Journal of
Alzheimer's Disease & Parkinsonism
ISSN: 2161-0460

Cacciottolo et al., J Alzheimers Dis Parkinsonism 2016, 6:6
DOI: 10.4172/2161-0460.1000287

Commentary OMICS International

Rust on the Brain from Microbleeds and Its Relevance to Alzheimer Studies: Invited Commentary on Cacciottolo Neurobiology of Aging, 2016

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Abstract

Cerebral microbleeds (MB) and small vessel disease (SVD) with congophilic arterial angiopathy (CAA) are increasingly recognized as a variable factor in AD cognitive impairments. This commentary on our recent report on sex-ApoE interactions in MBs published this February, briefly explores three aspects of MBs that could not be fully discussed therein: I, A possible gap between the prevalence of MBs as detected by MRI and post mortem analysis; II, The role of hemoglobin- degradation products in amyloid-attributed neurodegenerative changes; and III, Possible assessment of MB by cerebrospinal fluid (CSF) assays for iron-related markers to better screen patient subgroups for AD interventions.

Keywords: Microbleeds; Alzheimer disease; Transgenic mice; CSF heme

Cerebral microbleeds (MB) have increasing clinical interest because of their association with AD and with small vessel disease (SVD) [1-3]. Most convincing to us is the population-based Rotterdam Study, just published in August 2016. In this 6 year follow-up of 3257 clinically normal participants imaged with 1.5T MRI, those with one or more MBs had a 2-fold higher risk of clinical grade dementia; of all AD cases, about 50% had MBs [4]. The prevalence of MBs was about 10% in this total sample of average age 60 years, which is higher than the 5% prevalence of other similarly aged "general populations" in a meta-analysis [5]. Moreover, a brief comparison with other studies (Table 1) shows that the typical MRI strength of 1.5-3T may underestimate MB prevalence by >3-fold, as shown in exploratory studies by higher strength 7T MRI [6] and by postmortem histochemical analysis for iron deposits [7] (Table 2). The 7.5T MRI is not practical for most clinical studies because the prolonged scan times may induce claustrophobia. The MB numbers from postmortem 7.5T MRI of brain sections showed strong correspondence with MBs detected by iron histochemistry [7]. Mice are also shown in Table 3. Wild type C57BL/6 mice had very low levels at age 6 month that are strikingly increased by the introduction of human ApoE transgenes, and further boosted by FAD genes in EFAD mice [1].

The detection of MBs by Perls Prussian blue histochemistry represents extravasated heme ferric iron in ferritin and hemosiderin complexes. Intriguingly, heme and Aβ are colocalized at sites of extravasation in humans [8] and in ADtg mice transgene [1,9]. Of potential relevance to mechanisms in neurodegeneration, are interactions of the heme core with the human Aβ peptide (hAβ), which generates increased peroxidase activity with a broad range of substrates [10,11]. Importantly, hAβ has higher affinity for the heme core than rodent Aβ [11,12] due to specific amino acid differences (Arg5, Tyr10 and His13) in hAβ which differ critically from rodent Aβ (mouse or rat). We ask: could hAβ-attributed neurodegenerative changes in ADtg mice also represent promiscuous biochemical effects of the heme-hAβ complex that generate the oxidized proteins and lipids found in amyloid deposits? Heme-hAβ peroxidase bystander damage might be further studied with other knockouts and specific antioxidants.

Besides MBs, mice transgenic for human *ApoE3* or *E4*, but with wild type rodent APP, have age-related gross cerebral hemorrhages and cerebrovascular amyloid fibrils that are absent from aging C57BL/6 mice [13]. Because wild type rodent Aβ fibrillizes *in vitro*, yielding with equivalent Thioflavin fluorescence to hAβ [11], we need to consider other wildtype strains besides the C57BL/6 for spontaneous CAA and hemorrhages with aging.

Sex interactions merit further consideration. Cacciottolo et al. [1] and Vest and Pike [14] found that female AD mice have greater Aβ load than males. Because the greater accumulation of MBs [1] in female EFAD mice parallels their greater Aβ load and CAA, it seems important to resolve the independent vs. cooperative effects of Aβ and MBs, which could have shared or distinct pathways for AD-like neurodegenerative changes and cognitive decline.

In vivo detection of MBs currently depends on MRI. We ask: could there be a CSF marker related to extravasated iron in the brain? In subarachnoid hemorrhage (SAH), CSF ferritin levels were elevated >50-fold, attributed to intra-thecal production by microglia [15]. Hemopexin, another iron binding protein, was also elevated in about one-third of SAH patients [16]. Bilirubin, a heme degradation product, used as a CSF marker after SAH [17], was also elevated in AD patients by ~20% [18]. Intriguingly, in a 7 year longitudinal study of the ADNI cohort, CSF ferritin varied inversely with cognitive decline and predicted MCI conversion to AD [19].

Thus, CSF levels of bilirubin, ferritin, and hemopexin should be further analyzed in the extensive banks of CSF being collected in relation to MRI studies of older populations. Additionally, we suggest the study of CSF iron. Although serum iron, but not CSF-iron, was decreased in AD vs. healthy controls [20], a more comprehensive analysis of iron is warranted for serum and CSF in human samples; and

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Received November 04, 2016; **Accepted** November 17, 2016; **Published** November 24, 2016

Citation: Cacciottolo M, Morgan TE, Finch CE (2016) Rust on the Brain from Microbleeds and Its Relevance to Alzheimer Studies: Invited Commentary on Cacciottolo Neurobiology of Aging, 2016. J Alzheimers Dis Parkinsonism 6: 287. doi: 10.4172/2161-0460.1000287

Study [Reference]	Study (# participants)	Age, years; median (IQR)	Control/Population (%)	MCI (%)	AD (%)	VsD (%)	Dementia (%)
Cacciottolo et al. [1]	ADNI (658)	48-91 73 (67-78)	29	36	41		
	KIDS (488)	36-88 62 (57-66)	9.5	20	26		
Akoudad et al. [4]	Rotterdam Study (3257)	52-73 61 (59-63)	19		45		47
Chiang et al. [24]	ADNI (626)	69-89 76 (72-77)	21	65	13		
Cordonnier and van der Flier [5]	Meta-analysis (1411)	54-81 56 (53-81)	5	13	23		
Kester et al. [23]	Amsterdam Dementia Cohort (914)	50-76 67 (59-67)	14		21	77	
McAleese et al. [25]	KIDS (1504)	52-76 64 (57-68)	11	21	28	59	33
Average ± SD			**15.5 ± 8**	**31 ± 21**	**28 ± 11**	**68 ± 13**	**40 ± 10**

ADNI: Alzheimer Disease Neuroimaging Initiative; KIDS: Karolinska Imaging Dementia Study; MCI: Mild Cognitive Impairment; AD: Alzheimer Disease; VsD: Vascular Dementia

Table 1: Frequency of microbleeds in clinical studies.

Study [Reference]	Population (# participants)	MRI strength (T)	# MBs	% population with MB
Cacciottolo et al. [1]	ADNI (658)	1.5/3T	1	20
			2-4	8
			>4	4
	KIDS (488)	1.5/3T	1	8
			2-4	2
			>4	5
Akoudad et al. [4]	Rotterdam Study (3257)	1.5T	1	12
			2-4	4
			>4	2
Ni et al. [6]	unspecified (8)	1.5/3T	1	12.5
			2-10	50
			>10	12.5
		7T	1	12.5
			2-10	12.5
			>10	50

Table 2: Comparison of MB detection by MRI field strength.

Genotype	Microbleeds per 100 mm^2 of cerebral cortex (mean ± SD)
Wild type C57BL/6	0.6 ± 0.7
hApoE [1]	1.4 ± 1.37
EFAD [1]	22.6 ± 38.8

Number of microbleeds per 100 mm^2 of cerebral cortex identified by Perls Prussian Blue histochemistry on sagittal brain slices 25 µm thick. Both sexes: Wild type, 12 mice (independent analysis, not reported in [1]); hApoE (human ApoE): 16 mice; EFAD: 19 mice

Table 3: Mouse MB studies.

additionally in rodents, for iron in brain interstitial fluid. Further post mortem correlations are also warranted for CSF iron markers with brain MBs and CAA, and for brain region total iron which is also increased in some AD-vulnerable regions [20]. These assays could be paired with MRI for the early identification of MBs, paralleling the CSF-Aβ42 and PET imaging for in vivo amyloid [21].

We anticipate that inclusion of hematogenous parameters will add new iron-dependent mechanisms to the standard AD progression models based on amyloid and tau fibril accumulation [22]. The neurodegenerative mechanisms of amyloid may prove to involve downstream effects of Aβ-heme complexes as well as direct effects of oligomeric Aβ. In those AD patients with MB, CSF levels of Aβ42 were decreased [23,24], while non-AD subjects with MB showed increased tau [23]. If CSF- heme complexes or ferritin were found to precede the CSF-Aβ decline during AD [21], this could give a valuable pre-clinical marker for adjusting anti-coagulant dose and other therapeutics. Given findings from the Rotterdam Study that MBs are associated with higher AD risk and from ADNI that CSF ferritin increases MCI conversion, we suggest that MBs be given greater attention in the selection of patients for clinical trials. Lastly, we note the conclusion of a just published review: "*it must not be assumed that a primary hemorrhagic process produces all microbleeds or that the most severely affected vessels are the culprits*" [25].

Acknowledgement

CE Finch is grateful for grant support from the Cure Alzheimer's Fund and the National Institute on Aging: R01 AG051521; R21-AG040683.

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