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THROMBOSIS AND HEMOSTASIS

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New plugs for CCM bleeds

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In this issue of *Blood*, Lopez-Ramirez et al link hemorrhage associated with cerebral cavernous malformations (CCMs) to anticoagulant properties of lesion endothelial cells, suggesting novel ways to attenuate accompanying seizures and stroke.[1]

CCMs are central nervous system vascular lesions that affect ~1 in 200 humans. They are characterized by abnormal blood flow and hemorrhage that can lead to neurologic impairment, seizures, and stroke. Most CCM research has focused on the underlying genetic mutations and the cellular mechanisms leading to endothelial cell dysfunction, which results in the dilated and tortuous vessels that characterize the lesions.[2] It has been assumed that hemorrhage results from defective endothelial cell junctions (ie, a leaky bag) and/or rupture as a result of fragile vessels and disrupted blood flow. An intriguing new study by Lopez-Ramirez et al shifts focus to the coagulant properties of endothelial cells and shows that CCM lesions have increased anticoagulant proteins that contribute to the bleeding phenotype and may provide novel therapeutic targets.

CCM disease results from mutations in 1 of 3 genes (*CCM1/Krit1*, *CCM2*, *CCM3/PDCD10*) that are inherited as a single mutant allele in the familial form, with a second hit leading to somatic mutation of the remaining normal allele. The more common sporadic form of the disease also results from somatic mutation of *CCM* genes.[3] *CCM* mutations affect endothelial cell junctions, and evidence supports some form of transition from stable endothelium to a more active and migratory state called endothelial-to-mesenchymal transition (EndoMT) in CCM lesions.[4] A previous genome-wide transcriptomic analysis by the group[5] revealed that 2 components of a pathway that prevents blood clotting were increased in endothelial cells from mice carrying a *CCM1* mutation, *Thbd*, and endothelial protein C receptor (EPCR). *Thbd* encodes thrombomodulin (TM), a protein that binds to thrombin, prevents conversion of fibrinogen into insoluble fibrin, and enhances production of activated protein C (APC); EPCR also enhances generation of APC. APC is a potent natural anticoagulant, which led the authors to hypothesize that CCM-induced increase in endothelial cell anticoagulant status contributes to the hemorrhage associated with morbidity and mortality.

The authors first examined patient samples and found increased plasma levels of soluble TM in patients with CCM and increased TM and EPCR RNA and protein in human CCM lesions. They then analyzed 2 mouse models that recapitulate aspects of *CCM1* (*Krit1*^{iECKO} [inducible endothelial cell-knockout]) or *CCM3* (*Pdcd10*^{iECKO}) and found changes in the CCM lesions similar to those seen in humans. Moreover, the degree of hemorrhage correlated with TM levels in murine lesions, which suggested a causal relationship in vivo. Because the transcription factors KLF2 and KLF4 are known to regulate endothelial cell anticoagulant gene expression[6,7] and are upregulated in CCM,[8,9] the authors next asked whether these factors are upstream of anticoagulant expression using human umbilical vein and brain endothelial cells. They found that overexpression of both KLF factors upregulated TM but not EPCR, whereas reduced KLF levels prevented increased TM expression induced by reducing KRIT1 protein levels. To bring the study back into the mouse, the authors showed that reducing endothelial cell TM levels via gene inactivation in a *CCM* background (*Pdcd10*^{iECKO}; *Thbd*^{iECKO}) partially rescued the bleeding phenotype of the mice, and neutralizing antibodies against either TM or EPCR had a similar effect.

These exciting results suggest that, although the etiology of CCM resides in protein complexes that affect the stability of endothelial cell junctions, the effects of the mutations on other endothelial cell functions such as coagulation are also important and may be worth considering as novel therapeutic avenues. In this light, it is especially intriguing that systemic administration of blocking antibodies for TM or EPCR to mice partially rescued the bleeding phenotype that is thought to be causative of neurologic symptoms and morbidity in human patients. This work leads to several new questions that are important for a better understanding of the disease and for considering therapeutic interventions. Identification of upstream EPCR regulators will help us better understand how anticoagulant activity is regulated in CCM lesions. Although APC was increased in cultured endothelial cells with reduced KRIT1 protein levels, this study did not investigate the effect of protein C neutralization on CCM in vivo. Because APC is downstream of increased TM and EPCR in CCM, it may be a more effective therapeutic target. It will be important to determine whether systemic blockade of the anticoagulant pathway leads to abnormal coagulation in other parts of the body, which could complicate the use of anticoagulant blockers in managing CCM. The results from this study suggest that soluble TM may be a useful biomarker for CCM in human patients, especially given that imaging is the current modality for screening. Finally, taking a page from cancer therapeutics, a combination of both anticoagulant blockers and agents that restore endothelial

junction integrity may be more potent at preventing hemorrhage than single-modality therapeutics. For example, the RhoA pathway is activated downstream of *CCM* mutations to disrupt endothelial cell junction integrity,[10] so combinatorial treatment with RhoA pathway inhibitors and anticoagulant blockers may enhance treatment efficacy. Regardless, this new study suggests potential novel avenues for managing CCM, because hemorrhage is the proximal event leading to the most devastating sequelae of CCM disease.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Lilleness et al, page 215

AL amyloidosis cardiac staging updated using BNP

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The inability to perform gold-standard testing for cardiac involvement in immunoglobulin light chain amyloidosis (AL amyloidosis) remains a barrier in many centers as they try to rapidly identify and prognosticate the highest-risk patients. In this issue of *Blood*, Lilleness and colleagues sought to update the Mayo 2004 cardiac staging system by examining the utility of substituting the established N-terminal pro-brain natriuretic peptide (NT-proBNP) with the more widely available brain natriuretic peptide (BNP).[1] Importantly, the Boston University (BU) staging system defined in their article was built on a more contemporary cohort treated in an era in which proteasome inhibitors are now a mainstay of first-line therapy, and more stringent patient selection has optimized the use of high-dose chemotherapy and autologous stem cell transplantation.

The severity of cardiac involvement remains the most important prognostic factor in AL amyloidosis. Despite advances in therapy, many patients never live long enough to benefit from these improvements because of early cardiac death. More rapid determination of cardiac involvement helps identify those at highest risk, thus providing an opportunity to begin more aggressive supportive care strategies and even modify a given chemotherapeutic approach. Early work by the group at Mayo established the first broadly applicable tool to risk stratify patients. However, its dependence on NT-proBNP does limit its usefulness because many sites cannot run the test. Although BNP is more widely available, it cannot act directly as a substitute, hence the importance of the work presented by Lilleness et al.[2]

In the Lilleness et al study, the first step was to use a derivation cohort to identify the BNP level that best correlated with the NT-proBNP level of 332 pg/mL established in the original Mayo criteria.[3] Second, the cohort was used to determine a BNP cutoff indicative of cardiac involvement. Importantly, the authors defined that cutoff by incorporating modern diagnostics, including cardiac magnetic resonance imaging (MRI) and more detailed echocardiographic criteria now commonly used in practice but not captured in the 2005 consensus criteria.[4] It reflects a broader and more clinically relevant spectrum of cardiac involvement known to occur in AL amyloidosis.

Acknowledging the relative interchangeability of cardiac troponin I (TnI) and troponin T (TnT) established in the original Mayo publication, the authors chose the TnI threshold of 0.1 ng/mL as a partner for the BNP vs NT-proBNP analysis. Examining a cohort of 250 consecutively evaluated patients that had both NT-proBNP and BNP levels drawn at diagnosis, the BNP threshold of 81 pg/mL was identified as a match for the NT-proBNP level of 332 pg/mL noted in the original Mayo 2004 criteria. Using the same derivation cohort, the authors then went on to define both the BNP and NT-proBNP thresholds that correlate with cardiac involvement. Interestingly, the same BNP cutoff of 81 pg/mL was predictive of light chain cardiac deposition. In contrast, an NT-proBNP cutoff of 288 pg/mL was determined to be indicative of cardiac involvement, which was slightly lower than the level used for stratification in the Mayo staging system. This may be related to the initial study being designed primarily around survival rather than cardiac involvement as well as being done in an era without widespread availability of cardiac MRI.[4] Thus, both of these values may be useful as an initial screen at diagnosis to predict whether the heart might be involved and prompt more detailed workup when required.