Cerebrovascular and Alzheimer's disease biomarkers in dementia with Lewy bodies and other dementias

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Abstract

Co-pathologies are common in dementia with Lewy bodies and other dementia disorders. We investigated cerebrovascular and Alzheimer's disease co-pathologies in patients with dementia with Lewy bodies in comparison with patients with mild cognitive impairment, Alzheimer's disease, mixed dementia, vascular dementia, or Parkinson's disease with dementia and cognitively unimpaired participants. We assessed the association of biomarkers of cerebrovascular and Alzheimer's disease co-pathologies with medial temporal atrophy and global cognitive performance. Additionally, we evaluated whether the findings were specific to dementia with Lewy bodies.

We gathered a multi-cohort dataset of 4549 participants (dementia with Lewy bodies=331, cognitively unimpaired=1505, mild cognitive impairment=1489, Alzheimer's disease=708, mixed dementia=268, vascular dementia=148, Parkinson's disease with dementia=120) from the MemClin Study, Karolinska Imaging in Dementia Study, Gothenburg H70 Birth Cohort Studies, and the European DLB Consortium. Cerebrovascular co-pathology was assessed with visual ratings of white matter hyperintensities using the Fazekas scale through structural imaging. Alzheimer's disease biomarkers of β-amyloid and phosphorylated tau were assessed in the cerebrospinal fluid for a subsample (N=2191). Medial temporal atrophy was assessed with visual ratings and global cognition with the Mini Mental State Examination. Differences and associations were assessed through regression models, including interaction terms.

In dementia with Lewy bodies, 43% had a high white matter hyperintensity load, which was significantly higher than that in cognitively unimpaired (14%), mild cognitive impairment (26%), and Alzheimer's disease (27%), but lower than that in vascular dementia (62%). In dementia with Lewy bodies, white matter hyperintensities were associated with medial temporal atrophy, and the interaction term showed that this association was stronger than that in cognitively unimpaired and mixed dementia. However, the association between white matter hyperintensities and medial temporal atrophy was non-significant when β-amyloid was included in the model. Instead, β-amyloid predicted medial temporal atrophy in dementia with Lewy bodies, in contrast to the findings in mild cognitive impairment where medial temporal atrophy scores were independent of β-amyloid. Dementia with Lewy bodies had the lowest performance on global cognition, but this was not associated with white matter hyperintensities. In Alzheimer's disease, global cognitive performance was lower in patients with more white matter hyperintensities.

We conclude that white matter hyperintensities are common in dementia with Lewy bodies and are associated with more atrophy in medial temporal lobes, but this association depended on β-amyloid-related pathology in our cohort. The associations between biomarkers were overall stronger in dementia with Lewy bodies than in some of the other diagnostic groups.

Keywords: Imaging; Naturalistic cohort; Atrophy; Multi-cohort

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Introduction

Dementia with Lewy bodies (DLB) is a clinically heterogeneous disorder characterised by variability in symptoms primarily associated with Lewy body pathology. [1] However, some of this variability in symptoms has been associated with cerebrovascular and Alzheimer's disease (AD) co-pathologies. [2,3] This is not exclusive to DLB, as co-pathologies are also common in other dementias and contribute to their patterns of atrophy, clinical characteristics, and cognitive signatures. [3-7] Hence, it is essential to investigate these associations and contributions within DLB but also in comparison with other dementias.

Co-pathologies can be assessed *in vivo* through biomarkers. Cerebrovascular co-pathology is commonly assessed through white matter hyperintensities (WMHs) on magnetic resonance imaging (MRI). [8] In DLB, the frequency and effect of WMHs are debated. A recent review suggested that patients with DLB often have more WMHs than healthy controls and patients with AD, while their clinical contribution is not fully established. [2] Two recent studies reported an association between more WMHs and poorer cognition in DLB. [9,10] Moreover, WMHs have been widely investigated in populations other than DLB. For example, WMHs are associated with an increased risk for all-cause dementia, including AD and Vascular dementia (VaD), [11] and with poorer cognition. [8] Regarding AD co-pathology, β-amyloid (Aβ) and tau neurofibrillary tangles can be assessed in the cerebrospinal fluid (CSF) or on positron emission tomography. AD-biomarkers are positive in around 50% of patients with DLB and are associated with worse cognition. [3,12] However, to our knowledge, the association between WMHs and Aβ has not been studied in DLB before. In non-DLB populations, the review by Roseborough et al[13] suggested that the association between WMHs and Aβ remains unclear but WMHs may influence Aβ accumulation over time.

For these reasons, distinguishing DLB from other dementias can be challenging. In the clinical setting, the relative sparing of the medial temporal lobe is a supportive biomarker for the diagnosis of DLB. [1] Medial temporal atrophy (MTA) is commonly assessed in clinical practice with MRI or computer tomography (CT). [14] Although MTA can be supportive in distinguishing between DLB and AD patients, [15,16] DLB patients with AD co-pathology have more MTA than DLB patients without AD co-pathology. [17,18] However, atrophy has seldom been investigated together with biomarkers of cerebrovascular and AD co-pathologies. [19] Therefore, their associations with each other and whether those associations are specific to DLB remains largely unknown.

The first aim of our current study was to investigate the frequency of WMHs in DLB in comparison with other dementias, mild cognitive impairment (MCI), and cognitively unimpaired (CU) participants, and to elucidate if WMHs are associated with MTA, β-amyloid (Aβ) and phosphorylated tau (p-tau) biomarkers of AD, and cognitive performance. The second aim was to determine if these associations were specific to DLB by testing for statistical interactions with the other diagnostic groups. In-line with previous literature, we hypothesised that DLB patients would have a higher frequency of WMHs compared to CU, MCI, and AD, a lower frequency compared to VaD and mixed dementia (MD, i.e., AD plus VaD) groups, and a similar frequency compared to patients with Parkinson´s disease dementia (PDD). We further hypothesised that WMHs would be associated with more MTA, Aβ positivity, and worse cognitive performance in patients with DLB. Finally, we hypothesised that WMHs and AD biomarkers would independently contribute to MTA in patients with DLB. Regarding the specificity of the associations, we hypothesised that some associations found in DLB could be shared with AD and with groups with vascular aetiology such as VaD and MD. To address these aims and hypotheses, we assembled a large multi-cohort dataset of 4549 individuals including multiple diagnostic groups.

Materials and methods

Participants

Participants were enrolled from four large cohorts: the European DLB Consortium (E-DLB, N=546), [20] the Gothenburg H70 Birth Cohort Studies (H70, N=774), [21] the Karolinska Imaging Dementia Study (KIDS, N=1312), [22] and the MemClin Study (N=1917), [23] for a total of 4549 individuals as follows: DLB=331, CU=1505, MCI=1489, AD=708, MD=268, VaD=148, and PDD=120 (Table 1 and Supplementary Table 1). We included all eligible individuals with scores available on the Fazekas rating scale for WMHs (see next section), who were at least 45 years old and received one of the diagnoses of interest in this study, or were CU.

Diagnostic procedures for patient groups and CU were comparable across the four cohorts and are explained in the original publications[20-23] and detailed in Supplementary Table 2. Mixed dementia in this study refers to AD plus VaD as well as unspecified AD, and is included because it is a common diagnosis in the clinical setting as well as a common diagnostic group in the included cohorts as ICD-10 codes F00.2 and F00.9. Global cognition was assessed with the Mini Mental State Examination (MMSE). [24] Neuroimaging, CSF biomarkers, and MMSE were used in the diagnostic workup. However, neuroimaging was only used in an unstructured

manner for radiological assessment; cognitive impairment and establishment of cognitive profiles was done using extensive neuropsychological protocols above and beyond MMSE; and CSF biomarkers were supportive only for the dementia groups and were available for a subsample (N=2191). Final diagnosis was thus based on clinical judgement. Although neuroimaging, CSF biomarkers, and MMSE are the main variables of interest in this study, the risk for circularity is low and, if any, it would only affect one part of aim one, i.e. investigate the frequency of WMHs in DLB in comparison with other diagnostic groups, but it should not affect the hypotheses related to associations between measures.

Our present study received ethical approval from the Swedish Ethics Review Authority, and in addition, the included cohorts had their own ethical approvals. This research follows the Declaration of Helsinki

Neuroimaging measures of WMHs and MTA

Protocols for the acquisition of neuroimaging data in each cohort are described elsewhere. [20-23] Although protocols are standard and largely comparable between cohorts, due to the multiple scanners involved and the clinical focus of this study we favoured clinical measures of WMH and regional atrophy instead of more advanced research-oriented quantitative measures. Thus, WMHs were assessed with the Fazekas scale, [25] a radiological visual rating scale widely used in clinical settings as a measure of WMHs of presumed vascular origin. [8] Fazekas scores range from 0 to 3, with a score of 0 indicating no or few punctate white matter changes, a score of 1 indicating multiple punctate changes, a score of 2 indicating white matter changes that start to become confluent, and a score of 3 indicating changes that are fully confluent. [14] Following previous publications, [26] we determined Fazekas abnormality based on a cut point of 2, which provided two groups: Fazekas 0-1 vs. 2-3, with age adjustments performed in subsequent statistical analysis by including age as a covariate in the statistical models (please see 2.4 for a description of all the statistical models). MTA was assessed with the Scheltens' scale, [27] which is a visual rating scale that ranges from 0 to 4 and as the Fazekas scale, and it is also widely used in clinical settings. [14] An MTA score of 0 denotes a normal width of the temporal horn and choroid fissure as well as a normal hippocampus, a score of 1 denotes that the choroid fissure is slightly expanded, whilst in scores 2 to 4, the enlargement of choroid fissure and temporal horn, as well as the decreased hippocampal height, are progressively more pronounced. [14] We determined MTA abnormality using the cut points published in Ferreira et

al, [28] as follows: scores ≥ 1.5 for individuals below 75 years of age, ≥2 for individuals between 75 and 84 years and ≥2.5 for individuals older than 85.

Two neuroradiologists with long-time experience in rating scans clinically together applied the Fazekas scale in E-DLB, H70, and KIDS, as well as the MTA rating in E-DLB and KIDS. The two neuroradiologists have previously shown good inter-rater agreement between them on independent ratings. [29] Participants from H70 had MTA assessed with an artificial intelligence method trained on scores from one of our two neuroradiologists. [30] For MemClin, we used ratings from the radiological centre performing the scan, [23] which were performed either on MRI (n=657, 34% of the participants) or on CT (n=1258, 66%). Since previous studies have shown a good agreement for Fazekas and MTA scores across MRI and CT scans, [31] CT and MRI ratings from MemClin were combined for statistical analyses in our current study. Additionally, previous studies have shown a strong correlation between hyperintensities and hypointensities on MRI. [32] Hence, in this article we use the term white matter hyperintensities (WMHs), although they will appear hypointense on CT imaging for 1258 individuals from MemClin. This was done for simplicity and to better align with the current research terminology of WMHs of presumed vascular origin. [8] Otherwise, all ratings from E-DLB (N=546), H70 (N=774), and KIDS (N=1312) were performed on MRI scans. Imaging data was managed through theHiveDB. [33]

Cerebrospinal fluid biomarkers of AD pathology

In a subsample, we assessed Aβ and tau pathology through CSF biomarkers of Aβ42 and phosphorylated tau 181 (p-tau), respectively. See Table 1 for proportion of participants with CSF biomarkers and Supplementary Table 1 for that proportion in each cohort. We defined biomarker positivity based on centre-specific cut points to be able to combine the data from all four cohorts. Cohort-specific cut points and procedures are fully detailed elsewhere and summarized in Supplementary Table 3. [23,34-36]

Statistical analysis

We evaluated cohort characteristics using ANOVA for age and education and the Chi^2 test for sex distribution. All subsequent models were adjusted for age and sex, and MMSE analyses were additionally adjusted for years of education.

We used binary logistic regressions for dichotomous outcomes (i.e. WMHs, MTA, and CSF biomarkers) and multiple linear regression for continuous outcomes (i.e. MMSE).

Specifically, we compared the frequency of WMHs and MTA across diagnostic groups by performing binary logistic regressions, with WMHs and MTA as outcome variables in separated models and diagnostic group as a predictor. We also used binary logistic regression to evaluate the association of WMHs (predictor) with MTA, Aβ and p-tau (outcomes in three separate models), first in DLB patients alone and then across all diagnostic groups. Next, we assessed Aβ and tau in addition to WMHs in predicting MTA, with all predictors in the same binary logistic regression model. Furthermore, we evaluated WMHs in predicting MMSE through multiple linear regression, first in DLB patients alone and then across all diagnostic groups. Finally, to assess whether results were specific to DLB, we tested for the statistical interaction between the biomarker of interest and diagnostic group (DLB as the reference group compared to CU, MCI, AD, MD, VaD, and PDD). For example, for the association of WMHs with MTA, we fitted a model with an interaction term for WMHs by diagnostic group (in addition to WMHs and diagnostic group main effects as well as sex and age covariates as extra predictors) in predicting MTA. Post hoc tests after significant interaction terms were performed with the Chi^2 test and ANCOVA to assess differences pairwise for DLB vs. the other diagnostic groups, for categorical and continuous outcomes, respectively. Cramer V was used to estimate effect sizes after Chi^2 tests. Odds ratios are presented for the binary logistic regressions.

All statistical analyses were performed in R Studio and the alpha level was set to .05, with 95% confidence intervals.

Results

Cohort characteristics

Table 1 shows that compared to the DLB group, the CU and PDD groups were significantly younger, and the MD group was older. In terms of education, the CU and MCI groups had more years of education than the DLB group. Regarding cognition, the DLB group had the lowest MMSE scores. Compared to the DLB group, there were more women in the CU, MCI, AD, and MD groups and fewer women in the PDD group (Table 1). Thus, all subsequent analysis had both age and sex as covariates. Characteristics for the DLB group stratified by WMHs, MTA, and Aβ biomarker status are available in Supplementary Table 4.

WMHs across diagnoses

Results from logistic regression showed that the DLB group had significantly more WMHs than CU, MCI, and AD, but less WMHs than the VaD group (Table 1, Fig 1). We observed no significant differences in WMHs between DLB and the MD and PDD groups.

The association between WMHs and MTA

Table 1 and Fig 2 show the frequency of MTA across diagnoses. DLB had a significantly more MTA than the CU group, but less MTA than the AD and MD groups.

In the DLB group, more WMHs were significantly associated with more MTA (Table 2). To assess whether this association was DLB-specific, we tested for the statistical interaction between WMHs and diagnostic group in the whole sample, with MTA as the outcome variable (Table 3, Fig 3A). Compared to DLB, we observed a significant interaction between WMHs and diagnostic group for CU and MD groups. Specifically, DLB patients with more WMHs had more MTA compared to DLB patients with less WMHs (effect size by *Cramer V* = 0.24), whilst in CU and MD, MTA scores tended to be independent of WMH status (CU group: *Cramer V* = 0.08; MD group: *Cramer V*= 0.01).

The association between WMHs and AD biomarkers

AD biomarkers were available for a subsample of 726 individuals who were significantly younger and had less WMHs than individuals who did not have AD biomarkers ($P \leq 0.05$, data not shown). In contrast, there were no statistically significant differences in MMSE, MTA, or sex distribution ($P > 0.05$).

AD biomarkers across diagnoses are reported in Table 1. The DLB group had a significantly higher frequency of a positive Aβ biomarker than the CU group, but a lower frequency than the AD and MD groups. In terms of p-tau, the DLB group had a higher frequency of a positive p-tau biomarker than the CU and MCI groups, but a lower frequency than the AD group.

In DLB, a positive Aβ or p-tau biomarker was not associated with WMHs in separate models for Aβ or p-tau, respectively ($X^2(3, N=84)=3.966$, $p=0.265$ and $X^2(3, N=84)=3.964$, $p=0.265$). To understand if this finding was DLB-specific, we tested for the statistical interaction between WMHs and diagnostic group, with Aβ or p-tau as outcome variables. We did not find any significant interaction between WMHs and diagnostic group ($P > .05$).

WMHs, AD biomarkers and MTA

We then evaluated WMHs and AD biomarkers jointly in the prediction of MTA. For DLB, WMHs were no longer significantly associated with more MTA when Aβ and tau were included in the model, and only Aβ significantly predicted MTA in the presence of WMHs and tau (Table 2). To assess whether this finding was DLB-specific, we tested for the statistical interaction between Aβ and the diagnostic group in predicting MTA, retaining WMHs and tau in the model (Table 3, Fig 3B). We observed a significant interaction between DLB and the MCI group. Specifically, DLB patients with a positive Aβ biomarker had significantly more MTA than DLB patients with a negative Aβ biomarker ($P>.05$), whilst in MCI, MTA scores were independent of Aβ status ($P\leq.05$).

The association between WMHs and MMSE

We conducted a multiple linear regression with WMHs as the predictor and MMSE as the outcome variable. In the DLB group, WMHs were not significantly associated with MMSE scores (Table 2). To understand if this finding was DLB-specific, we tested for the statistical interaction between WMHs and the diagnostic group, with MMSE as the outcome variable (Table 3, Fig 4). We observed a significant interaction, where compared to the DLB group, the AD group showed a significant association between WMHs and MMSE. Specifically, in DLB, MMSE performance was independent of WMHs ($P>.05$), while in the AD group, patients with more WMHs performed worse in MMSE than those with less WMHs ($P\leq.05$).

Discussion

We investigated WMHs in relation to MTA, AD biomarkers, and cognition in DLB as well in comparison with other dementias, MCI, and CU. We first compared WMHs across diagnoses, showing that the DLB group had more WMHs than CU, MCI, and AD, whilst they had less WMHs than the VaD group. We then evaluated the association of WMHs with MTA, AD biomarkers, and cognition in DLB as well as in comparison with the other diagnostic groups through statistical interactions. Although several of the associations found in DLB were shared with the other diagnostic groups, we also observed some specific findings. In DLB, the association between WMHs and MTA was stronger than in CU and MD. Similarly, the

association between Aβ and MTA was stronger in DLB than in MCI. Finally, the association between WMHs and cognition was weaker in DLB than in AD.

DLB patients had more WMHs than CUs and patients with MCI or AD, whilst they had less WMHs than patients with VaD. We are aware of only one previous study that compared WMHs across multiple diagnostic groups, including DLB. [37] The authors found that patients with DLB had more WMHs than CUs and less WMHs than patients with VaD, in line with our findings. However, Koikkalainen and colleagues [37] did not find any statistically significant differences in WMHs between DLB and AD. Compared to our sample, Koikkalainen and colleagues[37] had a younger sample with less WMHs overall, which could explain the different results. Moreover, although we qualitatively observed more WMHs in DLB (43%) than in PDD (30%), this difference did not reach statistical significance in our cohort (p=0.13). The recent larger study by Gan and colleagues did reach a statistical significance, showing that DLB patients have more WMHs than PDD patients. [38] Previously, the review on DLB and PDD by Hijazi and colleagues[2] had highlighted inconclusive results with regards WMHs in DLB and PDD, potentially due to differing methods and moderate sample sizes (from 17 to 42 participants for DLB and from 20 to 88 participants for PDD). Therefore, our study and the study by Gan et al. [38] contribute to clarify that discussion by suggesting that in large cohorts, DLB patients seem to have more WMHs than in PDD patients. Altogether, the current evidence suggests that patients with DLB have more WMHs than CU, MCI, AD, and PDD and less WMHs than VaD, but similar levels as MD.

MRI was used in the diagnostic work up for our participants. This could have partly explained the finding on less WMHs in DLB than in VaD, but we do not expect any risk for circularity or bias in our findings for DLB versus CUs, MCI, AD, and PDD. In terms of biological mechanisms, WMHs are usually presumed to be of vascular origin, [8] but they may also be associated with neurodegenerative processes beyond vascular aetiology, at least in AD. [39,40] As such we encourage future WMH studies comparing multiple diagnostic groups, helping to fully understand differences and similarities between the different types of dementia, as the field is very limited at the moment. Increasing our understanding of the pathogenesis behind WMHs will ultimately inform on their clinical use, for example with implications for treatment decisions. That understanding can also have implications for the differential diagnosis in dementia, moving the field forward to acknowledge mixed forms of dementia beyond the well-established AD plus VaD mixed dementia (MD) form.

In terms of the association of WMHs with MTA, we found that DLB patients with WMHs had greater atrophy in the medial temporal lobe than DLB patients without WMHs. To

our knowledge, only Joki and colleagues[26] also investigated the association between WMH and MTA using visual rating scales as in our study. The authors found that periventricular hyperintensities were associated with MTA in DLB, but deep and subcortical hyperintensities were not associated with MTA. Hence, the association between WMHs and atrophy could be regional rather than global. In this line, three recent studies investigated regional measures of atrophy and semi or fully automated methods for global WMH. The measures of atrophy included brain areas that overlap only partially with the areas assessed by the MTA rating scale used in our study. [9,19,41] In Ferreira et al. [19] we found no statistically significant association between global WMHs and hippocampal volume in DLB. However, a significant association was observed for fusiform volume. [19] The fusiform gyrus is adjacent to medial temporal regions included in the MTA scale and atrophy in fusiform may thus contribute to width of temporal horn and choroid fissure, which are captured by the MTA scale. When assessing cortical thickness instead of volume, we could not find any statistically significant association for global WMHs with fusiform thickness in another DLB study. [9] We did not either find significant associations with thickness in other regions covered by the MTA scale or hippocampal volume. [9] The different results could be due to different sensitivity of the MRI methods used across these studies, but they could also partly be cohort-specific. In this line, these findings suggest that regional vulnerabilities could be expressed differently across DLB patients, who may be represented differently across cohorts. Indeed, in a recent publication we showed three distinct patterns of atrophy in DLB. Only the subgroup of DLB patients with more widespread atrophy including atrophy in medial temporal lobes, had a higher WMH volume. [41] Taking all these findings together, it is likely that WMHs are associated with atrophy in medial temporal areas in DLB, but the mechanism underlaying this association should be further investigated, ideally in studies including regional measures not only of both WMHs and atrophy.

Indeed, our models for WMHs and AD biomarkers predicting MTA could shed some light on the potential mechanism for WMHs and MTA. We demonstrated that WMHs were no longer significantly associated with MTA in DLB when AD biomarkers were also in the model. Instead, DLB patients with a positive Aβ biomarker showed more MTA. The medial temporal lobe is often spared in DLB and is used as a supportive biomarker in the diagnosis of DLB. [1] For example, the absence of MTA *in vivo* can distinguish pathologically-confirmed DLB from AD. [15,16] However, not all DLB patients have preserved medial temporal lobes. DLB patients with a positive Aβ biomarker had more MTA than DLB patients with a negative AD biomarker in a clinical sample, [17] and smaller hippocampus in pathologically-confirmed DLB patients. [18]

What it is not fully understood yet is the interplay between Aβ and WMHs in predicting MTA. A first attempt to answer that question is the study by Abdelnour et al[42], using E-DLB data. The authors identified subgroups of DLB patients based on AD biomarkers, lobar atrophy, and clinical features, and reported WMHs across subgroups. They found that DLB patients with Aβ positivity and more MTA had more WMHs. [42] However, to our knowledge, only our current study and the recent article by Ferreira et al[19] have explicitly modelled the interplay between Aβ and WMHs in predicting MTA in DLB. Using visual rating scales and CSF biomarkers, our findings suggest that whilst WMHs are associated with MTA, that association may partly depend on Aβ status. Using research-oriented MRI and PET biomarkers, Ferreira et al[19] showed that the volume of the fusiform gyrus could be predicted by a double mechanism including one path for global WMHs and one separate path for regional tau via regional Aβ. [19] The visual ratings and CSF biomarkers in our study are clinically available but lack the spatial granularity of MRI and PET measures in Ferreira et al[19]. These findings combined suggest that there is a complex interplay between WMHs, AD biomarkers, and MTA, while the exact biological mechanisms are not yet understood and require future investigation with both regional and global biomarker measures.

We found no statistically significant association between WMHs and MMSE in DLB. We previously showed that more WMHs had only a modest association with worse MMSE scores in DLB, using a different method for WMHs in a smaller sample minimally overlapped with our current sample. [9] The association of WMHs with cognition in DLB has been previously discussed in very few studies. [2] Chen et al[10] did find an association between more WMHs and cognitive impairment in DLB using a different cognitive test, i.e. MoCA. The MoCA test includes executive components to a larger extend than the MMSE test used in our study. In AD, WMHs are more associated with executive function than with memory. [43] Therefore, MMSE may be less sensitivity to WMH-related cognitive impairment than MoCA. Further, participants in Chen et al[10] were at a more severe cognitive stage and had less years of education than in our cohort, which could provide more variance in the data and thus explain the different result with our study.

To summarize, WMHs were common in DLB and were associated with MTA. However, when AD biomarkers were added to the model, WMHs were no longer statistically significantly associated with MTA, while a positive Aβ biomarker significantly predicted more MTA. WMHs were not associated with MMSE in our cohort. Our second main goal in this study was to elucidate whether these associations were DLB-specific, throughout testing for statistical interactions between biomarkers and diagnostic group in our models. We

demonstrated that for the association of WMHs with MTA, findings in the DLB group differed from those in the CU and MD groups. In DLB patients, the association between WMHs and MTA was statistically stronger than in both CU and MD. We observed that in the CU group, WMHs and MTA levels were low whilst in the MD group, WMHs and MTA levels were high, largely independently of WMH status. This suggests that MTA in DLB is partly influenced by WMH, while this does not seem to be the case in CU and MD, highlighting the specificity of this finding in DLB. We then evaluated the interaction between Aβ and diagnostic group in the prediction of MTA. We demonstrated that DLB patients with a positive Aβ biomarker had more MTA than patients with a negative Aβ biomarker, whilst MTA scores were independent of Aβ status in MCI. This interaction highlights the association between Aβ and MTA in DLB and may indicate different mechanisms of neurodegeneration than in MCI. Finally, the interaction for MMSE showed that while WMHs were not associated with MMSE in DLB, AD patients with more WMHs had worse MMSE scores. Despite these statistically significant interactions, we note that the other groups in the models (VaD, PDD) did not show any statistical interaction with DLB. Overall, this finding would suggest that the effect of WMHs is rather similar across diagnoses, but their load, regional placement, and interplay with alpha-synuclein and AD related pathological changes may differ. For example, whilst it is still debated whether there is a difference in the frequency of WMHs in DLB and PDD, it has been suggested that the regional distribution of WMHs does differ. [2] Similarly, differences in the regional distribution of WMHs have also been reported between DLB and AD. [2] Our study is a first step in understanding the interplay between WMHs, AD biomarkers, MTA, and MMSE. Future studies should evaluate these factors with methods that have high granularity, for example regional measures for WMHs or cognitive tests that assess specific cognitive domains in depth. Using cognitive tests with higher granularity would expand our current findings in several ways. Firstly, tests for global cognitive screening like MMSE may have limited ability to detect milder forms of cognitive impairment, while detailed neuropsychological tests would better characterise cognitive profiles specific to different types of dementia and MCI. Secondly, assessing cognition with detailed neuropsychological tests would allow for an increased understanding of the relationship and synergies of pathologies such as WMHs and AD biomarkers with cognition. Further, the inclusion of longitudinal data would also be important in understanding the sequence of cognitive impairments in relation to pathological and clinical trajectories across diagnoses.

This study has some limitations. Firstly, we used data from four cohorts, where one was a multi-centre study across Europe, one was a naturalistic multi-centre study including specialist clinics, one was a research-oriented specialised clinic, and one was population-based. Although this approach increases representativeness and generalisation of results, it may also introduce some variability in terms of measures and diagnostic procedures across cohorts, despite our efforts to harmonise the data for statistical analysis. To partly address this challenge, we ensured that diagnostic procedures were comparable and visual ratings were performed following established clinical guidelines across all cohorts. Secondly, diagnoses were primarily based on clinical judgment and lacked neuropathological confirmation. Previous studies have shown varied concordance between clinical and neuropathological diagnosis, [44,45] which should be considered when interpreting our findings. Thirdly, although DLB and PDD are often discussed to be part of the same spectrum of alpha-synuclein-related pathology, both are common clinical diagnoses in clinical settings and the cohorts used in this study. Therefore, we aimed to include both groups in our analyses and compare them to further clarify any potential differences in terms of WMHs, MTA, CSF biomarkers, and MMSE. The clinics had the information about the patients' onset of cognitive and motor symptoms for implementation of the one year rule for the differential diagnosis between DLB and PDD. [1] However, we could not access that specific data for reporting and analysis in our current study. Fourthly, we used Fazekas to assess WMHs. Whilst Fazekas is a clinically available and easy to use scale, it only reflects one aspect of cerebrovascular pathology, while there are imaging measures for the assessment of other aspects such as cerebral microbleeds or lacunes. [8] Additionally, Fazekas does not provide quantitative volume of WMHs nor detailed information on regional placement. In this line, three recent imaging studies characterized the regional pattern of cholinergic alterations in grey and white matter in DLB, [46-48] and we demonstrated an association between WMHs and atrophy in brain areas that receive prominent cholinergic input, in DLB. [9] Hence, an interesting prospect for the future would be to evaluate regional placement of WMHs on areas associated to cholinergic system in DLB. The Fazekas scale lacks granularity compared to automated volumetric methods for WMHs. However, Fazekas is less sensitive to variability in MRI scanner and processing methods common in multi-centre and multi-cohort studies, which guided method choice in our study. Finally, our sample was smaller for CSF analyses than for the main analysis, and it was younger and had less WMHs. This could in part reflect clinical decisions and the lower referral rate for lumbar punctures in older patients with more comorbidities. At the same time, this could have reduced our

possibilities to capture stronger associations of WMHs with AD biomarkers and atrophy, since all these increase with age in DLB. [9,12,49]

In conclusion, this study demonstrates that WMHs are more frequent in DLB than in CU, MCI, and AD, but less frequent than in VaD. In DLB, WMHs were associated with MTA, but this association could depend on Aβ positivity. We also observed several statistical interactions indicating partly DLB-specific results. The interactions overall suggest stronger associations in DLB in measures reflecting biological mechanisms (WMHs, Aβ, and MTA), which do not seem to translate to stronger associations in global cognitive performance assessed with MMSE. We believe these results reflect the added contribution of cerebrovascular and Aβ co-pathologies to DLB pathogenesis. While the biological contributions of WMHs may be similar across diagnoses, their effect may depend on the presence of co-pathologies, which in DLB have larger variability than in CU, MCI, and MD (the groups we captured biological interactions with). To advance the current field, it will be important to continue investigating the influence of these pathologies across multiple dementia diagnoses and their prodromal stages, elucidating potentially shared mechanisms but also distinct contributions to clinical presentations.

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Competing interests

A.R., U.E., S.S., L.R., J.S., A.Z., K.O., J.H., S. G-P., A.A., A.W.L., A.P., M.G.K., I.R., Z.W., J.S., M.P., L.B., T.G., I.S., L-O.W., & E.W – nothing to disclose.

S.K. has served at scientific advisory boards and / or as consultant for Geras Solutions, Optoceutics, Biogen and Bioarctic

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D.F. - consults for BioArctic and has received honoraria from Esteve.

Data availability

The data may be available upon reasonable request if legal and ethical requirements can be met. Included cohorts operate independently regarding data sharing and the corresponding authors can facilitate contact with each cohort.

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Figure legends

Figure 1. White matter hyperintensities across diagnoses

Fig 1. White matter hyperintensities (WMHs) across diagnoses. Odds ratios from a logistic regression model. Panel (A) shows examples of low and high WMHs. Panel (B) displays absolute frequencies of high WMH load (Fazekas scores 2 and 3). Panel (C) shows odds ratios based on logistic regression with the Dementia with Lewy Bodies (DLB) group as reference. Model adjusted for age and sex. $X^2(8, N=4549)= 634.82$, p<.001**.** In the logistic regression model, the WMHs variable is dichotomous and was coded as a high WMHs load (Fazekas scores 2 and 3) vs. a low WMHs load (Fazekas scores 0 and 1). Dot reflects the estimate (odds ratio) and whiskers the 95% confidence interval. Significant results compared to DLB does not cross the black line.

Figure 2. Medial temporal atrophy (MTA) across diagnoses

Fig 2. Medial temporal atrophy (MTA) across diagnoses. Odds ratios from a logistic regression model. Panel (A) shows examples of low and high MTA. Panel (B) display absolute frequencies of MTA (cut offs are age adjusted). Panel (C) shows odds ratios based on logistic regression with the Dementia with Lewy Bodies (DLB) group as reference. Absolute frequencies of MTA, odds ratios based on logistic regression with the Dementia with Lewy Bodies(DLB) as reference group. Model adjusted for age and sex. Model adjusted for age and sex. $X^2(8, N=3508)= 324.72$, p<.001**.** Dot reflects the estimate and whiskers the 95% confidence interval. Significant results compared to DLB does not cross the black line.

Figure 3. Interactions between biomarkers and diagnostic group on the prediction of MTA (Percentage with a high score of MTA)

Figure 3. Results from the logistic regression models in table 3. Interactions between biomarkers and diagnostic group on the prediction of MTA. Only statistically significant interactions displayed. Displayed is the percent in each category with a high degree of MTA. (A) Interaction of diagnostic group and WMHs on MTA; Dementia with Lewy bodies in comparison with Cognitively unimpaired and Mixed dementia. Model is adjusted for age and sex, and main effects of diagnostic group and WMHs are also fitted in addition to the interaction term. Omnibus statistics $X^2(15, N=3508)= 414.55$, p<.001 (B) Interaction of diagnostic group and Aβ on MTA; Dementia with Lewy bodies in comparison with Mild cognitive impairment. Model is adjusted for age and sex, and main effects of diagnostic group and MTA are also fitted in addition to the interaction term. Omnibus statistics X2(13, N=1477)= 137.77, p<.001. MTA=Medial temporal atrophy, WMH= White matter hyperintensities.

Figure 4. Interaction between WMHs and diagnosis on MMSE

Figure 4. Results from the linear regression models in table 3. Interaction between WMHs and diagnosis on MMSE. Only the statistically significant interaction displayed with fitted values of individual observations. Dementia with Lewy bodies in comparison to Alzheimer's disease. Model is adjusted for age, sex and education, and main effects of diagnosis and WMHs are also fitted in addition to the interaction term. Omnibus statistics $F(16, 2707)= 164.4$, Adjusted R^2 0.49, p<.001

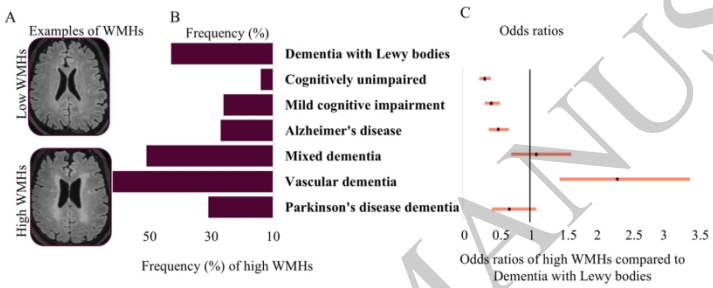

Figure 1. White matter hyperintensities across diagnoses

Fig 1. White matter hyperintensities (WMHs) across diagnoses. Odds ratios from a logistic regression model. Panel (A) shows examples of low and high WMHs. Panel (B) displays absolute frequencies of high WMH load (Fazekas scores 2 and 3). Panel (C) shows odds ratios based on logistic regression with the Dementia with Lewy Bodies (DLB) group as reference. Model adjusted for age and sex. X2(8, N=4549)= 634.82, p<.001. In the logistic regression model, the WMHs variable is dichotomous and was coded as a high WMHs load (Fazekas scores 2 and 3) vs. a low WMHs load (Fazekas scores 0 and 1). Dot reflects the estimate (odds ratio) and whiskers the 95% confidence interval. Significant results compared to DLB does not cross the black line.

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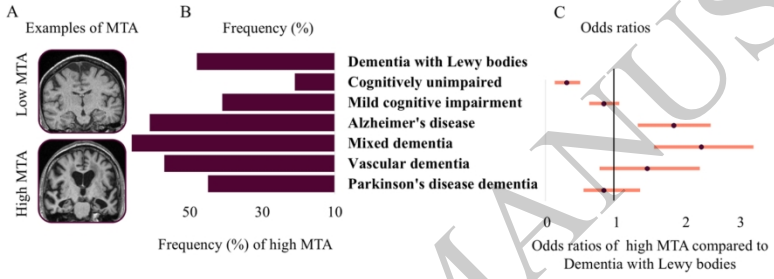

Figure 2. Medial temporal atrophy (MTA) across diagnoses

Fig 2. Medial temporal atrophy (MTA) across diagnoses. Odds ratios from a logistic regression model. Panel (A) shows examples of low and high MTA. Panel (B) display absolute frequencies of MTA (cut offs are age adjusted). Panel (C) shows odds ratios based on logistic regression with the Dementia with Lewy Bodies (DLB) group as reference. Absolute frequencies of MTA, odds ratios based on logistic regression with the Dementia with Lewy Bodies(DLB) as reference group. Model adjusted for age and sex. Model adjusted for age and sex. X2(8, N=3508)= 324.72, p<.001. Dot reflects the estimate and whiskers the 95% confidence interval. Significant results compared to DLB does not cross the black line.

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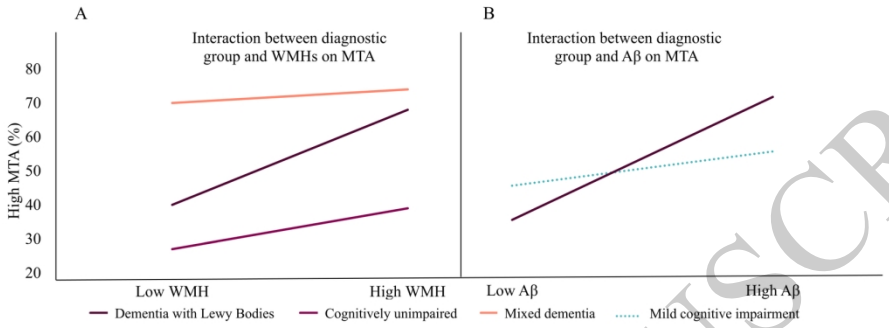

Figure 3. Interactions between biomarkers and diagnostic group on the prediction of MTA (Percentage with a high score of MTA)

Figure 3. Results from the logistic regression models in table 3. Interactions between biomarkers and diagnostic group on the prediction of MTA. Only statistically significant interactions displayed. Displayed is the percent in each category with a high degree of MTA. (A) Interaction of diagnostic group and WMHs on MTA; Dementia with Lewy bodies in comparison with Cognitively unimpaired and Mixed dementia. Model is adjusted for age and sex, and main effects of diagnostic group and WMHs are also fitted in addition to the interaction term. Omnibus statistics X2(15, N=3508)= 414.55, p<.001 (B) Interaction of diagnostic group and Aβ on MTA; Dementia with Lewy bodies in comparison with Mild cognitive impairment. Model is adjusted for age and sex, and main effects of diagnostic group and MTA are also fitted in addition to the interaction term. Omnibus statistics X2(13, N=1477)= 137.77, p<.001. MTA=Medial temporal atrophy, WMH= White matter hyperintensities.

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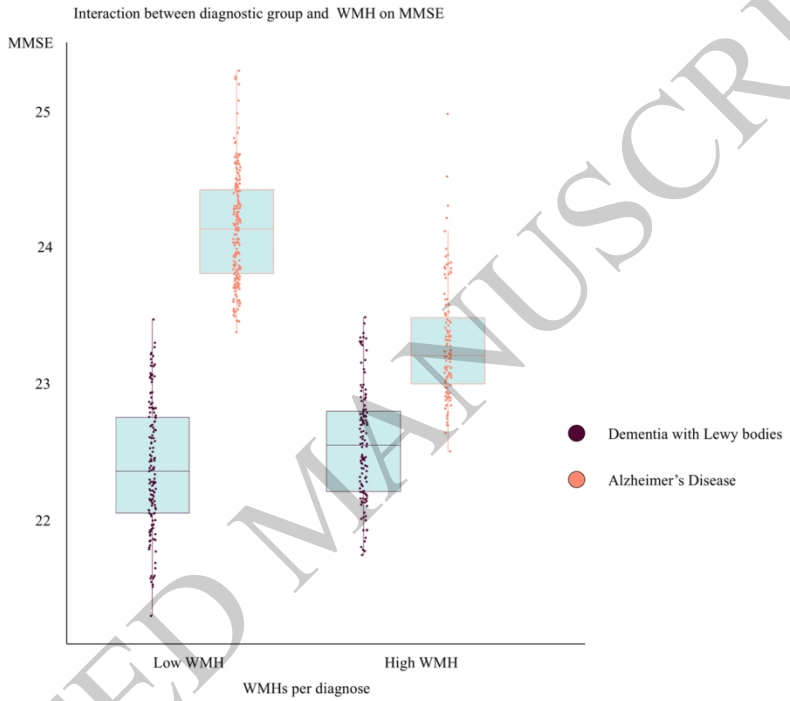

Figure 4. Interaction between WMHs and diagnosis on MMSE

Figure 4. Results from the linear regression models in table 3. Interaction between WMHs and diagnosis on MMSE. Only the statistically significant interaction displayed with fitted values of individual observations. Dementia with Lewy bodies in comparison to Alzheimer's disease. Model is adjusted for age, sex and education, and main effects of diagnosis and WMHs are also fitted in addition to the interaction term. Omnibus statistics F(16, 2707)= 164.4, Adjusted R2 0.49, p<.001

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Table 1. Cohort characteristics

	DLB	CU	MCI	AD	MD	VaD	PDD	Entire cohort
N	311	1505	1489	708	268	148	120	4549
Age, years	73.4 (8.1)	67.5 (7.9)	72.9 (9.7)	72.3 (9.1)	76.2 (8.1)	74.0 (10.3)	70.6 (7.4)	71.2 (9.3)
Women, N (%)	84 (38)	880 (59)	712 (48)	419 (59)	126 (47)	62 (42)	32 (27)	2350 (52)
Education, years	11.0 (4.0)	13.5 (4.0)	12.9 (6.6)	11.9 (3.6)	12.0 (3.6)	11.2 (3.3)	10.2 (4.3)	12.6 (3.9)
MMSE, total score	22.7 (4.0)	28.6 (2.1)	26.9 (2.6)	23.2 (4.0)	23.41 (4.8)	23.4 (4.5)	23.7 (4.0)	26.2 (3.9)
WMHs, High score, N (%)	135 (43)	215 (14)	388 (26)	188 (27)	136 (51)	92 (62)	37 (31)	1191 (26)
MTA, High score, N (%)	125 (48)	303 (21)	445 (41)	274 (61)	166 (66)	80 (57)	39 (45)	1441 (41)
Aβ, Positive, N (%)	28 (33)	108 (15)	228 (29)	246 (68)	95(61)	15 (24)	-	726 (33)
P-tau, Positive, N (%)	31 (37)	59 (8)	201 (27)	209 (58)	68 (44)	-	-	567 (26)

Data is reported as mean (SD), otherwise count (%) when frequencies are reported. Education N=2881, MMSE N= 2724, MTA N= 3508, Aβ and P-tau analysis N=2191. Analyses for Aβ and p-tau were only performed in groups with sufficient data (≥8 cases per cell): Parkinson disease with Dementia (PDD) had to be excluded from analysis of both Aβ and p-tau, and the Vascular dementia group (VaD) had to be excluded from the analysis of p-tau. For PDD, 28 individuals had available data, with only 6 individuals having a positive Aβ biomarker and 0 a positive p-tau biomarker. For VaD, 61 individuals had available data for p-tau, with only 3 individuals having a positive p-tau biomarker. Abbreviations: Aβ = β-amyloid, AD= Alzheimer's Disease, CU= Cognitively unimpaired, DLB= Dementia with Lewy bodies, MCI= Mild Cognitive Impairment, MD= Mixed Dementia, MMSE=Mini Mental State Examination, MTA=Medial temporal atrophy, PDD=Parkinson Disease with Dementia, p-tau = phosphorylated tau, VaD= Vascular Dementia, WMHs = White matter hyperintensities.

Table 2. Results for Dementia with Lewy body only analyses

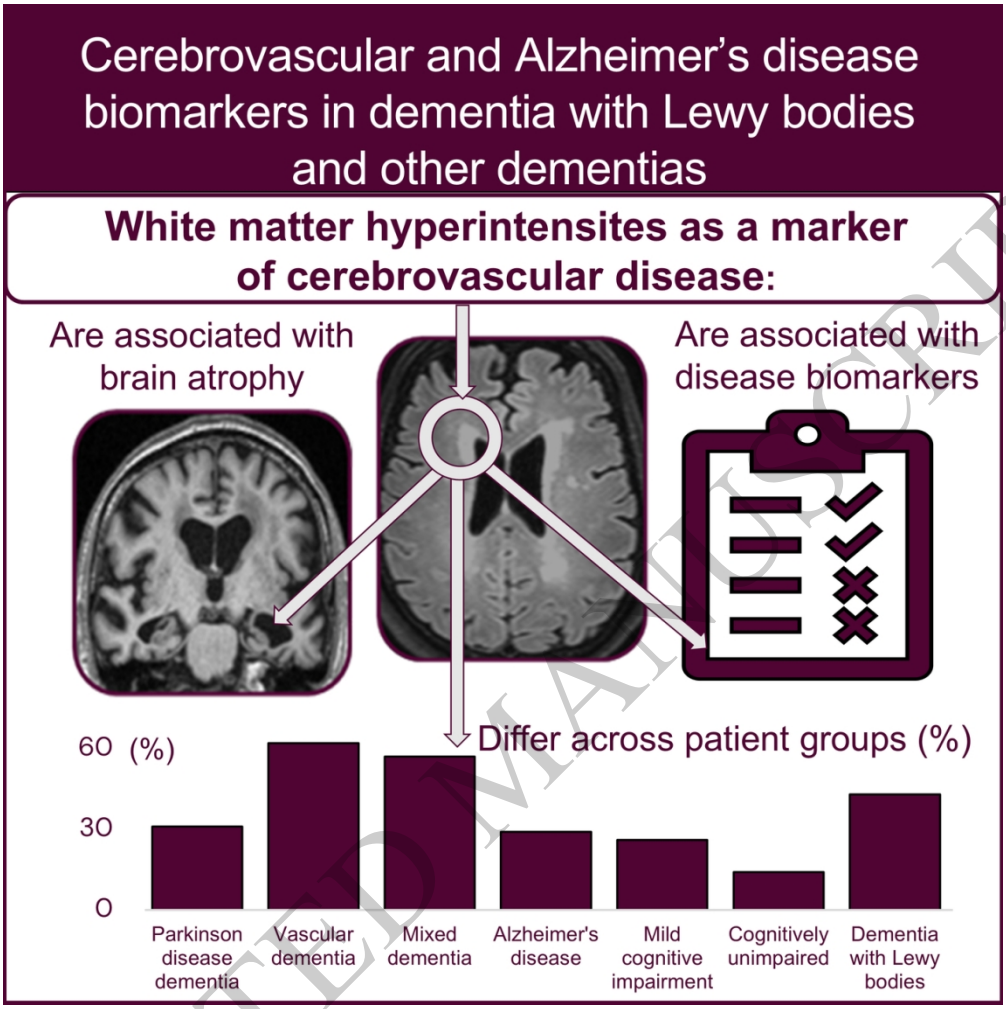
Model	Predictor	Estimate	95% confidence interval / *p*-value
Association of WMHs with MTA			
X^2(3, N=259)=27.701, p<0.001	WMHs	1.90	**1.09 - 3.32**
	Age	1.36	**1.13 - 1.64**
	Sex	1.02	0.60 - 1.74
Association of WMHs, Aβ and P-tau with MTA			
X^2(5, N=83)= 24.367, p<0.001	WMHs	1.53	0.51 - 4.62
	Aβ	3.98	**1.37 - 12.62**
	P-tau	0.47	0.16 - 1.37
	Age	1.87	**1.26 - 2.98**
	Sex	1.05	0.27 - 4.21
Association of WMHs with MMSE			
F(2, 258)=3.335,	WMHs	0.34	0.52
Adjusted R^2=0.035, p=0.01	Age	-0.01	0.86
	Sex	-0.24	0.64
	Education	0.22	**<0.001**

Odds ratios and 95% confidence intervals are reported for logistic regression models and beta estimates and p-values are reported for multiple linear regression models. For the logistic regressions, the odds ratio for age is presented per 5 years of age. Reference groups are negative biomarkers and male sex. Significant predictors are bold. Abbreviations: Aβ = β-amyloid, MMSE=Mini Mental State Examination MTA=Medial temporal atrophy, P-tau = phosphorylated tau. WMHs = White matter hyperintensities.

Table 3. Results for analyses across diagnoses and interactions with the Dementia with Lewy bodies group.

Model	Predictor	Estimate	95% confidence interval / *p*-value
Association of WMHs with MTA - WMHs by diagnosis interaction			
X^2(15, N=3508)= 414.55, p<.001	WMHs*diagnosis (CU)	0.52	**0.28 - 0.96**
	WMHs*diagnosis (MCI)	0.64	0.36 - 1.13
	WMHs*diagnosis (AD)	0.95	0.48 - 1.88
	WMHs*diagnosis (MD)	0.36	**0.17 - 0.74**
	WMHs*diagnosis (VaD)	0.71	0.30 - 1.71
	WMHs*diagnosis (PDD)	0.66	0.24 - 1.85
	Age	0.93	**0.88 - 0.97**
	Sex	0.63	**0.55 - 0.73**
Association of WMHs, AD-biomarkers with MTA - interaction WMHs and Aβ			
X^2(13, N=1477)= 137.77, p<.001	Aβ *diagnosis (CU)	0.34	0.11 - 1.00
	Aβ *diagnosis (MCI)	0.32	**0.11 - 0.91**
	Aβ *diagnosis (AD)	0.53	0.17 - 1.60
	Aβ *diagnosis (MD)	0.43	0.12 – 1.44
	Age	0.95	0.88 - 1.01
	Sex	0.66	**0.53 - 0.823**
Association of WMHs with MMSE			
F(16, 2707)= 164.4, Adjusted R^2 0.49, p<.001			
	WMHs*diagnosis (CU)	-0.01	0.97
	WMHs*diagnosis (MCI)	-0.35	0.29
	WMHs*diagnosis (AD)	-0.87	**0.03**
	WMHs*diagnosis (MD)	-0.33	0.520
	WMHs*diagnosis (VaD)	-0.35	0.581
	WMHs*diagnosis (PDD)	0.12	0.844
	Age	0.02	**0.01**
	Sex	0.03	0.77
	Education	0.11	**<.001**

Odds ratios and 95% confidence intervals are reported for logistic regression models and beta estimates and p-values are reported for multiple linear regression models. For the logistic regressions, the odds ratio for age is presented per 5 years of age. Models are fitted with the main effect of diagnostic group and biomarker of interest and interaction term of interest, e.g. the model for Association of WMHs with MTA - WMHs by diagnosis interaction additionally contain the predictors WMH and diagnosis which are not displayed in the interest of brevity. Reference groups are Dementia with Lewy bodies, negative biomarkers, and male sex. Significant predictors are bold. Abbreviations: Aβ = β-amyloid, AD= Alzheimer's disease, CU= Cognitively unimpaired, MCI=Mild Cognitive Impairment, MD= Mixed dementia, MMSE=Mini Mental State Examination, MTA=Medial temporal atrophy, PDD = Parkinson disease with dementia, P-tau = phosphorylated tau, VaD=Vascular dementia, WMHs = White matter hyperintensities.



Graphical Abstract

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