The Significance of Cortical Cerebellar Microbleeds and Microinfarcts in Neurodegenerative and Cerebrovascular Diseases

A Post-Mortem 7.0-Tesla Magnetic Resonance Study with Neuropathological Correlates

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Key Words

Postmortem 7.0-tesla magnetic resonance imaging · Cortical cerebellar microbleeds · Cortical cerebellar microinfarcts · Atherosclerotic cerebrovascular disease · Cerebral amyloid angiopathy · Neurodegenerative diseases · Vascular dementia

Abstract

Background: As cortical microbleeds and microinfarcts in neurodegenerative and cerebrovascular diseases have been studied predominantly at the level of the cerebral hemispheres and linked to the presence of cerebral amyloid angiopathy (CAA), we aimed at determining with 7.0-tesla magnetic resonance imaging (MRI) whether the causes and the frequency of cortical cerebellar microbleeds (CCeMBs) and microinfarcts (CCeMIs) are the same. **Materials and Methods:** Hundred and four postmortem brains, composed of 29 with pure Alzheimer's disease (AD), 9 with AD associated to CAA, 10 with frontotemporal lobar degeneration, 9 with amyotrophic lateral sclerosis, 10 with Lewy body dis-

ease, 12 with progressive supranuclear palsy, 9 with vascular dementia (VaD), and 16 controls, were examined. On a horizontal section of a cerebellar hemisphere examined with 7.0-tesla MRI, the number CCeMBs and CCeMIs were compared between the different disease groups and the control group. The MRI findings were also compared with the corresponding mean values observed on histological examination of a separate standard horizontal section of a cerebellar hemisphere, used for diagnostic purpose. **Results:** CCeMBs and CCeMIs were only significantly increased in the VaD group. When comparing the diseased patients with and without CAA mutually and with those with arterial hypertension and severe atherosclerotic cerebrovascular disease, only in the latter an increase of CCeMBs and CCeMIs was observed. There was an excellent correlation between the MRI and the neuropathological findings. **Conclusions:** CCeMBs and CCeMIs are mainly due to atherosclerotic cerebrovascular disease and not due to CAA. Their increased presence cannot be included to the Boston diagnostic criteria for CAA.

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Introduction

Magnetic resonance imaging (MRI) has shown a high incidence of cortical microbleeds (CMBs) in patients with small-vessel diseases such as cerebral amyloid angiopathy (CAA) and lipohyalinosis [1–3]. They are also found in asymptomatic persons and in those with arterial hypertension and white matter changes [4, 5]. CMBs are frequently observed in brains of patients suffering from Alzheimer dementia (AD) associated to CAA [6, 7]. In the absence of CAA, they are mainly linked to disturbances of the blood-brain barrier associated to the neurodegenerative disease itself and mainly consist of small perivascular bleeds [8]. Although nearly visible on naked-eye examination of postmortem brains, they can easily be detected on 7.0-tesla T2*-weighted magnetic resonance imaging (MRI) [9].

Until recently, cortical microinfarcts (CMIs) were considered the invisible lesions in clinical-radiological correlation studies that rely on conventional structural MRI [10]. They are also nearly visible on naked eye examination of postmortem brains and best detected by light-microscopic examination [11]. Recently, *in vivo* detection of CMIs has been demonstrated with high-resolution 7.0-tesla MRI [12]. They usually occur at the latest stage of CAA or of arteriosclerosis [13] and significantly affect cognition in brain aging [14, 15]. In our recent postmortem 7.0-tesla MRI study, they were significantly prevalent not only in CAA brains and mainly in patients with vascular dementia, but also to a lesser degree in those with Alzheimer dementia and with Lewy body disease [16].

As the impact of cortical CMBs and CMIs in neurodegenerative and cerebrovascular diseases has mainly been studied at the level of the cerebral hemispheres, this study aims at determining with 7.0-tesla MRI and its neuropathological correlates whether the causes and the frequency of cortical cerebellar microbleeds (CCeMBs) and microinfarcts (CCeMIs) are the same.

Materials and Methods

Patients and Materials

Hundred and four patients, who were followed up mainly at the Lille University Hospital or at the Wattrelos Hospital, underwent an autopsy. The final disease diagnosis was made according to the validated neuropathological criteria [17]. The cohorts consisted of 38 patients with Alzheimer's disease (AD), 10 with frontotemporal lobar degeneration (FTLD), 9 with amyotrophic lateral sclerosis (ALS), 10 with Lewy body disease (LBD), 12 with progressive supranuclear palsy (PSP), 9 with vascular dementia (VaD), and 16 controls who had no clinical history of dementia or stroke (C).

The diagnosis of VaD was made according to recently published neuropathological criteria [13]. In two AD brains and in one LBD brain, the additional severe arteriosclerotic cerebrovascular lesions allowed them being considered as mixed VaD cases.

Moderate to severe CAA was present in 9 AD brains (AD-CAA), compared with 29 AD brains without or with mild CAA, in 5 of the 9 VaD ones, in 1 of the 10 LBD ones, and in none in the other groups.

A previous obtained informed consent of the patients or from the nearest family allowed an autopsy for diagnostic and scientific purposes. The brain tissue samples were acquired from the Lille Neuro-Bank of Lille University, federated to the Centre des Ressources Biologiques that acted as an institutional review board.

One fresh cerebral hemisphere was deeply frozen for biochemical examination. The remaining hemisphere, the brainstem, and a cerebellar hemisphere were fixed in formalin for 3 weeks.

The patients with neurodegenerative diseases and VaD were compared regarding the incidence of CCeMBs and CCeMIs to the control group. The overall group of patients with moderate to severe CAA was also compared with the non-CAA diseased group and with the group with pure and mixed atherosclerotic VaD.

Neuropathological Examination

The standard procedure for diagnosis of the type of neurodegenerative disease consisted of examining samples from the primary motor cortex, the associated frontal, temporal and parietal cortex, the primary and secondary visual cortex, the cingulate gyrus, the basal nucleus of Meynert, the amygdaloid body, the hippocampus, basal ganglia, mesencephalon, pons, medulla, and cerebellum. Slides from paraffin-embedded sections were immunostained for protein tau, β -amyloid, α -synuclein, prion protein, TDP-43, and ubiquitin.

Similar to the CERAD criteria, brains were classified as CAA, when a majority of β -amyloid-stained vessels were present in at least three of the four examined samples and as not-CAA, when absent or scarce, in case of a few stained vessels in one or two slides [18].

A quantitative evaluation of the number of CCeMBs and CCeMIs was performed on a standard horizontal section through a cerebellar hemisphere at the level of the dentate nucleus, according to a previously described method [19]. The mean ranking scores in AD, AD-CAA, FTLD, ALS, LBD, PSP, and VaD brains were compared with the controls.

MRI Examination

From each brain one separate horizontal section of a cerebellar hemisphere at the level of the dentate nucleus was submitted to MRI.

We used a 7.0-T MRI Bruker BioSpin SA with an issuer-receiver cylinder coil of 72-mm inner diameter (Ettlingen, Germany), according to a previously described method [9]. The cerebellar section was placed in a plastic box filled with salt-free water, the size of which did not allow significant tissue movements. Three MRI sequences were used: a positioning sequence, a T2 sequence, and a T2* sequence.

The CCeMBs were detected as focal areas of increased signals on the T2* sequence, whereas CCeMIs were observed as small sharply demarcated zones of increased signals on the T2 sequence without signal loss on the T2*-weighed gradient-echo sequence.

The mean prevalence of CCeMBs and CCeMIs in the different groups of neurodegenerative diseases and VaD was compared with the control group.

Table 1. Comparison of median age (interquartile range), gender distribution (%) and vascular risk factors (%) between the control and the disease groups

Items	C (n = 16)	AD (n = 29)	AD-CAA (n = 9)	FTLD (n = 10)	ALS (n = 9)	LBD (n = 10)	PSP (n = 12)	VaD (n = 9)
Age, years (IQR)	68 (50–88)	73 (63–88)	68 (63–85)	68 (62–74)	65 (57–76)	82* (75–97)	75 (72–82)	68 (59–86)
Male, %	50	55	56	60	56	70	33	33
Arterial hypertension	13	10	40	20	20	40	30	70**
Diabetes	13	10	10	10	10	0	10	30
Hypercholesterolemia	13	20	17	20	10	20	10	30
Smoking	0	20	17	10	10	10	10	30
Antithrombotic drugs	19	40*	40	0	10	40	30	70**

C = Control; AD = Alzheimer's disease; AD-CAA = Alzheimer's disease with cerebral amyloid angiopathy; FTLD = frontotemporal lobar degeneration; ALS = amyotrophic lateral sclerosis; LBD = lewy body disease; PSP = progressive supranuclear palsy; VaD = vascular dementia.

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

The 15 brains with CAA were also compared with those diseased brains without CAA, and separately with 8 non-CAA ones with pure and mixed VaD.

The total number of CCeMBs and CCeMIs of each brain was determined by consensus evaluation by three observers (JDR, FA, and ND), blinded to the neuropathological examination. The inter-rater reliability resulted in an interclass correlation coefficient of 0.81.

Statistical Analyses

Univariate comparisons of unpaired groups were performed with the Fisher's exact test for categorical data. The non-parametric Mann-Whitney U test was used to compare continuous variables. The significance level, two-tailed, was set at ≤ 0.05 , for marginally significant, ≤ 0.01 for significant, and ≤ 0.001 for highly significant.

Results

No differences were observed regarding the median age and gender distribution between the control and the disease groups except for the LBD patients, who were somewhat older. Arterial hypertension and the use of antithrombotic agents were significantly more frequent in the VaD patients compared with the controls, whereas antithrombotic agents were also more used in the AD patients (table 1).

On neuropathological examination of the cerebellar hemisphere, in addition to the increased mean values of CCeMBs and CCeMIs, more arteriosclerotic leptomeningeal vessels were observed in the VaD brains (fig. 1). On the separate cerebellar section examined by MRI, only the VaD brains had significantly more CCeMBs and CCeMIs compared with the controls (fig. 2). The percentage num-

ber of VaD brains with CCeMBs and CCeMIs was also significantly higher compared with the control brains with 78 and 100% in the former and 31 and 19% in the latter group, respectively (table 2).

The CAA brains had no more CCeMBs and CCeMIs than the overall groups of diseased brains without CAA. Also, when comparing CAA brains with those with pure and mixed VaD without CAA, the latter group had significantly higher mean values of CCeMBs and CCeMIs (table 3).

Discussion

This study shows that an increased number of CCeMBs and CCeMIs is not correlated to the presence of CAA, but is mainly found in patients with high vascular risk factors, in particular in those with arterial hypertension and severe arteriosclerotic cerebrovascular disease. Although some groups of neurodegenerative diseases are rather small, we had to make this subdivision as the incidence of CMBs and CMIs in the cerebral hemispheres is quite different between them [16, 17, 19].

There is an excellent correlation between our findings on neuropathological examination and those on MRI. Although there are some differences in the mean numbers, they are not statistically different with a p value of 0.11 for the CCeMBs and 0.21 for the CCeMIs.

We did not examine subtypes of CCeMIs, as done in a previous study [16] as the cerebellar arterial angioarchitecture is completely different from that in cerebral cortex, with only a single type of cortical arterial branches [20].

Fig. 1. Histological sections of cerebellar cortex in the brain of a patient with vascular dementia showing a microbleed (arrow) (a) and an old infarct, near a arteriosclerotic leptomeningeal vessel (arrow) (b).

Fig. 2. MRI of a horizontal section of a cerebellar hemisphere in a patient with atherosclerotic vascular dementia. The T2* sequence shows a cortical microbleed (arrow). Note the iron accumulation around the dentate nucleus and in some leptomeningeal postmortem thrombi. T2 MRI of a horizontal section of a cerebellar hemisphere in another patient with atherosclerotic vascular dementia, showing a cortical microinfarct (arrow).

Table 2. Comparison the mean number (standard deviations) of the cortical cerebellar microbleeds and microinfarcts between the control and disease groups on neuropathological examination and on magnetic resonance imaging

Items	C (n = 16)	AD (n = 29)	AD-CAA (n = 9)	FTLD (n = 10)	ALS (n = 9)	LBD (n = 10)	PSP (n = 12)	VaD (n = 9)
<i>Neuropathology</i>								
Microbleeds, SD	0.3 (1.3)	0.2 (0.7)	0.7 (2.0)	0.1 (0.4)	0.0 (0.0)	0.0 (0.0)	0.2 (0.6)	1.2** (1.3)
Microinfarcts, SD	0.2 (0.9)	0.3 (0.8)	0.9 (1.5)	0.1 (0.4)	0.0 (0.0)	0.8 (1.3)	0.9 (1.8)	3.4** (3.0)
<i>Magnetic resonance imaging</i>								
% Brains								
Microbleeds	31	28	33	20	0	40	42	78**
Microinfarcts	19	28	33	30	11	50	58*	100***
Mean number								
Microbleeds, SD	0.3 (0.6)	0.4 (0.7)	0.6 (1.1)	0.2 (0.4)	0.0 (0.0)	0.4 (0.5)	0.6 (0.5)	3.4** (2.9)
Microinfarcts, SD	0.2 (0.4)	0.3 (0.6)	0.8 (1.2)	0.3 (0.5)	0.2 (0.7)	0.5 (0.5)	0.8 (1.1)	2.3** (0.9)

C = Control; AD = Alzheimer's disease; AD-CAA = Alzheimer's disease with cerebral amyloid Angiopathy; FTLD = frontotemporal lobar degeneration; ALS = amyotrophic lateral sclerosis; LBD = lewy body disease; PSP = progressive supranuclear palsy; VaD = vascular dementia.

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Table 3. Mutual comparison on magnetic resonance imaging of the mean number (standard deviations) of cortical cerebellar microbleeds and microinfarcts in the brains of patients with neurodegenerative diseases, without and with cerebral amyloid angiopathy (CAA), and with pure or mixed vascular dementia due to atherosclerotic disease (AtVaD)

Items	Non-CAA (n = 73)	CAA (n = 15)	AtVaD (n = 8)
Microbleeds	0.7 (1.2)	1.0 (1.2)	4.5 (2.9)**
Microinfarcts	0.9 (1.3)	1.4 (1.2)	3.0 (1.3)*

* $p \leq 0.05$; ** $p \leq 0.01$.

In contrast to the high incidence of CMBs and CMI in the cerebral hemispheres of brains with CAA [16], the latter is not a significant cause of CCEMBs and CCEMIs. The latter are also not increased in the different types of pure neurodegenerative diseases.

Microbleeds are, on the other hand, frequently observed around the dentate nucleus of the cerebellum in AD-CAA and PSP brains, mainly due to the neurodegenerative process itself [17].

A hierarchical sequence study of the vascular pathology in AD showed a difference in progression between CAA and arteriosclerotic disease, the latter affecting more frequently and earlier the cerebellum [21].

CCEMIs are considered mainly border zone infarcts due to focal cerebellar hypoperfusion either by large vessel occlusive disease or by brain embolism [22]. This is in

contrast to small deep microinfarcts and microbleeds, which are more frequently associated to small-artery disease [23].

Isolated cerebellar infarcts may result in subtle cognitive changes that are primarily related to working memory deficit [24] and can contribute to further cognitive impairment in patients with neurodegenerative diseases [25].

In conclusion, CCEMBs and CCEMIs are mainly due to arteriosclerotic cerebrovascular disease and not to CAA. Their increased presence cannot be included in the Boston diagnostic criteria for CAA [26].

Disclosure Statement

The authors report no conflicts of interest.

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