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Vascular Permeability and Iron Deposition Biomarkers in Longitudinal Follow-up of Cerebral Cavernous Malformations

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Abstract

OBJECTIVE.—Vascular permeability and iron leak are central features of cerebral cavernous malformations (CCM) pathogenesis. We aimed to correlate prospective clinical behavior of CCM lesions with longitudinal changes in biomarkers of dynamic contrast enhanced quantitative permeability (DCEQP) and quantitative susceptibility mapping (QSM) assessed by magnetic resonance imaging.

METHODS.—Patients with CCMs (N=46) underwent 2 or more permeability and/or susceptibility studies in conjunction with baseline and follow-up imaging and clinical surveillance during a mean 12.05 months of follow-up (range 2.4 to 31.27 months). Based on clinical and

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Author Contributions

All authors were involved in the concept, design, drafting and critical revision of the manuscript. MW and YC contributed to the statistical analysis. RG, MDF, HAZ, MJ, AM, CS, RS and IAA were involved in patient recruitment, data collection and analysis and interpretation of results. RG, HAZ, AM, CS, HBWL, HT and NH were involved in the permeability and QSM processing. HAZ, MDF, MJ, GAC and IAA reviewed the medical records for relevant information.

Patient consent

Obtained

Ethics approval

This study was approved by The University of Chicago Institutional Review Board.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

imaging features, cases/lesions were classified as *stable*, *unstable*, or *recovering*. We investigated associated and predictive changes in quantitative permeability and susceptibility.

RESULTS.—Lesional mean permeability and QSM values were not significantly different in *stable* versus *unstable* lesions at baseline. Mean lesional permeability in *unstable* CCMs with lesional bleeding or growth increased significantly (+85.9% change; $p=0.005$), while permeability in *stable* and *recovering* lesions did not significantly change. Mean lesional QSM values significantly increased in *unstable* lesions (+44.1 % change; $p=0.01$), decreased slightly with statistical significance in *stable* lesions (−3.2 % change; $p=0.002$) and did not significantly change in *recovering* lesions. Among familial cases developing new lesions during the follow-up period showed a higher background brain permeability at baseline ($p=0.001$), and higher regional permeability ($p=0.003$) in the area that would later develop a new lesion than in homologous contralateral brain region.

CONCLUSIONS.—*In vivo* assessment of vascular permeability and iron deposition on MRI can serve as objective and quantifiable biomarkers of disease activity in CCMs. This may be applied in natural history studies, and may help calibrate clinical trials. The two techniques are likely applicable in other disorders of vascular integrity and iron leak such as aging, hemorrhagic microangiopathy and traumatic brain injury.

Keywords

Cerebral Cavernous Malformation; Biomarker; DCEQP; QSM; Iron; Permeability

Cerebral cavernous malformations (CCM) are a common neurovascular disorder affecting 0.5–1% of the population. Two clinical subtypes have been described. Sporadic CCM account for almost two thirds of cases and are characterized by a solitary lesion, frequently associated with a developmental venous anomaly.^{1,27,32} The familial disease manifests multifocal lesions, developing throughout life in different regions of the brain, in association with mutations at three identifiable gene loci, inherited in an autosomal dominant pattern.^{11,20,36} the CCM can result in intracerebral hemorrhage, progressive focal neurological deficits or seizures, but the clinical activity of individual lesions remains largely unpredictable.^{18,33}

Identification of the three *CCM* genes uncovered a hallmark mechanistic feature, involving RhoA kinase (ROCK) mediated disruption of endothelial cell-cell junctions and vascular hyperpermeability.^{4,36,39,43} Hemorrhage is another fundamental feature of the CCM lesion, with evidence of thrombus of varying ages, hemosiderin and chronic deposition of non-heme iron in and around CCM lesions regardless of genotype.^{38,40} Gradient recalled echo and susceptibility weighted magnetic resonance imaging (MRI) sequences, sensitive to the paramagnetic effects of iron content in lesions, have greatly enhanced sensitivity of lesion detection.^{6,8}

Our group had previously optimized and reported retrospective analyses of two novel imaging techniques, the T₁-weighted dynamic contrast-enhanced quantitative perfusion (DCEQP) and quantitative susceptibility mapping (QSM), as respective measures of regional vascular permeability and mean iron concentration in CCM.^{30,31,40,41,43} We herein report

the first prospective longitudinal analysis of these two biomarkers in relation to CCM clinical behavior. We hypothesize that changes in vascular permeability and QSM reflect the clinical instability of CCM lesions, and can be used as *in vivo* biomarkers of disease activity. The objective of this study is to validate DCEQP and QSM as biomarkers of clinical behavior of CCM lesions.

Methods

Subjects

This prospective case controlled study included patients with confirmed sporadic or familial CCM disease of any genotype, who consented to undergo two or more MRI studies involving DCEQP and QSM protocols in conjunction with their initial routine clinical and MRI evaluation, and later longitudinal follow-up(s) at a single site referral and clinical research center. The routine MRI sequences included T₁-weighted pre and post contrast images, T₂-weighted images, susceptibility weighted images (SWI) and T₂*-weighted images performed at 3 Tesla with similar acquisition parameters described previously.^{30,31,40,41,43} Patients with partial or complete CCM lesion resection or any prior brain irradiation were not included in this study. A written informed consent was obtained from all participants in accordance to the Declaration of Helsinki, and approved by The University of Chicago Institutional Review Board (IRB). The ethical principles guiding the IRB are consistent with The Belmont Report, and comply with the rules and regulations of The Federal Policy for the Protection of Human Subjects (56 FR 28003).

From November 2012 to August 2015, DCEQP and QSM studies were performed in 116 CCM patients who participated in the IRB approved Advanced Imaging in Cerebral Cavernous Malformations study at the University of Chicago Medical Center. Of all eligible patients during the same period, four refused to participate. Follow-up studies were performed at scheduled 1 year intervals in the majority of cases, or sooner if new symptoms arose. Epochs between two consecutive imaging studies during follow up ranged from 1.5 to 12.96 months (mean 10.26 months).

Of the above cases 87 subjects had two or more permeability follow-up scans within a year of their baseline scans. Among the permeability scans, 14 scans (from 14 patients) were excluded due to bad input functions derived from the contrast signal or improper contrast injection flow, five (from five patients) due to head motion, and one due to the improper selection of image slices during acquisition. Another 26 scans (from 26 patients) were excluded due to subsequently discovered concomitant non-CCM brain disease (i.e. cerebral amyloid angiopathy, hemorrhagic telangiectasias, lacunar strokes, etc.). Finally, 41 CCM subjects with at least two MRI permeability studies were analyzed in this study accounting for 104 scans.

Eighty-three patients had QSM follow-up scans within a year of their baseline scans. Of the QSM acquisitions, 16 scans (from 16 patients) were excluded due to head motion during the data acquisition, 22 (from 22 patients) due to technical issues in post-processing procedures, and 12 (from 12 patients) because of non-CCM disease diagnosis. Finally, 33 CCM subjects with at least two QSM acquisitions performed during their routine clinical and MRI

evaluation were included in the analysis accounting for 82 scans. Twenty four patients had both QCEQP and QSM studies during follow-up.

A CCM symptomatic hemorrhage was defined according to accepted adjudicated criteria.³ CCM growth was identified as a change in lesion diameter by 2 or more millimeters on comparable T₁-weighted or T₂-weighted sequences, adjudicated by a neuroradiologist. Patients and lesions were classified as *stable* (no CCM symptomatic hemorrhage or demonstrated growth in the year preceding the first scan, nor at follow-up scan), *unstable* (CCM symptomatic hemorrhage or demonstrated lesion growth occurring between the initial and follow-up scan), or *recovering* (from a CCM symptomatic hemorrhage or growth preceding the initial scan). Cases with familial disease were further considered for the presence of new lesions on follow-up comparable sequence 3 Tesla MRI scans, most typically noted on susceptibility sequences.^{6,8}

Data Acquisition and Processing

DCEQP and QSM scans were obtained in conjunction with the routine clinical evaluation and follow-up MRI, obtained as part of the patients' clinical care. Vascular permeability was measured via a DCEQP protocol involving a gadolinium-based dynamic scan following a pre-contrast T₁-weighted scan (acquisition time 8 minutes, with another approximate 1 minutes for set up, slice selection and image alignment). Five axial slices were acquired during each scan. This data was then processed in MATLAB to generate and calculate a permeability map.²² Interobserver and other validations of this technique were previously reported, including rationale and optimization of region of interest selection in lesion and background brain.^{30,31}

QSM acquisitions were generated using a single 3-dimensional, multi-echo, gradient recalled echo T₂*-weighted, spoiled gradient echo sequence (acquisition time 11 minutes). A morphology-enabled dipole inversion algorithm was used to reconstruct the QSM images.^{24,25} Interobserver and other validations of QSM measures have been published, including precise correlations with iron concentration in resected CCM lesions and phantom solutions of molecular iron.⁴⁰

Regions of interest were selected for both permeability and QSM as previously reported, as illustrated in Fig. 1.³¹

The DCEQP and QSM dataset were acquired and post-processed by two experienced imaging scientists (RG, HT) and two research clinical fellows (HAZ, AGM) following a high intra- and interobserver consistent established protocol.^{30,41} The operators were blinded to the clinical status of the patients throughout the image analysis. The electronic medical records of the patients were reviewed and adjudicated by the senior author with experience in the care of CCM, for abstracting in the database, blinded to DCEQP and QSM results (IAA). Two research clinical fellows (MDF and HAZ) classified patients into the respective categories based on information in the clinical database and pre-articulated criteria (*stable*, *unstable* or *recovering*), and they were also blinded to the DCEQP and QSM results. Finally, the identification of the *stable*, *unstable* and *recovering* CCM lesions matching on the

baseline and follow-up scans was conducted conjointly with the experienced imaging scientist (RG) and research clinical fellows (MDF and HAZ).

Statistical Analysis

The difference in age at baseline scan inclusion, the time between the two follow-up MRI scans protocols and baseline permeability or QSM values among cases considered either as *stable*, *unstable* or *recovering* were tested using ANOVA. A Chi-square test, and Fisher's exact test when the sample size was less than 5, were used to compare proportions in repartition in genotype and ethnicity between pairs of groups. Linear regression models were conducted to detect a modification of lesional permeability and QSM in the three groups (*stable*, *unstable*, *recovering*). A Generalized Estimating Equation approach estimated the parameters in the regression models to take into account the longitudinal structure of the dataset.¹⁶ Paired t-test compared the difference of mean lesional permeability and QSM values, white matter far from the lesion (WMF), and white matter near lesions (WMN) between baseline and follow-up scans in *stable*, *unstable* and *recovering* groups respectively. The F test evaluated the variances between two unpaired groups. The differences between these two groups were compared using Student's t-test with equal variances and Welch's correction with unequal variances.

A linear combination of mean lesional permeability percent change and mean lesional QSM percent change was generated using the Canonical linear discriminant analysis,^{19,34} with the equation shown below:

Combination = $0.30 \times \text{Mean lesional permeability \% change} + 1.45 \times \text{Mean lesional QSM \% change}$

Receiver operating characteristic (ROC) curves were generated and area under curves (AUC) were calculated to evaluate mean lesional permeability percent change, mean lesional QSM percent change and their combination's ability to detect being *unstable* rather than *stable*.^{19,34} Predictive thresholds (cut off value) for the *unstable* group were determined by the value achieving the best sensitivity and specificity together. The concordance (agreement) between lesional permeability increase and QSM increase was assessed by kappa test. Kappa coefficients (K) were calculated for quantifying inter-rater agreement^{29,37} and interpreted according to Landis and Koch criteria.^{21,35} Kappa is a concordance index ranging from zero to one, with the maximum value of one indicating a perfect agreement, and 0 corresponding to a complete absence of agreement. Statistical analyses were performed using SAS9.4 (SAS Institute Inc., Cary, NC) and GraphPad Prism4.0 (GraphPad Software Inc., La Jolla, CA). All p values were considered to be statistically significant at $p < 0.05$.

For more information on Methods and Materials refer to Supplementary Material

Results

Demographic and CCM lesion characteristics

In all, 46 patients had completed two or more technically satisfactory DCEQP or QSM studies, and included in this analysis. Total follow-up periods of these patients ranged from 2.4 to 31.27 months (mean 12.05 months), with 11 subjects undergoing more than two clinical and imaging follow-ups. There were no significant differences in the age at the time of baseline scan among cases who would behave as *stable*, *unstable* or *recovering*, nor any significant differences in baseline permeability or QSM between the three groups (Table 1). Mean interval time between scans was 10.67 months in stable lesions, 9.00 months in unstable lesions, and 9.05 months in recovering lesions (differences not significant). Each CCM lesion was referenced separately as *stable*, *unstable* or *recovering* for the follow-up analysis on lesional permeability and QSM. Supplementary Table 1 summarizes the respective stability categories of CCM patients and lesions undergoing follow-up DCEQP and QSM studies. The clinical and radiological correlates of the unstable lesions are summarized in Supplementary Table 2.

For the analysis of new lesion formation, 15 new lesions were detected by MRI at the time of the follow-up clinical evaluation. Among these 15 CCM patients, 10 had permeability maps covering the region in which the new lesion formed later on.

Lesional Permeability and QSM Increase in *Unstable* Lesions

There was a significant increase in mean lesional permeability (Fig. 2A) between the two consecutive scans in *unstable* CCM lesions (+85.9 %, $p=0.005$, $t\text{-score}=-3.38$, $df=8$; one-tailed paired t -test), with no significant differences observed in mean lesional permeability in the *stable* and *recovering* cohorts (Fig. 2B and C).

An increase was also observed in mean lesional susceptibility in *unstable* lesions (Fig. 2D) (+44.1 % change, $p=0.01$, $t\text{-score}=-2.68$, $df=8$; one-tailed paired t -test). *Stable* lesions demonstrated a slight but significant decrease in lesional susceptibility values (−3.2 % change, $p=0.003$, $t\text{-score}=-2.86$, $df=95$; one-tailed paired t -test) (Fig. 2E). No significant difference between scans was observed in the *recovering* group (Fig. 2F).

ROC curves were generated based on the percent change across the two MRI scans in mean lesional DCEQP and QSM values. The AUC was calculated to evaluate the accuracy of these two MR techniques in association with lesional instability.⁴⁴ The predictive threshold value was estimated (39.59% and 5.81% for permeability and QSM respectively) with sensitivity of 78.72% and 82.29% for permeability and QSM, respectively; and specificity of 88.89% and 88.89% for permeability and QSM, respectively (Fig. 3A and B). The ROC curves can be interpreted as showing a “good” accuracy for both DCEQP and QSM techniques to distinguish *unstable* from *stable* lesions (AUC=0.81, $p=0.004$ and AUC=0.86, $p=0.0004$ for permeability and QSM respectively).

Combined DCEQP and QSM Enhances Sensitivity and Specificity of Distinguishing Unstable CCM Lesions

We performed a Kappa coefficient test to determine how mean lesional DCEQP and QSM concurred as in-vivo biomarkers of lesional behavior. In the *unstable* group, the analysis displayed a perfect agreement (Kappa coefficient =1) between the two techniques, while it was slight in *stable* (Kappa coefficient=0.14, z-score=0.76, p=0.45, dl=1) and *recovering* groups (Kappa coefficient=-0.17, z-score=-0.44, p=0.66, dl=1). ROC analysis combining percent change of mean lesional permeability and susceptibility values showed a better accuracy (AUC=0.94, p=0.005) to detect *unstable* CCM lesions than either technique alone, with enhanced sensitivity (87.88%) and specificity (100%) (Fig. 3C).

Higher Brain Permeability in Cases with De Novo Lesion Formation

For this analysis, we considered the cohort who developed new lesions at the time of the follow-up clinical evaluation, and as expected these were all familial cases. At the initial MRI scan, the baseline mean background brain permeability, WMF, was higher (p=0.001, t=3.327, df=22; one-tailed unpaired t-test) in patients who developed new lesions during the follow-up evaluation (Fig. 4A) compared to familial patients with no new lesion formation. Moreover, the baseline mean permeability was also higher (p=0.003, t-value=3.55, df=9; one-tailed paired t-test) in the cerebral matter region where the lesion later developed compared to the symmetric contralateral brain region on the same scan (Fig. 4B).

Refer to Supplementary Material for more information on results on background permeability in unstable patients

Discussion

In the 20 years since first identification of the first *CCM* gene locus, much has been learned about the pathogenesis of the disease.^{4,15} Familial cases carry a heterozygous germ line mutation at one of 3 known *CCM* genes, affecting all cells in the body, while lesional endothelial cells exhibit somatic biallelic homozygous mutations, consistent with Knudsonian two-hit hypothesis.^{2,10,12} Similar somatic biallelic mutations have been recently identified in sporadic CCM lesions, indicating a common genetic basis for lesion pathogenesis.²⁷ The loss of any of the three *CCM* genes in endothelial cells results in ROCK mediated vascular hyperpermeability.^{36,39,42} ROCK activity has been also documented in endothelial cells lining human sporadic and familial CCM lesions.^{36,39} The *Ccm* heterozygous mice in fact demonstrate a subtle but significant vascular leak in skin, brain and lungs, even in the absence of lesions, and this hyperpermeability is rescued by ROCK inhibition and statins.^{39,42} When these mice are genetically sensitized for enhanced rate of somatic mutations, they develop a rich repertoire of CCM lesions throughout their brain, with all phenotypic features recapitulating the human disease.²⁶ Pharmacologic ROCK inhibition has been shown to blunt CCM lesion development and hemorrhage in these models, as predicted mechanistically.^{26,28}

Beyond these biologic advances in understanding disease mechanisms, there have been recent discoveries regarding the natural history of CCM. Some *CCM* disease categories (familial versus sporadic), and familial genotypes (*CCM3* versus *CCM1* and *CCM2*) indeed

manifest inherently more aggressive disease behavior,^{11,36} and pro-inflammatory genes may act as disease modifiers enhancing clinical penetrance in some familial cases.⁵ Lesions that have bled previously and possibly brainstem lesions are known to have greater hemorrhage rates.¹⁸ Our group and others have also reported variations with disease activity in correlation with serum cholesterol, vitamin D levels and seasonal variation that require further study.^{9,13,14} Despite these advances, there remains wide variability and unpredictability in the clinical behavior of individual lesions.

MRI has revolutionized the management of CCM disease by improving lesion detection rates with gradient echo and susceptibility sequences,^{8,23} and by demonstrating acute hemorrhage features and lesion growth in correlation with specific symptoms, allowing monitoring of lesional activity. Routine MRI sequences do not measure permeability in the background brain or CCM lesions, nor quantify the iron leak in lesions, as postulated mechanistically. These features are critical to CCM pathogenesis and may reflect features of aggressive lesions or the impact of novel therapies.

The advent of DCEQP and QSM MRI sequences and their application in this disease has added a new dimension in CCM research.^{7,17,22} Our group has recently applied and validated these novel techniques in CCM disease subjects.^{30,31,40,41} These studies have shown excellent inter-observer and cross platform agreement and close correlation of lesional mean QSM with actual iron concentration in resected CCM specimens and in phantoms.⁴⁰ They also demonstrated greater lesional QSM in lesions with higher DCEQP,³¹ and higher mean lesional QSM with increasing age and in lesions with past history of overt clinical hemorrhages.⁴¹ Changes in mean lesional QSM were independent of lesion size alone, and did not always reflect new bleeding by conventional MRI criteria.⁴¹ The results also confirmed higher background brain permeability, most notably in white matter away from lesions, in familial CCM cases as compared to sporadic subjects and non-CCM controls, consistent with germ line heterozygosity in the former cases.³⁰ There was higher background brain permeability in cases with past aggressive disease behavior, and lower permeability in subjects receiving statin therapy for incidental indications unrelated to CCM.³⁰ While confirming the likely validity of DCEQP and QSM, these studies did not address the critical questions of changes with disease activity in longitudinal study, hallmarks of biomarker sensitivity and specificity. In this report we attempt to describe the changes of these two imaging biomarkers in correlation with prospective clinical activity over time, and also query the predictive value of these biomarkers with subsequent clinical activity.

Our results illustrate that neither permeability in background brain or lesions at baseline, nor baseline QSM was predictive of subsequent lesional hemorrhage or growth. However, significant lesional permeability increases at follow-up correlated with interval hemorrhage or growth. This is consistent with the hypothesis that enhanced vascular permeability is associated with, and may drive CCM hemorrhagic proliferation. Similarly, mean lesional QSM increased in lesions manifesting clinical hemorrhage or growth, indicating greater deposit of magnetic susceptible iron products in unstable lesions. In the *stable* lesions, there was a measurable small but significant mean lesional QSM decrease likely representing the body's inherent mechanism of clearing iron deposit, or a change into a form with lesser magnetic susceptibility.⁴⁰ Sensitivity and specificity of DCEQP and QSM for unstable

lesions were good, and further enhanced when combining the two biomarkers, reaching 88% sensitivity and 100% specificity. It is unclear what is the meaning of some instances of observed increases in DCEQP or QSM in the absence of overt clinical instability, and whether this reflects occult hemorrhage not manifested clinically. It is also unclear why some lesions manifested overt clinical activity without significant detectable change on DCEQP or QSM. Sub-threshold changes with either technique may be postulated to carry clinical significance, undemonstrated in this study. The variable timing of imaging in relation to clinical activity may have also impacted on imperfect sensitivity or specificity of the two techniques.

New lesion formation, a significant feature of disease aggressiveness in familial cases,³⁶ was studied separately using DCEQP. In a previous study by our group, we had reported that familial CCM patients possess higher background brain permeability compared to patients with sporadic CCM lesion, a finding consistent with their germ line heterozygosity, and we showed that familial cases with more aggressive past disease manifestations (lesion burden and number of bleeds) manifested greater background brain permeability.³⁰ Results from the current study now illustrate prospectively that familial CCM patients who develop interval *de novo* lesion genesis have a significantly higher baseline brain permeability compared to other familial patients whose lesion burden remains stable. Additionally, we showed higher regional brain permeability than contralateral homologous regions in anatomical locations initially lacking CCM lesions by high MRI sensitivity technique, which later developed *de novo* lesions. This denotes a predictive and causative role of higher brain permeability in the development of new lesions. Unfortunately, the small number of cases with *de novo* lesion genesis did not allow meaningful analysis of sensitivity, specificity or positive predictive value.

We have pointed out the imperfect sensitivity and specificity, and unknown significance of sub-threshold changes in biomarker activity. The study was further limited by the small number of subjects with individual features of disease aggressiveness (hemorrhage versus growth and clinical correlates), and potential biases of referral and follow-up inherent to a single research site. Yet we achieved a notably high enrollment rate and follow-up among CCM subjects in our practice, and there were no major demographic or baseline differences among cases that would later behave as unstable or otherwise.

Of the patients who had multiple studies done within the first year, only roughly half had acceptable DCEQP scans and less than half of the patients had acceptable QSM follow-up scans to analyze. The reasons were multifactorial but most of the unusable scans were done at the beginning (within the first year) of this 4 years protocol. Once optimized, the unusable DCEQP and QSM scans were largely reduced. This learning period is quite common when a new technique is deployed. At the current time, all MRI technicians in our institution are comfortable with the technique and the standardized protocol.

An additional approximate 20 minutes of added in-scanner time are needed beyond a regular clinical MRI scan of the brain for both biomarker sequences, including image acquisition and set up. Both techniques can be applied to a regular 3T MRI unit without any instrument modifications. We utilized proprietary software for post-processing, although QSM analysis

software is available commercially through major MRI instrument manufacturers. We utilized a dual-observer approach for research rigor and other analyses of inter-observer and cross platform validation, although the post-processing would roughly require an additional 1 hour and can be performed by a trained imaging technician with access to a high speed computing/image processing platform. The added cost cannot be quantified at this time, as it will be dictated by bundling and reimbursement factors. Ultimately, as with many novel techniques, the generalizability to clinical settings will be dictated by the team's interests/expertise, volume of cases, etc.. This would be similar to MR spectroscopy and functional MRI, etc. which require a similar level of image acquisition and processing sophistication, and ultimately have found their roles at large specialized clinical centers.

Conclusions

This is the largest study of its kind, and the first report on prospectively enrolled, longitudinally assessed cases with CCM in correlation with novel mechanistically linked imaging biomarkers over time. The findings provide several first proofs of mechanistic concepts, and generate novel hypotheses for future testing of these techniques as quantifiable *in vivo* biomarkers of CCM disease activity. It remains to be shown if they will potentially aid in prognostication, tailoring management and monitoring therapeutic effects of novel therapies. DCEQP is most likely to be applied for dose calibration of novel permeability therapies.^{30,39,42,43} As well, preclinical studies have shown an impact of ROCK inhibition therapy on iron leak in CCM,²⁸ so the QSM technique may be used to assess potential therapeutic impact on hemorrhage in CCM lesions.

DCEQP and QSM sequences are likely to be applicable in other disorders of vascular integrity and iron leak such as aging, hemorrhagic microangiopathy and traumatic brain injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Illustration of assessment of brain permeability and QSM values in CCM patient. **(A)** The lesional permeability estimation is done by selecting the CCM lesion, including the hypo-intense hemosiderin rim, observed on T₂-weighted images acquired simultaneously then superimposed on the DCEQP post-processed maps. The normal background brain permeability is estimated by an average of 16 pixels square-shaped regions of interest (ROIs) for white matter near (WMN) and far (WMF) from CCM lesions, areas devoid of CCM lesions on any sequence. **(B)** In CCM familial patients harboring new lesions at the time of

the follow up, the new lesion area was identified using SWI or T₂-weighted images. Then on the prior scan, the permeability of the area where the lesion developed, and the symmetric contralateral area were assessed separately by averaging 10 to 50 ROIs of brain matter depending on the quantity of white matter available for analysis in the slice.

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FIG. 2.

CCM lesions with demonstrated growth or symptomatic hemorrhage (*unstable*) increase in lesional permeability and QSM values over time. The mean permeability doubled ($p=0.005$) in *unstable* lesions (A), in contrast to *stable* (B) and *recovering* (C) lesions, which showed no difference in permeability. Similarly, the lesional QSM value increased ($p=0.01$) in (D) *unstable* lesions, (E) slightly decreased ($p=0.002$) in *stable* lesions and was (F) unchanged in *recovering* lesions. All p values were considered to be statistically significant at $*p < 0.05$ or $**p < 0.01$.

FIG. 3.

The combination of DCQEP and QSM techniques are indicators of CCM lesional instability.

(A) The receiver operating characteristic (ROC) curve generated for percentage of permeability change was associated with lesional instability [area under curve (AUC)=0.81, $p=0.004$] with a sensitivity of 78.72% and specificity of 88.89% at a 39.59% threshold change. (B) Similarly, QSM change was associated with CCM instability (AUC=0.86, $p=0.0004$) with a sensitivity of 82.29% and specificity of 88.89% at a 5.81% threshold change. (C) The combination of the two biomarkers reached a better sensitivity (87.88%) and specificity (100%) to detect CCM lesional instability (AUC=0.94, $p=0.005$) (at a threshold of 65.43). All p values were considered to be statistically significant at $p < 0.05$.

FIG. 4.

Subsequent new lesion formation is associated with a higher background and local brain permeability at a baseline DCEQP exam. **(Left)** CCM familial patients harboring new lesions at the time of the follow-up exam showed higher mean background permeability ($p=0.0015$) at baseline examination than familial CCM cases without de novo lesion formation. **(Right)** Within the same patient at the time of the baseline exam, the region of new lesion formation demonstrated a higher permeability ($p=0.003$) than in the symmetric contralateral brain area. All p values were considered to be statistically significant at $**p<0.01$.

TABLE 1.

CCM patients baseline features and demographics

	Patient classification*				Recovering	New Lesion Formation
	Stable	Unstable				
Sample Size	26	11	9	15		
Mean age (years) ± SD	34.71 ± 19.93	29.79 ± 11.44	43.10 ± 18.96	35.38 ± 19.13		
Range (years)	13.49 – 50.75	21.63 – 35.80	26.65 – 58.09	13.49–55.90		
Genotype						
Familial (%)	15 (58)	9 (82)	7 (78)	15 (100)		
Sporadic (%) [‡]	11(42)	2 (18)	2 (22)	NA		
Mean time between scans (months) ± SD	10.67 ± 3.15	9.00 ± 4.21	9.05 ± 3.95	12.50 ± 0.66		
Ethnicity						
White/Caucasian (%) [‡]	19 (73)	7 (64)	7 (78)	11 (73)		
African American (%)	3 (12)	2 (18)	1 (11)	0		
Hispanic (%)	4 (15)	2 (18)	1 (11)	4 (27)		
Baseline Permeability values (ml/100g/min) ± SD						
Lesional	0.50 ± 0.38	0.33 ± 0.11	0.56 ± 0.64	NA		
WMF	0.17 ± 0.09	0.15 ± 0.08	0.17 ± 0.05	0.25 ± 0.03		
WMN	0.19 ± 0.14	0.15 ± 0.07	0.15 ± 0.13	NA		
Baseline Lesional QSM values (ppm) ± SD	0.38 ± 0.12	0.36 ± 0.15	0.44 ± 0.14	NA		

min = minute

ml = milliliter

NA = not applicable

ppm = parts per million

SD = standard deviation

WMF = white matter far

WMN = white matter near

ppm = parts per million

* If a patient harbors more than one lesion, classification was based on the most active lesion

[‡] Significant difference in proportions between stable and unstable ($p=0.02$), and between stable and recovering ($p=0.02$)