Tumor-associated macrophage infiltration in meningioma

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Abstract

Background. Meningioma, a most common brain tumor, has a high rate of recurrence. Tumor-associated macrophages (TAMs) are the most abundant immune cell type in meningioma. TAMs display functional phenotypic diversity and may establish either an inflammatory and anti-tumoral or an immunosuppressive and pro-tumoral microenvironment. TAM subtypes present in meningioma and potential contribution to growth and recurrence is unknown.

Methods. Immunofluorescence staining was used to quantify M1 and M2 TAM populations in tissues obtained from 30 meningioma patients. Associations between M1 and M2 cells, M1:M2 cell ratio to tumor characteristics, WHO grade, recurrence, size, location, peri-tumoral edema, and patient demographics such as age and sex were examined.

Results. TAM cells accounted for ~18% of all cells in meningioma tissues. More than 80% of infiltrating TAMs were found to be of pro-tumoral M2 phenotype and correlated to tumor size (P = .0409). M1:M2 cell ratio was significantly decreased in WHO grade II, compared to grade I tumors (P = .009). Furthermore, a 2.3-fold difference in M1:M2 ratio between primary (0.14) and recurrent (0.06) tumors was observed (n = 18 and 12 respectively, P = .044).

Conclusion. This study is the first to confirm existence of pro-tumoral M2 TAMs in the meningioma microenvironment, emphasizing its potential role in tumor growth and recurrence.

Key Points

1. Macrophages in meningioma are predominantly of M2 subtype.

2. The M1:M2 ratio may well be an indicator of tumor recurrence.

Meningioma is the most common primary brain tumor in adults, with incidences increasing with age.[1] Often labeled as benign, the lifetime tumor recurrence rates for WHO grade I, II, and III meningioma are 30%, 50%, and 95%, respectively.[2] Additional risk factors for tumor recurrence include tumor size, multiplicity, extent of resection, and genetics.[3–6] However, pathological mechanisms that contribute to tumor recurrences and progression are incompletely understood.[7] Tumor microenvironment plays a critical role in recurrence and prognosis for many solid tumors[8,9] and is the focus of rigorous investigation for evolving cancer treatments.[10,11] How the tumor microenvironment contributes to meningioma behavior is incompletely understood.

Importance of the Study

Meningioma, often considered a benign brain tumor, has a lifetime recurrence rate of 30% for WHO grade I, 50% for WHO grade II, and 95% for WHO grade III. In addition, on recurrence, meningioma may transform to a higher grade. In this study, we identified for the first time that tumor-associated macrophages (TAMs) in meningioma microenvironment are predominantly of the M2 subtype (not M1). TAM quantities were positively associated with tumor size but did not correlate with tumor recurrence. Instead, the M1:M2 ratio was found to correlate with tumor recurrence and which indeed has been validated as a novel prognostic indicator for many cancers. Accordingly, the M1:M2 ratio may be an important biomarker of meningioma behavior and propensity for recurrence and progression.

Tumor and immune cell markers indicative of an immune suppressive environment are consistently identified in meningioma tissues,[12–17] including the expression of immune checkpoint proteins B7-H3, CTLA-4, LIM, PD-1, PD-L1, PD-L2, and Tim-3 on tumor cells and infiltrating lymphocytes.[12–18] The composition of infiltrating immune cells in meningioma microenvironment supporting immune suppression has also been noted. Tumor-associated macrophages (TAMs) are the most abundant immune cell type present in large numbers in the meningioma microenvironment..[12–14,16,18] TAM infiltration varies noticeably between cases although a trend toward higher numbers of cells in tumors with monosomy for 22 (NF2 tumors)[19] and with higher WHO grade is reported. TAMs may be scattered in single or groups of cells and may be dense in number at the tumor-brain interface often with a component of brain invasion.[19,20] The composition of TAM populations in meningioma is not known.

TAMs are highly plastic, existing in a spectrum of polymerized states, but often dichotomized by an M1 or M2 phenotype.[21–23] M1 TAMs have *anti-tumoral* effects but M2 macrophages are *pro-tumoral*.[21,22] M2 TAMs are associated with poor prognosis and decreased time to recurrence for a number of cancers.[24] This contrasts with M1 TAMs that are linked to improved prognosis.[25–27] Increasing TAM numbers correlate to poor outcome for most cancers including brain tumors as does high M2:M1 cell ratio.[28–32]

In this immunofluorescence staining study of meningioma, we identified TAMs in the meningioma microenvironment to be predominantly of the M2 subtype. TAM quantities were positively associated with tumor size and increased with WHO grade but were not correlated with tumor recurrences. Instead, the M1:M2 ratio was found to correlate with tumor recurrence and indeed has been validated as a novel prognostic indicator for many cancers.[28–32] Accordingly, this may also be an important biomarker of meningioma behavior and propensity for recurrence and progression.

Methods

Patient Characteristics

This study was approved by the Conjoint Health Research Ethics Board—University of Calgary. Informed written consent was obtained from each patient. A total of 30 patients (16 Grade I, 12 Grade II, and 2 Grade III) were included. For each case, histopathologic subtype and final WHO grading were obtained from Neuropathology report. Patient information (age, sex) and detailed medical history (past resections, comorbidities including cancer, other treatment, radiotherapy), baseline cognitive data and information specific to the tumor (size, location, surgical procedure, pre- and post-surgery MR imaging, extent of resection and severity of peri-tumoral edema) were also acquired (Table 1). Tumor size was estimated from MR images as the maximal cross-sectional distance in cm. A semi-quantitative assessment of the severity of peri-tumoral edema was performed. No peri-tumoral edema (scale = 0), mild peri-tumoral edema (scale = 1), was classified as edema adjacent to the tumor less than 1 cm in margin. In moderate peri-tumoral edema (scale = 2), edema had a margin of 1-2 cm. Peri-tumoral edema with a margin greater than 3 cm was classified as severe (scale = 3). Tumors were characterized as recurrent if the tissue was collected from resection of a recurrent tumor.

Immunofluorescence Staining

Immunofluorescence staining was performed as described previously.[17] Briefly, 4 μm tissue sections were cut in series from formalin-fixed paraffin-embedded tissue blocks for each meningioma case. Standard H&E staining was performed for each tumor and histological features of each section were reviewed by a neuropathologist establishing the WHO grade (Neuropathology report). For immunofluorescence, sections were stained overnight with primary antibodies; mouse anti-CD68 antibodies (1:200, (KP1) conjugated to Alexa-488 Santa Cruz Biotechnology), mouse anti-CD80 (1:100, (R&D Systems), mouse anti-CD86 (1;100, D-6 Santa Cruz Biotechnology together with either rabbit anti-CD163 (1:200, EPR19518, Abcam), rabbit anti-CD206 (1:1000, ab64693, Abcam), or rabbit anti-iNOS (1:200, Abcam). Secondary goat anti-rabbit or anti-mouse Alexa-555 & -488 antibodies (Molecular probes) were applied to sections and tissue auto-fluorescence was blocked with 30-min incubation in 0.02% Sudan black in 70% EtOH. Sections were then coverslipped using hard-set mounting media containing DAPI (Vectashield).

Table 1. Patient characteristics

Case ID	Age	Gender	Location	Size - cm	Edema	WHO grade	Simpson grade	Histological subtype	Recurrence
M2	63	F	R. mid parafalx	1.8	Moderate	I	1	Meningo/Angio	N
M3	62	F	L. cerebellopontine angle	3.5	Nil	I	4	Secretary	N
M4	33	F	R. Frontoparietal convexity	4.4	Moderate	II	1	Atypical	N
M5	71	F	L. frontotemporal convexity	2.2	Moderate	III	1	Anaplastic/Rhabdoid	Y
M8	52	M	R. convexity	6.7	Mild	II	4	Atypical	Y
M9	58	F	L. Frontal-parietal Parafalx	6.6	Nil	II	2	Transitional	N
M11	79	F	L. cerebellopontine angle	4.8	Mild	I	4	Meningothelial	N
M12	68	M	L. frontal medial sphenoid wing	5	Mild	I	4	Psammomatous	N
M13	57	F	L. sphenoid wing	2.6	Mild	I	1	Meningiothelial	N
M14	58	F	Tuberculum sellae	1.8	Nil	I	1	Psammomatous	Y
M15	75	F	L. mid posterior parafalx	7.4	Moderate	II	4	Atypical	Y
M17	46	F	L. parietal parafalx	1.6	Nil	I	4	Fibrous	N
M18	28	F	Midline Parafalx (R. > L.)	7.5	Moderate	II	2	Atypical	Y
M19	28	F	L. sphenoid wing	3	Moderate	I	4	Meningothelial	N
M23	59	F	Posterior-frontal parafalx	2.9	Nil	I	1	Transitional	N
M27	64	F	L. frontal convexity	4.4	Mild	I	1	Transitional	Y
M28	41	M	Posterior Parafalx (R. > L.)	6.5	Moderate	I	4	Transitional	Y
M29	74	M	Petroclival	6.5	Moderate	I	4	Meningothelial	Y
M30	56	M	Olfactory groove	8.5	Nil	II	4	Chordoid	Y
M31	62	F	R. paritotemporal convexity	3.9	Nil	II	1	Atypical	N
M32	81	F	Tuberculum sellae	2.9	Nil	I	4	Transitional	N
M34	68	F	L. frontal convexity	3.9	Mild	II	1	Atypical	N
M35	62	M	R. tentorial meningioma	2	Nil	I	4	Transitional	N
M38	51	M	R. CP angle meningioma	1.5	Nil	I	1	Transitional	N
M39	70	M	L. Cerebellar Convexity	2.7	Nil	I	1	Unknown	N
M40	71	M	R. tentorial	3.2	Moderate	II	1	Atypical	N
M45	36	F	R. & L. parietal meningioma	7.5	Nil	II	4	Atypical	Y
M46	83	M	R. frontal convexity	6.4	Severe	II	4	Atypical	N
M47	73	F	Post-midline parafalx	4.3	Severe	II	1	Atypical	Y
M65	69	F	L. frontal convexity	7.8	Moderate	III	1	Rhabdoid	N

TAM Quantification

Tissue sections were imaged at 10× and 40× magnifications with a fluorescence microscope (Olympus BX51) with developer's software. M1 and M2 distribution and association with tissue structures was assessed qualitatively at 10× and 40×. M1 and M2 cells were quantified in 5 regions of interest (ROI) per stained meningioma tissue section, under 40× magnification using a cell counter plugin in ImageJ (https://fiji.sc/). TAM quantifications were performed by 2 blinded independent observers. M2 macrophages were classified as CD68+/CD163+ double-stained cells. M1 macrophages were classified as CD68+-only cells in CD68 and CD163 co-stained sections or as CD68+/iNOS+ double-positive cells. Total number of cells in an ROI was calculated with the analyze particles plugin in Image J to count DAPI-labeled nuclei. Cell nuclei counts were used to calculate percentage of TAMs. TAM infiltration density was assessed in each ROI by correcting for regions and structures associate with vasculature. This was done by normalizing TAM counts according to total numbers of cells (DAPI-stained nuclei) in each ROI. Additional staining of M1 markers, CD80, and CD86 as well as an M2 marker CD206 was assessed in a small selection of cases in the study ($n = 6$).

Statistical Analysis

Correlations between TAM number (M1 + M2, ie, CD68+ cells) or TAM percentage of total cells or M1/M2 ratio (M2 = CD68+/CD163+ cells) to clinical data variables: tumor size, peri-tumoral edema severity, and age were evaluated and a Spearman rank correlation test was used in correlation analyses. A Welch's *t*-test was used for comparing means between 2 groups for TAM number (CD68+ cells) or TAM percentage of total cells or M1:M2 ratio according to WHO grade, tumor location (skull base or convexity), gender, and recurrence status. One-way ANOVA was performed to evaluate differences in TAM number (CD68+ cells) between histological subtypes. Data in bar graphs are represented as group means ± SEM. Statistical significance was achieved with *P*-value ≤ .05 and provided as an absolute number. We excluded WHO grade III group from analyses as only 2 cases were included in the study. However, individual data points for these 2 cases are still included in results graphs.

Results

TAM Subtypes and Distribution in Meningioma

The TAM subtypes present in the tumor microenvironment are presented in Figures 1 and 2. M2 TAMs as indicated by (CD68+/CD163+ staining) were identified in all tissues studied, whereas a few M1, (CD68+/iNOS+) cells, were present in most (Figures 1 and 2 and Supplementary Figures S1–S2). Staining of additional M2 marker (CD206) and M1 markers (CD80 and CD86) was performed on 6 cases and showed comparable results to CD163 and iNOS staining. Majority of TAMs had high CD163 and CD206
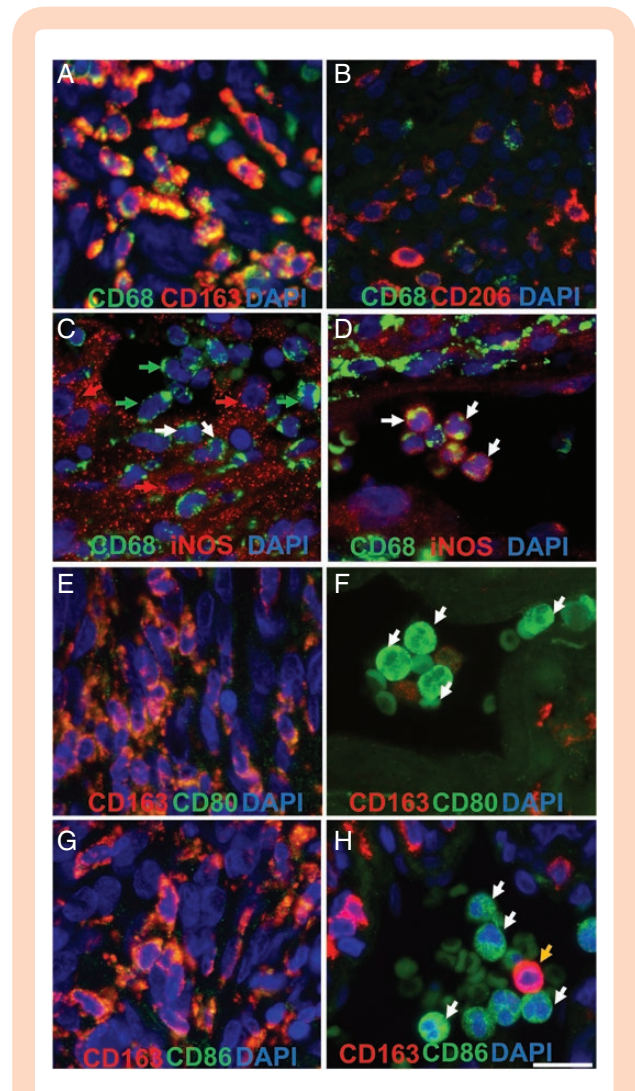
Figure 1. Representative CD68, CD163, CD206, iNOS, CD80, and CD86 staining in meningioma tissue sections. **(A)** CD68 (green), CD163 (red) tissue section double staining. CD68+/CD163+ cells are characterized as M2 tumor-associated macrophages (TAMs) **(B)** CD68 (green), CD206 (red) tissue section double staining. **(C)** CD68 (green), iNOS (red) tissue section double staining. Red and white arrows depict CD68−/iNOS+ tumor cells and CD68+/iNOS+ M1 cells respectively. CD68+/iNOS− M2 cells are labeled by green arrows. **(D)** Example CD68+/iNOS+ monocytes are labeled by white arrows. CD68+/iNOS− perivascular M2 cells are also present. **(E and F)** CD80 (green), CD163 (red) tissue section double staining. White arrows in **F** depict CD80+ monocytes. **(G and H)** CD86 (green), CD163 (red) tissue section double staining. White arrows in **H** depict CD86+ monocytes and a CD163+ monocyte is labeled by an orange arrow. Scale bars represent 15 μm.

staining and low CD80, CD86, and iNOS staining (Figure 1 and Supplementary Figures S4–S6). TAMs (CD68+ cells) accounted for on average 18 ± 2% of all cells (Figure 2K). CD68+/CD163+ double-positive cells were the most abundant TAM cell-type in tissues (89 ± 2% of TAMs). Fewer CD68+/CD206+ double labeled cells were present than CD68+/CD163+ cells leading to a higher M1:M2 ratio 0.2 to 0.1 respectively in $n = 6$ examined (Supplementary Table 1).
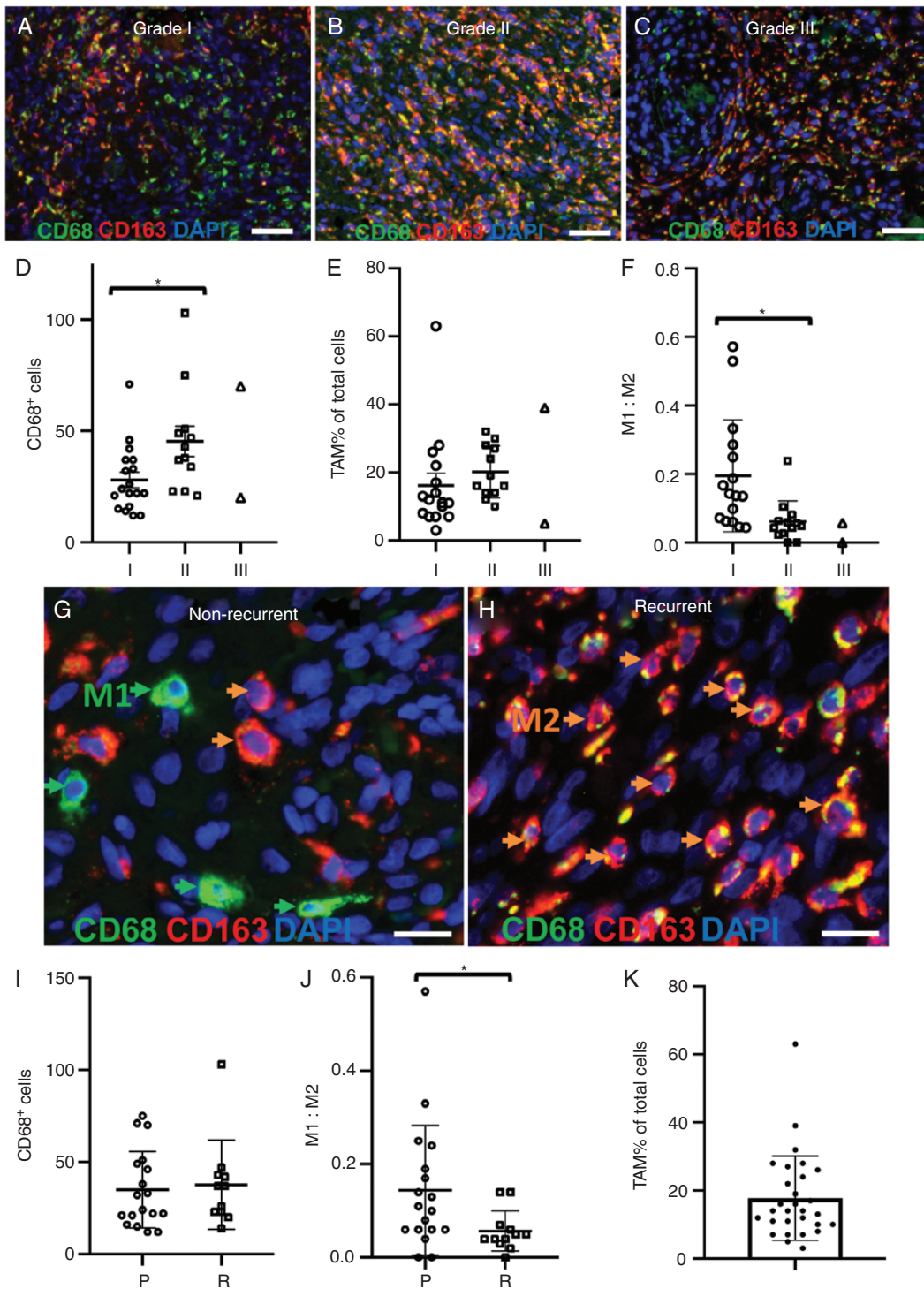
Figure 2. Representative fluorescence immunohistochemistry images of tumor-associated macrophages in meningioma tissue sections according to WHO grades I–III, primary and recurrent tumor groups. (**A**–**C**) Representative images of CD68 (green) and CD163 (red) staining according to WHO grades I (n = 16), II (n = 12), and III (n = 2). (**D**) Mean tumor-associated macrophage (TAM) cell counts (CD68$^+$ cells) for each case are plotted according to WHO grade. (**E**) TAMs as a percentage of total cells for each case are quantified according to WHO grade. (**F**) M1:M2 ratios for each case are quantified according to WHO grade. (**G** and **H**) Representative TAM cell staining in primary (n = 18) and recurrent (n = 12) meningioma tissue sections. Example M1 and M2 cells are labeled by arrows (**I**) Total TAMs (CD68$^+$ cells) for each case are quantified according to recurrence status. (**J**) M1:M2 ratios for each case according to recurrence group. (**K**) Average value of TAMs as a percentage of total cells for each case. A whorl with little TAM infiltration is present in top left section of image in panel C. Bar represent mean TAM percentage (n = 30). Error bars in each graph represent ± SEM. Scale bars represent 200 µm (A–C) and 15 µm (G and H). *P < .05.

Although a large discrepancy between CD163- and CD206-labeled cells in patient M65 largely accounted for this difference. CD68⁻/iNOS⁺ and CD68⁻/CD163⁺ positive cells were also present in tissues (Supplementary Figure S3). For most cases, TAM infiltration was observed throughout the tissue section (Figures 2A–C). Infiltration of single and groups of cells was present (Figures 1 and A–C, G, H). A high density of perivascular macrophages around vessels was common (Figure 1D Supplementary Figure S7). TAM infiltration was observed in whorls, but many whorls with limited TAM cell infiltration were also encountered (Figure 2C). Interestingly, in tumors with high numbers of CD68⁺/CD163⁻ M1 cells, a regional distinction between populations of M1 and M2 cells was commonly observed (Figure 2A).

Total Number of TAMs and TAM Profiling According to WHO Grade and Histological Subtype

M1 and M2 cell infiltration was evident in tissues of each tumor grade. Mean total number of TAMs (CD68+ cells) was statistically higher in grade II (45 ± 7 cells/ROI, $n = 12$) compared with grade I tumors (28 ± 4 cells/ROI, $n = 16$; $P = .0195$; Figure 2D). However, when we corrected TAM infiltration according to total numbers of cells in each ROI (to account for tissue regions and structures associated with vasculature), no significant difference was observed (Figure 2E). The composition of TAM phenotypes (represented as M1:M2 cell ratio) according to WHO grade was next examined. The M1:M2 cell ratio differed significantly between grade I (0.20 ± 0.04/ROI, $n = 16$) compared with grade II tumors (0.06 ± 0.02/ROI, $n = 12$; $P = .009$; Figure 2F). No differences in total TAM numbers were recorded between histological subtype groups ($P = .46$; Supplementary Figure S8).

TAM Profiles and Meningioma Recurrence

The number of CD68⁺ TAM cells was compared between primary and recurrent tumor groups ($n = 18$ and 12 cases respectively). Figure 2G and H demonstrates representative M1 and M2 cell infiltration staining for each group. TAM numbers (CD68⁺ cells) between these 2 groups were not statistically significant ($P = .75$; Figure 2I). However, the M1:M2 cell ratio differed significantly between primary tumor (0.14 ± 0.03/ROI, $n = 18$) and recurrent tumor groups (0.06 ± 0.01/ROI, $n = 12$; $P = .0448$; Figure 2J). No effect of extent of resection (Simpson grade) on recurrence was observed ($P = .39$; Supplementary Figure S9).

TAM Profiles According to Clinical Data and Patient Demographics (Age, Sex, Tumor Location, Size, Edema)

Several clinical variables and patient demographics have been associated with tumor recurrence in meningioma. We investigated the potential association between TAM subtype profiles (M1:M2 ratio), total number of TAMs and the percentage of TAMs of total cells to patient age and gender as well as tumor location, size, and severity of peri-tumoral edema (Figure 3). Numbers of TAMs, TAM percentage of total cells and M1:M2 ratio each correlated to tumor size ($r = .3819$, $P = .0409$, $r = .3677$, $P = .0497$, and $r = -.4366$, $P = .0179$, respectively; Figure 3A). No correlation was observed between TAM profile variables and age (Figure 3A) and TAM profiles did not differ significantly between male or female patients (Figure 3B). Similarly, no association was observed between TAMs and severity of peri-tumoral edema and no statistically significant differences were measured between skull base and convexity tumor location groups (Figures 3A and C).

Discussion

This study is the first to confirm and quantify the existence and abundance of pro-tumoral M2 TAMs in meningioma microenvironment. Interestingly, we found greater than 80% of infiltrating TAMs were of polarized M2-like phenotype and that the number of TAMs was positively associated with tumor size. Of note, TAM subtype profiles differed significantly between primary and recurrent meningioma. Specifically, the M1:M2 cell ratio was decreased in recurrent tumors. Accordingly, the results identify a potential role for infiltrating TAM cells in promoting meningioma growth and recurrence.

Infiltrating immune cells have an integral role in the tumor microenvironment, influencing the growth and progression of most solid tumors and the functional phenotype of immune cells is often a reliable indicator of tumor behavior. Macrophages are the most abundant infiltrating immune cell type in meningioma and are likely to be a major contributor to an apparent immunosuppressive environment.[12–14,16,18] Interestingly, TAMs isolated from meningioma were demonstrated to have an immunosuppressive effect on *in vitro* T cell proliferation and also express PD-L1 immune checkpoint protein.[18] Total number of TAMs has been correlated to poor prognosis for many solid tumors[28–30] and a positive association between numbers of TAMs and tumor grade has been reported for meningioma.[19] Adding to this, we have now shown that different TAM phenotypes may contribute to tumor behavior and recurrences.

Previous studies showed meningioma tissues stain positive for the M2 marker CD163, suggesting TAMs of an M2 phenotype are likely present.[33–35] CD163 expression was reported in cells of macrophage-like morphology in the stroma of some meningioma tissues[33] and in 2 cases of chordoid meningioma.[35] However, these findings were not confirmed, and previous work on meningioma has mainly focused on CD163 expression in tumor cells. Moreover, mRNA patterns in flow-cytometry harvested immune cells have indicated TAMs present in meningioma tissues are predominantly of the M1 type, where infiltration patterns may differ according to genetic subtype.[19] However, no confirmatory method such as immunohistochemistry was performed to substantiate the transcript findings.

In this investigation, we have confirmed that both M1 and M2 TAMs are present in the meningioma
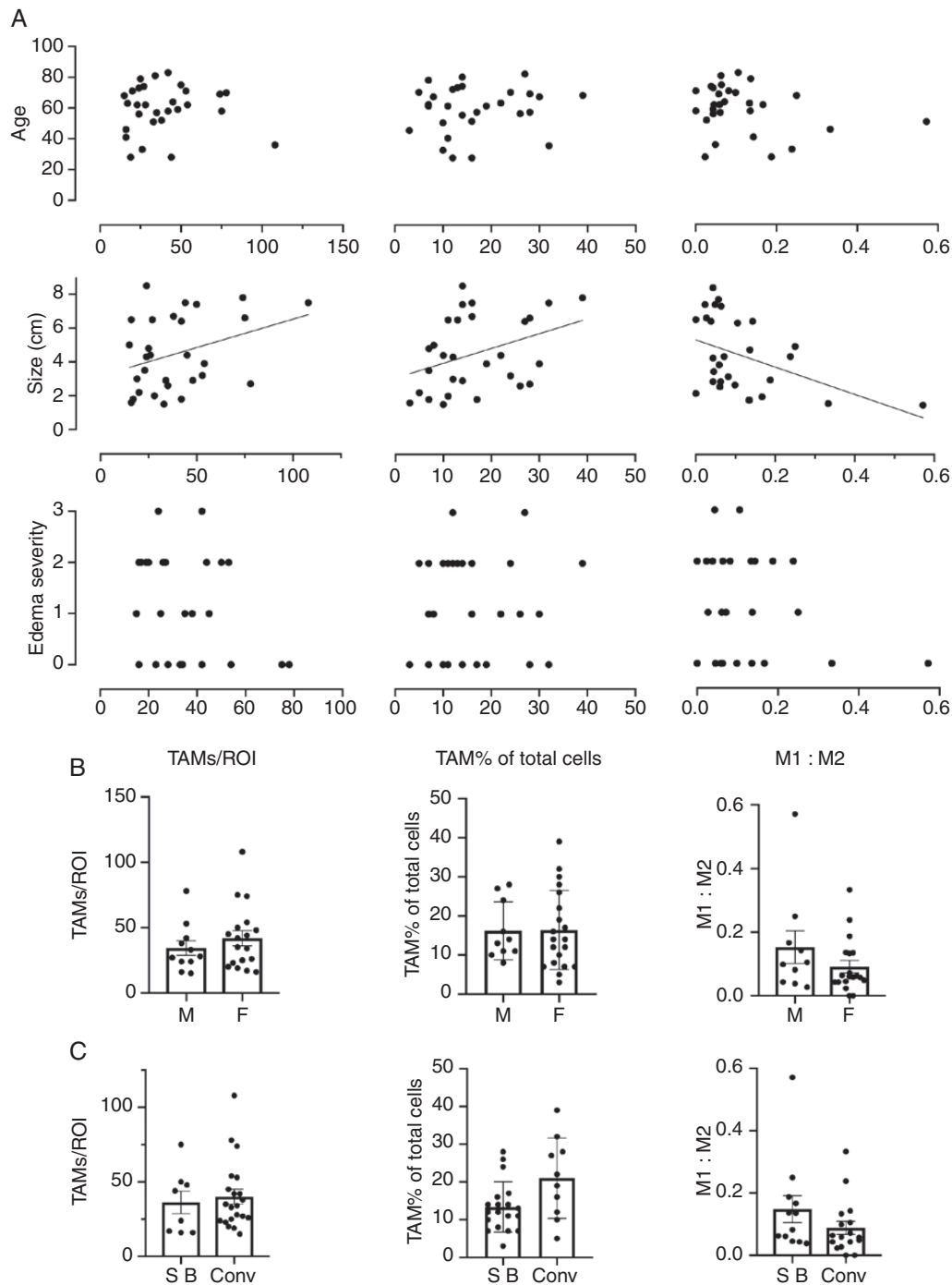
Figure 3. Association of total tumor-associated macrophages (TAM) number, TAM percentage of total cells and M1/M2 profiles to patient demographics and clinical variables. (**A**) Scatter plots for patient age, tumor size, and peri-tumoral edema severity according to TAM profile variables. Peri-tumoral edema severity in A; (0 = nil, 1 = mild, 2 = moderate, 3 = severe). (**B** and **C**) TAM profile variables according to sex (M—male, F—female) and tumor location (SB—skull base, Conv—convexity) are quantified in bar graphs. Bars represent mean. Error bars in each graph represent ± SEM.

microenvironment using a well-established and accepted dual macrophage marker immunostaining method for differentiating TAM subtypes. Indeed, TAMs accounted for ~18% of all cells and their numbers increased with WHO grade. These finding were consistent with previous reports.[16,36] We did not observe any significant difference between TAM infiltration in skull base and convexity meningioma groups despite reported differences in genetics and tumor behavior in these locations. The number of TAMs in meningioma did positively correlate to tumor size.

This is in line with recent findings in other cancers, and current classification of M2 TAMs as tumorigenic, capable of supporting tumor cell growth.[35, 37–40] However, it was the reduced M1:M2 ratio, and not total number of TAMs that was associated with tumor recurrence.

The M1:M2 cell ratio has been demonstrated to have prognostic value in several tumor types, including glioma, melanoma, ovarian, colorectal, and liver cancer.[28–32] A higher M1:M2 ratio has been associated with decreased tumor severity and grade[26,38] and an association between higher M1:M2 ratios and longer progression-free survival has been observed.[31,32] Improved progression-free survival is attributable to M1 TAMs, which may function as tumoricidal cells by promoting inflammation via phagocytosis and cytotoxic cytokine release and recruiting immunostimulating leukocytes to impede growth.[25–27] Thus, higher ratios of M1 to M2 TAMs likely decreases risk of recurrence.[26] Conversely, an inverse correlation between M2 TAM density and 5-year overall survival has been reported.[24] Overall, our findings in meningioma indicate that M1:M2 TAM ratio may prove to be an effective indicator of meningioma growth and recurrence. However, to establish this more conclusively, a larger patient cohort with long-term clinical outcome that includes robust statistical analyses would be necessary.

Investigators have reported an association between TAM density in meningioma tissues and WHO grade.[16,41] Although we also detected higher numbers of TAMs in grade II tumors compared to grade I, when correcting for TAM infiltration by area of tissue in each ROI removing regions and structures associate with vasculature, no significant difference was observed. Furthermore, TAM numbers did not differ between primary and recurrent tumors. These findings are consistent with previous reports on other tumor types, showing little association between the number of M1 or M2 or total TAM numbers to tumor behavior.[28–32] A more specific approach to the quantification of TAM infiltration by tumor region has shown TAM abundance in tumor parenchyma, and this may be a reliable predictor for tumor progression.[37,42] It will be important in future studies on meningioma tissues to quantify numbers of TAMs in stromal and tumor parenchyma compartments.

A number of biological events are described in meningioma that could regulate TAM behavior and explain the profiles and distribution patterns of TAMs in these tumors. In many tumors including glioblastoma, hypoxia has been considered a key contributor to induction of TAMs to an M2 subtype.[43–46] The spatiotemporal distribution of TAMs throughout a solid tumor is often thought to be a direct result of high density of TAMs in hypoxic regions.[45,47] Similarly, TAMs are also attracted to necrotic regions.[44,48] Although necrosis is not a common pathology of meningioma, it can be a feature of high-grade and aggressive subtypes.[49] The number of hypoxic regions in meningioma also increases with grade.[50,51] These events could explain the increase in density of TAMs with tumor grade. The location of these pathological features may also contribute to the variable TAM distribution observed in meningioma, that is from single and sparse infiltration to dense groups of cells when in close proximity to hypoxic tissue.

Limitations and Conclusion

While a small patient cohort, the findings presented here offer unique insight into the potential pathogenic processes that contribute to tumor growth, progression, and recurrence of meningioma. However, macrophage plasticity and that TAMs in meningioma may have phenotypes anywhere on the spectrum between the 2 extremes of the M1 and M2 dichotomy need to be recognized.[21] The TAM subtype quantification was performed on only a single whole-tumor slice, and heterogeneity in patterns of immune cell infiltration in other regions of the tumor that may exist was not detected. Similarly, although we did not observe a difference in TAM infiltration between skull base and convexity meningioma, there may be differences between histological subtypes that we were not able to establish due to the small sample size.

We have used the commonly accepted marker of macrophages, CD68, and the iNOS and CD163 for identifying and quantifying M1 and M2 macrophages respectively.[29,52,53] However, it should be noted that both iNOS and CD163 macrophage markers may also be expressed in tumor cells.[33–35] Furthermore, high expression of CD68 in select tumor cells has been observed in a few cases of rare xanthomatous meningioma and also in a small number of atypical meningioma case reports.[54–56] Indeed, the expression of CD68⁻/CD163⁺ tumor cells was commonly present in the cases studied, as was the expression of iNOS and CD206 in tumor cells. When differentiating and quantifying M1 and M2 cells in meningioma, these factors must be taken into consideration. Interestingly, the aberrant expression of macrophage markers in tumor cells has been observed in other cancers and is associated with more aggressive behavior of the tumor cell. Given immune marker expression in tumor cells was more frequently attributed to higher-grade cases, a similar aggressive behavior of these cells may be a characteristic of meningioma biology.

It may now be concluded that macrophages in meningioma are predominantly of M2 subtype, as evidenced by high expression of CD163 and CD206 and low expression of CD80, CD86, and iNOS. Furthermore, an M1:M2 ratio may be an indicator of tumor recurrence. These findings are important as the presence of M2 macrophages, considered to be pro-tumoral in nature, could well contribute to tumor progression and recurrence. Furthermore, this could account for immunosuppressive events present in meningioma, establishing the M2 macrophage as a novel target for future therapy.

Supplementary Material

Supplementary data are available at *Neuro-Oncology Advances* online.

Keywords

macrophage | meningioma | microenvironment | M1:M2 TAM ratio | tumor recurrence.

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