Prospective longitudinal evaluation of cytokines in mild cognitive impairment due to AD and Lewy body disease

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Objectives: We conducted a prospective longitudinal study of plasma cytokines during the Mild Cognitive Impairment (MCI) stage of Lewy body disease and Alzheimer’s disease, hypothesizing that cytokine levels would decrease over time and that this would be correlated with decline in cognition.

Methods: Older (≥60) people with MCI were recruited from memory services in healthcare trusts in North East England, UK. MCI was diagnosed as due to Alzheimer’s disease (MCI-AD) or Lewy body disease (MCI-LB). Baseline and repeat annual clinical and cognitive assessments were undertaken and plasma samples were obtained at the same time. Cytokine assays were performed on all samples using the Meso Scale Discovery V-Plex Plus Proinflammatory Panel 1, which included IFNγ, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-13 and TNFα.

Results: Fifty-six patients (21 MCI-AD, 35 MCI-LB) completed prospective evaluations and provided samples up to 3 years after baseline. Six cytokines (IFNγ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNFα) showed highly significant (P < .002) decreases over time. AD and LB did not differ in rate of decrease nor were there any effects related to age or general morbidity. Decrease in five of these cytokines (IFNγ, IL-1β, IL-2, IL-4, and IL-10) was highly correlated with decrease in cognition (P < .003).

Conclusions: Peripheral inflammation decreased in both disease groups during MCI suggesting this may be a therapeutic window for future anti-inflammatory agents.

KEYWORDS
Alzheimer’s disease, cytokines, dementia with Lewy bodies, inflammation, MCI

1 | INTRODUCTION

Alzheimer’s disease (AD) and dementia with Lewy bodies (DLB) are the two commonest causes of neurodegenerative dementia. Extensive evidence indicates their pathophysiology involves central and peripheral inflammation. A meta-analysis of 40 studies found increases in pro-inflammatory cytokines TNFα, IL-6, IL-1β, IL-12 and IL-18 in AD compared with controls.1 GWAS in AD have identified that genetic polymorphisms involved in inflammatory processes are risk factors for AD,2 including for genes coding for IL1β, HLA-DR, CLU, TREM2 and CR1. PET imaging with PK11195, a measure of cerebral microglial activation, has reported increased binding in cortical regions in AD compared with controls,3,4 supporting microglial activation in AD. Microglia surround, react to and phagocytose Amyloid-beta (Aβ) in cell

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Inflammatory cytokines were previously observed to be elevated in MCI relative to both healthy older adults and those with dementia.

This was the case in both Lewy body and Alzheimer's diseases.

In this follow-up study, the increased inflammation in MCI subsided as this progressed in severity.

Inflammatory processes may represent a potential early therapeutic target in common neurodegenerative diseases.

2.1 Patient assessments

The MDS Unified Parkinson's Disease Rating Scale - Motor Examination (UPDRS-III), Epworth Sleepiness Scale (ESS), and Geriatric Depression Scale (GDS) were administered to patients. The Instrumental Activities of Daily Living (IADL) scale, North-East Visual Hallucinations Inventory (NEVHI), Neuropsychiatric Inventory (NPI), Mayo Sleep Questionnaire (MSQ), Clinician Assessment of Fluctuation (CAF), and Dementia Cognitive Fluctuation Scale (DCFS) were administered to informants. Clinical Dementia Rating scale (CDR) and Cumulative Illness Rating Scale for Geriatrics (CIRS-G) were completed based on the clinical history. A detailed neuropsychological evaluation was also carried out as reported previously which included the ACE-R. All subjects were offered dopaminergic imaging with FP-CIT SPECT at baseline, including those with MCI-AD. Images were randomized, coded, and then visually rated as normal/abnormal by an experienced consensus panel blind to clinical information and diagnosis as reported earlier and incorporated into diagnoses.

2.2 Clinical diagnosis

A three-person consensus clinical panel of experienced Board Certified old age psychiatrists (AJT, PCD, JPT) independently reviewed clinical notes taken from the baseline assessment and confirmed diagnoses of MCI according to NIA-AA criteria. This was based on evidence of minimal functional impairment (independent living was maintained) and a CDR of 0 or 0.5, and the presence of subjective and objective cognitive decline. Anyone with dementia was excluded. To determine the etiology, the presence or absence of core LB symptoms were also rated by the panel, in accordance with the fourth consensus criteria for DLB. Based on health service clinical notes and imaging results, those with possible significant vascular or frontotemporal...
etiolologies, or parkinsonism pre-dating cognitive impairment by more than 1 year, were also excluded. Where possible, an informant was sought (spouse, friend, or family member) to provide additional information. FP-CIT findings were later incorporated into diagnoses but the panel decisions on MCI and symptoms were made blind to these findings.

Participants received a diagnosis of MCI with probable Alzheimer’s disease (MCI-AD) when they had no core LB symptoms, a normal FP-CIT scan and evidence of decline which was characteristic of AD, with no evidence for another etiology, that is, they met the additional NIA-AA criterion of “etiology of MCI consistent with AD pathophysiologic process.” MCI with Lewy bodies (MCI-LB) was diagnosed when at least one core feature was present or the patient had an abnormal FP-CIT scan, in accordance with current consensus research criteria for the diagnosis of MCI-LB. One participant did not consent to dopaminergic transporter imaging but had sufficient clinical LB symptomology for a MCI-LB diagnosis (two core features) without confirmatory biomarkers.

2.3 Prospective evaluation

Participants were re-assessed every 12 months by a research nurse and/or doctor in a prospective longitudinal design, with the above assessment instruments repeated each year. Clinical assessments were undertaken blind to results of cytokine assessments. Core LB symptom presence and severity of cognitive impairment (MCI or dementia) were re-appraised at annual follow-ups by the clinical diagnostic panel and blood samples were repeated each year.

2.4 Cytokine analyses

Baseline and repeat venous blood samples were taken from all patients using EDTA tubes, which were then centrifuged and the plasma removed. Samples were stored at −80°C until assays were performed. Cytokine assays were performed using the Meso Scale Discovery V-Plex Plus Proinflammatory Panel 1, which included IFNγ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNFα. Assays were performed at the Newcastle University BioScreening Core Facility according to the manufacturer’s protocol, and samples were processed in triplicates. Samples which were under the limit of detection for a particular cytokine (concentration in pg/mL < 0.05 for IFN gamma, <0.03 for IL-13, <0.02 for IL-12p70 and <0.01 for all other cytokines) had cytokine levels which were low enough to be indistinguishable from background noise, and therefore these samples were treated as having “zero” levels of that cytokine.

2.5 Statistical analyses

Longitudinal analyses were undertaken with R software packages lme4 and lmerTest. Repeated measures correlations were analyzed with the rmcorr package to account for non-independence of observations within individuals, and providing greater statistical power than alternative methods (eg, averaging across repeated observations, or assessing correlations at each time-point). Linear mixed-effects models assessed the effects of time (continuous) on cytokine levels, controlling for the effects of age, gender, concurrent illness (CIRS-G score), use of anti-inflammatory medications (non-steroidal anti-inflammatory drugs or steroids), and MCI disease group. The effects of controlling for baseline cognitive function (ACE-R total score) and indices of local deprivation were also assessed, but their inclusion did not improve model fit in any cases. Improvements in model fit were assessed by likelihood ratio tests, with an alpha level of P < .05. Random intercept and time-slopes were included at the subject level, allowing for correlation between these where appropriate; fixed and random non-linear terms for time were also assessed, but did not improve fit over the linear-only term. Cytokine levels were log transformed as at baseline, and continuous covariates were mean-centered. For testing our two planned hypotheses in all eight cytokines (16 planned tests), alpha level was adjusted by 0.05/16 = 0.0031. For all other factors we applied a Bonferroni correction to adjust the significance level to <0.001.

The study has ethical approval from a UK Research Ethics Committee (NRES Committee North East - Newcastle & North Tyneside 2, reference: 12/NE/0290). Written informed consent was obtained from all study participants in accordance with this approval.

3 RESULTS

Seventy-seven MCI patients (21 MCI-AD, 56 MCI-LB) completed the baseline assessment and provided blood samples, as reported previously. Of these, 56 (21 MCI-AD, 35 MCI-LB) completed at least one follow-up assessment and provided blood samples. Those who were not available for repeated samples had a significantly lower baseline cognitive function (ACE-R) score (t[33] = −2.854, P = .007), but did not significantly differ in their baseline cytokine levels. Increased retention of AD likely reflected the more severe course of DLB. Differential diagnoses were stable over this time (ie, there were no cases which changed from MCI-LB to MCI-AD), but of those included who provided two or more samples, 10 MCI-AD (48%) and 13 MCI-LB (37%) converted to dementia over the course of assessment. The baseline characteristics of these 56 patients are provided in Table 1. MCI-LB were slightly younger and as expected there were sex differences between the disease groups (more females in AD group) and minor differences in neuropsychiatric symptoms (higher in the MCI-LB patients), with scores being modest and consistent with early stage disease. The two groups did not differ in their levels of motor impairment as assessed with the UPDRS-III, though in the full cohort as previously described including those lost to follow-up, MCI-LB had significantly higher scores in this measure. As impairments were “rated as seen,” higher UPDRS-III scores in MCI-AD most likely reflect non-parkinsonian age-related motor impairments; this is supported by a moderate association between increasing age and UPDRS-III scores
in the MCI-AD group ($r = 0.56, P = .01$), but not in MCI-LB ($r = 0.23, P = .18$). Sixteen (46%) of the MCI-LB group had abnormal FP-CIT imaging, while none of the MCI-AD group did. Patient baseline inflammatory markers are shown in Table 2. As with the larger baseline group, there were no significant differences in baseline cytokine levels between the diagnostic groups.

### 3.1 | Prospective evaluation of cytokines in MCI

We repeated at least one annual review for each patient with the longest follow-up being at 3 years after baseline assessment (four assessments and samples). The mean (SD) time from baseline sample to final sample was 1.51(0.66) years and we obtained a median of two samples per patient (minimum 2 and maximum 4). Of the 10 cytokines evaluated in the Meso Scale panel, two were not included in statistical analyses because either too many samples had undetectable levels (IL-13) or the inter-assay variability was too high (IL-12p70). For the remaining cytokines, the inter-assay % coefficients of variation were: IFN-γ: 5.99%; IL-10: 9.59%; IL-1β: 10.45%; IL-2: 11.69%; IL-4: 9.73%; IL-6: 8.12%; IL-8: 8.95% and TNF-alpha: 7.61%.

### 3.2 | Change in cytokines over time

Figure 1 shows the change in the remaining eight cytokines over this period and Table 3 shows the findings from the linear mixed effects models. Six cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-6 and IL-10) showed highly significant decreases over time. Four of these (IL-1β, IL-2, IL-4 and IL-10) had been significantly higher than controls at baseline. IFN-γ and IL-6 were not significantly different from controls at baseline but there was high variability in these cytokines.
reflected in large standard deviations. Both IL-8 and TNFα were not different at baseline, and had no significant increase or decrease over time in MCI. Consistent with the baseline information, the MCI diagnostic groups did not significantly differ in their inflammatory trajectories.

3.3 | Correlations of cytokines with decrease in cognition

Examining our second hypothesis, in the overall group we found significant positive correlations within individuals (r values between 0.35
<table>
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<th>df</th>
<th>t</th>
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<td>Anti-inflammatory use</td>
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<td>49.60</td>
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<td>.533</td>
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<td>0.01</td>
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<td>.995</td>
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<td>0.11</td>
<td>82.19</td>
<td>−0.460</td>
<td>.646</td>
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<tr>
<td><strong>IL-2</strong></td>
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<tr>
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<td>99.72</td>
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<td>.808</td>
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<td>MCI-LB vs MCI-AD</td>
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<td><strong>IL-6</strong></td>
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<td>Intercept (MCI-AD)</td>
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and 0.57) between worsening of cognitive impairment (decrease in ACE-R) and decrease in cytokine levels for IFNγ, IL-1β, IL-2, IL-4 and IL-10 (see Table 4). IL-8 had a weak non-significant correlation in the opposite direction (increasing cytokine level associated with lower cognition) and there was no correlation for IL-6 and TNFα.

Regarding other potential relationships with cytokine changes, there were no differences between the disease groups in any of the changes in cytokine levels nor were there significant effects due to age or sex or general illness burden (as measured by the CIRS-G) or use of anti-inflammatory drugs. There was also no correlation between change in cytokine levels and severity of parkinsonism (measured using UPDRS).

The data that support the findings of this study are available by request through Dementias Platform UK at https://portal.dementiasplatform.uk/DAMatrix.27

### DISCUSSION

Our primary hypotheses based on previous literature, that there would be a decline in plasma cytokine levels in people with MCI due to AD and DLB over time and that such a decrease would correlate with the accompanying decline in cognition, were both supported by our analyses. These findings suggest there may be a therapeutic window for use of anti-inflammatory drugs in these two major neurodegenerative diseases including this MCI stage of these illnesses.

Both AD and DLB have cholinergic deficits and respond well to cholinesterase inhibitors and both show modest benefits with memantine.28 However, there are no disease modifying treatments available for either disease, with a large number of trials having failed to demonstrate benefits.29,30 Identification of new therapies for these devastating diseases is a major priority and targeting neuroinflammation is an attractive alternative therapeutic approach. Previous evidence from genetics in AD,2 and Lewy body disease14 and
peripheral measures of inflammation in milder AD and LBD supports a role for inflammatory processes in both diseases. We previously reported that whilst the dementia stage cytokine levels had returned to the level of healthy controls, they were elevated at the MCI stage in both diseases. This is consistent with other evidence that inflammatory markers appear to be increased years before the development of AD dementia, though no studies seem to have assessed MCI-AD. To our knowledge there have been no previous prospective studies of inflammation in DLB, and the only previous prospective studies in PD have been from our centers in people without cognitive impairment, which have supported a role for peripheral inflammation in disease progression in PD. In addition, a CSF study in PD which did not include anyone with dementia but found increasing levels of the inflammatory marker YKL-40, a marker of macrophages, which correlated with cognitive performance. Uncertainty about the activation state the microglia are in and the specificity of these PET microglial markers makes detailed interpretation and comparison of these studies difficult and may contribute to inconsistencies in findings, as may the modest patient numbers in such studies. However, overall a large body of evidence suggests that in diseases such as AD and DLB there is both central and peripheral inflammation and that systemic inflammation can drive neurodegeneration and exacerbate clinical symptoms. Indeed, Perry and Teeling have described a concept where increased peripheral inflammation can accelerate neurodegeneration via a number of different mechanisms.

There is now increasing focus on targeting inflammation as a treatment for AD and our findings suggest such treatments could be extended to DLB. Several epidemiological studies have demonstrated the protective effects of non-steroidal anti-inflammatory drugs in reducing incidence of AD and LBD. and DLB. In the latter study cerebral inflammation was higher in mild compared with moderate/severe DLB and the microglial activation was also found to be positively associated with cognitive performance. Uncertainty about the activation state the microglia are in and the specificity of these PET microglial markers makes detailed interpretation and comparison of these studies difficult and may contribute to inconsistencies in findings, as may the modest patient numbers in such studies. However, overall a large body of evidence suggests that in diseases such as AD and DLB there is both central and peripheral inflammation and that systemic inflammation can drive neurodegeneration and exacerbate clinical symptoms. Indeed, Perry and Teeling have described a concept where increased peripheral inflammation can accelerate neurodegeneration via a number of different mechanisms.

The few PET studies of neuroinflammation have used microglial markers and found evidence for inflammation in the brain in AD and DLB. In the latter study cerebral inflammation was higher in mild compared with moderate/severe DLB and the microglial activation was also found to be positively associated with cognitive performance. Uncertainty about the activation state the microglia are in and the specificity of these PET microglial markers makes detailed interpretation and comparison of these studies difficult and may contribute to inconsistencies in findings, as may the modest patient numbers in such studies. However, overall a large body of evidence suggests that in diseases such as AD and DLB there is both central and peripheral inflammation and that systemic inflammation can drive neurodegeneration and exacerbate clinical symptoms. Indeed, Perry and Teeling have described a concept where increased peripheral inflammation can accelerate neurodegeneration via a number of different mechanisms.

While there is no reason to believe that these biomarkers progress in a linear fashion within individuals, non-linear time terms were not supported in this sample, likely due to the limited number of observations per person. Future work may benefit from considering the possibility of heterogeneity within a sample such as this; the declining trajectory of inflammation observed in this study may describe the later stages of MCI after peripheral inflammation peaks, while those at the earlier stages may feature a different inflammatory trajectory (eg. increasing before decreasing in a non-linear fashion). With sufficient observations and timespan, different trajectory directions and shapes may be identifiable with a data-driven longitudinal clustering method, for example, latent class mixed modeling.

In summary, we found strong evidence for a decrease in peripheral inflammation in both AD and LBD from elevated levels of cytokines at the MCI stage towards healthy control levels typical of the dementia stages of these diseases, findings which have important implications for the design, conduct and outcome measures of future anti-dementia trials involving putative anti-inflammatory agents.

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CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available by request through Dementias Platform UK at https://portal.dementiasplatform.uk/DAMatrix.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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