schwannomas and 16 vestibular schwannomas from 15 patients with proven neurofibromatosis type 2 (NF2). Their immunoreactivity data demonstrated a robust protein expression for hypoxia-inducible factor (HIF)-1α, erythropoietin (Epo), Epo receptor (EpoR), and bcl-2, but no correlation between HIF-1α, Epo, EpoR or bcl-2 expression and microvessel density, tumor size, sex, or age. Vascular endothelial growth factor (VEGF) is one of the most potent mediators of angiogenesis. In 2003, the research group at the Department of Otorhinolaryngology at the Gentofte University Hospital in Copenhagen examined vestibular schwannomas from 18 patients using a polyclonal antibody against VEGF [25]. They found that staining intensity varied from tumor to tumor, and semiquantification revealed that VEGF expression correlated significantly with tumor growth rate, but not with symptom duration or tumor size. The same group pursued their investigations in this specific field on 27 patients with vestibular schwannomas found growing on repeated MRI [26]. Using ELISA, VEGF and VEGF receptor 1 (VEGFR-1) concentrations were measured in tumor homogenates, and found to correlate with tumor growth rates, but not with tumor size or symptom duration. In 2007, at Mainz University, surgical specimens from 17 patients with sporadic vestibular schwannoma were also tested for basic fibroblast growth factor (bFGF), and microvessel density was assessed from CD31 staining [27]. The researchers found that mRNA expression and protein expression correlated with tumor volume, tumor growth rate, and microvessel density. In 2012, de Vries et al. [28] immunohistochemically evaluated a series of sporadic vestibular schwannomas for cell proliferation (histone H3 and Ki-67), microvessel density (CD31), and inflammation (CD45 and CD68). They found that microvessel density and inflammation were positively correlated with tumor size and tumor growth index. Very recently, the same research group of Leiden University investigated the cytokines M-CSF and IL-34 in vestibular schwannomas also to see if their expression was related to angiogenesis, macrophage numbers, cystic degeneration, and volumetric tumor progression [29]. They concluded that M-CSF was related to macrophage activity and tumor progression, making it a potential target for therapy; on the other hand, there was no significant relation between microvessel density and M-CSF expression. Yener et al. [30] compared 6 cases of sporadic vestibular schwannoma with 4 NF2-associated cases in terms of angiogenic capacity using corneal angiogenesis assay: the NF2-associated tumors showed a significantly lower angiogenic potential than the sporadic ones.

Hong et al. [31] said that tumor microvessel density assessed with CD105 staining is the gold standard for judging tumor angiogenesis in the clinic, and a recent meta-analysis by Zhang et al. [32] concluded that a high microvessel density assessed with CD105 in patients with breast cancer, esophagus cancer, gynecologic cancer, or head and neck squamous cell carcinomas is of remarkable prognostic value. There is a dearth of information, however, regarding the role of CD105 as a marker of angiogenesis in schwannomas. There is an anecdotal report of a case of trigeminal schwannoma involving multiple intracranial fossae and the extracranial compartment having a high microvessel density detected on immunohistochemical staining with endoglin [33]. Very recently, our group preliminarily investigated CD105 expression in a series of NF2-associated vestibular nerve schwannomas [34]. A positive correlation emerged between tumor growth rate (measured on contrast-enhanced MRI) and vessel density. To the best of our knowledge, the present study is the first to have investigated sporadic vestibular nerve schwannoma neoangiogenesis using CD105 staining in a reasonably large consecutive case series. The main strengths of the present study lie in that: (i) only sporadic VIII CN schwannomas were considered; (ii) the series of tumors was consecutive; (iii) the indication for surgical treatment was decided by the same surgical team; (iv) the surgical treatment was performed by the same team; (v) the histological diagnosis was confirmed by the same pathologist (SB); and (vi) the cases under observation according to a wait-and-scan protocol were examined by the same team. In addition, a computer-based IA system was used to ensure a highly accurate and reproducible analysis of the immunostained slides. Over the course of 15 years [35], the pathologists in our research group have developed a solid experience in assessing the immunohistochemical expression of CD105 in head and neck neoplasms using various IA techniques. The main weaknesses of the present study, on the other hand, concern its retrospective setting, and the limited number of cases available (especially for the purpose of calculating tumor growth rate).

Based on CD105 staining, the present study identified a significant positive correlation between tumor size at the time of surgery and vessel cross-sectional area and density. Vessel cross-sectional area and density were both significantly greater in tumors measuring ≥ 10 mm in size than in those < 10 mm (Fig. 4). These preliminary results are in line with reports in the literature of a higher CD105 expression in tumor-activated endothelial cells correlating with faster tumor growth (suggesting a pro-angiogenic role for CD105), and a downregulation of CD105 being associated with a more limited tumor angiogenesis and growth [36]. No significant correlation emerged, however, between tumor growth rate and vessel cross-sectional area or density assessed with CD105 in our subcohort of patients whose tumor growth rate could be calculated on serial ceMRI findings (38 cases, 10 of them with a schwannoma growth rate of 0 mm/month). Nor did the neoangiogenesis variables investigated differ significantly when only the 28 patients showing tumor growth were considered (median growth rate ≥ 0.22 mm/month *vs* < 0.22 mm/month). These last preliminary findings may be due to our limited number of patients with two or more preoperative ceMRI needed to calculate tumor growth rates, however.

5. Conclusions

Also in the light of information available from the latest translational literature, our preliminary results point to the need to examine larger series of sporadic vestibular schwannomas, preferably by means of multicenter studies, focusing on the following issues.

(i) The release of circulating endoglin (Sol-ENG) produced by activated endothelial cells as well as neoplastic cells: this form of endoglin serves as a marker of a poor prognosis in patients with malignancies [36,37]. The diagnostic potential of Sol-ENG in benign head and neck tumors was recently investigated in a small series of head and neck paragangliomas [38]. The authors identified a positive correlation between preoperative Sol-ENG levels and tumor size. Levels of serum Sol-ENG (or endoglin mRNA) should be investigated as a serological biomarker of schwannoma growth. This could help to orient the choice between early surgery and a rational wait-and-scan policy to monitor sporadic VIII CN schwannoma growth.

(ii) Although anti-VEGF bevacizumab has been associated with a shrinkage of most growing vestibular schwannomas [32], evidence of antibodies against CD105 reacting preferentially with active endothelial cells of angiogenic tissues could be extremely useful with a view to vascular targeting. Several studies using different anti-endoglin antibodies (including monoclonal antibodies, immunotoxin-conjugated antibodies or radiolabeled antibodies) have all demonstrated good anti-angiogenic and antitumor responses [13]. One new idea concerns the use of a chimeric IgG1 anti-CD105 monoclonal antibody (TRC105) that avidly binds human endoglin: it seems to inhibit angiogenesis and tumor growth by slowing endothelial cell growth, causing antibody-dependent cellular cytotoxicity and apoptosis, and complementing VEGF inhibitors [36,39]. In the area of gene therapies, silencing endoglin by interfering with RNA is seen as a potential alternative approach to endoglin targeting.

Contributors

Study design: GM, EZ, SB, LN.
Data collection: CP, LN.
Data analysis and interpretation: GM, EZ, SB, LN, VG.
Statistical analysis: LG.
Drafting the article: GM, EZ; LN, CP, DC.
Critical revision of the article: all the authors.
Final approval of the version to be published: all the authors.

Financial disclosure

This study was partly supported by grant No. DOR1778819/17 (G. Marioni) from the University of Padova, Italy.

Declaration of Competing Interest

None declared.

Acknowledgments

The authors thank Frances Coburn for correcting the English version of this paper.

References

[1] M. Kirchmann, K. Karnov, S. Hansen, T. Dethloff, S.E. Stangerup, P. Caye-Thomasen, Ten-year follow-up on tumor growth and hearing in patients observed with an intracanalicular vestibular schwannoma, Neurosurgery 80 (2017) 49–56.
[2] E. Zanoletti, D. Cazzador, C. Faccioli, S. Gallo, L. Denaro, D. D'Avella, A. Martini, A. Mazzoni, Multi-option therapy vs observation for small acoustic neuroma: hearing-focused management, Acta Otorhinolaryngol. Ital. 38 (2018) 384–392.
[3] E. Zanoletti, A. Mazzoni, D. D'Avella, Hearing preservation in small acoustic neuroma: observation or active therapy? Literature review and institutional experience, Acta Neurochir. (Wien) 161 (2019) 79–83.
[4] A. Mazzoni, E. Zanoletti, L. Denaro, A. Martini, D. D'Avella, Retrolabyrinthine meatotomy as part of retrosigmoid approach to expose the whole internal auditory canal: rationale, technique, and outcome in hearing preservation surgery for vestibular schwannoma, Oper. Neurosurg. (Hagerstown) 14 (2018) 36–44.
[5] S.E. Stangerup, P. Caye-Thomasen, M. Tos, J. Thomsen, The natural history of vestibular schwannoma, Otol. Neurotol. 27 (2006) 547–552.
[6] A. Martini, G. Marioni, E. Zanoletti, R. Cappellesso, R. Stramare, E. Fasanaro, C. Faccioli, L. Giacomelli, L. Denaro, D. D'Avella, A. Mazzoni, A. Fassina, YAP, TAZ and AREG expression in eighth cranial nerve schwannoma, Int. J. Biol. Markers 32 (2017) e319–e324.
[7] M. Lionello, A. Staffieri, G. Marioni, Potential prognostic and therapeutic role for angiogenesis markers in laryngeal carcinoma, Acta Otolaryngol. 132 (2012) 574–582.
[8] J. Folkman, E. Merler, C. Abernathy, G. Williams, Isolation of a tumor factor responsible for angiogenesis, J. Exp. Med. 133 (1971) 275–288.
[9] J. Folkman, Tumor angiogenesis: therapeutic implications, N. Engl. J. Med. 285 (1971) 1182–1186.
[10] G. Marioni, F. Marino, S. Blandamura, E. D'Alessandro, L. Giacomelli, V. Guzzardo, M. Lionello, C. De Filippis, A. Staffieri, Neoangiogenesis in laryngeal carcinoma: angiogenin and CD105 expression is related to carcinoma recurrence rate and disease-free survival, Histopathology 57 (2010) 535–543.
[11] G. Marioni, R. Nucci, F. Marino, L. Giacomelli, M. Rugge, R. Pareschi, A. Martini, Neoangiogenesis in temporal bone carcinoma: the prognostic role of CD105, Otol. Neurotol. 33 (2012) 843–848.
[12] J. Zhang, L. Zhang, Q. Lin, W. Ren, G. Xu, Prognostic value of endoglin-assessed microvessel density in cancer patients: a systematic review and meta-analysis, Oncotarget 9 (2017) 7660–7671.
[13] T. Li, G. Kang, T. Wang, H. Huang, Tumor angiogenesis and anti-angiogenic gene therapy for cancer, Oncol. Lett. 16 (2018) 687–702.
[14] G. Marioni, F. Marino, L. Giacomelli, C. Staffieri, M.L. Mariuzzi, E. Violino, C. De Filippis, Endoglin expression is associated with poor oncologic outcome in oral and oropharyngeal carcinoma, Acta Otolaryngol. 126 (2006) 633–639.
[15] G. Marioni, E. D'Alessandro, L. Giacomelli, A. Staffieri, CD105 is a marker of tumour vasculature and a potential target for the treatment of head and neck squamous cell carcinoma, J. Oral Pathol. Med. 39 (2010) 361–367.
[16] F. Tanaka, Y. Otake, K. Yanagihara, Y. Kawano, R. Miyahara, M. Li, T. Yamada, N. Hanaoka, K. Inui, H. Wada, Evaluation of angiogenesis in non-small cell lung cancer: comparison between anti-CD34 antibody and anti-CD105 antibody, Clin. Cancer Res. 7 (2001) 3410–3415.
[17] G. Marioni, E. D'Alessandro, L. Giacomelli, C. De Filippis, N. Calgaro, M. Sari, A. Staffieri, S. Blandamura, Maspin nuclear localization is related to reduced density of tumour-associated micro-vessels in laryngeal carcinoma, Anticancer Res. 26 (2006) 4927–4932.
[18] C. Fan, J. Zhang, Z. Liu, M. He, T. Kang, T. Du, Y. Song, Y. Fan, J. Xu, Prognostic role of microvessel density in patients with glioma, Medicine (Baltimore) 98 (2019) e14695.
[19] J. Kanzaki, M. Tos, M. Sanna, D.A. Moffat, E.M. Monsell, K.I. Berliner, New and modified reporting systems from the Consensus Meeting on systems for reporting results in vestibular schwannoma, Otol. Neurotol. 24 (2003) 642–649.
[20] Committee on Hearing and Equilibrium guidelines for the evaluation of hearing preservation in acoustic neuroma (vestibular schwannoma). American Academy of Otolaryngology-Head and Neck Surgery Foundation, INC, Otolaryngol. Head. Neck Surg. 113 (1995) (1995) 179–180.
[21] L. Nicolè, R. Cappellesso, T. Sanavia, V. Guzzardo, A. Fassina, MiR-21 over-expression and programmed cell death 4 down-regulation features malignant pleural mesothelioma, Oncotarget 9 (2018) 17300–17308.
[22] E. Zanoletti, A. Mazzoni, A. Martini, et al., Surgery of the lateral skull base: a 50-year endeavour, Acta Otorhinolaryngol. Ital. 39 (SUPPL.1) (2019) S1–S146.
[23] C. Labit-Bouvier, B. Crebassa, C. Bouvier, L. Andrac-Meyer, J. Magnan, C. Charpin, Clinicopathologic growth factors in vestibular schwannomas: a morphological and immunohistochemical study of 69 tumours, Acta Otolaryngol. 120 (2000) 950–954.
[24] M. Diensthuber, T. Ilner, T. Rodt, M. Samii, A. Brandis, T. Lenarz, T. Stöver, Erythropoietin and erythropoietin receptor expression in vestibular schwannoma: potential role in tumor progression, Otol. Neurotol. 28 (2007) 559–565.
[25] P. Cayé-Thomasen, L. Baandrup, G.K. Jacobsen, J. Thomsen, S.E. Stangerup, Immunohistochemical demonstration of vascular endothelial growth factor in vestibular schwannomas correlates to tumor growth rate, Laryngoscope 113 (2003) 2129–2134.
[26] P. Cayé-Thomasen, K. Werther, A. Nalla, T.C. Bøg-Hansen, H.J. Nielsen, S.E. Stangerup, J. Thomsen, VEGF and VEGF receptor-1 concentration in vestibular schwannoma homogenates correlates to tumor growth rate, Otol. Neurotol. 26 (2005) 98–101.
[27] D. Koutsimpelas, T. Stripf, U.R. Heinrich, W.J. Mann, J. Brieger, Expression of vascular endothelial growth factor and basic fibroblast growth factor in sporadic vestibular schwannomas correlates to growth characteristics, Otol. Neurotol. 28 (2007) 1094–1099.
[28] W.M. de Vries, P.C.W. Hogendoorn, I.H. Briaire-de Bruyn, M.J.A. Malessy, A.G.L. van der Mey, Intratumoral hemorrhage, vessel density, and the inflammatory reaction contribute to volume increase of sporadic vestibular schwannomas, Virchows Arch. 460 (2012) 629–636.
[29] W.M. de Vries, I.H. Briaire-de Bruijn, P.P.G. van Benthem, A.G.L. van der Mey, P.C.W. Hogendoorn, M-CSF and IL-34 expression as indicators for growth in sporadic vestibular schwannoma, Virchows Arch. 474 (2019) 375–381.
[30] U. Yener, T. Avsar, E. Akgün, A. Şeker, Y. Bayri, T. Kılıç, Assessment of anti-angiogenic effect of imatinib mesylate on vestibular schwannoma tumors using in

vivo corneal angiogenesis assay, J. Neurosurg. 117 (2012) 697–704.
[31] H. Hong, Y. Yang, Y. Zhang, J.W. Engle, T.E. Barnhart, R.J. Nickles, B.R. Leigh, W. Cai, Positron emission tomography imaging of CD105 expression during tumor angiogenesis, Eur. J. Nucl. Med. Mol. Imaging 38 (2011) 1335–1343.
[32] N. Zhang, J. Chen, G.B. Ferraro, L. Wu, M. Datta, R.K. Jain, S.R. Plotkin, A. Stemmer-Rachamimov, L. Xu, Anti-VEGF treatment improves neurological function in tumors of the nervous system, Exp. Neurol. 299 (Pt B) (2018) 326–333.
[33] C. Alafaci, M. Caffo, V. Barresi, M. Cutugno, M.A. Pino, F. Granata, F.S. De Ponte, F.M. Salpietro, F. Tomasello, Large trigeminal schwannoma of the infratemporal fossa: evaluation of neoangiogenesis in this rare neoplasm, Head Neck 35 (2013) E272–276.
[34] G. Marioni, L. Nicolè, D. Cazzador, C. Pavone, D. D'Avella, A. Martini, A. Mazzoni, E. Zanoletti, Endoglin (CD105) expression in neurofibromatosis type 2 vestibular schwannoma, Head Neck 41 (2019) 3612–3617.
[35] G. Marioni, E. Gaio, L. Giacomelli, R. Marchese-Ragona, C. Staffieri, A. Staffieri, F. Marino, Endoglin (CD105) expression in head and neck basaloid squamous cell carcinoma, Acta Otolaryngol. 125 (2005) 307–311.
[36] A. Kasprzak, A. Adamek, Role of endoglin (CD105) in the progression of hepatocellular carcinoma and anti-angiogenic therapy, Int. J. Mol. Sci. 19 (2018) 3887.
[37] C.H. Chen, H.C. Chuang, Y.T. Lin, F.M. Fang, C.C. Huang, C.M. Chen, H. Lu, C.Y. Chien, Circulating CD105 shows significant impact in patients of oral cancer and promotes malignancy of cancer cells via CCL20, Tumour Biol. 37 (2016) 1995–2005.
[38] M. Litwiniuk, K. Niemczyk, J. Niderla-Bielińska, I. Łukawska-Popieluch, T. Grzela, Soluble endoglin (CD105) serum level as a potential marker in the management of head and neck paragangliomas, Ann. Otol. Rhinol. Laryngol. 126 (2017) 717–721.
[39] Y. Liu, H. Tian, G.C. Blobe, C.P. Theuer, H.I. Hurwitz, A.B. Nixon, Effects of the combination of TRC105 and bevacizumab on endothelial cell biology, Invest. New Drugs 32 (2014) 851–859.