Comparison of quantification methodologies used to assess the pathological hallmarks of neurodegenerative age-related diseases

Holly Stewart, Supervisor: Dr Johannes Attems, Co-supervisors: Dr Lauren Walker, Mary Johnson

Institute of Neuroscience, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne, NE4 5PL

h.e.stewart1@ncl.ac.uk

Newcastle University

Institute of Neuroscience

Introduction

Alzheimer's disease (AD) and Lewy Body disease (LBD) are some of the most prevalent age-related neurodegenerative diseases in the world population (1). The clinical AD manifestations associated with said hallmarks include impairment in memory, executive dysfunction and aphasia (2). Hallmark lesions associated with AD include extracellular deposits of aggregated amyloid-β protein (Aβ) such as senile plaques, figure 1a, and fleecy amyloid. Other lesions include neuritic plaques (3). Hyperphosphorylated tau **(**HP-т) is another aggregated protein associated with AD and it forms hallmark lesions such as neurofibrillary tangles and neuropil threads.

LBD encompasses Parkinson disease dementia, dementia with Lewy bodies and Parkinson disease without dementia. Their overlapping clinical manifestations are perceptual abnormalities and a fluctuating cognition (4). LBD hallmarks are intracellular aggregates of α-synuclein (α-syn), figure 1c. These are Lewy bodies Lewy neurites.

Mixed AD/DLB cases exhibit pathology from both diseases so much so that either of the pathologies could have caused clinical dementia but quantitative analysis will highlight the differences in pathology load (2).

Quantification methods

Neuropathological assessment *Post-mortem* is often semi-quantitative which can be very subjective and the stage given can differ between researchers and research institutes. Quantitative analysis has progressed from manual counting to more uniform techniques using software which uses universal algorithms and premade thresholds to give a numerical value as to the degree of pathology (2, 5). Quantitative analysis can be conducted using the slide produced from the original tissue block, a diagnostic slide, or a (Tissue micro-array) TMA. TMAs are paraffin blocks containing tissue cores from different sections of the original tissue blocks (6).

Methods

A selection of fixed pure AD, pure DLB, mixed AD/DLB and control cases were extracted from the Newcastle Brain Tissue Resource (NBTR) (total, 45; control, 10; AD, 13; LBD, 12; Mixed AD/DLB, 10).

Paraffin embedded blocks were produced from the transentorhinal cortex, prefrontal lobe and cingulate. These were first sectioned and mounted onto glass slides to produce the diagnostic slides. They were then used to make TMAs by removing tissue cores from predefined areas and these tissue cores were placed in a pre-made recipient TMA paraffin block. TMA blocks were then processed into glass slides. The slides were stained with AT8, 4G8 and α-syn which stain HP-т, Aβ and α-syn respectively.

Quantitative analysis was conducted upon the TMA and diagnostic sections, calculating the mean percentage area covered by the pathology (protein load).

Semi quantitative analysis was conducted upon the diagnostic slides by estimating the quantity of pathology of each section. The semi quantitative score given was between 1 and 3 (1 and 4 for α-syn) with 1 being mild and the severity increased with an increase in number.

Statistical analysis

Correlation tests (Pearson's rank and Spearman's rank) and statistical difference tests (Wilcoxon signed ranks Test (WSRT) and paired samples T-Test) were used. They tested the correlation and statistical difference between protein load determined by TMA, diagnostic slides and semi quantitative analysis.

Aim and hypothesis

The aim was to investigate semi quantitative and quantitative methods of quantification of neuropathology; assessing their accuracy and precision in quantifying Aβ HP-т α-syn load in the frontal cortex, entorhinal cortex and cingulate respectively.

The hypothesis was that quantitative analysis would be more accurate and representative of the tissue than semi quantitative analysis. Also, TMAs would provide experimental uniformity during quantification.

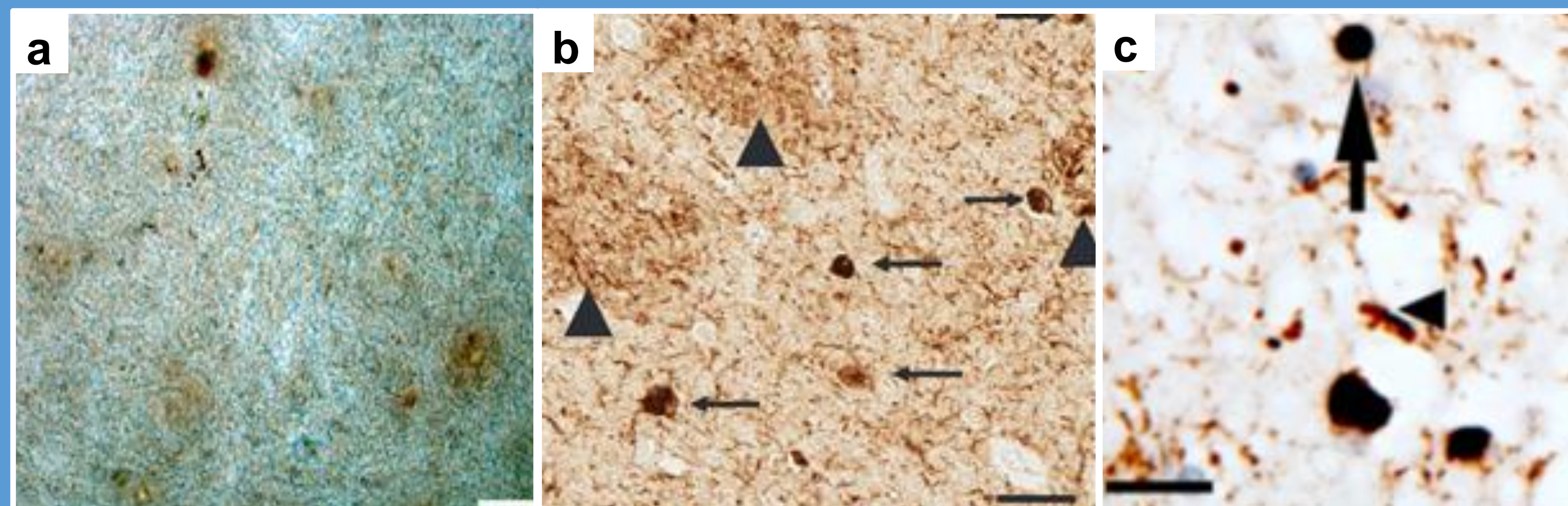

Figure 1a – An image of Aβ in a tissue section from the entorhinal cortex of a patient with AD (1).**1b -** An image of an area of the brain visualised using a microscope and phosphor-tau Immunohistochemistry (2). The arrowheads highlight NPs. The arrows highlight NFTs.**1c -** Intracellular α-syn aggregates in the nervous system (5).

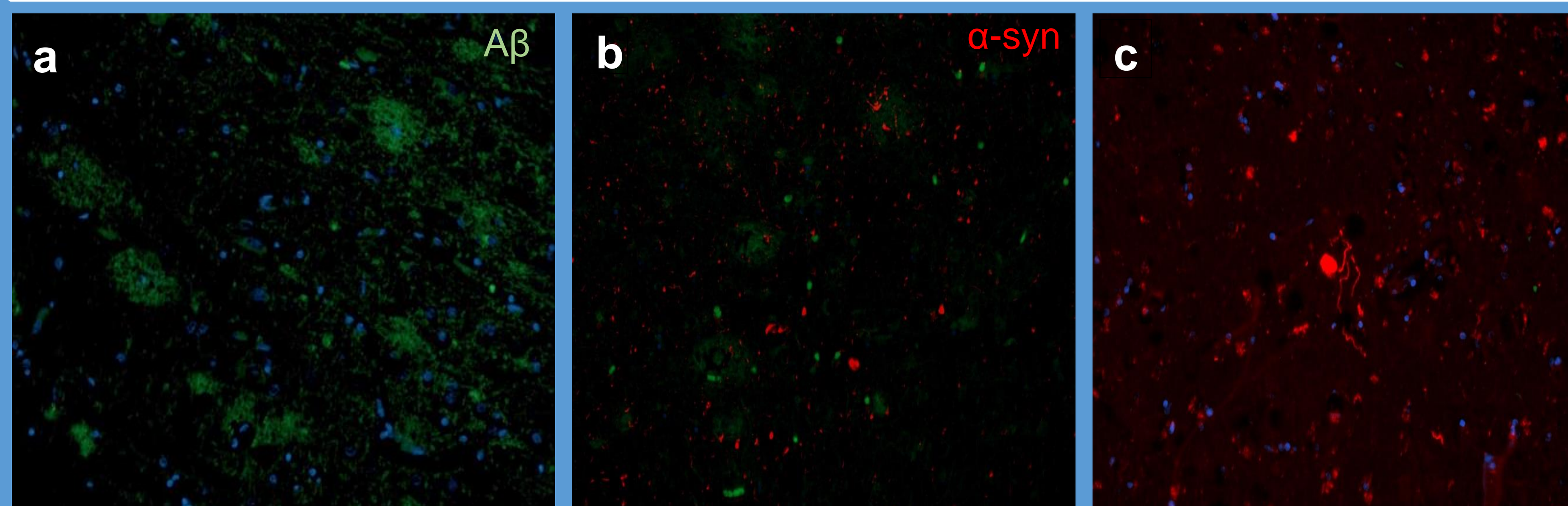

Figure 2 - Immunofluorescent labelling of a diagnostic slide of a mixed AD/DLB *post-mortem* brain temporal section. **2a –** Stained in green is the Aβ pathology and blue is the nuclei. **2b –** Stained in red is the α-syn pathology, blue is the nuclei and green is the Aβ pathology. **2c -** Stained in red is the α-syn pathology, blue is the nuclei.

Results

Whole cohort analysis indicated there was a significant different ($p<0.05$) between the mean HP-т load of the diagnostic and TMA slides of the entorhinal cortex but no statistical difference ($p>0.05$) between them for Aβ load in the frontal cortex and α-syn load in the cingulate. Correlation analysis revealed a medium correlation for between TMA and diagnostic slides for Aβ and HP-т load but no correlation for α-syn load.

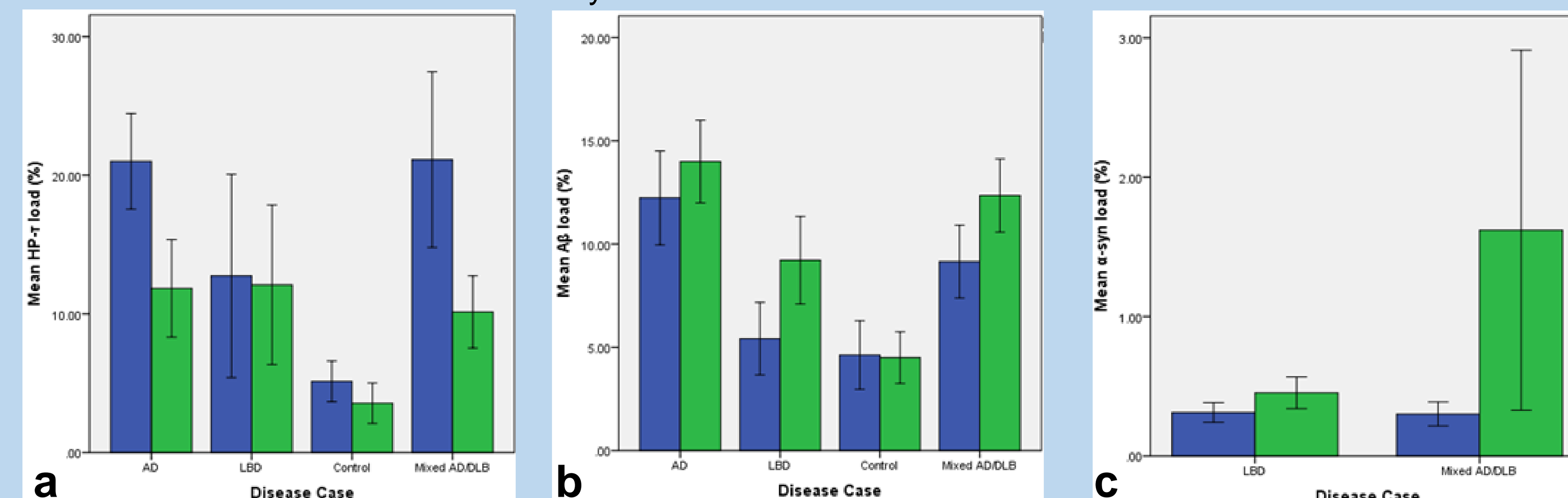

Figure 4 – Graphs showing the mean load for the three proteins analysed. Green bars represent the mean protein load of the diagnostic slides and the blue bars represent the mean protein load of the TMA slides. For all comparisons of TMA and diagnostic analysis for each protein for the individual disease cases, there was no statistical difference ($p>0.05$). Correlation analysis for the individual disease case cohorts gave mixed data with some not showing any correlation ($p>0.05$) or the correlation being too weak (correlation coefficient<0.40). This is likely to be due to the low case number.

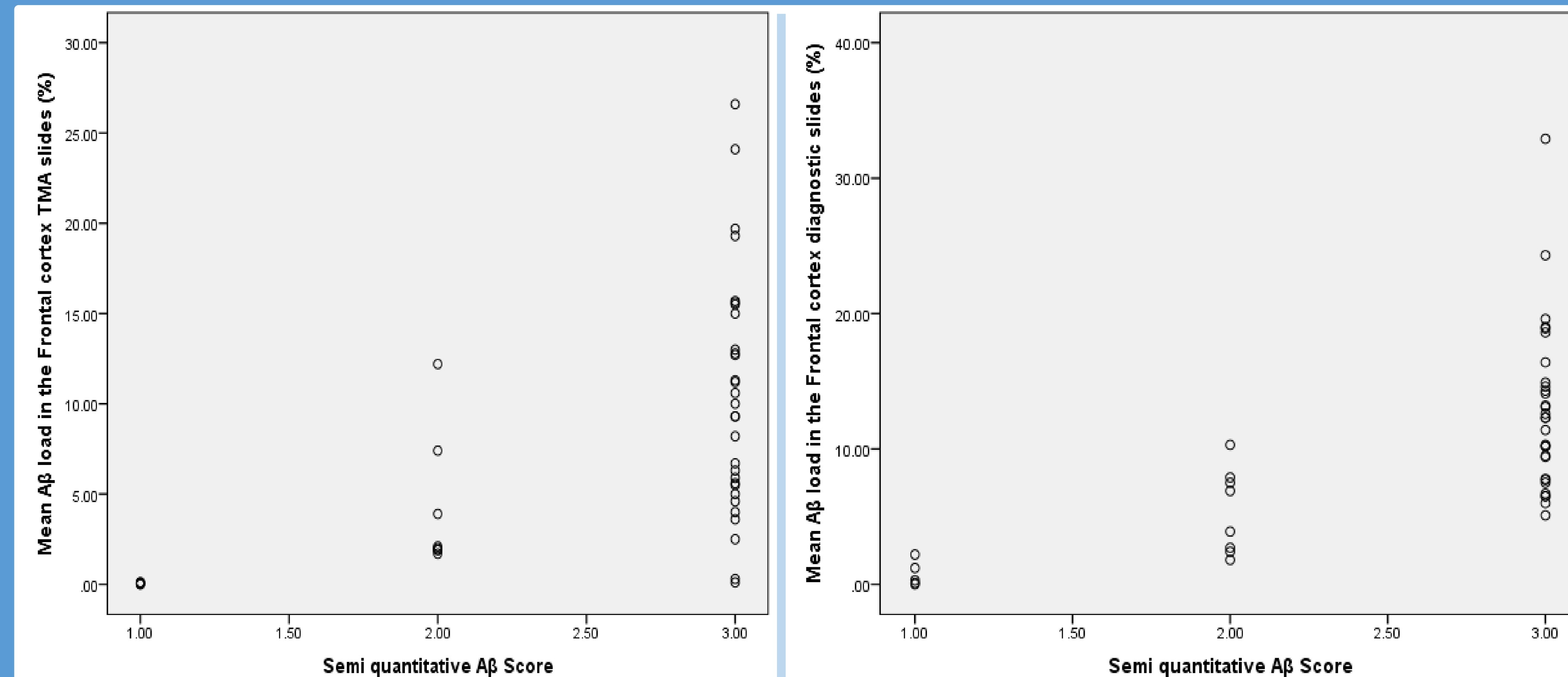

Figure 5 – Scatter graphs comparing semi quantitative analysis of Aβ to the calculated mean protein load using the diagnostic (right) and TMA (left) slides in quantitative analysis. There is a strong correlation between semi quantitative and the diagnostic and TMA slides (SRCC=0.70, $p<0.01$ and SRCC=0.64, $p<0.01$ respectively). For HP-т load correlation, both exhibited a correlation, diagnostic slides had a stronger correlation (SRCC=0.68, $p<0.01$) with the TMA having a weak correlation (SRCC=0.33, $p<0.05$). For α-syn load, there was no correlation in comparison to TMA analysis but there was a strong correlation with diagnostics slides (SRCC=0.54, $p<0.01$).

Discussion

The results support the hypothesis that semi quantitative analysis can give accurate data which is representative of the pathology in the tissue, allowing it to be an important tool in diagnostics but, it does not allow identification of the subtle differences in pathology which quantitative methods can (7).

The use of TMA analysis instead of diagnostic slide analysis would be a useful substitute, enabling researchers to utilise TMA analysis as a high throughput technique (8). This would allow an increase in case load and a decrease in time taken for quantification as well as reducing the amount of the tissue needed which is critical when there is limited tissue available (8).

Furthermore, the use of TMA slides would increase experimental uniformity through the use of tissue cores from the same parts of the tissue and all the samples would be stained at the same time in the same conditions (6) but, this study has shown that the methodology still needs to be improved to allow it to be as accurate as is necessary.

The focal nature of α-syn may have caused α-syn pathology in the TMA samples to not be representative of the whole sample. The heterogeneity of the tissue has been highlighted in other studies as an issue with TMA analysis and it has been suggested that larger tissue cores should be used, increasing their diameter to allow more of the sample to be included. Studies still need to be conducted to decide if this is an appropriate improvement as drawbacks include increased damage to the donor block and a decreased number of tissue cores which can be placed in the recipient block which would be more likely to have a negative effect upon the precision of the data(9). An alternative could be increasing the number of samples collected from the tissue for the TMA analysis could improve its accuracy (6).

To further this research, studies could be conducted to analyse if increasing the diameter of the tissue core or increasing the number of tissue cores extracted from the donor block has an effect on the precision of the data.

References

1.Checkoway H, Lundin JI, Kelada SN. Neurodegenerative diseases. IARC scientific publications. 2011(163):407-19.
2.Walker L, McAleese KE, Thomas AJ, Johnson M, Martin-Ruiz C, Parker C, et al. Neuropathologically mixed Alzheimer's and Lewy body disease: burden of pathological protein aggregates differs between clinical phenotypes. Acta neuropathologica. 2015;129(5):729-48.
3.Ly PT, Cai F, Song W. Detection of neuritic plaques in Alzheimer's disease mouse model. Journal of visualized experiments : JoVE. 2011(53).
4.Troster AI. Neuropsychological characteristics of dementia with Lewy bodies and Parkinson's disease with dementia: differentiation, early detection, and implications for "mild cognitive impairment" and biomarkers. Neuropsychology review. 2008;18(1):103-19.
5.Byrne UT, Ross JM, Faull RL, Dragunow M. High-throughput quantification of Alzheimer's disease pathological markers in the post-mortem human brain. Journal of neuroscience methods. 2009;176(2):298-309.
6.Jawhar NM. Tissue Microarray: A rapidly evolving diagnostic and research tool. Annals of Saudi medicine. 2009;29(2):123-7.
7.Attems J, Neltner JH, Nelson PT. Quantitative neuropathological assessment to investigate cerebral multi-morbidity. Alzheimer's research & therapy. 2014;6(9):85.
8.Mark D Gustavson DLRMD-F. Tissue microarrays: leaping the gap between research and clinical adoption. Future Medicine. 2013;10(5):441-51.
9.Wang H, Wang H, Zhang W, Fuller GN. Tissue microarrays: applications in neuropathology research, diagnosis, and education. Brain pathology (Zurich, Switzerland). 2002;12(1):95-107.