Correlation of Preoperative Von Willebrand Factor with Magnetic Resonance Imaging Perfusion and Permeability Parameters as Predictors of Prognosis in Glioblastoma

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BACKGROUND: **Angiogenesis has been shown to be strictly related to tumor malignancy. Glioblastoma (GBM) is highly vascularized and von Willebrand Factor (VWF) plays a potent proangiogenic role. Dynamic contrast-enhanced and dynamic susceptibility contrast magnetic resonance imaging (MRI) represent a widely accepted method to assess GBM microvasculature. Our aim was to investigate the correlation between plasma VWF:Ag, permeability, and perfusion MRI parameters and examine their potential in predicting GBM patient prognosis.**

METHODS: **We retrospectively analyzed preoperative dynamic contrast-enhanced, dynamic susceptibility contrast MRI, and VWF:Ag level of 26 patients with GBM. We assessed the maximum values of relative cerebral blood flow and volume, volume transfer constant K^{trans}, plasma volume (V_p) and reflux rate constant between fractional volume of the extravascular space and blood plasma (K_{ep}). Nonparametric Mann-Whitney test and Kaplan-Meier survival analyses were conducted and a *P* value < 0.05 was considered statistically significant.**

RESULTS: **The median VWF:Ag value was 248 IU/dL and the median follow-up duration was about 13 months. We divided patients according to low-VWF:Ag and high-VWF:Ag and we found significant differences in the median follow-up duration (19 months vs. 10 months; $P = 0.04$) and in K^{trans} (0.31/minute vs. 0.53/minute; $P = 0.02$), and K_{ep} (1.79/minute vs. 3.89/minute; $P = 0.005$) values. The cumulative 1-year survival was significantly shorter in patients with high-VWF:Ag and high-K_{ep} compared with patients with low-VWF:Ag and low-K_{ep} (37.5% vs. 68%; $P = 0.05$).**

CONCLUSIONS: **These findings, in a small group of patients, suggest a role for VWF:Ag, similar to K^{trans}, and K_{ep} as a prognostic indicator of postoperative survival of patients with GBM.**

INTRODUCTION

Glioblastoma (GBM) is the most common and fatal human primary intracranial tumor, accounting for some 45.6% of all malignant central nervous system tumors.[1] Despite standard treatment (cytoreductive surgery followed by radiotherapy plus chemotherapy with temozolomide) and

Key words
- Angiogenesis
- GBM
- K_{ep}
- K^{trans}
- MRI
- VWF

Abbreviations and Acronyms
CBF: Cerebral blood flow
CBV: Cerebral blood volume
DCE: Dynamic contrast-enhanced
DSC: Dynamic susceptibility contrast
GBM: Glioblastoma
IQR: Interquartile range
K_{ep}: Flux rate constant
K^{trans}: Volume transfer constant
KPS: Karnofsky Performance Status
MRI: Magnetic resonance imaging
rCBF: Relative cerebral blood flow
rCBV: Relative cerebral blood volume
ROI: Region of interest
V_p: Plasma volume
V_e: Extravascular extracellular volume
VWF: Von Willebrand factor

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extensive research into new therapeutic strategies, the prognosis remains extremely poor, with a mean survival of <14 months.[2] GBM is characterized by uncontrolled proliferation, diffuse infiltration of adjacent tissues, high genomic instability, and resistance to chemoradiotherapy, all features prognostic for poor overall survival. Unlike lower-grade gliomas such as oligodendrogliomas and anaplastic astrocytomas, GBM expresses an aggressive neoangiogenesis that renders it the most highly vascularized brain tumor.[3] Although originally described as simply the formation of new capillaries from preexisting vessels, it has recently been shown that the endothelium in GBM is morphologically and functionally different from low-grade glioma and meningiomas.[4]

The neoangiogenic process by which solid tumors obtain oxygen and nutrients to supply their growth involves a complex interplay of events with a decisive role even in the earliest stages of GBM growth.[5] An imbalance between proangiogenic and antiangiogenic factors induces an "angiogenic switch",[6] which triggers increased secretion of proangiogenic mediators marking a fundamental step in the progression of the disease, which is strictly correlated with GBM malignancy and poor clinical prognosis. The von Willebrand factor (VWF) has recently come to be considered a potential circulating marker for tumor angiogenesis in different types of cancer,[7] including brain tumors.[8] VWF is synthesized and stored in the Weibel-Palade bodies of endothelial cells and can be rapidly released into the bloodstream on stimulation.[9] It is a multimeric glycoprotein that carries coagulation factor VIII in plasma and thus participates in the coagulation process, as well as hemostasis and modulation of angiogenesis. Besides these roles, VWF may also participate in proangiogenic processes, regulating different linked pathways involved in angiogenesis modulation and able to influence vascular endothelial growth factor signaling, the most important and targeted in GBM disease.[10]

In clinical practice, dynamic susceptibility contrast (DSC) and dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) provide high spatial resolution techniques for assessing tumor vasculature via models of perfusion and permeability in terms of parameters such as cerebral blood volume (CBV), cerebral blood flow (CBF), flux rate constant (K_{ep}) and volume transfer constant (K^{trans}), and plasma volume (V_p) and extravascular extracellular volume (V_e) fractions. The existence of a correlation between tumor vascularity and maximum tumor blood volume was shown >15 years ago.[11] Although K^{trans} is defined as the volume transfer constant of contrast agent between plasma and interstitial spaces,[11] its physiologic significance depends on the balance between capillary permeability and blood flow in the tissue of interest. K_{ep} represents a similar concept for the return from the extravascular to vascular spaces. In high-grade tumors such as GBM, neoangiogenesis gives rise to an increased number of vessels, a high proportion of which are immature and thus have increased endothelial permeability. This process facilitates the bidirectional transfer of gadolinium chelate contrast agents between plasma and the extracellular space. When the blood-brain barrier is intact, K^{trans} and K_{ep} are effectively zero (or unmeasurable by MRI techniques) irrespective of blood flow. With loss of blood-brain barrier integrity, K^{trans} and K_{ep} are associated with the leakiness of the vessels and the total surface of the leaky capillaries. Reflecting these vascular changes, CBV and K^{trans} have shown good discriminative power in distinguishing low-grade and high-grade tumors[12] and in predicting prognosis.[13-15] However, to date, there has been little examination of how imaging markers of vascularity relate to the factors that determine neoangiogenesis. Such research would provide data on the clinical usefulness of perfusion and permeability MRI parameters in GBM in combination with plasma VWF:Ag levels, allow a deeper understanding of the roles of VWF in the complex tumoral angiogenic process, and possibly improve prediction of the clinical outcome of patients with GBM.

The aim of this work is to investigate the correlation between plasma VWF:Ag levels and permeability and perfusion MRI parameters in patients with GBM and examine their potential in predicting patient prognosis.

METHODS

This retrospective study was conducted with the approval of the hospital's institutional review board. Written informed consent was obtained from all patients for contrast-enhanced MRI, blood collection, and molecular analysis according to clinical routine, and the institutional review board waived the need for retrospective research use of these data.

After surgical resection of the tumor mass, all patients with GBM received a 6-week treatment by radiotherapy with concomitant systemic therapy using the alkylating agent temozolomide. Standard treatment involved the administration total of 60 Gy in 30–35 fractions of 1.8–2.0 Gy, in sessions performed 5 days a week. Concomitant temozolomide was administered at 75 mg/m^2/day during radiotherapy and thereafter at 150–200 mg/m^2/day for 5 days every 28 days for 12 cycles.[16] All patients in our cohort were operated on by 1 of 3 neurosurgeons, each with established series of neurosurgical procedures for brain tumor removal (>250 in the last 5 years).

Patient Population

Patients of both sexes with newly diagnosed malignant brain tumors who underwent surgery for tumor excision at our institution were eligible for this study. Inclusion criteria were: 1) 18–80 years of age; 2) Karnofsky Performance Status (KPS) >70; 3) DSC-MRI and DCE-MRI acquisitions within 2 weeks of surgery; 4) availability of a preoperative blood sample; 5) signed consent for the study; and 6) histologically proven diagnosis of GBM according to the World Health Organization classification on review by 2 independent pathologists. Exclusion criteria were: 1) previous brain surgery for other intracranial malignancies, 2) concomitant life-threatening disease, 3) history or presence of other malignancies, and 4) refusal or inability to consent to the study protocol.

Clinical and Demographic Data

Demographic and clinical data covering the interval from the date of diagnosis to death or the last follow-up visit were collected from the patient's records. Specifically, KPS, Ki67 positivity, and O-6 methylguanine methyltransferase promoter methylation were recorded.

MRI Protocol

Acquisition Protocol. The MRI scans were performed no more than 14 days before blood sampling and surgery on 1.5-T or 3-T scanners (Achieva, Software Release 2.6 [Philips Healthcare, Best, The Netherlands]) using a dedicated volumetric head coil. Brain MRI was performed according to the clinical routine protocol for preoperative and first examination in intracranial expansive disease, which includes T1-weighted spin-echo, T2-weighted turbo spin-echo, volumetric fluid-attenuated inversion recovery, T2*-weighted gradient-echo, spin-echo echo-planar diffusion-weighted, volumetric T1-weighted DCE permeability mapping, dynamic gradient-echo echo-planar DSC perfusion mapping and volumetric postcontrast T1-weighted gradient-echo sequences. Apparent diffusion coefficient maps were calculated from the diffusion-weighted images on the basis of b value 0 and 1000 second/mm^2 images.

Images for permeability and perfusion mapping were each acquired serially to follow the first passage of a contrast agent bolus consisting of 0.1 mL/kg of gadobenate dimeglumine (MultiHance [Bracco Diagnostic, Milan, Italy]) injected by a pneumatically driven injection pump at an injection rate of 5 mL/second.

Perfusion and Permeability Assessment. Generation of the perfusion and permeability maps was performed offline using dedicated software (Olea Sphere, version 2.3 [Olea Medical, La Ciotat, France]). From the DSC-MRI images, relative CBV (rCBV) and relative CBF (rCBF) maps were obtained using the deconvolution technique. From the DCE-MRI, the transport constant related to the permeability-surface area (K^{trans}), the reflux rate constant between the extracellular, extravascular space, and blood plasma (K_{ep}), and the fractional plasma and extracellular, extravascular space volumes (V_p and V_e), were calculated using the extended Tofts model.

Tumor Volume and Region of Interest Measurement

All MRI scans were reviewed by an experienced (>20 years) neuroradiologist (A.C.) and a neuroradiologist in training (F.M.D.) with 3 years' experience, who were blinded to clinical data. All images were evaluated to define tumor location and to identify confounding regions (vessels, meninges, artefacts, and necrotic region) for exclusion from measurements of the maps derived from perfusion-weighted imaging. Cases of disagreement in measurement or region of interest (ROI) selection were resolved by consensus.

The tumor volume of each GBM was calculated from the postcontrast T1-weighted images using the approximate ellipsoid volume equation (A×B×C)/2, in which A, B, and C represent the 3 dimensions of the enhancing lesion.[17] If >1 lesion was present, the largest was considered.

The fluid-attenuated inversion recovery and diffusion-weighted images and apparent diffusion coefficient maps were coregistered, along with the DSC-MRI and DCE-MRI images and derived maps, to the postcontrast volumetric T1-weighted image on a workstation with dedicated software (Olea Sphere, version 2.3 [Olea Medical]). ROIs for derivation of representative perfusion and permeability values for each tumor were defined on contrast-enhancing areas of 3 slices of the lesions (corresponding to the first, second, and the third quartile of the lesions z-axis extent). Contralateral normal white matter ROIs were defined to provide reference values. The pixel-wise values from the perfusion and permeability maps were exported for all ROIs and the mean and the maximum values of CBF, CBV, K^{trans}, K_{ep}, V_p, and V_e were calculated for the primary GBM lesion in each patient.

Blood Sample Collection and VWF:Ag Levels Measurement

Peripheral blood samples were taken from each patient the day before surgery. Blood was drawn into tubes with sodium citrate anticoagulant, centrifuged twice at 350*g* for 5 minutes, and the supernatants were stored at −80°C. VWF:Ag levels were determined blind to the clinical and imaging data using enzyme-linked immunosorbent assay (Helena Bioscience Ltd, Gateshead, United Kingdom) following the manufacturer's instructions.

Statistical Analyses

Clinical and demographic data are shown as median ± interquartile range (IQR). Patients were divided in 2 equally sized groups based on their plasma VWF:Ag levels (high and low), using the median value (248 IU/dL) as the cutoff. The reliability of the parameters calculated by neuroradiologists was estimated by Cronbach α value and the interrater variability was calculated by the intraclass correlation coefficient.

On testing of the neuroimaging parameters for normality using the Kolmogorov-Smirnov test, the permeability parameters K^{trans}, K_{ep}, and V_e were nonnormally distributed. The Mann-Whitney U test was therefore used to test differences in the neuroimaging parameters between the groups with high and low plasma VWF:Ag levels. The Pearson correlation coefficient was calculated to determine the relationship between the perfusion and permeability parameters and plasma biomarker. Kaplan-Meier survival curves were plotted for patients with high and low plasma VWF:Ag levels. In addition, survival curves of the patient subgroups with high (and similarly with low) values of both VWF:Ag and K^{trans} or K_{ep}, and were analyzed by using the Gehan-Breslow-Wilcoxon test. For the survival curves, death was the event, and follow-up duration was the time variable. The follow-up duration for each patient was calculated from the date of diagnosis to death or the last visit at time of medical files review. Mortality with 95% confidence intervals were calculated as the number of events per person-year. Statistical analyses were performed using SPSS software (version 25.0 [IBM Corp., Armonk, New York, USA]) and GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, California, USA). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Between May 2014 and February 2017, a series of 133 consecutive patients underwent resection of an intracranial brain tumor, of which 94 (71%) were GBMs. The required DSC-MRI and DCE-MRI sequences and preoperative blood samples were available in 26 patients (male/female ratio, 18:8) (28%), all of whom were included in the study (**Figure 1**).

A gross total resection of the lesion was obtained for all 26 patients. Patient demographics, performance status, and clinical data before surgery are reported in **Table 1** along with tumor size and follow-up duration. The mean age of patients was 63 years

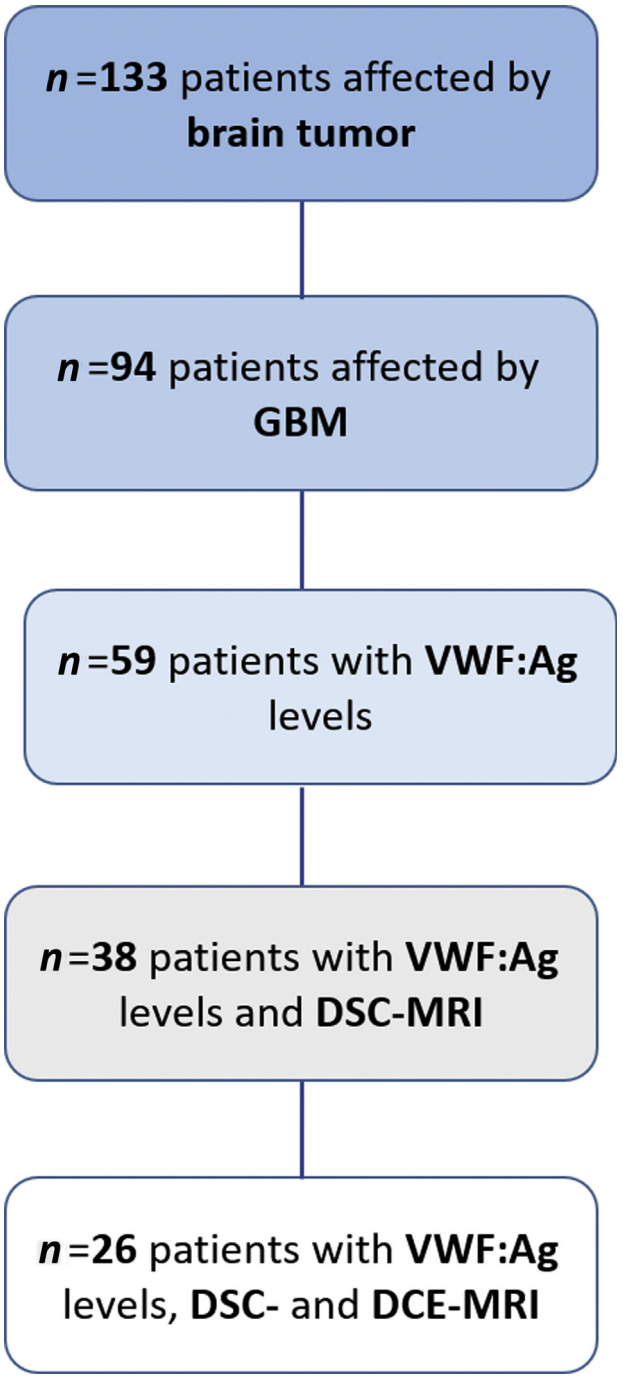

Figure 1. Breakdown of patient inclusion criteria. In this retrospective study, of 133 patients operated on for brain tumor, 94 were found to have glioblastoma (GBM), and among these, preoperative plasma von Willebrand factor:Ag (VWF:Ag) levels were available in 59 cases, of which 28 had also undergone preoperative perfusion (dynamic susceptibility contrast [DSC]) and permeability (dynamic contrast-enhanced [DCE]) magnetic resonance imaging (MRI) examinations.

Table 1. Main Clinical Characteristics of the Patients with Glioblastoma (n = 26) in the Study

Parameter	Value
Male sex (%)	69
Age at study entry (years), median (IQR)	63 (58–69)
Preoperative Karnofsky Performance Status, median (IQR)	80 (70–90)
Ki-67 (%), median (IQR)	27.5 (20–39)
Percentage of O-6 methylguanine methyltransferase promoter methylation, median (IQR)	14 (4–34)
von Willebrand factor:Ag levels (IU/dL), median (IQR)	248 (164–415)
Tumor volume (cm^3), median (IQR)	32.5 (19.8–72.9)
Follow-up duration (years), median (IQR)*	1.1 (0.7–1.4)

IQR, interquartile range.
*Follow-up duration was calculated from the date of diagnosis to date of death or last follow-up visit, whichever came first.

(range, 58-69 years) and the median preoperative KPS was 80 (IQR, 70–90). The median O-6 methylguanine methyltransferase promoter methylation was 14% (IQR, 4–34). The median overall survival was 11 months (IQR, 7.5–10.5) from the day of the surgical procedure.

MRI Findings

To assess the reliability of the parameters estimated by neuroradiologists, Cronbach and interrater variability were calculated and they were 0.86 and 71%, respectively, indicating substantial agreement. The median of the tumor volumes, calculated from MRI, was 32.5 cm^3 (IQR, 17.56–72.05). The group-wise median of the maximum DSC-MRI–derived and DCE-MRI–derived vascular permeability parameters, including rCBV, rCBF, K^{trans}, K_{ep}, V_p, and V_e are shown in Table 2. As expected, the perfusion and permeability maps of brain (CBV, CBF, K^{trans}, K_{ep}, V_p, and V_e) showed heterogeneous patterns in the GBMs (Figure 2).

Impact of Plasma VWF Levels in Patients with GBM

The median plasma VWF:Ag level across all patients with GBM was 248 IU/dL (IQR, 171–410). This value was used as a cutoff to categorize patients into 2 groups (High [H-] and Low [L-]) based on plasma VWF:Ag levels (Table 3). In the L-VWF:Ag (<248 IU/dL) and H-VWF:Ag (≥248 IU/dL) GBM patient groups, the median VWF:Ag was 165 IU/dL (IQR, 150–200) and 410 IU/dL (IQR, 361–524), respectively. Among the clinical parameters, a significant difference ($P = 0.04$) was seen between these groups only for the median follow-up duration: 19 (IQR, 11–22) months vs 10 (IQR, 7–13) months (Table 3).

Relationship Between VWF:Ag and Magnetic Resonance Pharmacodynamic Parameters

As shown in Table 4, significant differences were found between the L-VWF:Ag and H-VWF:Ag groups for K^{trans} ($P = 0.022$) and

Table 2. Group-Wise Median of the Individual Patient Mean and Maximum Perfusion and Permeability Characteristics of the Patients with Glioblastoma (n = 26) in the Study

	Median of Maximum (Interquartile Range)
Relative cerebral blood volume (mL/100mL)	15.84 (11.04–20.03)
Relative cerebral blood flow (mL/100mL/min)	109.5 (73.45–145.3)
K^{trans} (/minute)*	0.385 (0.240–0.635)
K_{ep} (/minute)†	2.435 (1.740–4.783)
V_p‡	6.500 (4.003–11.11)
V_e§	25.00 (5.150–100.0)

*Transport constant related to the permeability-surface area.
†Reflux rate constant between V_e and V_p.
‡Fractional plasma volume.
§Fractional volume of the extravascular, extracellular space.

K_{ep} levels ($P = 0.005$). No significant differences were observed for rCBV, rCBF, V_p, and V_e. There was a strong correlation between K^{trans} and K_{ep} ($r = 0.70$; $P = 0.00005$), and a moderate correlation was seen between VWF:Ag and both K^{trans} ($r = 0.48$; $P = 0.023$) (Figure 3A) and K_{ep} ($r = 0.42$; $P = 0.033$) (Figure 3B).

Outcome Dependence on VWF:Ag and Magnetic Resonance Pharmacodynamics Parameters

By the end of the follow-up period, 15 deaths had occurred (58%), of which 9 (60%) were in the H-VWF:Ag group. The cumulative 1-year survival was significantly lower ($P = 0.05$) in patients with H-VWF:Ag levels (31%) than in the L-VWF:Ag group (65%) (Figure 4).

As for VWF:Ag, we also formed patient groups based on the mean values of K^{trans} (0.61/minute), thus defining a low (L-) K^{trans} and high (H-) K^{trans} group, and similarly for K_{ep} (3.44/minute) to define a low (L-) K_{ep} and high (H-) K_{ep} group. Taking the intersections of these groups with the H-VWF:A and L-VWF:Ag groups, we compared survival of the subgroups having both H-VWF:Ag and H-K^{trans} with those having L-VWF:Ag and L-K^{trans}, and similarly, comparing those having H-VWF:Ag and H-K_{ep} with those having L-VWF:Ag and L-K_{ep}. We found no statistically significant difference in survival between the H-VWF:Ag + H-K^{trans} and L-VWF:Ag + L-K^{trans} subgroups ($P = 0.03$), likely because of the small sample number of patients with H-VWF:Ag + H-K^{trans}. However, a statistically significant difference in survival was seen between the H-VWF:Ag + H-K_{ep} and L-VWF:Ag + L-K_{ep} subgroups ($P = 0.03$), with the H-VWF:Ag + H-K_{ep} subgroup having a survival almost half that of the L-VWF:Ag + L-K_{ep} subgroup (8.5 months; IQR, 7.0–12.5 vs. 15 months; IQR, 11–22, respectively; $P < 0.05$). The cumulative 1-year survival was 37.5% for H-VWF:Ag + H-K_{ep} and 68% for L-VWF:Ag + L-K_{ep} (Figure 5). Further, the H-VWF:Ag + H-K_{ep} patients had a higher hazard of mortality (hazard ratio, 3.16;

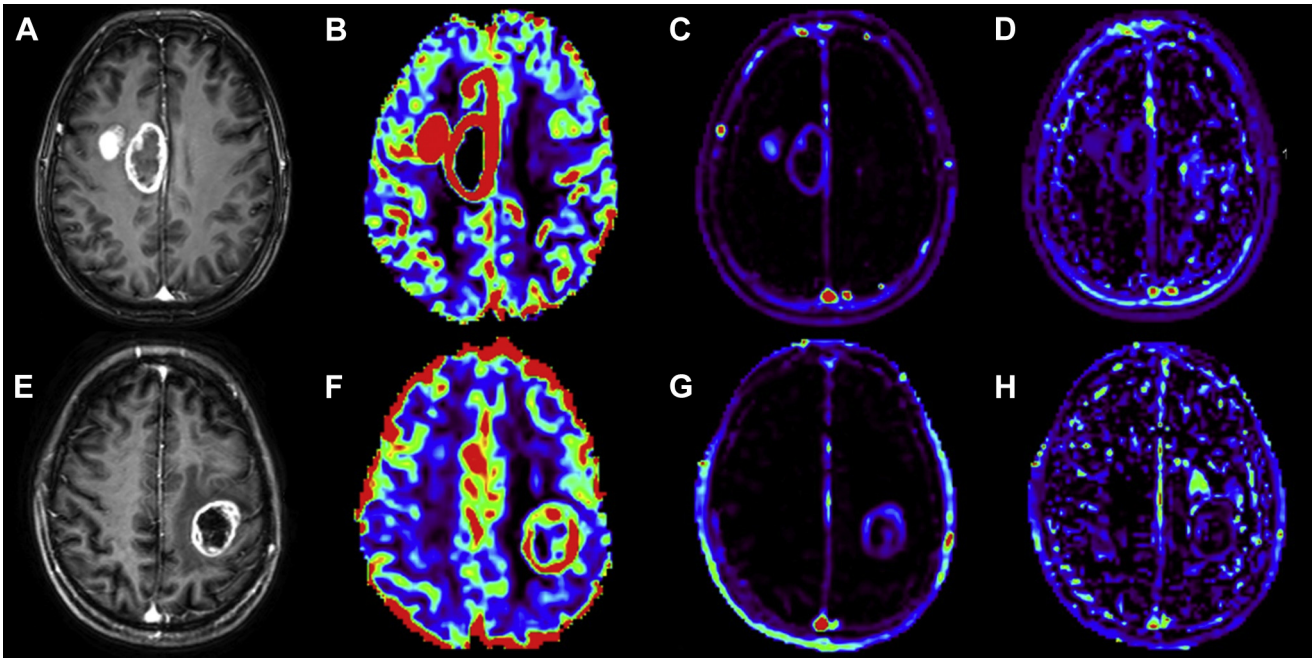

Figure 2. Images from patients with glioblastoma with high and a low plasma von Willebrand factor:Ag levels. (**A, E**) T1-weighted imaging postcontrast, (**B, F**) relative cerebral blood volume maps, (**C, G**) volume transfer constant maps, (**D, H**) flux rate constant maps of patients with glioblastoma with low (**A**–**D**) and high (**E**–**H**) plasma von Willebrand factor:Ag levels.

Table 3. Comparison of Clinical Parameters Between Patients with Glioblastoma with Low and High Plasma von Willebrand Factor:Ag Levels

Parameter	L-VWF:Ag Glioblastomas (<248 IU/dL)	H-VWF:Ag Glioblastomas (>248 IU/dL)	*P* Value
Male sex (%)	76	62	ns
Age at study entry (years), median (IQR)	66 (60—71)	62 (56—64)	ns
Preoperative KPS, median (IQR)	80 (70—90)	80 (70—90)	ns
Ki67 positivity, median (IQR)	36 (20—65)	32 (16—46)	ns
Percentage of O-6 methylguanine methyltransferase promoter methylation, median (IQR)	12 (4—37)	14 (4—32)	ns
VWF:Ag levels (IU/dL), median (IQR)	165 (137—201)	410 (329—528)	—
Tumor volume (cm^3), median (IQR)	32.4 (12.8—64.2)	32.5 (23.1—79.5)	ns
Follow-up duration (months), median (IQR)*	19 (11—22)	10 (7—13)	0.04

All quantitative parameters are given as medians and IQRs; KPS and Ki67 are given as percentage. All *P* values are 2-sided for the Mann-Whitney *U* test.
VWF:Ag, von Willebrand factor:Ag; ns, not significant; IQR, interquartile range.
*Follow-up duration was calculated from the date of diagnosis to date of death or last follow-up visit, whichever came first.

95% confidence interval, 0.914—10.94) compared with L-VWF:Ag + L-K_{ep} patients. The survival of this latter subgroup was superimposable with H-VWF:Ag + H-K_{ep} group.

DISCUSSION

The present study examined the association between DSC-MRI—derived and DCE-MRI—derived vascular parameters of perfusion and permeability, and plasma levels of VWF:Ag, to determine whether they are correlated, and whether they are potentially complementary as prognostic factors in patients with GBM.

Our principal finding is that both K^{trans} and K_{ep} were significantly higher in H-VWF:Ag patients with GBM than in those with L-VWF:Ag ($P < 0.05$) (Table 4) with both K^{trans} and K_{ep} being significantly correlated with VWF:Ag (Figure 3). Other perfusion and permeability parameters, and in particular, rCBV did not show significant correlations with VWF:Ag levels.

That rCBV was not statistically different in patients with GBM with H-VWF:Ag and L-VWF:Ag is not entirely an expected finding. The DSC-MRI perfusion parameter rCBV has been well reported as providing a means of differentiating patients with low and high grade of gliomas[18,19] but its usefulness in the diagnosis and prognosis of gliomas is limited, in part because it is a semi-quantitative measurement but also because it can be influenced by postprocessing steps as the choice of the normal contralateral white matter. The rCBV obtained from the normal contralateral white matter has been shown to have significant interscanner variability.[20] The lack of difference in rCBV between the

Table 4. Microvascular Values and Association Between Patients with Glioblastoma with Low and High Plasma von Willebrand Factor:Ag Levels

Parameter	Low von Willebrand Factor:Ag Glioblastomas (<248 IU/dL)	High von Willebrand Factor:Ag Glioblastomas (>248 IU/dL)	*P* Value
Relative cerebral blood volume (mL/100 mL)	5.10 (3.86—6.71)	5.94 (3.98—6.83)	ns
Relative cerebral blood flow (mL/100 mL/minute)	4.29 (3.29—6-55)	4.47 (3.97—5.87)	ns
K^{trans} (/minute)*	0.31 (0.19—0.44)	0.53 (0.35—0.97)	0.022
K_{ep} (/minute)†	1.79 (1.23—2.70)	3.89 (2.42—7.15)	0.005
V_p‡	7.00 (3.50—10.50)	6.00 (5.20—10.75)	ns
V_e§	33.33 (16.67—100)	16.67 (4.50—75)	ns

All quantitative parameters are given as means ± standard deviations. All *P* values are 2-sided for the Mann-Whitney *U* test.
Ns, not significant.
*Transport constant related to the permeability-surface area.
†Reflux rate constant between V_e and V_p.
‡Fractional plasma volume.
§Fractional volume of the extravascular, extracellular space.

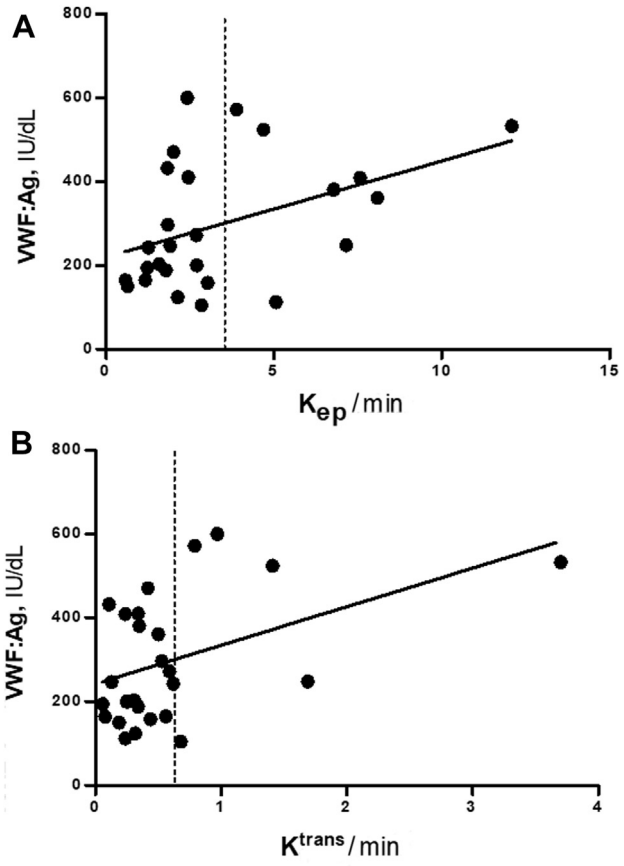

Figure 3. Correlation between plasma von Willebrand factor:Ag (VWF:Ag) level and flux rate constant (K_{ep}) and volume transfer constant (K^{trans}) in patients with glioblastoma. Scatter plots and fitted regression line of (**A**) K_{ep} vs VWF:Ag and (**B**) K^{trans} versus VWF:Ag in patients with glioblastoma. The Pearson correlation coefficient between VWF:Ag and K^{trans} was $r = 0.48$, with $P = 0.023$ and between VWF:Ag and K_{ep} was $r = 0.42$ with $P = 0.033$.

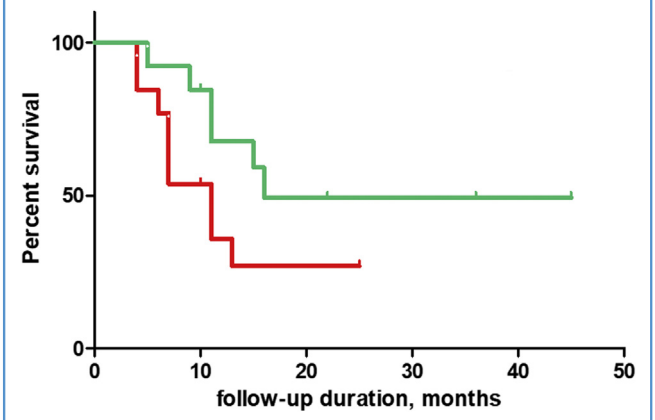

Figure 4. Survival among patients with glioblastoma (GBM) compared on the basis of plasma von Willebrand factor:Ag (VWF:Ag) levels. The Kaplan-Meier curves of overall survival in patients with GBM who underwent gross total resection followed by standard-of-care radiation therapy and chemotherapy show significantly shorter survival in patients with GBM with VWF:Ag levels >248 IU/dL (*red line*) relative to patients with VWF:Ag levels <248 IU/dL (*green line*) (Gehan-Breslow-Wilcoxon test, $P = 0.05$). K_{ep}, flux rate constant; K^{trans}, volume transfer constant.

H-VWF:Ag and L-VWF:Ag groups in our cohort may be because all our patients had supranormal VWF:Ag values, making it difficult to find a statistically significant difference.

To date, in patients with GBM, the prognostic value of DCE-MRI has not been well studied. K^{trans}, from DCE-MRI is often used as a synonym for permeability and blood flow in the tissue of interest. Estimated in a noninvasive manner,[21] it is influenced by CBF and vascular permeability,[22] so high K^{trans} tends to be higher in the presence of angiogenesis and a sign of greater biologic aggressiveness and prognostic of shorter survival.[23] Increased microvascular permeability allows plasma protein extravasation into the extravascular space, leading to profound alterations in the extracellular matrix that, in turn, favor angiogenesis. Comparing our results with previous studies, a role for K^{trans} as a prognostic factor was proposed by Kim et al.,[24] who found that the mean K^{trans} was higher in the early disease progression group than in the nonprogression group.

K^{trans} is known to predict glioma grading.[25,26] Similarly, K_{ep} is strictly related to vascular permeability and aggressive tumors that are characterized by a rapid enhancement followed by a rapid washout, providing insight into the nature of the bulk tissue features.[27] K_{ep} is considered a more robust parameter than K^{trans} because it is not so dependent on the T1 values of the tissue or V_e.[27] K_{ep} showed a significant predictive value for overall survival ($P = 0.035$) in our cohort, whereas K^{trans} failed to achieve significance ($P = 0.12$) in prediction of overall survival (data not shown).

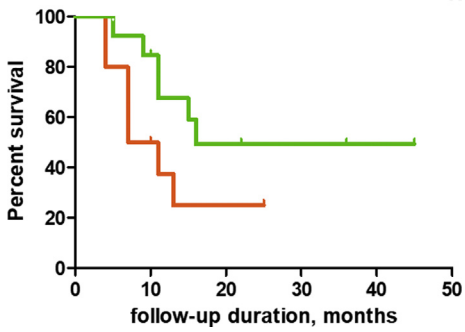

Figure 5. Survival among patients with glioblastoma compared on the basis of von Willebrand factor:Ag (VWF:Ag) and K_{ep} (flux rate constant) values. Kaplan-Meier curves show that overall survival in patients with glioblastoma with H-VWF:Ag and H-K_{ep} (*orange line*) was significantly shorter compared with patients with L-VWF:Ag and L-K_{ep} values (*green line*). Gehan-Breslow-Wilcoxon test, $P = 0.04$.

Consistent with findings in a previous study,[8] VWF:Ag level showed a significant association with survival and follow-up duration, expressed in months (Table 1). In particular, L-VWF:Ag patients had a follow-up duration of 19 months, as opposed to just 10 months in H-VWF:Ag patients. Moreover, survival analysis showed that the cumulative 1-year survival was significantly shorter in H-VWF:Ag versus L-VWF:Ag patients. These data support the potential role of VWF:Ag as a circulating biomarker useful for prognosis. Importantly, none of our patients underwent antivessel treatment, which is known to cause alterations in perfusion parameters[28] such as K^{trans} and V_e. In many institutions, qualitative analysis remains the dominant means of assessing permeability and perfusion maps because of the considerable heterogeneity (Figure 2) and sensitivity to various acquisition and processing choices (see later discussion on limitations). This situation makes a potentially stable reproducible metric of angiogenic activity attractive.

Our study is subject to some limitations. First, we are able to report on only a few patients, because GBM is a rare cancer and the analysis was limited to newly diagnosed patients. Second, DCE-MRI and DSC-MRI biomarkers depend on the details of the pulse sequences, magnetic field strength, and contrast agent used during scanning and the analysis algorithm in postprocessing,[26] which can weaken intercenter correlation. In our study, all patients were examined within a single center using the same contrast agent and postprocessing was performed within the OLEA postprocessing package (using the extended Tofts model for DCE data, and normalizing the DSC results with the anatomically symmetric normal-appearing contralateral white matter), which should serve to limit variability. However, 2 different magnetic field strengths were used and this can be expected to lead to increased variability in the perfusion and permeability values. Third, many patients with GBM received steroids at the time of admission; this treatment might have introduced a measurement bias on the pharmacokinetic parameters. Treatment with steroids has been reported to decrease contrast enhancement in malignant gliomas and to reduce total fractional blood volume.[29] In the 2 patients in our cohort who did not receive corticosteroids at admission, the perfusion and permeability values were within the ranges of values of the patients who received steroids and thus are unlikely to have biased the results. Fourth, because VWF is an acute phase inflammatory marker, it is often difficult to establish exactly whether increased levels are attributable to a cancer-associated or an inflammatory process. We recorded also the values of C-reactive protein, a protein produced in the liver during an acute inflammatory state. The results did not show a significant increase in C-reactive protein in the patients enrolled. We examined VWF at a single time point (preoperative). Although this point is likely to best correlate with the DCE and DSC metrics of the vasculature, it is not sufficient to address the important issue of whether VWF can provide insight into choice of therapy to achieve the greatest inhibitory effect on tumor neovascularization and growth. Future works will need to provide clinical validation in a larger series of patients with GBM including all stages of disease and over the course of therapy.

CONCLUSIONS

There is a clinical need in neuro-oncology for an accessible and reproducible tumoral marker to optimize neuroimaging diagnosis and follow-up and to help the clinician in choosing the therapeutic strategy. Our results suggest a role for VWF:Ag values in classification of tumor malignancy as shown by survival, associated with tumor vascularization as supported by the correlations between VWF:Ag and K^{trans} and K_{ep}. Because VWF:Ag measurements are easily accessible and reproducible, they are well suited to serve as a readout throughout the progression from diagnosis to relapse. Although limited because of the small sample size and monocentric nature, the present study may open a new means of confronting the complexity of predicting the successful use of antiangiogenic therapies in GBM.

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REFERENCES

1. Wen PY, Kesari S. Malignant gliomas in adults. *N Engl J Med*. 2008;359:492-507.
2. Ahmed R, Oborski MJ, Hwang M, Lieberman FS, Mountz JM. Malignant gliomas: current perspectives in diagnosis, treatment, and early response assessment using advanced quantitative imaging methods. *Cancer Manag Res*. 2014;6:149-170.
3. Hardee ME, Zagzag D. Mechanisms of glioma-associated neovascularization. *Am J Pathol*. 2012;181:1126-1141.
4. Guarnaccia L, Navone SE, Trombetta E, Cordiglieri C, Cherubini A, Crisà FM, et al. Angiogenesis in human brain tumors: screening of drug response through a patient-specific cell platform for personalized therapy. *Sci Rep*. 2018;8:8748.
5. Mao JM, Liu J, Guo G, Mao XG, Li CX. Glioblastoma vasculogenic mimicry: signaling pathways progression and potential anti-angiogenesis targets. *Biomark Res*. 2015;3:8.
6. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell*. 1996;86:353-364.
7. Yang X, Sun HJ, Li ZR, Zhang H, Yang WJ, Ni B, et al. Gastric cancer-associated enhancement of von Willebrand factor is regulated by vascular endothelial growth factor and related to disease severity. *BMC Cancer*. 2015;15:80.
8. Marfia G, Navone SE, Fanizzi C, Tabano S, Pesenti C, Abdel Hadi L, et al. Prognostic value of preoperative von Willebrand factor plasma levels in patients with Glioblastoma. *Cancer Med*. 2016;5:1783-1790.
9. Sporn LA, Marder VJ, Wagner DD. Inducible secretion of large, biologically potent von Willebrand factor multimers. *Cell*. 1986;46:185-190.

10. Randi AM, Laffan MA, Starke RD. Von Willebrand factor, angiodysplasia and angiogenesis. *Mediterr J Hematol Infect Dis*. 2013;5:e2013060.

11. Tofts PS, Brix G, Buckley DL, Evelhoch JL, Henderson E, Knopp MV, et al. Estimating kinetic parameters from dynamic contrast-enhanced T(1)-weighted MRI of a diffusable tracer: standardized quantities and symbols. *J Magn Reson Imaging*. 1999;10:223-232.

12. Patankar TF, Haroon HA, Mills SJ, Balériaux D, Buckley DL, Parker GJ, et al. Is volume transfer coefficient (Ktrans) related to histologic grade in human gliomas? *AJNR Am J Neuroradiol*. 2005;26:2455-2465.

13. Mills SJ, Patankar TA, Haroon HA, Balériaux D, Swindell R, Jackson A. Do cerebral blood volume and contrast transfer coefficient predict prognosis in human glioma? *AJNR Am J Neuroradiol*. 2006;27:853-858.

14. Bisdas S, Kirkpatrick M, Giglio P, Welsh C, Spampinato MV, Rumboldt Z. Cerebral blood volume measurements by perfusion-weighted MR imaging in gliomas: ready for prime time in predicting short-term outcome and recurrent disease? *AJNR Am J Neuroradiol*. 2009;30:681-688.

15. Cao Y, Tsien CI, Nagesh V, Junck L, Ten Haken R, Ross BD, et al. Survival prediction in high-grade gliomas by MRI perfusion before and during early stage of RT [corrected]. *Int J Radiat Oncol Biol Phys*. 2006;64:876-885.

16. Stupp R, Roila F, ESMO Guidelines Working Group. Malignant glioma: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol*. 2009;(suppl 4):126-128.

17. Kothari RU, Brott T, Broderick JP, Barsan WG, Sauerbeck LR, Zuccarello M, et al. The ABCs of measuring intracerebral hemorrhage volumes. *Stroke*. 1996;27:1304-1305.

18. Law M, Young RJ, Babb JS, Peccerelli N, Chheang S, Gruber ML, et al. Gliomas: predicting time to progression or survival with cerebral blood volume measurements at dynamic susceptibility-weighted contrast-enhanced perfusion MR imaging. *Radiology*. 2008;247:490-499.

19. Hirai T, Murakami R, Nakamura H, Kitajima M, Fukuoka H, Sasao A, et al. Prognostic value of perfusion MR imaging of high-grade astrocytomas: long-term follow-up study. *AJNR Am J Neuroradiol*. 2008;29:1505-1510.

20. Boxerman JL, Schmainda KM, Weisskoff RM. Relative cerebral blood volume maps corrected for contrast agent extravasation significantly correlate with glioma tumor grade, whereas uncorrected maps do not. *AJNR Am J Neuroradiol*. 2006;27:859-867.

21. Cha S, Yang L, Johnson G, Lai A, Chen MH, Tihan T, et al. Comparison of microvascular permeability measurements, K(trans), determined with conventional steady-state T1-weighted and first-pass T2*-weighted MR imaging methods in gliomas and meningiomas. *AJNR Am J Neuroradiol*. 2006;27:409-417.

22. Alcaide-Leon P, Pareto D, Martinez-Saez E, Auger C, Bharatha A, Rovira A. Pixel-by-pixel comparison of volume transfer constant and estimates of cerebral blood volume from dynamic contrast-enhanced and dynamic susceptibility contrast-enhanced MR imaging in high-grade gliomas. *AJNR Am J Neuroradiol*. 2015;36:871-876.

23. Henderson E, Sykes J, Drost D, Weinmann HJ, Rutt BK, Lee TY. Simultaneous MRI measurement of blood flow, blood volume, and capillary permeability in mammary tumors using two different contrast agents. *J Magn Reson Imaging*. 2000;12:991-1003.

24. Kim R, Choi SH, Yun TJ, Lee ST, Park CK, Kim TM, et al. Prognosis prediction of non-enhancing T2 high signal intensity lesions in glioblastoma patients after standard treatment: application of dynamic contrast-enhanced MR imaging. *Eur Radiol*. 2017;27:1176-1185.

25. Jung SC, Yeom JA, Kim JH, Ryoo I, Kim SC, Shin H, et al. Glioma: application of histogram analysis of pharmacokinetic parameters from T1-weighted dynamic contrast-enhanced MR imaging to tumor grading. *Am J Neuroradiol*. 2014;35:1103-1110.

26. Nguyen TB, Cron GO, Perdrizet K, Bezzina K, Torres CH, Chakraborty S, et al. Comparison of the diagnostic accuracy of DSC- and dynamic contrast-enhanced MRI in the preoperative grading of astrocytomas. *AJNR Am J Neuroradiol*. 2015;36:2017-2022.

27. Choyke PL, Dwyer AJ, Knopp MV. Functional tumor imaging with dynamic contrast-enhanced magnetic resonance imaging. *J Magn Reson Imaging*. 2003;17:509-520.

28. Yang J, Liao C, Liu Y, Yang G, Ke T, Ding Y, et al. MR imaging biomarkers evaluating vascular normalization window after anti-vessel treatment. *Oncotarget*. 2017;21:11964-11976.

29. Zaki HS, Jenkinson MD, Du Plessis DG, Smith T, Rainov NG. Vanishing contrast enhancement in malignant glioma after corticosteroid treatment. *Acta Neurochir (Wien)*. 2004;146:841-845.

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