Vascular permeability and iron deposition biomarkers in longitudinal follow-up of cerebral cavernous malformations

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OBJECTIVE Vascular permeability and iron leakage are central features of cerebral cavernous malformation (CCM) pathogenesis. The authors 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 MRI.

METHODS Forty-six patients with CCMs 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 2.4–31.27 months). Based on clinical and imaging features, cases/lesions were classified as stable, unstable, or recovering. Associated and predictive changes in quantitative permeability and susceptibility were investigated.

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 mean 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 = 0.003), and did not significantly change in recovering lesions. Familial cases developing new lesions during the follow-up period showed a higher background brain permeability at baseline (p = 0.001), as well as higher regional permeability (p = 0.003) in the area that would later develop a new lesion as compared with the 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 2 techniques are likely applicable in other disorders of vascular integrity and iron leakage such as aging, hemorrhagic microangiopathy, and traumatic brain injury.

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KEY WORDS cerebral cavernous malformation; biomarker; dynamic contrast-enhanced quantitative permeability; quantitative susceptibility mapping; iron; vascular disorders
Cerebral cavernous malformations (CCMs) are a common neurovascular disorder affecting 0.5%–1% of the population. Two clinical subtypes have been described. Sporadic CCM accounts for almost two-thirds of cases and is characterized by a solitary lesion, frequently associated with a developmental venous anomaly.1,27,32 The familial type of CCM manifests multifocal lesions developing throughout life in different regions of the brain, in association with mutations at 3 identifiable gene loci that are 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 3 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 MRI sequences, sensitive to the paramagnetic effects of iron content in lesions, have greatly enhanced lesion detection.6,8

Our group had previously optimized and reported retrospective analyses of 2 novel imaging techniques, 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 In this paper we report the first prospective longitudinal analysis of these 2 biomarkers in relation to CCM clinical behavior. We hypothesized 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 was to validate DCEQP and QSM as biomarkers of clinical behavior of CCM lesions.

Methods
Study Participants
This prospective case-controlled study included patients with confirmed sporadic or familial CCM disease of any genotype who consented to undergo 2 or more MRI studies involving DCEQP and QSM protocols in conjunction with their initial routine clinical and MRI evaluation, and later longitudinal follow-up evaluations at a single-site referral and clinical research center. The routine MRI sequences included T1-weighted pre- and postcontrast images, T2-weighted images, susceptibility-weighted imaging (SWI) images, and T2*-weighted images performed at 3-tesla with similar acquisition parameters as previously described.30,31,40,41,43 Patients with partial or complete CCM lesion resection or any prior brain irradiation were not included in this study. Written informed consent was obtained from all participants in accordance with 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 patients with CCMs 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, 4 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 2 consecutive imaging studies during follow-up ranged from 1.5 to 12.96 months (mean 10.26 months).

Of the above cases, 87 patients had 2 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, 5 (from 5 patients) due to head motion, and 1 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 patients with CCMs and at least 2 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 postprocessing procedures, and 12 (from 12 patients) because of non-CCM disease diagnosis. Finally, 33 patients with CCM and at least 2 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.1 CCM growth was identified as a change in lesion diameter by 2 or more millimeters on comparable T1- 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 SWI sequences.6,8

Data Acquisition and Processing
DCEQP and QSM scans were obtained in conjunction with routine clinical evaluation and follow-up MRI at 3-tesla field strength, obtained as part of the patients’ clinical care. Vascular permeability was measured via a DCEQP protocol involving a gadolinium-based dynamic scan following a precontrast T1-weighted scan (acquisition time 8 minutes, with another approximately 1 minute...
for setup, slice selection, and image alignment). Five axial slices were acquired during each scan. These data were then processed in MATLAB to generate and calculate a permeability map. Interobserver and other validations of this technique have been previously reported, including rationale and optimization of region of interest (ROI) selection in the lesion and background brain (white matter far from the lesion [WMF]).

QSM acquisitions were generated using a single 3D, multiecho, spoiled gradient recalled echo T2*-weighted sequence (acquisition time 11 minutes). A morphology enabled dipole inversion algorithm was used to reconstruct the QSM images. 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. ROIs were selected for both permeability and QSM as previously reported, and as illustrated in Fig. 1.

The DCEQP and QSM data sets were acquired and postprocessed by 2 experienced imaging scientists (R.G., H.T.) and 2 research clinical fellows (H.A.Z., A.G.M.) following an established protocol with high intra- and interobserver agreement. The operators were blinded to the clinical status of the patients throughout the image analysis. The senior author (I.A.A.), with experience in the treatment of CCM, reviewed and adjudicated the electronic medical records of the patients for abstracting in the database, and was blinded to the DCEQP and QSM results. Two clinical research fellows (M.D.F., H.A.Z.) classified patients into the respective categories based on information in the clinical database and prearticulated criteria (stable, unstable, or recovering), and they were also blinded to the DCEQP and QSM results. Finally, the individual stable, unstable, and recovering CCM lesions were matched on the baseline and follow-up scans conjointly by the experienced imaging scientist (R.G.) and clinical research fellows (M.D.F., H.A.Z.).

Statistical Analysis

The difference in age at baseline scan inclusion, the time between the 2 follow-up MRI scans protocols, and baseline permeability or QSM values among cases considered stable, unstable, or recovering were tested using an ANOVA. A chi-square test, or a Fisher’s exact test when the sample size was less than 5, was used to compare proportions in repartition in genotype and ethnicity between pairs of groups. Linear regression models were constructed to detect a modification of lesonal permeability and QSM in the 3 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 data set. A paired t-test compared the difference of mean lesonal permeability and QSM values, WMF, and white matter near the lesion (WMN) between baseline and follow-up scans in stable, unstable, and recovering groups, respectively. The F test evaluated the variances between 2 unpaired groups. The differences between these 2 groups were compared using a Student t-test with equal variances and Welch’s correction with unequal variances.

A linear combination of mean lesonal permeability percentage change and mean lesonal QSM percentage change was generated using the canonical linear discriminant analysis, with the following equation:

Combination = 0.30 × mean lesonal permeability % change + 1.45 × mean lesonal QSM % change.

Receiver operating characteristic (ROC) curves were generated and areas under the curves (AUCs) were calculated to evaluate mean lesonal permeability percentage change, mean lesonal QSM percentage change, and their...
combination’s ability to detect being unstable rather than stable. Predictive thresholds (cutoff values) for the unstable group were determined by the value achieving the best sensitivity and specificity together. The concordance (agreement) between leional permeability increase and QSM increase was assessed by the $\kappa$ test. $\kappa$ coefficients were calculated for quantifying interrater agreement and interpreted according to Landis and Koch criteria. $\kappa$ is a concordance index ranging from 0 to 1, with the maximum value of 1 indicating a perfect agreement, and 0 corresponding to a complete absence of agreement.

Statistical analyses were performed using SAS (version 9.4, SAS Institute Inc.) and GraphPad Prism (version 4.0, GraphPad Software Inc.). All $p$ values were considered to be statistically significant at $p < 0.05$. For more information on methods and materials, please refer to the Supplementary Material.

Results

Demographic and CCM Lesion Characteristics

In all, 46 patients had completed 2 or more technically satisfactory DCEQP or QSM studies, and were included in this analysis. Total follow-up durations of these patients ranged from 2.4 to 31.27 months (mean 12.05 months), with 11 patients undergoing more than 2 clinical and imaging follow-up evaluations. There were no significant differences in age at the time of the baseline scans among cases that would be classified as stable, unstable, or recovering, nor any significant differences in baseline permeability or QSM between the 3 groups (Table 1). The mean interval duration 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 leional 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.

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

Lesional Permeability and QSM in Unstable Lesions

There was a significant increase in mean leional permeability (Fig. 2A) between the 2 consecutive scans in unstable CCM lesions (+85.9%, $p = 0.005$, t-score = -3.38, degrees of freedom [df] = 8; 1-tailed paired t-test), with no significant differences observed in mean leional permeability in the stable and recovering cohorts (Fig. 2B and C).

An increase was also observed in mean leional susceptibility in unstable lesions (Fig. 2D; +44.1% change, $p = 0.01$, t-score = -2.68, df = 8; 1-tailed paired t-test). Stable lesions demonstrated a slight but significant decrease in leional susceptibility values (-3.2% change, $p = 0.003$, t-score = -2.86, df = 95; 1-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 percentage change across the 2 MRI scans in mean leional DCEQP and QSM values. The AUC was calculated to evaluate the accuracy of these 2 MRI techniques in association with leional instability. 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.

### Table 1. Baseline features and demographics of patients with CCM

<table>
<thead>
<tr>
<th>Patient Classification*</th>
<th>Stable</th>
<th>Unstable</th>
<th>Recovering</th>
<th>New Lesion Formation</th>
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</thead>
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<tr>
<td>Sample size</td>
<td>26</td>
<td>11</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Mean age (yrs) ± SD</td>
<td>34.71 ± 19.93</td>
<td>29.79 ± 11.44</td>
<td>43.10 ± 18.96</td>
<td>35.38 ± 19.13</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial (%)</td>
<td>15 (58)</td>
<td>9 (82)</td>
<td>7 (78)</td>
<td>15 (100)</td>
</tr>
<tr>
<td>Sporadic (%)†</td>
<td>11 (42)</td>
<td>2 (18)</td>
<td>2 (22)</td>
<td>NA</td>
</tr>
<tr>
<td>Mean time btwn scans (mos) ± SD</td>
<td>10.67 ± 3.15</td>
<td>9.00 ± 4.21</td>
<td>9.05 ± 3.95</td>
<td>12.50 ± 0.66</td>
</tr>
<tr>
<td>Ethnicity</td>
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<tr>
<td>White/Caucasian (%)†</td>
<td>19 (73)</td>
<td>7 (64)</td>
<td>7 (78)</td>
<td>11 (73)</td>
</tr>
<tr>
<td>African American (%)</td>
<td>3 (12)</td>
<td>2 (18)</td>
<td>1 (11)</td>
<td>0</td>
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<td>Hispanic (%)</td>
<td>4 (15)</td>
<td>2 (18)</td>
<td>1 (11)</td>
<td>4 (27)</td>
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<td>Baseline permeability values (ml/100 g/min) ± SD</td>
<td></td>
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<tr>
<td>Lesional</td>
<td>0.50 ± 0.38</td>
<td>0.33 ± 0.11</td>
<td>0.56 ± 0.64</td>
<td>NA</td>
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<tr>
<td>WMF</td>
<td>0.17 ± 0.09</td>
<td>0.15 ± 0.08</td>
<td>0.17 ± 0.05</td>
<td>0.25 ± 0.03</td>
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<tr>
<td>WMN</td>
<td>0.19 ± 0.14</td>
<td>0.15 ± 0.07</td>
<td>0.15 ± 0.13</td>
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<tr>
<td>Baseline leional QSM values (ppm) ± SD</td>
<td>0.38 ± 0.12</td>
<td>0.36 ± 0.15</td>
<td>0.44 ± 0.14</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not applicable, ppm = parts per million.
* If a patient harbored more than 1 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).
tively (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**

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 2 techniques, while the agreement was low in stable (\( \kappa \) coefficient = 0.14, \( z \)-score = 0.76, \( p = 0.45 \), df = 1) and recovering groups (\( \kappa \) coefficient = -0.17, \( z \)-score = -0.44, \( p = 0.66 \), df = 1). ROC analysis combining percentage change of mean lesional permeability and susceptibility values showed a better accuracy to detect unstable CCM lesions than either technique alone (AUC = 0.94, \( p = 0.005 \)), with enhanced sensitivity (87.88%) and specificity (100%; Fig. 3C).

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 significantly increased (\( p = 0.01 \)) in unstable lesions (D), slightly decreased (\( p = 0.002 \)) in stable lesions (E), and was unchanged in recovering lesions (F). All p values were considered to be statistically significant at *\( p < 0.05 \) or **\( p < 0.01 \).

FIG. 3. The combination of DCEQP and QSM techniques is an indicator of CCM lesional instability. A: The ROC curve generated for percentage of permeability change was associated with lesional instability (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 62.29% and specificity of 88.89% at a 5.81% threshold change. C: The combination of the 2 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 \( p < 0.05 \).
Brain Permeability in Cases With De Novo Lesion Formation

For this analysis, we considered the cohort that 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 significantly higher (p = 0.001, t = 3.327, df = 22; 1-tailed unpaired t-test) in patients who developed new lesions during the follow-up evaluation (Fig. 4A) compared with patients with familial CCM and no new lesion formation. Moreover, the baseline mean permeability was also significantly higher (p = 0.003, t-value = 3.55, df = 9; 1-tailed paired t-test) in the cerebral matter region where the lesion later developed compared with the symmetric contralateral brain region on the same scan (Fig. 4B). Please refer to the Supplementary Material for more information on results of background permeability in unstable patients.

Discussion

In the 20 years since the 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 1 of 3 known CCM genes, affecting all cells in the body, while lesional endothelial cells exhibit somatic bi-allelic homozygous mutations, consistent with the Knudsonian 2-hit hypothesis.2,10,12 Similar somatic bi-allelic mutations have been recently identified in sporadic CCM lesions, indicating a common genetic basis for lesion pathogenesis.27 The loss of any of the 3 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 an 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 Pharmacological ROCK inhibition has been shown to blunt CCM lesion development and hemorrhage in these models, as predicted mechanistically.26,28

Beyond these biological 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 vs CCM1 and CCM2) indeed manifest inherently more aggressive disease behavior.11,36 and proinflammatory genes may act as disease modifiers enhancing clinical penetrance in some familial cases.5 Lesions that have bleed 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 remain 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 leakage 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 patients with CCM disease.30,31,40,41 These studies have shown excellent interobserver and cross-platform agreement and close correlation of lesional mean QSM, with actual iron concentration in resected CCM specimens and in phantoms.26 These studies also demonstrated greater lesional QSM in lesions with higher DCEQP,31 and higher mean lesional QSM with increasing age and in lesions with a history of overt clini-
Changes in mean lesional QSM were independent of lesion size alone and did not always reflect new bleeding by conventional MRI criteria. The results also confirmed higher background brain permeability, most notably in white matter away from lesions, in familial CCM cases as compared with sporadic CCM cases and non-CCM controls, consistent with germ-line heterozygosity in the former cases. We found higher background brain permeability in cases with past aggressive disease behavior, and lower permeability in patients receiving statin therapy for incidental indications unrelated to CCM. 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 attempted to describe the changes of these 2 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 finding 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 deposits, or a change into a form with lesser magnetic susceptibility.

Sensitivity and specificity of DCEQP and QSM for unstable lesions were good, and further enhanced when combining the 2 biomarkers, reaching 88% sensitivity and 100% specificity. It is unclear as to 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. Subthreshold changes with either technique may be postulated to involve clinical significance, which was not demonstrated in this study. The variable timing of imaging in relation to clinical activity may have also affected the imperfect sensitivity or specificity of the 2 techniques.

New lesion formation, a significant feature of disease aggressiveness in familial cases, was studied separately using DCEQP. In a previous study by our group, we reported that patients with familial CCM possess higher background brain permeability compared with patients with sporadic CCM lesions, a finding consistent with their germ-line heterozygosity, and we showed that familial cases of CCM with more aggressive past disease manifestations (lesion burden and number of bleeds) manifested greater background brain permeability. Results from the current study now illustrate prospectively that patients with familial CCM who develop interval de novo lesion genesis have a significantly higher baseline brain permeability compared with other familial patients whose lesion burdens remain stable. Additionally, using a high MRI sensitivity technique, we showed higher regional brain permeability than contralateral homologous regions in anatomical locations initially lacking CCM lesions, 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 indicated the imperfect sensitivity and specificity, and unknown significance, of subthreshold changes in biomarker activity. The study was further limited by the small number of patients with individual features of disease aggressiveness (hemorrhage vs 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 patients with CCM 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 completed multiple studies within the first year, only approximately 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 completed at the beginning (within the first year) of this 4-year 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 time within the scanner is needed beyond a regular clinical MRI scan of the brain for both biomarker sequences, including image acquisition and setup. Both techniques can be applied to a regular 3-tesla MRI unit without any instrument modifications. We used proprietary software for postprocessing, although QSM analysis software is available commercially through major MRI instrument manufacturers. We used a dual-observer approach for research rigor and other analyses of interobserver and cross-platform validation, although the postprocessing would approximately require an additional 1 hour and could 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, which require a similar level of image acquisition and processing sophistication, and that 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 mechanisti-
cally 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 to dose calibration of novel permeability therapies. As well, preclinical studies have shown an impact of ROCK inhibition therapy on iron leakage such as aging, hemorrhagic microangiopathy, CCM lesions. DCEQP and QSM sequences are likely to be applicable in other disorders of vascular integrity and iron leakage such as aging, hemorrhagic microangiopathy, and traumatic brain injury.

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References


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

Author Contributions
Conception and design: all authors. Acquisition of data: Awad, Girard, Fam, Zeineddine, Tan, Mikati, Shi, Jesselson, Shenkar, Hobson, Larsson, Christoforidis. Analysis and interpretation of data: all authors. Drafting the article: all authors. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Statistical analysis: Wu, Cao. Study supervision: Awad.

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