Plasma Biomarkers of Inflammation and Angiogenesis Predict Cerebral Cavernous Malformation Symptomatic Hemorrhage or Lesional Growth

Short Communication

Romuald Girard,* Hussein A. Zeineddine,* Janne Koskimäki, Maged D. Fam, Ying Cao, Changbin Shi, Thomas Moore, Rhonda Lightle, Agnieszka Stadnik, Kiranj Chaudagar, Sean Polster, Robert Shenkar, Ryan Duggan, David Leclerc, Kevin J. Whitehead, Dean Y. Li, Issam A. Awad

Rationale: **The clinical course of cerebral cavernous malformations is highly unpredictable, with few cross-sectional studies correlating proinflammatory genotypes and plasma biomarkers with prior disease severity.**

Objective: **We hypothesize that a panel of 24 candidate plasma biomarkers, with a reported role in the physiopathology of cerebral cavernous malformations, may predict subsequent clinically relevant disease activity.**

Methods and Results: **Plasma biomarkers were assessed in nonfasting peripheral venous blood collected from consecutive cerebral cavernous malformation subjects followed for 1 year after initial sample collection. A first cohort (N=49) was used to define the best model of biomarker level combinations to predict a subsequent symptomatic lesional hemorrhagic expansion within a year after the blood sample. We generated the receiver operating characteristic curves and area under the curve for each biomarker individually and each weighted linear combination of relevant biomarkers. The best model to predict lesional activity was selected as that minimizing the Akaike information criterion. In this cohort, 11 subjects experienced symptomatic lesional hemorrhagic expansion (5 bleeds and 10 lesional growths) within a year after the blood draw. Subjects had lower soluble CD14 (cluster of differentiation 14; *P*=0.05), IL (interleukin)-6 (*P*=0.04), and VEGF (vascular endothelial growth factor; *P*=0.0003) levels along with higher plasma levels of IL-1β (*P*=0.008) and soluble ROBO4 (roundabout guidance receptor 4; *P*=0.03). Among the 31 weighted linear combinations of these 5 biomarkers, the best model (with the lowest Akaike information criterion value, 25.3) was the weighted linear combination including soluble CD14, IL-1β, VEGF, and soluble ROBO4, predicting a symptomatic hemorrhagic expansion with a sensitivity of 86% and specificity of 88% (area under the curve, 0.90; *P*<0.0001). We then validated our best model in the second sequential independent cohort (N=28).**

Conclusions: **This is the first study reporting a predictive association between plasma biomarkers and subsequent cerebral cavernous malformation disease clinical activity. This may be applied in clinical prognostication and stratification of cases in clinical trials. (*Circ Res*. 2018;122:1716-1721. DOI: 10.1161/CIRCRESAHA.118.312680.)**

Key Words: biomarkers ■ cerebrovascular disorders ■ hemangioma, cavernous, central nervous system ■ ROC curve ■ stroke

Cerebral cavernous malformations (CCMs) are a common cerebrovascular pathology predisposing 0.5% of the population, predisposing to a lifetime risk of intracerebral hemorrhage, seizures, and progressive focal neurological deficits.[1,2] A sporadic form of the disease, with solitary lesions, accounts for almost two thirds of cases, whereas a familial form manifests multifocal lesions developing throughout the patient's lifetime in different regions of the brain. The familial phenotype

Meet the First Author, see p 1626

Original received January 5, 2018; revision received April 25, 2018; accepted April 30, 2018. In March 2018, the average time from submission to first decision for all original research papers submitted to *Circulation Research* was 10.69 days.

From the Section of Neurosurgery, Department of Surgery, University of Chicago Medicine and Biological Sciences, IL (R.G., H.A.Z., J.K., M.D.F., Y.C., C.S., T.M., R.L., A.S., K.C., S.P., R.S., I.A.A.); Cytometry and Antibody Technology, Biological Sciences Division, Office of Shared Research Facilities, University of Chicago, IL (R.D., D.L.); and Division of Cardiology, Department of Medicine (K.J.W., D.Y.L.), University of Utah School of Medicine, Salt Lake City.

*These authors contributed equally to this article.

The online-only Data Supplement is available with this article at http://circres.ahajournals.org/lookup/suppl/doi:10.1161/CIRCRESAHA.118.312680/-/DC1.

Correspondence to Issam A. Awad, MD, Section of Neurosurgery, University of Chicago Medicine and Biological Sciences, 5841 S Maryland Ave, Chicago, IL 60637. E-mail iawad@uchicago.edu

Novelty and Significance

What Is Known?

- Cerebral cavernous malformation (CCM) is a common neurovascular pathology affecting 0.5% of the population worldwide.
- The clinical course of CCMs is highly unpredictable.
- The central and systemic inflammatory processes influence the pathogenesis and progression of CCMs.

What New Information Does This Article Contribute?

- It is the first study reporting a mathematical model predicting short-term subsequent clinical CCM lesional activity.
- This model may play a direct role in selecting patients with CCM for aggressive therapies and in the stratification of cohorts in clinical trials.
- These results may be critical to define biological targets for therapies.

Herein, we report a predictive association between plasma biomarkers and subsequent lesional clinical activity. Our results support an extensive literature on the influence of inflammatory and angiogenic processes in the pathobiology of CCM disease. They also define a likelihood-based computational model to predict short-term future clinical activity based on a panel of preselected inflammatory and angiogenic cytokines. If validated in multisite studies, this model may play a direct role in selecting patients for aggressive therapies and in the stratification of cohorts in clinical trials. The same approach may be useful in other cerebrovascular pathologies, including hemorrhagic microangiopathy, amyloid angiopathy, and aging, where similar mechanisms have been postulated.

Nonstandard Abbreviations and Acronyms

AUC	area under the curve
CCM	cerebral cavernous malformations
CD14	cluster of differentiation 14
IL	interleukin
ROBO4	roundabout guidance receptor 4
ROCK	RhoA-associated kinase
sCD14	soluble cluster of differentiation 14
sROBO4	soluble roundabout guidance receptor 4
VEGF	vascular endothelial growth factor

is associated with an autosomal dominant Mendelian inheritance with germ line mutations at 1 of the 3 *CCM* gene loci (*CCM1*/*KRIT1*, *CCM2*/*MGC4607*, and *CCM3*/*PDCD10*).[3]

The lesions harbored in the 2 forms are histologically indistinguishable and consist of clusters of thin-walled blood-filled vascular caverns lined by endothelium and lacking mature vessel wall angioarchitecture.[4,5] Endothelium of sporadic and familial lesions harbors somatic mutations in 1 of the 3 documented *CCM* genes, indicating common pathogenesis mechanisms.[5] *CCM* genes encode proteins involved in maintaining endothelial barrier integrity by inhibiting ROCK (RhoA-associated kinase) activation.[6] A loss of *CCM* gene function results in increased ROCK activity leading to endothelial cell–cell junction dysregulation and defective vascular permeability.[3] Experimental evidence also suggests a complex interplay between vascular permeability, inflammatory, and angiogenic processes in the neuroglial milieu as central features of CCM.[7]

Acute lesional bleeding or proliferative hemorrhagic expansion results in clinically significant sequelae. However, the molecular mechanisms underlying such relevant lesional activity remain unclear, and the behavior of CCMs remains highly variable and unpredictable. Proinflammatory gene variants have been associated with higher lesion counts in cases with common familial *CCM1* (Q455X) mutation.[8,9] Our group has also reported associations of brain vascular permeability[1,10] and clustered proinflammatory biomarkers in peripheral blood plasma[7,11] with cumulative CCM disease severity during a patient's life. Yet specific biomarker signatures predicting subsequent clinically relevant disease behavior would be the most relevant clinically and have remained elusive. Herein, we studied the prognostic association between the plasma levels of a panel of inflammatory and angiogenic proteins previously related to CCM disease pathobiology[7] and the occurrence of a CCM-related clinically significant event in the subsequent year.

Methods

Because of the sensitive nature of the data collected for this study, requests to access the data set from qualified researchers trained in human subject confidentiality protocols may be sent to the University of Chicago Medicine to I.A.A. (e-mail: iawad@uchicago.edu).

Patient Recruitment

From July 2014 to February 2018, 172 consecutive CCM subjects were evaluated clinically at a single referral center and enrolled in biomarker studies (www.uchospitals.edu/ccm). Of these, 77 CCM subjects (42 sporadic and 35 familial) were followed for 1 year (±30 days) after biomarker collection and considered as 2 independent cohorts based on their period of enrollment. The first cohort (group 1 algorithm definition) included 49 patients enrolled and biomarkers collected between July 2014 and May 2016. The second cohort entitled group 2 algorithm testing included an additional 28 patients enrolled between June 2016 and February 2018 (Online Figure I; Online Table I).

All patients involved in this study gave written informed consent in accordance to the Declaration of Helsinki and approved by the University of Chicago Institutional Review Board. The ethical principles guiding the institutional review board 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).

As per currently accepted disease categorization, cases were classified as sporadic if they harbored a solitary lesion on the most sensitive susceptibility-weighted magnetic resonance imaging sequences or a cluster of lesions associated with a developmental venous anomaly. They were classified as familial if they harbored multifocal CCMs, a family history of CCM in a first-degree blood relative, or a mutation genotyped at a CCM gene locus. Patients with partial or complete CCM lesion resection or any prior brain irradiation were excluded.[1,7,10]

The best weighted combination of biomarkers to predict subsequent disease activity within 1 year (±30 days) was defined in group 1 algorithm definition and then tested in group 2 algorithm testing.

For each patient enrolled in the study, the aforementioned features were assessed during the clinical follow-up visit. These were reviewed and adjudicated by the senior author with experience in the care of CCM (I.A.A.), blinded to any knowledge about the biomarker levels and electronically stored in a secure database for subsequent analysis. For more methodological details, refer to the Online Data Supplement.

Results

Demographic and CCM Lesion Characteristics

In the first cohort (group 1 algorithm definition), 11 of the 49 subjects manifested a hemorrhagic expansion. One subject experienced a new symptomatic hemorrhage from a known CCM per adjudicated criteria,[12] 5 developed lesional growth by >3 mm diameter on T_2-weighted magnetic resonance imaging sequences, and 5 had both symptomatic hemorrhage and lesional growth during the subsequent year after biomarker collection.[2,10] Thirty-eight subjects (78%) did not experience any change in CCM lesions during the same period and were defined as stable.

In the second cohort (group 2 algorithm testing), 7 of the 28 subjects experienced hemorrhagic expansion as described previously (2 subjects experienced a new symptomatic hemorrhage; 3, a lesional growth; and 2, both) within 1 year after biomarker collection.

Among the familial cohort, 8 subjects developed new lesions on the most sensitive magnetic resonance imaging susceptibility–weighted imaging. Cases with hemorrhagic expansion had higher T_2-weighted (P=0.001) and total (P=0.002) lesion counts and nonsignificant trends toward higher prevalence of brain stem lesion location and recent symptomatic hemorrhage in the prior year. There were no significant differences in the age, sex, or mean follow-up time between the stable subjects and the ones who experienced a hemorrhagic expansion within the year after the blood sample. However, the cohort of familial cases that developed new lesions within a year was older than familial patients that did not (P=0.02).

Soluble CD14 (cluster of differentiation 14; sCD14), VEGF (vascular endothelial growth factor), and IL (interleukin)-6 plasma levels were lower, whereas IL-1β and soluble ROBO4 (roundabout guidance receptor 4; sROBO4) were higher in subjects who experienced clinical lesional activity within the year after the initial blood sample.

After correction for batch effect when appropriate, subjects experiencing a hemorrhagic expansion showed lower plasma levels of sCD14 (P=0.05), IL-6 (P=0.04), and VEGF (P=0.0003), along with higher IL-1β (P=0.008) and sROBO4 (P=0.03) plasma levels (Online Figure II). There were no potential confounders affecting the plasma levels of the 24 biomarkers, such as age of enrollment, phenotype (solitary/sporadic or multifocal/familial), genotype (sporadic, *CCM1*, *CCM2*, or *CCM3*), or sex (male or female). In addition, plasma levels of these biomarkers were not associated with brain stem lesion location or symptomatic bleed within the preceding year, known risk factors of lesional activity.[4] We did not identify significant association between the formation of new CCMs and the plasma levels of any of the selected biomarkers in the familial cohort.

The combination of sCD14, IL-1β, VEGF, and sROBO4 is the best predictor of a future lesional activity.

The receiver operating characteristic curves for sCD14 (area under the curve [AUC], 0.68; P=0.04) and IL-6 (AUC, 0.66; P=0.007) showed poor accuracy. The accuracies to predict lesional activity were considered as fair for sROBO4 (AUC, 0.76; P=0.004) and VEGF (AUC, 0.77; P=0.01), whereas it was good for IL-1β (AUC, 0.82; P=0.0003; Online Figure II). Further analysis showed that among the 31 possible weighted linear combinations of these 5 biomarkers, the best model (Equation 1) defined by the lowest Akaike information criterion value (Akaike information criterion, 25.3; Figure [A]) was achieved by combining sCD14, IL-1β, VEGF, and sROBO4 (AUC, 0.90; P<0.0001).

$$-0.135\times[\text{sCD14}]+7.73\times[\text{IL-1}\beta]-0.775\times[\text{VEGF}]+0.658\times[\text{sROBO4}]$$

The receiver operating characteristic analysis differentiated subjects who experienced a lesional hemorrhagic expansion from stable subjects with a sensitivity of 86% and specificity of 88% (Figure [B]). Further analysis showed that the mean estimated combination value (Equation 1) was 5-fold higher (P<0.0001) in subjects who experienced hemorrhagic expansion (mean estimated value, 1.67±1.13) within the following year compared with subjects who remained

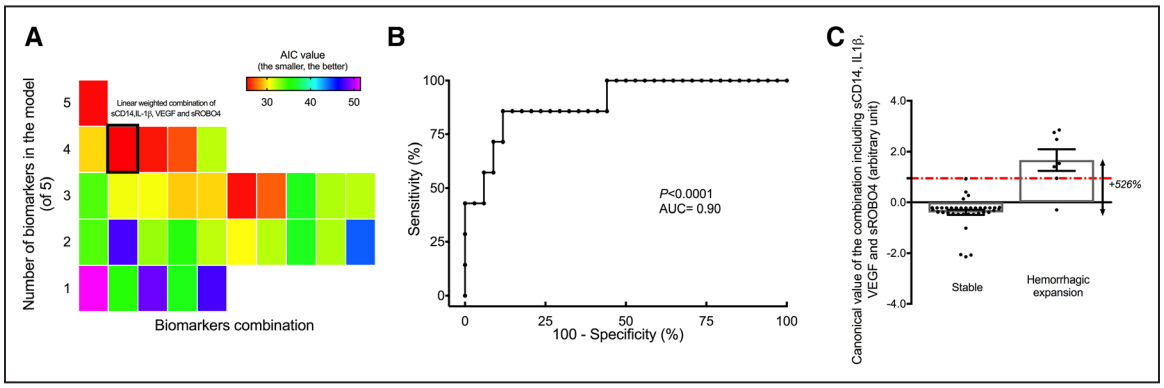

Figure. The linear combination, including soluble CD14 (soluble cluster of differentiation 14; sCD14), IL (interleukin)-1β, VEGF (vascular endothelial growth factor), and soluble ROBO4 (roundabout guidance receptor 4; sROBO4), was the best predictor of future lesional activity within 1 y. A, Each colored square represents an optimal weighted combination of biomarkers with its associated Akaike information criterion (AIC) value. The lowest AIC value (25.3) was achieved with –0.135×[sCD14]+7.73×[IL-1β]–0.775×[VEGF]+0.658×[sROBO4]. **B**, The receiver operating characteristic curve generated for the best linear combination was able to predict a clinical lesional activity within a year (±30 d; area under the curve [AUC], 0.90; P=0.0001) with a sensitivity of 86% and specificity of 88%. **C**, The mean estimated combination value was 5-fold higher in subjects who experienced a hemorrhagic expansion than stable cases (P<0.0001).

stable (mean estimated value, −0.39±0.59; Figure [C]). The mean estimated combination value calculated with the best model was also 3-fold higher ($P<0.01$) in patients of the independent cohort (ie, group 2 algorithm testing) who experienced a subsequent hemorrhagic expansion. In addition, the logistic regression analysis showed that the best linear combination was able to correctly predict the subsequent respective stable or hemorrhagic expansion status in 83.3% and 75.0% of the cases in the independent cohort (Online Table III). This result was also supported by receiver operating characteristic analysis, which showed 90% sensitivity and 71% specificity (Online Figure III) of the weighted biomarker equation predicting a hemorrhagic expansion within 1 year (±30 days) in the independent cohort.

For more results, refer to the Online Data Supplement.

Discussion

We evaluated the prognostic association between the plasma levels of 24 biomarkers and the occurrence of an impending clinically relevant lesional activity during the subsequent year. The biomarkers were selected based on a systematic literature review of demonstrated associations with relevant disease mechanisms in CCM or brain hemorrhage (Online Table IV).[7] Results showed that the plasma levels of sCD14, IL-1β, sROBO4, VEGF, and IL-6 were differently expressed in subjects who experienced a CCM-related event in the year after the initial blood sample. We then calculated the Akaike information criterion—a robust and traditional likelihood-based model selection—to determine the best linear combination to predict future lesional activity. The best model was achieved by the linear weighted combination, including sCD14, IL-1β, sROBO4, and VEGF.

The influence of proinflammatory genotypes and neuroinflammation in the physiopathology and clinical course of CCM disease has been described in recent years.[7–9] Single-nucleotide polymorphisms of *CD14*, *IL1B*, and *IL6* genes have been associated with aggressive phenotypes of hemorrhagic cerebrovascular disease, including CCM, brain arteriovenous malformation, and aneurysm.[8,9,13] Recently, Tang et al[9] in collaboration with our group showed that functional gene variants causing increased expression of CD14-anchored membrane glycoprotein are associated with greater lesion counts in familial CCM1 sharing the same mutation (Q455X). Here, we report lower levels of sCD14 as a predictor of subsequent lesional activity. The association between the levels of circulating CD14 and the anchored membrane form remains unclear and may not be correlated.[14] Higher levels of sCD14 may have anti-inflammatory effects by inhibiting lipopolysaccharide-mediated functional responses.[14–16]

IL-1β is a proinflammatory cytokine with a role in mediating inflammation-induced angiogenic responses that indirectly regulates the synthesis of proangiogenic factors and facilitates endothelial cell migration, proliferation, and organization into blood vessel-like structures.[17,18] IL-1β has also been shown to increase the endothelial permeability promoting leukocyte transmigration via multiple direct and indirect pathways.[19,20] We recently reported that an increased permeability assessed by magnetic resonance imaging at follow-up correlated with symptomatic lesional hemorrhage or growth.[10] IL-6 is a multifunctional cytokine mediating proinflammatory and anti-inflammatory processes, as well as regenerative, neural, and metabolic pathways.[21] As with *CD14*, SNP (single nucleotide polymorphisms) in *IL6R* gene has been correlated with a greater number of CCM lesions.[8] The roles of IL-6 versus IL-6 receptor in CCM lesion development and hemorrhage will require further investigation. Our results showing higher IL-1β and lower IL-6 predicting subsequent CCM clinical activity support the hypothesis that an increased endothelial permeability may occur via inflammatory mechanisms, resulting in imminent symptomatic lesional bleeding or expansion.

ROBO4 is an endogenous inhibitor of VEGF signaling expressed by vascular endothelial cells.[22,23] This protein has been shown to dynamically maintain vascular network stability, during pathological angiogenesis and proinflammatory processes[24–26] by modulating the expression of tight junction proteins, such as ZO-1 (zona occludens-1), occludin, and claudin-5.[27] In CCM disease, lesions harbor structurally defective tight junctions[28] and decreased mRNA expression of occludin, claudin-5, and ZO-1.[29] An increase in sROBO4 may reflect proinflammatory processes enhancing endothelial permeability, consistent with its prognostic association with CCM bleeding and growth.

VEGF has direct mitogenic effects on endothelial cells and is a key regulator of angiogenesis.[17] Dysregulation of VEGF expression has been widely studied in CCM disease and has been shown to worsen the blood–brain barrier permeability and promote progression of CCM lesions.[6,30] A modulatory effect of VEGF in the hemorrhagic brain has also been described.[7,31,32] However, no difference in lesional VEGF expression was observed in a study comparing unstable versus CCM lesions resected surgically.[33] Here, we observed that decreased plasma level of VEGF is a predictor of subsequent lesional hemorrhage or growth in CCM disease. This is somewhat counterintuitive and motivates a hypothesis about how lower VEGF plasma levels might reflect an imbalance in vascular integrity, associated with subsequent aggressive lesion behavior.

The combined model including weighted contribution of 4 biomarkers sCD14, IL-1β, VEGF, and sROBO4 was the best predictor of future lesional activity. The absence of co-correlation among these 4 protein levels suggests that their relative contribution to CCM lesional activity is independent and additive (Online Table V).

Previous studies had shown that brain stem lesion location and recent symptomatic hemorrhage are predictors of future bleeding risk.[4] Also, familial cases with aggressive genotype are associated with greater lesion burden and hemorrhagic risk.[3] In our study, patients with these risk factors were also more likely to manifest prospective symptomatic hemorrhage or lesional growth. However, we noted no association of the biomarkers with these or other baseline clinical features of disease, suggesting that the biomarker prognostication was independent of these clinical features. To best explore these interactions, future studies will need to be powered to detect prognostic associations of the weakest biomarker in the smallest relevant disease subgroup.

None of the biomarkers showed association with de novo lesion genesis in familial cases. We had previously shown an association of focal brain vascular hyperpermeability with

subsequent lesion genesis in that same brain region.[10] Our study included a small number of familial cases with lesion growth. If confirmed in a larger cohort, it is possible that such focal brain vascular hyperpermeability may not be reflected in our assayed biomarkers.

Our single-site study does not exclude referral bias, and cases with biomarker sampling may have undergone lesion resection or otherwise not been available for prospective follow-up at our center. These cases without follow-up did not have significantly different biomarker levels or clinical features, reassuring us that follow-up bias did not likely impact our results. Beyond larger sample sizes, with sufficient number of cases in relevant subgroups, future multisite studies will be designed to better control for these potential biases.

Notwithstanding these limitations, this study is the largest to date in this rare disease, and this is the first report of a strong prognostic association of a peripheral blood biomarker with clinical activity in a defined cerebrovascular pathology. We will pursue validation of these results in conjunction with multisite clinical trial readiness project already underway, funded by the United States National Institutes of Health, and in other prospective question-driven studies. These will consider specific clinical scenarios and risk versus benefit of biomarker clinical application.[34] In addition, the assay methodologies and batch effect correction have generated arbitrary units that could not be extended directly into clinical practice. Future studies will assess the differences in plasma levels between healthy controls and cohorts of patients with CCM with stable and unstable clinical activity, providing reference ranges of biomarker values applicable to positive and negative clinical prognostic risk.

The correlations herein do not imply a specific causality related to CCM disease, but they generate cogent hypotheses about mechanism of disease risk, to be pursued in future laboratory and clinical studies. Finally, the chosen biomarkers were selected based on current knowledge about CCM disease mechanims.[17] More recent investigations have highlighted additional key molecules involved in pathobiology.[9,35] Respective biomarkers related to these novel mechanisms may further refine the predictive power of clinical behavior in this disease.

Summary

Our results support an extensive literature on the influence of inflammatory and angiogenic processes in the pathobiology of CCM disease. They define a mechanistic-based conceptual model to predict short-term future clinical activity based on a panel of inflammatory and angiogenic cytokines. If validated in multisite studies, this model may play a direct role in selecting patients for aggressive therapies and in the stratification of cohorts in clinical trials. This same approach may be useful in other cerebrovascular pathologies, including hemorrhagic microangiopathy, amyloid angiopathy, and aging where similar mechanisms have been postulated.[36]

Acknowledgments

We would like to thank Mark H. Ginsberg from the University of California, San Diego for helpful discussions.

Sources of Funding

This work was partially supported by a grant from the National Institutes of Health (R21NS087328) to I.A. Awad, by the University of Chicago Medicine Comprehensive Cancer Center Support Grant (P30 CA14599), by the William and Judith Davis Fund in Neurovascular Surgery Research, and by the Safadi Translational Fellowship to R. Girard. Funding sources played no role in the formulation of research questions or the interpretation of results.

Disclosures

None.

References

1. Mikati AG, Khanna O, Zhang L, Girard R, Shenkar R, Guo X, Shah A, Larsson HB, Tan H, Li L, Wishnoff MS, Shi C, Christoforidis GA, Awad IA. Vascular permeability in cerebral cavernous malformations. *J Cereb Blood Flow Metab*. 2015;35:1632–1639. doi: 10.1038/jcbfm.2015.98.
2. Akers A, Al-Shahi Salman R, A Awad I, et al. Synopsis of guidelines for the clinical management of cerebral cavernous malformations: consensus recommendations based on systematic literature review by the Angioma Alliance Scientific Advisory Board Clinical Experts Panel. *Neurosurgery*. 2017;80:665–680. doi: 10.1093/neuros/nyx091.
3. Shenkar R, Shi C, Rebeiz T, et al. Exceptional aggressiveness of cerebral cavernous malformation disease associated with PDCD10 mutations. *Genet Med*. 2015;17:188–196. doi: 10.1038/gim.2014.97.
4. Al-Shahi Salman R, Hall JM, Horne MA, Moultrie F, Josephson CB, Bhattacharya JJ, Counsell CE, Murray GD, Papanastassiou V, Ritchie V, Roberts RC, Sellar RJ, Warlow CP; Scottish Audit of Intracranial Vascular Malformations (SAIVMs) Collaborators. Untreated clinical course of cerebral cavernous malformations: a prospective, population-based cohort study. *Lancet Neurol*. 2012;11:217–224. doi: 10.1016/S1474-4422(12)70004-2.
5. McDonald DA, Shi C, Shenkar R, Gallione CJ, Akers AL, Li S, De Castro N, Berg MJ, Corcoran DL, Awad IA, Marchuk DA. Lesions from patients with sporadic cerebral cavernous malformations harbor somatic mutations in the CCM genes: evidence for a common biochemical pathway for CCM pathogenesis. *Hum Mol Genet*. 2014;23:4357–4370. doi: 10.1093/hmg/ddu153.
6. Whitehead KJ, Chan AC, Navankasattusas S, Koh W, London NR, Ling J, Mayo AH, Drakos SG, Jones CA, Zhu W, Marchuk DA, Davis GE, Li DY. The cerebral cavernous malformation signaling pathway promotes vascular integrity via Rho GTPases. *Nat Med*. 2009;15:177–184. doi: 10.1038/nm.1911.
7. Girard R, Zeineddine HA, Fam MD, et al. Plasma biomarkers of inflammation reflect seizures and hemorrhagic activity of cerebral cavernous malformations. *Transl Stroke Res*. 2018;9:34–43. doi: 10.1007/s12975-017-0561-3.
8. Choquet H, Pawlikowska L, Nelson J, McCulloch CE, Akers A, Baca B, Khan Y, Hart B, Morrison L, Kim H; Brain Vascular Malformation Consortium (BVMC) Study. Polymorphisms in inflammatory and immune response genes associated with cerebral cavernous malformation type 1 severity. *Cerebrovasc Dis*. 2014;38:433–440. doi: 10.1159/000369200.
9. Tang AT, Choi JP, Kotzin JJ, et al. Endothelial TLR4 and the microbiome drive cerebral cavernous malformations. *Nature*. 2017;545:305–310. doi: 10.1038/nature22075.
10. Girard R, Fam MD, Zeineddine HA, Tan H, Mikati AG, Shi C, Jesselson M, Shenkar R, Wu M, Cao Y, Hobson N, Larsson HBW, Christoforidis GA, Awad IA. Vascular permeability and iron deposition biomarkers in longitudinal follow-up of cerebral cavernous malformations. *J Neurosurg*. 2017;127:102–110. doi: 10.3171/2016.5.JNS16687.
11. Girard R, Khanna O, Shenkar R, Zhang L, Wu M, Jesselson M, Zeineddine HA, Gangal A, Fam MD, Gibson CC, Whitehead KJ, Li DY, Liao JK, Shi C, Awad IA. Peripheral plasma vitamin D and non-HDL cholesterol reflect the severity of cerebral cavernous malformation disease. *Biomark Med*. 2016;10:255–264. doi: 10.2217/bmm.15.118.
12. Al-Shahi Salman R, Berg MJ, Morrison L, Awad IA; Angioma Alliance Scientific Advisory Board. Hemorrhage from cavernous malformations of the brain: definition and reporting standards. Angioma Alliance Scientific Advisory Board. *Stroke*. 2008;39:3222–3230. doi: 10.1161/STROKEAHA.108.515544.
13. Kim H, Hysi PG, Pawlikowska L, Poon A, Burchard EG, Zaroff JG, Sidney S, Ko NU, Achrol AS, Lawton MT, McCulloch CE, Kwok PY, Young WL.

Common variants in interleukin-1-Beta gene are associated with intracranial hemorrhage and susceptibility to brain arteriovenous malformation. *Cerebrovasc Dis*. 2009;27:176–182. doi: 10.1159/000185609.
14. Lloyd-Jones KL, Kelly MM, Kubes P. Varying importance of soluble and membrane CD14 in endothelial detection of lipopolysaccharide. *J Immunol*. 2008;181:1446–1453.
15. Lévêque M, Simonin-Le Jeune K, Jouneau S, Moulis S, Desrues B, Belleguic C, Brinchault G, Le Trionnaire S, Gangneux JP, Dimanche-Boitrel MT, Martin-Chouly C. Soluble CD14 acts as a DAMP in human macrophages: origin and involvement in inflammatory cytokine/chemokine production. *FASEB J*. 2017;31:1891–1902. doi: 10.1096/fj.201600772R.
16. Haziot A, Rong GW, Bazil V, Silver J, Goyert SM. Recombinant soluble CD14 inhibits LPS-induced tumor necrosis factor-alpha production by cells in whole blood. *J Immunol*. 1994;152:5868–5876.
17. Voronov E, Carmi Y, Apte RN. The role IL-1 in tumor-mediated angiogenesis. *Front Physiol*. 2014;5:114. doi: 10.3389/fphys.2014.00114.
18. Carmi Y, Voronov E, Dotan S, Lahat N, Rahat MA, Fogel M, Huszar M, White MR, Dinarello CA, Apte RN. The role of macrophage-derived IL-1 in induction and maintenance of angiogenesis. *J Immunol*. 2009;183:4705–4714. doi: 10.4049/jimmunol.0901511.
19. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. *Neurobiol Dis*. 2010;37:13–25. doi: 10.1016/j.nbd.2009.07.030.
20. Wang Y, Jin S, Sonobe Y, Cheng Y, Horiuchi H, Parajuli B, Kawanokuchi J, Mizuno T, Takeuchi H, Suzumura A. Interleukin-1β induces blood-brain barrier disruption by downregulating Sonic hedgehog in astrocytes. *PLoS One*. 2014;9:e110024. doi: 10.1371/journal.pone.0110024.
21. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta*. 2011;1813:878–888. doi: 10.1016/j.bbamcr.2011.01.034.
22. Acevedo LM, Weis SM, Cheresh DA. Robo4 counteracts VEGF signaling. *Nat Med*. 2008;14:372–373. doi: 10.1038/nm0408-372.
23. Koch AW, Mathivet T, Larrivée B, et al. Robo4 maintains vessel integrity and inhibits angiogenesis by interacting with UNC5B. *Dev Cell*. 2011;20:33–46. doi: 10.1016/j.devcel.2010.12.001.
24. London NR, Li DY. Robo4-dependent Slit signaling stabilizes the vasculature during pathologic angiogenesis and cytokine storm. *Curr Opin Hematol*. 2011;18:186–190. doi: 10.1097/MOH.0b013e328345a4b9.
25. Yadav SS, Narayan G. Role of ROBO4 signalling in developmental and pathological angiogenesis. *Biomed Res Int*. 2014;2014:683025. doi: 10.1155/2014/683025.
26. Shirakura K, Ishiba R, Kashio T, Sakai M, Fukushima Y, Yamamoto N, Manabe S, Shigesada N, Tanaka T, Hino N, Aird WC, Doi T, Okada Y. Endothelial Robo4 regulates IL-6 production by endothelial cells and monocytes via a crosstalk mechanism in inflammation. *Biochem Biophys Res Commun*. 2018;495:801–806. doi: 10.1016/j.bbrc.2017.11.067.
27. Cai H, Liu W, Xue Y, Shang X, Liu J, Li Z, Wang P, Liu L, Hu Y, Liu Y. Roundabout 4 regulates blood-tumor barrier permeability through the modulation of ZO-1, occludin, and claudin-5 expression. *J Neuropathol Exp Neurol*. 2015;74:25–37. doi: 10.1097/NEN.0000000000000146.
28. Dejana E, Tournier-Lasserve E, Weinstein BM. The control of vascular integrity by endothelial cell junctions: molecular basis and pathological implications. *Dev Cell*. 2009;16:209–221. doi: 10.1016/j.devcel.2009.01.004.
29. Schneider H, Errede M, Ulrich NH, Virgintino D, Frei K, Bertalanffy H. Impairment of tight junctions and glucose transport in endothelial cells of human cerebral cavernous malformations. *J Neuropathol Exp Neurol*. 2011;70:417–429. doi: 10.1097/NEN.0b013e31821bc40e.
30. Zhu Y, Wu Q, Fass M, Xu JF, You C, Müller O, Sandalcioglu IE, Zhang JM, Sure U. In vitro characterization of the angiogenic phenotype and genotype of the endothelia derived from sporadic cerebral cavernous malformations. *Neurosurgery*. 2011;69:722–731; discussion 731. doi: 10.1227/NEU.0b013e318219569f.
31. Matsuo R, Ago T, Kamouchi M, et al. Clinical significance of plasma VEGF value in ischemic stroke - research for biomarkers in ischemic stroke (REBIOS) study. *BMC Neurol*. 2013;13:32. doi: 10.1186/1471-2377-13-32.
32. Jung KH, Chu K, Jeong SW, Park HK, Bae HJ, Yoon BW. Cerebral cavernous malformations with dynamic and progressive course: correlation study with vascular endothelial growth factor. *Arch Neurol*. 2003;60:1613–1618. doi: 10.1001/archneur.60.11.1613.
33. Maiuri F, Cappabianca P, Gangemi M, De Caro Mdel B, Esposito F, Pettinato G, de Divitiis O, Mignogna C, Strazzullo V, de Divitiis E. Clinical progression and familial occurrence of cerebral cavernous angiomas: the role of angiogenic and growth factors. *Neurosurg Focus*. 2006;21:e3.
34. Leptak C, Menetski JP, Wagner JA, et al. What evidence do we need for biomarker qualification? *Sci Transl Med*. 2017;9:eaal4599. doi: 10.1126/scitranslmed.aal4599.
35. Zhou Z, Tang AT, Wong WY, et al. Cerebral cavernous malformations arise from endothelial gain of MEKK3-KLF2/4 signalling. *Nature*. 2016;532:122–126. doi: 10.1038/nature17178.
36. Poggesi A, Pasi M, Pescini F, Pantoni L, Inzitari D. Circulating biologic markers of endothelial dysfunction in cerebral small vessel disease: a review. *J Cereb Blood Flow Metab*. 2016;36:72–94. doi: 10.1038/jcbfm.2015.116.