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Incident Risk and Progression of Cerebral Microbleeds in Healthy Adults: A Multi-Occasion Longitudinal Study

Ana M. Daugherty, Ph.D.^{a,*} and Naftali Raz, Ph.D.^{b,c}

^aBeckman Institute for Advanced Science and Technology, University of Illinois Urbana-Champaign, 405 N Matthews Dr., Urbana, IL 61801

^bDepartment of Psychology and Institute of Gerontology, Wayne State University, 87 E. Ferry St., 226 Knapp Bldg., Detroit, MI 48201

^cMax Planck Institute for Human Development, Lentzeallee, 94, Berlin D-14195, Germany

Abstract

Decline in cerebrovascular health complicates brain aging and development of cerebral microbleeds (CMBs) is one of its prominent indicators. In a large sample of healthy adults (N = 251, age 18–78 years at baseline, 70% women), the contributions of chronological age and vascular health indicators to risk of developing a CMB was examined in a prospective eight-year longitudinal study, as well as change in CMB size and iron content from susceptibility weighted imaging. Twenty-six persons (10.4%), most of whom were 40 years of age or older, had at least one CMB during the study. Older age was associated with greater risk for developing a CMB (odds ratio 1.03), and elevation of combined metabolic syndrome indicators ($b = 0.15$, $p = 0.001$) that conferred additional risk (odds ratio 1.02). High body mass index worsened risk associated with poor vascular health ($b = 0.75$, $p < 0.001$) and frequent exercise mitigated it ($b = -0.46$, $p = 0.03$). CMBs persisted over time, yet their volume decreased (mean change = -0.32 , $p < 0.05$), while their relative iron content remained stable (mean change = -0.14 , $p = 0.05$). We conclude that although developing a CMB is unlikely during normal aging, risk increases with declining vascular health, which is modifiable via behavioral and pharmaceutical intervention.

Keywords

aging; metabolic syndrome; inflammation; latent variable modeling; susceptibility weighted imaging

*Corresponding author: adaugher@illinois.edu; phone: +01 217-244-1176; fax: +01 217-333-2922.

Conflicts of interest: none.

The study was approved by the Wayne State University Institutional Review Board and conducted in accordance with guidelines and procedures for human subject safety. All participants provided written informed consent.

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1.0 Introduction

¹ Brain aging is complicated by decline in cerebrovascular health (Raz and Rodrigue, 2006; Warsch and Wright, 2010) and one of its prominent indicators is development of cerebral microbleeds (CMBs)—deposits of blood within the brain, typically no more than 10 mm in diameter. CMB incidence in the general population is low (5–38%) but increases with advanced age and frailty (Greenberg et al., 2009b; Yates et al., 2014; Chung et al., 2016). As compared to healthy adults, CMBs are more prevalent in patients with cognitive disorders—e.g., vascular dementia and Alzheimer’s disease (Pettersen et al., 2008; Seo et al., 2007; Kirsch et al., 2009; Hanyu et al., 2003; Haller et al., 2010; Cordonnier et al., 2006)—and CMB burden predicts progressive cognitive declines (Akoudad et al., 2016). Thus, in asymptomatic persons, CMBs may portend neural and cognitive deterioration that share vascular and inflammatory antecedents (Jeon et al., 2007; Michikawa et al., 2009; Fan et al., 2003; Bokura et al., 2011; Thijs et al., 2010). In recent years, development of MRI sequences that are sensitive to heme iron, such as T2*-weighted gradient-recalled echo or susceptibility weighted imaging (SWI; Haacke et al., 2005), facilitated *in vivo* study of risk associated with CMBs (Greenberg et al., 2009b).

Given their vascular nature, it stands to reason that greater incidence of CMB would be linked to poor vascular health, and increase in vascular risk indicators indeed predicts CMB prevalence. For example, CMBs are more common among carriers of the apolipoprotein E (ApoE) ε4 allele, which is associated with suboptimal cholesterol clearance and increased risk for Alzheimer’s disease (Maxwell et al., 2011). Arterial hypertension or elevated systolic blood pressure (Greenberg et al., 2009b; Jeon et al., 2007; Poels et al., 2011; Jia et al., 2014; Liu et al., 2012) and high fasting blood glucose (Viswanathan et al., 2006) increase risk for CMBs, although evidence regarding cholesterol is mixed (Amarencu and Labreuche, 2009; Hackam et al., 2011). Notably, each of the listed CMB risk indicators is a constituent of metabolic syndrome (Grundy et al., 2005). Progressive, cumulative decline in vascular health is common during aging (Warsch and Wright, 2010) and risk for metabolic syndrome rises after the fifth decade (Grundy et al., 2005). Thus, age-related increase in CMB prevalence may reflect age-related decline in cardiovascular health and not chronological age *per se*. Inflammation, as a promoter of CMBs and one of the putative mechanisms of neural declines (Gouw et al., 2011; Rosidi et al., 2011), further complicates the relation between advanced age and vascular insults. Moreover, CMBs may be affected by modifiable lifestyle factors that can be manipulated through medical and behavioral interventions: frequent aerobic activity, body mass index (BMI) and smoking (Greenberg et al., 2009b; Yates et al., 2014; Grundy et al., 2005).

Although most studies have considered the surveyed risk factors as isolated correlates of CMB load, their interdependency and intertwined time course need to be tested in a

¹AIC—Akaike information criterion; BIC—sample size-adjusted Bayesian information criterion; BP—blood pressure; BMI—body mass index; BS 95% CI—bias-corrected bootstrapped 95% confidence intervals; CES-D—Center for Epidemiological Studies Depression Scale-Revised; CFI—comparative fit index; CMB—cerebral microbleed; FIML—full information maximum likelihood estimation; LCSM—latent change score model; mIP—minimum intensity projection map; MMSE—Mini-Mental State Exam; RMSEA—root mean square error of approximation; SRMR—standardized root mean residual; SWI—susceptibility weighted imaging; QSM—quantitative susceptibility mapping

longitudinal study. Recently, several longitudinal studies of patients have confirmed age and metabolic syndrome components among the strongest predictors of CMB incidence (Kirsch et al., 2009; Bokura et al., 2011; Poels et al., 2010; 2011; Lee et al., 2011; Chen et al., 2006). Yet, the predictors of CMB development in the course of non-pathological aging remain unclear (Greenberg et al., 2009b; Yates et al., 2014). In one cohort, 24% of older adults had a single CMB at baseline and 10% developed a new CMB three years later, with worse vascular health predicting greater eventual CMB burden (Poels et al., 2010; 2011). These two reports are based on the same sample and are limited to two assessments, thus an independent study over a longer period is warranted. Further, a longitudinal study over a longer period could account for progressive accumulation of age-related vascular risk apart from calendar age. Here, we aimed to address the outlined limitations by examining a sizable lifespan sample of healthy adults who were assessed up to five times over eight years. In addition to estimating risk for developing a CMB in a latent survival analysis, we tested latent composite metabolic syndrome and inflammation risk scores of, as well as age, sex, BMI and exercise frequency as predictors of risk. In secondary analyses, change in CMB volume and iron content measured from SWI scans was evaluated in latent change score models. Several longitudinal studies report persistent presentation of a CMB over subsequent measurement occasions (Poels et al., 2010; 2011; Goos et al., 2010; Gregoire et al., 2010), but to our knowledge, none have modeled the change in CMB severity (volume and iron content) over time and individual differences therein.

2.0 Material and Methods

2.1. Participants

Data were collected in two on-going longitudinal studies of neural and cognitive correlates of aging from healthy residents of the Metro Detroit area. Studies were approved by the Institutional Review Board and all participants provided informed consent. This sample partially overlaps with the samples used in our prior reports of longitudinal changes in MRI indices of brain structure and cognitive function (i.e., Persson et al., 2016; Bender and Raz, 2015; Daugherty et al., 2015; Daugherty and Raz, 2017). Present analyses include participants from these on-going studies that were scanned with the necessary MRI sequence (SWI) for the study of CMBs. At baseline, 251 participants (age 18–78 years, 70% female; $n = 148$ with MRI) were enrolled. The sample (details in Table 1) was assessed up to five times over eight years, with the following delays (mean $\pm$ SD, months): first follow-up = 26.39 ± 3.41 ; second follow-up = 29.78 ± 5.79 ; third follow-up = 26.93 ± 4.09 ; fourth follow-up = 25.33 ± 1.59 . Participants were recruited based on several criteria. They were right-hand dominant and spoke English as a primary language. They reported no head trauma with loss of consciousness greater than 5 min; no history of neurological, cardiovascular, endocrine or psychiatric disease; scored at least 26 on the Mini-Mental State Examination (MMSE; Folstein et al., 1975) and less than 16 on the Center for Epidemiological Studies Depression Scale-Revised (CES-D; Radloff, 1977; mean = 5.39, SD = 4.96). Four participants with MRI were removed from analysis because of violating enrollment criteria determined retrospectively: 1 was left-handed, 1 was on anti-psychotic medication, 1 reported head trauma, and 1 exceeded the CES-D cut-off. Another had an incidental finding—venous angioma. In the present longitudinal study, participants had a

mixed pattern of missing data, determined by availability of the necessary MRI sequence, participant drop-out, or pending the next occasion in an on-going study (see Table 1). Missingness correlated with age ($t(249) = -5.53, p < 0.001$) but not other demographic variables (all $p > 0.05$). Missing data were handled via full information maximum likelihood (FIML), a non-imputation method that leverages all available data during latent model estimation and the recommended statistical procedure for longitudinal analyses (Little and Card, 2013). The inclusion of baseline age as a covariate further off-set bias under the assumption of data missing at random, thus minimizing the impact of attrition on the reported analyses.

2.2 MRI: CMB Detection and Measurement

Participants underwent MRI in a 3 Tesla Verio scanner (Siemens GmbH, Erlangen, Germany). In addition to several structural brain scans, we acquired an SWI sequence with the following parameters: 11 echoes, echo time TE = 5.68–31.38 ms (spacing = 2.57 ms), repetition time TR = 37 ms; GRAPPA factor = 2; voxel = $0.5 \times 0.5 \times 2 \text{ mm}^3$; 64 axial slices. From the single sequence, quantitative susceptibility maps (QSM) of multi-echo phase were created with SPIN (<http://mrinnovations.com/spin-lite>; last accessed 6/14/16) using a SHARP filter (Barbosa et al., 2015), as well as T2* maps. The magnitude image from the 15.96 ms echo served for anatomical reference and minimum intensity projection (mIP) maps were created from the same echo (collapsing every 4th slice). CMBs were identified as circular objects, smaller than 10 mm in diameter, that appeared hyperintense (bright) on QSM, and confirmed with similar visualization on the T2* map. Vascular objects were excluded by also reviewing the mIP. The criteria and localization of the CMB were adopted from extant protocols (Greenberg et al., 2009a; 2009b; Gregoire et al., 2009; Cordonnier et al., 2009). CMBs were manually traced on the QSM (magnified $\times 4$), and their volume and susceptibility (ppb) were measured by a single operator. Within-rater test-retest reliability (15 cases, 10 with CMB, a 4-week delay) was: classification (Cohen $\kappa = 1.00$), volume (intra-class correlation coefficient, ICC(3) = 0.99) and susceptibility (ICC(3) = 0.98). During primary SWI scan review and measurement of CMBs, the rater was blind to measurement occasion and participant demographics. In post-hoc analysis, the rater confirmed the persistence of a CMB based on anatomical location in reference to scans from prior time points.

2.3 Vascular Health Indices

All vascular health indicators were assessed at each longitudinal measurement occasion, but only baseline measures were included as covariates to predict the likelihood of developing a CMB over time. On the day of MRI, circulating blood markers of vascular health and inflammation were obtained after a 12-hour overnight fast. A latent composite risk score of metabolic syndrome (Grundy et al., 2005) included serum high density lipoprotein cholesterol and glucose determined enzymatically, as well as waist-to-hip ratio, diagnosis of hypertension, and systolic blood pressure (averaged over 3–4 measurements taken on separate days). Blood pressure was measured by mercury sphygmomanometer (BMS 12-S25) using a standard cuff (Omron Professional) on the left arm with the participants seated, resting their forearm on a table. A latent composite risk score of inflammation included plasma total homocysteine determined by fluorescence polarization immunoassay, C-

reactive protein from a high-sensitivity assay, and folate. Whole blood samples (20 cc) were collected by venipuncture in serum separator tubes, with homocysteine collected in EDTA tubes, centrifuged and placed on ice. All analyses were performed at Detroit Medical Center laboratories. In addition to these indicators, BMI was determined from height and weight measures. Participants reported weekly frequency of exercise.

2.4 Latent Survival Analysis and Latent Change Score Models

Cumulative longitudinal risk for developing a CMB was modeled in a latent, discrete-time survival analysis (Muthén and Massyn, 2005) with FIML estimation in Mplus software (v7; Muthén and Muthén). Similar to logistic regression, survival analysis determines the hazard odds ratio of an event ($-\tau$) in relation to predictors, but it takes time course into account. Latent factors were created from multiple indicators, and the unique and shared effects of risk factors were explicitly modeled. Covariates in the survival analysis were baseline measures of age, sex, BMI, weekly exercise frequency, and latent composite risk scores of metabolic syndrome and inflammation; non-significant effects among covariates were constrained in the final model (Figure 2). Correlated changes in CMB volume and susceptibility were modeled as latent change scores (LCSM; McArdle and Nesselroade, 1994; McArdle, 2009) from single indicators, which is conceptually similar to a difference score, and models included baseline age and sex as covariates. Paucity of individuals who developed a CMB throughout study limited the number of testable parameters. The LCSM was bootstrapped with bias-correction (5000 draws; Hayes and Scharkow, 2013) to produce 95% confidence intervals (BS 95% CI). Model fit was evaluated by multiple goodness-of-fit indices: non-significance of normal theory weighted chi-square; comparative fit index (CFI > 0.90), root mean square error of approximation (RMSEA = 0.05) and standardized root mean residual (SRMR < 0.10). For nested models, relatively lower Akaike information criterion (AIC) and sample size-adjusted Bayesian information criterion (BIC) indicate improved fit.

3.0 Results

3.1 Incidence of Cerebral Microbleeds

During the study, 26 (10.4%) participants, most of whom were 40 years or older at baseline, had at least one CMB (see Figure 3). Participants incurred CMB over the course of study: 11 participants presented with a CMB on the baseline scan, another four developed a first CMB at the two-year follow-up, eight at the 4th year follow-up, and three at the 6th yr follow-up; no participant developed a new CMB at the 8th yr follow-up. Six of these participants had two CMBs at a single occasion and, after the first was identified, only three individuals developed a second CMB over time. For an illustrated summary of CMB location observed in the 26 individuals see Figure 4.

3.2 Longitudinal Cumulative Risk for Developing a Cerebral Microbleed

Participants who had incurred a CMB during study had similar demographic characteristics to those who had not (Table 2), yet the cumulative effect of risk and protective factors may explain the likelihood of developing an CMB over the lifespan. We examine this possibility by including several covariates and their interactions to predict latent longitudinal risk of

CMB development. At the final assessment (eight years after baseline) no remaining participants had developed a new CMB; thus, the latent survival analysis estimated longitudinal risk for developing the first CMB over four assessments spanning up to 6 years. Because CMB were uncommon among participants who were younger than 40 years at the inception of the study, the analyses of age and age-related risk factors for developing a CMB were conducted in a sample restricted to baseline age 40 years and older ($N = 169$), as well as in the total sample (age 18–78 years, $N = 251$). The hazard model without covariates in both the total and age-restricted samples had acceptable fit ($\chi^2(11) = 8.71$, $p = 0.59$), and the covariate model with time-invariant effects was chosen for better fit ($AIC = 8960.86$; $BIC = 8972.93$) as compared to an alternative model with time varying effects ($AIC = 8972.84$; $BIC = 8988.94$).

In the sample restricted to age 40 years and older, the frequency of CMB at each measurement occasions was 8–12%. None of the predictors of developing a first CMB was statistically significant (Table 3) but the cumulative risk can still be considered. In the total sample, older age predicted greater risk for developing a CMB. Advanced age was also associated with greater metabolic syndrome risk score ($b = 0.15$, $p = 0.001$), which conferred additional age-related risk for CMB in the total sample. Thus, the cumulative age-related risk for a CMB was in part explained by age-related increase in cardiovascular health risk. When considering the age-restricted sample of participants age 40 years and older at baseline, these same effects were not statistically significant (all $p > 0.27$), further underscoring the interdependency of chronological age and age-related increase in vascular health risk. Accounting for age effects and minimal metabolic syndrome risk, the longitudinal incidence of CMB was 1–10% among 40–80 year-olds, and added average metabolic syndrome risk increased progressive incidence upwards of 25% (Figure 5). High BMI ($b = 0.75$ and 0.54 , $p < 0.001$, total and restricted samples, respectively) exacerbated this trend, but it was mitigated by frequent exercise ($b = -0.46$, $p = 0.03$ and $b = -0.29$, $p = 0.10$, total and restricted samples, respectively). Moreover, men were at greater risk for developing a CMB, which was in part explained by higher metabolic syndrome risk scores ($b = 8.52$ and 6.29 , $p < 0.001$, total and restricted samples, respectively). Inflammation composite risk score was unrelated to other covariates (all $p > 0.10$, constrained) or to CMB risk.

3.3. Persistence of Cerebral Microbleeds

Among persons with CMB ($n = 26$), 13 had MRI scans at subsequent occasions, which were used to evaluate the persistence and severity of a CMB over time. A CMB was determined to be the same if the anatomical location was the same as in prior time points. In all but one case, the CMB was still visualized two years, and up to four years, later. LCSM of CMB volume and magnetic susceptibility over two years, including age and sex as covariates, had excellent fit: $\chi^2(7) = 6.56$, $p = 0.48$; CFI = 1.00; RMSEA = 0.00; SRMR = 0.16. CMB volume decreased over two years (mean change = -0.32 , $p < 0.05$; BS 95% CI: $-0.55/-0.14$; $d = -0.34$) but susceptibility did not (mean change = -0.14 , $p = 0.05$; BS 95% CI: $-0.54/0.26$; $d = -0.15$). Individuals varied in change of both CMB volume (0.23 , $p < 0.05$) and susceptibility (1.17 , $p < 0.05$), yet this variability was unrelated to age or sex (all $p > 0.64$).

4.0 Discussion

We demonstrated that sub-clinical elevation in metabolic syndrome risk increased the likelihood of developing a cerebral microbleed in normal aging. Low CMB frequency in this healthy lifespan sample (~10% among adults 40 years and older) is in line with extant reports on healthy older adults (Greenberg et al., 2009b; Yates et al., 2014). Although CMBs were more frequent in older persons, greater metabolic syndrome composite risk score at baseline was a stronger predictor of cumulative risk over time as compared to chronological age.

Extant studies identify age and vascular health indicators as prominent predictors of CMBs (Jeon et al., 2007; Poels et al., 2010; 2011; Jia et al., 2014; Liu et al., 2012), but leave unclear the conjoint effect of age and age-related deterioration of vascular health. We report that middle-aged adults with subclinical elevation in metabolic syndrome risk are at greater risk for a CMB over time than older adults who had maintained good health. Whereas age accounted for 1–10% cumulative risk for CMB occurrence over time, adding average metabolic syndrome risk score increased progressive risk upwards of 25%. After considering these factors together, we reproduce the reported range of incidence rates in healthy adults (Greenberg et al., 2009b; Yates et al., 2014). As expected, CMB were more frequent among older adults, but elevation in metabolic syndrome risk increased likelihood for CMB throughout adulthood. In addition, BMI worsened metabolic syndrome score, which in turn accounted for greater risk of CMB. Notably, frequent exercise could alleviate this source of risk. Because declines in vascular health are usually progressive and cumulative across the lifespan, the importance of lifestyle factors that mitigate risk in later life cannot be overstated. As demonstrated in the analyses of age-restricted sample, the effect of metabolic syndrome risk on CMB incidence was mitigated in persons older than 40 years, in part due to the number of older adults in the study who had maintained good health. Thus, risk for developing a CMB that is attributed to chronological age is partially due to decline in vascular health, which is a plausible target for health intervention.

Despite the potential for mitigating CMBs by lifestyle, we observed no reduction in CMB burden over time. Once a CMB became evident, it persisted at each subsequent assessment, in accord with many (Poels et al., 2010; 2011; Goos et al., 2010; Gregoire et al., 2010) though not all (Asif et al., 2010) longitudinal observations. Nevertheless, there may be grounds for optimism, as the size of the CMB declined over time. Identifying and locating CMB may assist in determining risk of hemorrhage, ischemia (Jouvent et al., 2016) or neurodegenerative disease (Pettersen et al., 2008; Seo et al., 2007; Kirsch et al., 2009; Hanyu et al., 2003; Haller et al., 2010; Cordonnier et al., 2006; Zivadinov et al., 2016). Thus, treatments reducing CMB may decrease risk of these correlated phenomena.

Important to note, however, is that CMB shrinkage paired with stable iron content (susceptibility) would produce a relative increase in local iron concentration and ensuing oxidative stress, which promotes neural deterioration (Daugherty and Raz, 2015). This may be a plausible mechanism of CMB contribution to cognitive declines (Mills et al., 2010; Zecca et al., 2004). CMB incidence has been linked to cognitive deficits in the development of neurodegenerative diseases (Pettersen et al., 2008; Seo et al., 2007; Kirsch et al., 2009;

Hanyu et al., 2003; Haller et al., 2010; Cordonnier et al., 2006). Based on their anatomical location, CMB appear to selectively impair relevant motor and cognitive functions (Huang et al., 2015; Park et al., 2009), in line with known functional specialization of the brain. Moreover, decline in cardiovascular health exacerbates age-related neural and cognitive declines (Raz and Rodrigue, 2006; Warsch and Wright, 2010). Yet, it is unclear if a CMB directly contributes to neural and cognitive deterioration (Greenberg et al., 2009b; Yates et al., 2014) or is merely a marker of common vascular and inflammatory antecedents of decline (Warsch and Wright, 2010). Due to the low incidence rate, the sample size is insufficient to test CMB development as a predictor of changes in cognition in the same period. The sample evidenced notable variability in the CMB location, and thus a mixed pattern of CMB-related cognitive deficit is expected. We can only speculate about the role of CMB in cognitive dysfunction. Prior publications on the studies from which this sample was drawn have demonstrated subcortical iron accumulation that predicts regional shrinkage and cognitive decline two years (Daugherty, Haacke, Raz, 2015; Daugherty and Raz, 2017) and up to seven years later (Daugherty and Raz, 2016). Thus, it is plausible that CMB as a source of iron-related oxidative stress and indicator of vascular health risk may contribute to cognitive declines during aging.

Inflammation is another suggested mechanism of CMB-related impairment in brain function (Gouw et al., 2011), but in our sample, inflammation composite risk score did not explain CMB burden. This may be due to limited assessment of systemic inflammation markers or due to the minimal effects in a sample selected for good health. Prospective longitudinal study in samples that are not as strictly selected for health, and in which multiple inflammation biomarkers are assessed is necessary to further evaluate these hypotheses.

This report should be interpreted with consideration of additional limitations. First, the sample size is relatively small to detect a low-base-rate phenomenon. Although this is a sizable, lifespan sample with multiple measurement occasions, the group that had developed a CMB during the study was too small to evaluate multiple covariates and cognitive outcomes. Due to attrition and introducing the SWI sequence mid-way in an ongoing study, some cases were missing observations. Although missing data were handled via FIML, the current recommended practice for longitudinal studies (Little and Card, 2013), we cannot completely discount the possibility of bias related to missingness. Second, the magnitude of age-related effects may be affected by sample selection. Stringent health screening criteria applied to avoid confounding age and concomitant vascular disease produced a sample with middle aged adults who were at risk for disease, but below clinical criteria for diagnosis. They might have been in relatively worse health than older adults who met the same qualifying criteria at enrollment. Nevertheless, as the hazard frequency across occasions was similar to the majority of the extant literature (Greenberg et al., 2009b; Yates et al., 2014), we believe that this bias was negligible. Third, SWI is a sensitive method for detection and measurement of iron-related susceptibility, and this source of image inhomogeneity may cause over-estimation of CMB volume. The use of QSM as a method that localizes susceptibility estimates may mitigate this source of bias (Haacke et al., 2015). Moreover, if this were a prominent influence on the estimates of CMB volume, changes in volume and susceptibility should occur simultaneously, which was not the case: we find decline in volume but no change in susceptibility. In addition, longitudinal estimates of CMB volume

and iron content may be affected by variability in the scanning environment over time. A CMB is relatively small and differences in head placement in the scanner with respect to the image slice thickness may impact the visualization of a CMB and its measurement. Nonetheless, in all but one case, CMB persisted on subsequent MRI scans with relatively stable iron content over time. Fourth, a single rater reviewed the MRI scans; the rater was highly reliable, but possible individual rater bias should be taken into consideration. Finally, without additional measures, we can only speculate about the role of CMB in neural and cognitive decline.

4.1 Conclusion

Although developing a CMB is unlikely during normal aging, risk is increased by declines in vascular health. Importantly, behavioral modifiers of vascular risk affect CMB burden and may be harnessed for its mitigation. Association of CMBs with neural and cognitive declines in aging warrants a longitudinal study of a large cohort.

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Highlights

- Cerebral microbleeds (CMB) were assessed in vivo in an adult lifespan sample
- 10% of healthy adults presented with one cerebral microbleed over eight years
- Metabolic syndrome risk partially accounted for age-related risk for CMB occurrence
- High body mass index exacerbates risk and frequent exercise mitigates it
- CMBs persisted over eight years and volume, but not iron content, decreased

Figure 1.

Example MRI visualization of a CMB with susceptibility weighted imaging. Images are shown at $\times 2$ magnification and insets are $\times 4$ magnification. The CMB is marked by an arrowhead; it appears bright on QSM images (brighter intensity values indicate proportionally greater iron content), and dark on magnitude and mIP images. QSM—quantitative susceptibility mapping (11 echo; $0.5 \times 0.5 \times 2$ mm); magnitude ($0.5 \times 0.5 \times 2$ mm); mIP—minimum intensity projection map ($0.5 \times 0.5 \times 2$ mm).

Figure 2.

Schematic presentation of latent survival analysis. The analysis models the longitudinal risk for developing the first CMB from baseline ($-\tau_0$) to six years later ($-\tau_3$). $C(1)$ labels the latent, cumulative hazard odds for the entire sample and the latent f is a tool to hold all covariate effects time invariant. All covariates are baseline measures and can be interpreted as predictors of future risk. Indicators fixed to 1 were used to identify the scale for latent constructs. The same model was tested in the entire sample (age 18–78 years at baseline) and in a restricted sample of middle-aged and older adults (age 40–78 years at baseline). Age—baseline age; Met Syn—latent metabolic syndrome composite risk score (systolic blood pressure, fasting blood glucose, high-density lipoprotein, waist-hip ratio, hypertension diagnosis); Inflam—latent inflammation composite risk score (homocysteine, high sensitivity c-reactive protein, folate); BMI—body mass index.

Figure 3.

Frequency of cerebral microbleed (CMB) incidence. From the total sample (N=251), 26 persons presented with at least one CMB in the course of study.

Figure 4.

Illustration of CMB location observed in the 26 persons in the course of study. The relative size of each CMB is illustrated by the grayscale. Schematic diagram of the brain was adapted from Gregoire et al. (2009).

Figure 5.

Cumulative risk probability of developing a CMB over six years by age and composite metabolic syndrome (Met Syn) composite risk score. Greater cumulative risk probability indicates greater likelihood of developing the first CMB. Solid shapes correspond to risk attributed to chronological age alone and open shapes, the additional risk from sub-clinical elevation in metabolic syndrome risk score at baseline. Hazard risk effects were calculated from the age-restricted sample (40–78 years at baseline) and to illustrate effects of age and metabolic syndrome composite risk score, all other covariate effects were held at baseline hazard odds (i.e., contributed no risk).

Table 1

Description of sample demographics.

	Baseline	2nd-yr Follow-up	4th-yr Follow-up	6th-yr Follow-up	8th-yr Follow-up
N	251	76	84	47	15
MRI (n)	148	64	81	39	13
Hypertensive (n and percentage)	37 (15%)	13 (17%)	30 (36%)	11 (24%)	4 (27%)
Age (years)	46.98 ± 17.42	52.80 ± 15.72	59.88 ± 13.11	62.40 ± 10.83	61.27 ± 13.71
MMSE	28.80 ± 1.02	28.75 ± 1.05	28.91 ± 1.10	28.98 ± 0.99	29.00 ± 0.85
BMI	26.31 ± 5.59	27.12 ± 5.10	27.73 ± 5.87	26.59 ± 4.87	25.70 ± 4.93
Waist-Hip Ratio	0.87 ± 0.09	0.88 ± 0.07	0.88 ± 0.08	0.90 ± 0.09	0.90 ± 0.08
Exercise Frequency (days per week) *	3.20 ± 2.11	3.70 ± 2.32	3.38 ± 2.21	3.76 ± 2.23	
Avg. Systolic (mmHg)	120.05 ± 12.66	119.52 ± 12.76	124.98 ± 13.28	125.00 ± 12.64	125.25 ± 16.67
Avg. Diastolic (mmHg)	75.13 ± 7.75	74.99 ± 9.16	76.72 ± 8.45	76.56 ± 7.16	75.08 ± 7.64
Glucose (mg/dL)	86.84 ± 14.99	87.74 ± 12.06	92.45 ± 17.17	94.27 ± 21.31	91.53 ± 16.66
HDL (mg/dL)	56.60 ± 15.74	57.68 ± 15.92	56.61 ± 16.40	57.59 ± 14.52	61.13 ± 11.53
CRP (mg/L)	2.33 ± 3.86	2.65 ± 2.63	2.76 ± 3.86	2.51 ± 2.50	2.02 ± 1.84
Homocysteine (μmol/L)	9.28 ± 2.56	9.45 ± 1.96	10.50 ± 3.25	10.27 ± 2.56	11.66 ± 3.26
Folate (μmol/L)	18.50 ± 5.28	19.73 ± 4.82	20.14 ± 5.07	19.42 ± 5.01	18.36 ± 6.75

Note: Sample means ± standard deviations are reported. N—sample enrolled in study, MRI (n)—the number of individuals with a SWI scan for CMB measurement; MMSE—mini-mental state exam (cut-off > 25); BMI—body mass index; HDL—high density lipoprotein; CRP—high sensitivity C-reactive protein; blood pressure measures are an average of 3–4 measurements; circulating blood markers were taken from a fasting blood test.

* format of reporting exercise frequency changed at the 8-year follow-up and cannot be compared. Only baseline measures are used for covariate analyses.

Table 2

Baseline demographic and health descriptors of persons with and without a cerebral microbleed in the course of study.

	With CMB	Without CMB
N	26	225
Age (years)	58.62 ± 9.86	47.40 ± 18.61
MMSE	28.68 ± 1.18	28.81 ± 1.00
n Female (percentage)	16 (61.54%)	160 (71.11%)
Hypertensive (n and percentage)	6 (23.08%)	37 (16.44%)
Smoking (n and percentage)	0 (0%)	46 (20.44%)
BMI	27.97 ± 3.98	26.29 ± 5.69
Waist-Hip Ratio	0.90 ± 0.10	0.88 ± 0.08
Exercise Frequency (days per week)	3.67 ± 1.70	3.14 ± 2.05
Avg. Systolic (mmHg)	123.36 ± 11.29	120.09 ± 13.28
Avg. Diastolic (mmHg)	77.74 ± 6.15	74.94 ± 7.61
Glucose (mg/dL)	89.12 ± 8.06	87.49 ± 13.64
HDL (mg/dL)	56.56 ± 18.23	57.15 ± 16.07
CRP (mg/L)	2.59 ± 2.37	2.28 ± 3.69
Homocysteine (μmol/L)	9.94 ± 2.17	9.59 ± 2.76
Folate (μmol/L)	20.11 ± 5.13	18.89 ± 5.12

Table 3

Covariate effects predicting risk of developing the first cerebral microbleed.

Covariate	Age 18–78 years				Age 40–78 years			
	Odds Ratio	Hazard Effect	95% CI	p	Odds Ratio	Hazard Effect	95% CI	p
Age	1.03	0.03	0.003/0.06	0.06	0.99	−0.01	−0.04/0.03	0.75
Sex (Male)	1.28	0.25	−0.76/1.25	0.69	1.70	0.53	−0.53/1.59	0.41
Met Syn	1.02	0.02	−0.06/0.10	0.68	1.02	0.02	−0.10/0.14	0.82
Inflam	0.59	−0.53	−1.12/0.07	0.14	0.69	−0.38	−0.93/0.17	0.26

Note: Metabolic Syndrome (Met Syn) and Inflammation (Inflam) were latent composite risk scores of multiple indicators (see Materials and Methods for details). 95% confidence intervals (CI) are reported for all effects.