Review of: "Concomitant AD and DLB pathologies shape subfield microglia responses in the hippocampus"

Natalie Zahr[1]

1 Stanford University

Potential competing interests: The author(s) declared that no potential competing interests exist.

This manuscript presents data from 10 Alzheimer's disease (AD), 8 Dementia with Lewy Bodies (DLB), and 11 healthy-control postmortem brain tissue samples with analyses focused on Amyloid beta (Ab), Tau, and P-Synuclein immunohistochemistry and the relationship between these markers and microglial morphology in the human hippocampus. The authors have developed an automated analyses procedure called the "Microglia and Immune Cells Morphologies Analyser and Classifier (MIC-MAC)" pipeline. In addition to confocal microscopy analyses to determine volumes of Ab, Tau, and P-Synuclein in CA1-CA4 hippocampal tissue, MIC-MAC was used to classify thousands of microglia per sample. Tau was found to be highest in CA1 (lowest in CA4); Ab lowest in CA1 (highest in CA4); P-Synuclein showed negligible staining (highest in CA1, lowest in CA4); this staining pattern/distribution was similar in AD and DLB with the only notable difference being a higher degree/severity in AD relative to DLB. Microglia in the diseased (i.e., AD and DLB) relative to healthy control hippocampal tissue were more compact and less ramified. It is suggested that the "severity, distribution, and overlap of co-occurrence of Ab, Tau, and P-Synuclein impact microglia phenotypic heterogeneity" but the data as presented are challenging to follow. In general, the manuscript requires editing for clarification. Methods need to be better detailed. The MIC-MAC pipeline should be better presented - with evidence for its validity and reliability - in the Introduction. Both the methods and results need to be re-written so that readers can better grasp and potentially replicate the current findings. In the discussion, comments regarding whether the observed microglial morphological changes are beneficial or detrimental should be provided.

In the Title, AD and DLB must be spelled out.

Abstract

Abstract is not clear. For example, what is meant by, "pathologies ***composing*** the various local microenvironment of the hippocampus across AD and DLB". Is the process referred to that of the CA1 being more affected in AD than DLB? "How microglia are transformed" Is it meant: how microglial morphology is altered by disease processes? Abstract should explicitly state that experiments were performed in "post-mortem brain tissue".

“P-Tau, Aß and P-Syn burdens were significantly exacerbated in AD” relative to DLB?
Language use has to be more precise to convey the goals of the project.

Introduction

More general introduction of DLB and AD, e.g., in addition to smaller CA1, are those with AD cognitively worse than those with DLB? (is memory impairment for severe?)

Please expand on relevant previous work and results in references 58–63 .

More information must be provided regarding the Microglia and Immune Cells Morphologies Analyser and Classifier (MIC-MAC) pipeline.

Needs review for grammar, clarity.

Methods

AD and DLB, as well as from age matched CTLs > were the groups matched on any other demographic characteristics? SES? Years of education? Were there any heavy alcohol drinkers in any of the samples? Were the samples from individuals with no DSM diagnoses and free of substance use disorders?

More women in control group; older age in control group (83.1 vs. 77.3 years in DLB group not significant?)

Not enough information/unclear:
How many samples were stained with NFT versus alpha-synuclein?
How many samples were staged by Braak, ABC, versus McKeith measures?

In table 1, please include same number of decimal points for all numbers.

How the different sections were distributed for P-Syn, Ab, and Tau antibodies is not clear. Was each slice immunostained for all 3 markers? Or were consecutive slices stained for the different markers?

Where is description of Iba-1 staining?
Can more information be provided regarding the validity, CVs, and other parameters of the MIC-MAC pipeline relative to human scoring of microglial morphology?

Results

Some parts of the Resutsl belong in the Methods or Introduction (i.e., details regarding MIC_MAC).

Language needs to be clarified so comparisons can be better understood.

Not clear if covariates such as age, PMI, sex, etc were considered in the analyses.

Words like “interestingly” and “surprisingly” do not belong in a Results section, or in a scientific paper in general. More appropriate and specific language should be used.

This is a cross-sectional study, so “higher” and “lower” are more appropriate terms than “increasing” or “decreasing”.

The presentation of the results of Cluster Data analysis referred to in Figure 4 is confusing and hard to follow.

Discussion

“pathologies show a specific subfield pattern” is not supported by the findings which instead show the same kind of pathology in AD and DLB but with differing severities. Confusion may be due to authors using the term, “pathology” to refer to Ab, Tau, and P-Synuclein staining.

Limitations of the study should be grouped together in a single paragraph.
Limitations of small sample size and consequent heterogeneity, and lack of generalizability of the current findings – especially in the context of DLB with many AD features – need to be acknowledged.