In a Prospective Observational Case-Control Study Kärkkäinen et al. aimed to identify neuroinflammation-related proteins in tear fluid (TF) as potential biomarkers for early-stage Alzheimer's disease (AD). The novelty lies in using a non-invasive biofluid (TF) and applying high-resolution proteomics.

2. Strengths Non-invasive approach: Tear fluid collection via Schirmer strips offers a safe, patient-friendly method ideal for elderly populations.

Mass spectrometry-based proteomics: The use of label-free quantitative proteomics enhances the detection of subtle changes in protein expression without bias toward known candidates.

Clear case-control design: The inclusion of well-defined mild AD patients (CDR 0.5–1, MMSE 23.8 ± 2.8) and cognitively healthy controls (MMSE 28.9 ± 1.4) allows for meaningful comparisons.

Focus on neuroinflammation: Targeting neuroinflammatory pathways aligns with current hypotheses that inflammation plays a central role in early Alzheimer's disease pathogenesis.

Identified candidate markers: The study reports 14 differentially expressed proteins, several of which (e.g., SERPINA3, ORM1, SPARCL1) have known links to inflammatory and neurodegenerative processes.

3. Limitations Small sample size: With only 19 AD cases and 34 controls, the study is underpowered for broad generalization or robust statistical correction for confounding variables.

Cross-sectional nature: Being observational and cross-sectional, it does not address causality or longitudinal stability of these biomarkers.

Lack of external validation: The findings are not validated in an independent cohort or in cerebrospinal fluid/plasma, limiting translational relevance.

Omission of confounding factors: Factors like dry eye syndrome, medications, systemic inflammation, or comorbidities were not extensively controlled for, which could significantly affect TF protein composition.

Overinterpretation risk: While the link between neuroinflammation and AD is established, asserting that these TF proteins are early biomarkers of AD is premature without longitudinal or mechanistic validation.

Lack of machine learning or biomarker signature development: Despite the proteomic data, no predictive models or ROC analyses were reported to evaluate diagnostic utility.

4. Contribution to the Field This study provides preliminary evidence that tear fluid proteins may reflect neuroinflammatory changes in early AD. It adds to a growing interest in peripheral biomarkers and supports the exploration of eye-brain connections in neurodegeneration.

5. Recommendations for Future Research Conduct longitudinal studies to assess temporal evolution of TF biomarkers in preclinical and prodromal AD.

Validate the identified proteins in larger, multicentric cohorts.

Correlate TF protein changes with CSF biomarkers (Aβ, tau) and neuroimaging findings.

Explore multiplex assays or ELISA-based panels for potential clinical translation.

Include confounder analysis (e.g., ocular surface disease, systemic inflammation).

Conclusion

This study is a promising proof-of-concept for using tear fluid as a diagnostic window into neurodegeneration, particularly AD. However, the small sample size, lack of validation, and cross-sectional design limit its current clinical utility. It should be viewed as a hypothesis-generating work that warrants more rigorous, larger-scale follow-up studies [1)]

1)
Kärkkäinen V, Saari T, Rusanen M, Uusitalo H, Leinonen V, Thiede B, Kaarniranta K, Koivisto AM, Utheim TP. Neuroinflammation Markers in Tear Fluid of Mild Alzheimer's Disease. J Mol Neurosci. 2025 Jun 5;75(2):73. doi: 10.1007/s12031-025-02368-x. PMID: 40471493.