

Is the SMI34 antibody a reliable marker of axonal loss in Alzheimer's disease?

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Introduction

- Alzheimer's disease (AD) is the most frequent common age-related neurodegenerative disease and is the leading contributor to dementia, a condition characterised by deteriorating brain function (1). Microscopically *post mortem* AD brains can be characterised by clumped proteins made up of amyloid-beta ($A\beta$) plaques and intracellular tau tangles which can cause death to brain cells known as neurones (2).
- The brain consists of 2 main types of tissue; the outer grey matter containing the cell body and dendrites of the neuron, and the white matter (WM) which is primarily made up of axons. AD is usually considered a disease of the outer grey matter of the brain however a common feature seen in AD brains are white matter hyperintensities (WMH) which are areas of high intensity, visualised using MRI in both *pre-* and *post mortem* (3).
- The antibody SMI34 has been shown to react with both tau in a clumped state and with the hyperphosphorylated neurofilament heavy (NFH), a key component of the axon which is responsible for carrying information away from the neuron (4). Hyperphosphorylated NFH is present in axons that are elongating from AD plaques and neurofibrillary tangles in the grey matter (5). This suggests that SMI34 would be an appropriate marker of white matter axonal damage in AD caused by the diseased grey matter.
- The development of this technique could provide an accurate means to measure axonal dysfunction in the WM associated with neurones affected by intracellular tau deposits as a consequence of AD in the grey matter.

Aims: Optimize this antibody staining (immunohistochemical) protocol and assess the reliability as SMI34 antibody as a marker of axonal damage in *post-mortem* fixed brain tissue.

Methods

Cohort: 15 *post-mortem* brains (Mean age: 84.6 ± 7.8 years; AD=4, control=11; Male=9, Female=6)

Post-mortem MRI: T2 MRI images were produced of the right hemisphere prior to dissection and were graded according to the ARWMC scale. Data obtained from Newcastle Brain Tissue Resource (NBTR).

Tissue microarray (TMA): Brains were dissected, embedded in paraffin and sliced at a thickness of $7\mu\text{m}$. Paraffin blocks containing 40 2mm cylindrical samples of tissue from different brain regions were used for this investigation. 4 of these were WM samples (2 frontal, 1 parietal, 1 occipital) and were analysed for this study. Shown by **Figure 1**. Scale bar represents $200\mu\text{m}$.

Neuropathology: Quantitative data on tau amount in the grey matter. Data provided by the NBTR.

Immunohistochemistry and Histochemistry: TMA sections were immunohistochemically stained with SMI34 antibody at a 1/5000 dilution and a Bielchowskys silver stain was also applied.

Image analysis of TMA: At a magnification of 200X a 3X3 image was taken using NIS elements software (Nikon). Thresholds for positive structures (e.g. damaged axons) were produced and were modified according to the level of background. **Figure 2A** and **2B** show what images were taken of and **Figure 3A** and **3B** show after the threshold was applied to these images. Positivity was measured as a binary area fraction. SMI34 values were divided by Bielchowskys figures in order to control for axonal loss. Scale bars represent $50\mu\text{m}$ on **Figure 2A** and **2B** and $200\mu\text{m}$ on **Figure 3A** and **3B**.

Sclerotic index (SI): Quantitative data on blood vessel damage. Data provided by the NBTR.

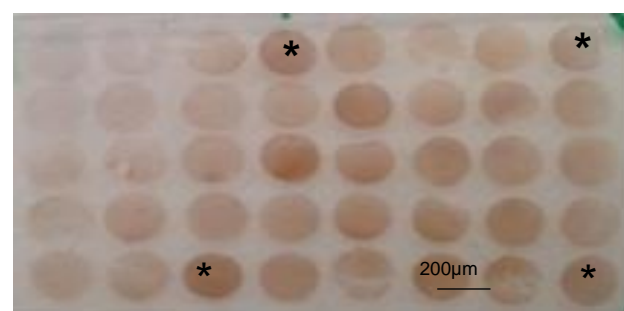


Figure 1: Photograph showing the layout of TMA slides. Punches marked with an asterisk correspond to WM.

Figure 2: Individual punches shown at a magnification of 20X. **A** is stained with SMI34 whilst **B** is stained with Bielchowskys silver. An outline of the picture taken is outlined.

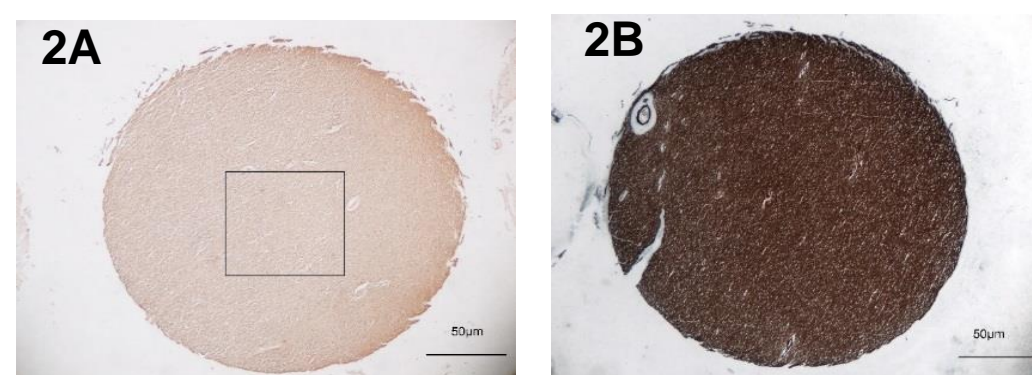
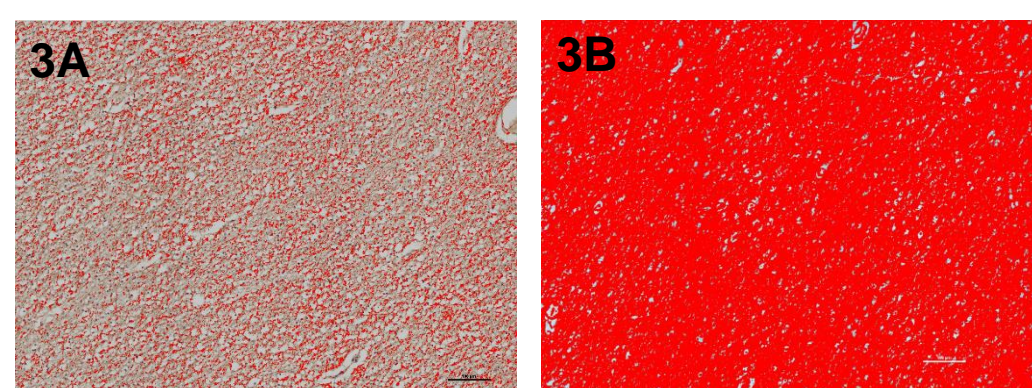


Figure 3: A screenshot of image taken. **A** shows before the threshold was applied and **B** afterwards.



Results

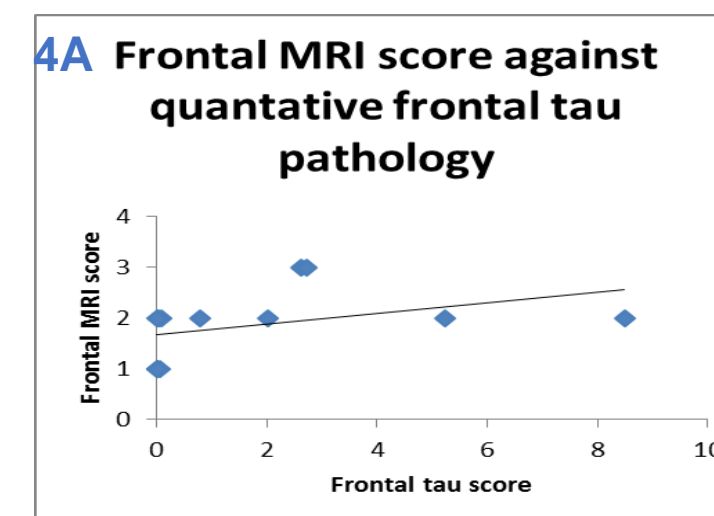


Figure 4: A significant correlation was found between MRI scores against tau pathology in the frontal (**4A**) ($Rho=0.664$, $p=0.007$ **), parietal (**4B**) ($Rho=0.512$, $p=0.037$ *) but not in the occipital lobe (**4C**) ($Rho=0.281$, $p=0.176$).

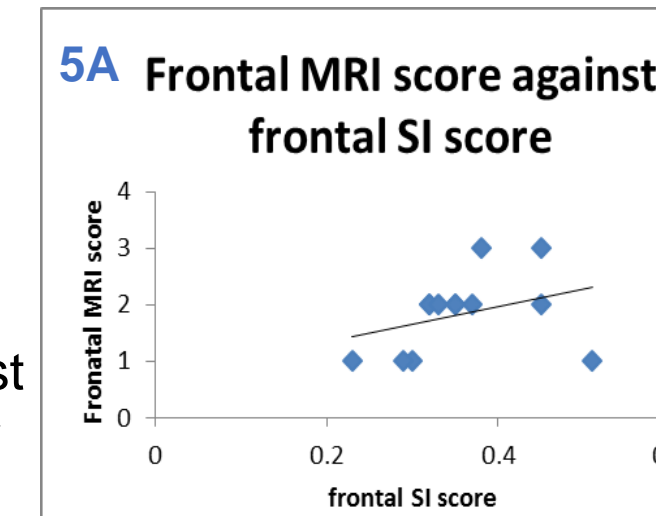
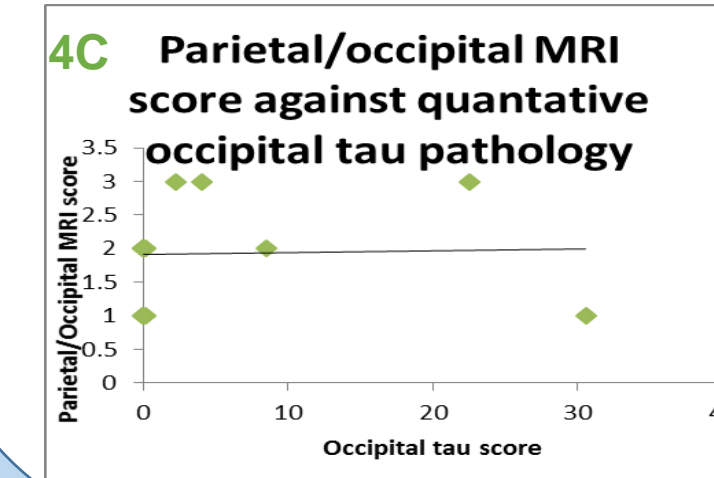
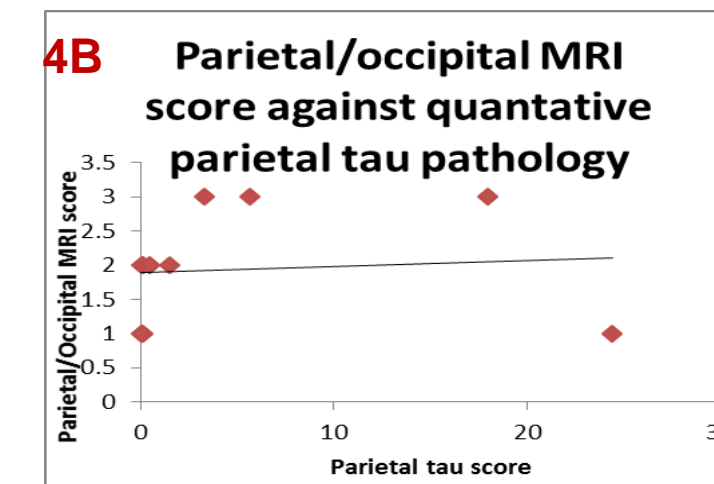


Figure 5: Positive correlations were also found between MRI scores and SI scores in the frontal (**5A**) ($Rho=0.508$, $p=0.038$ *), parietal (**5B**) ($Rho=0.683$, $p=0.005$ **) but once again not in the occipital lobe (**5C**) ($Rho=0.456$, $p=0.64$).

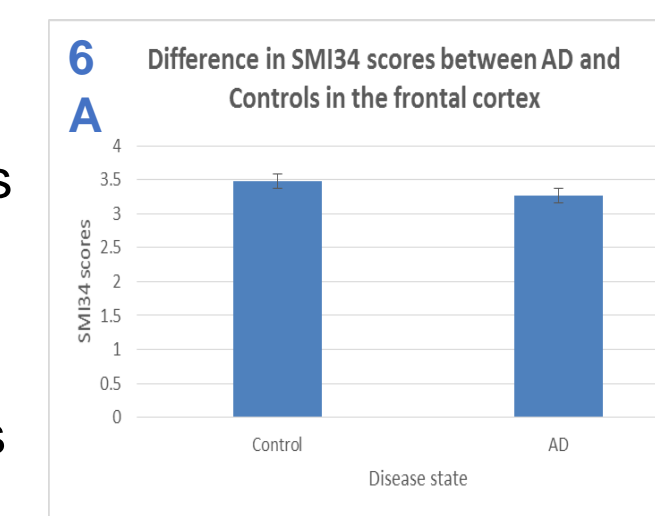
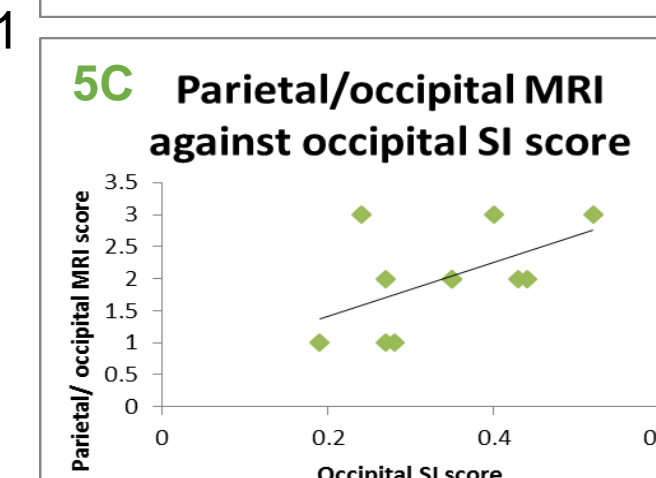
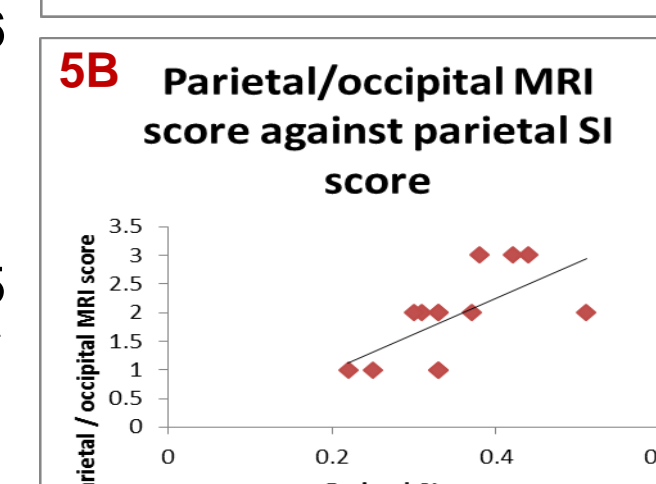


Figure 6: No significant difference was found between control and AD groups in the frontal (**A**) ($p=0.851$), parietal (**B**) ($p=0.571$) and occipital white matter (**C**) ($p=0.945$).

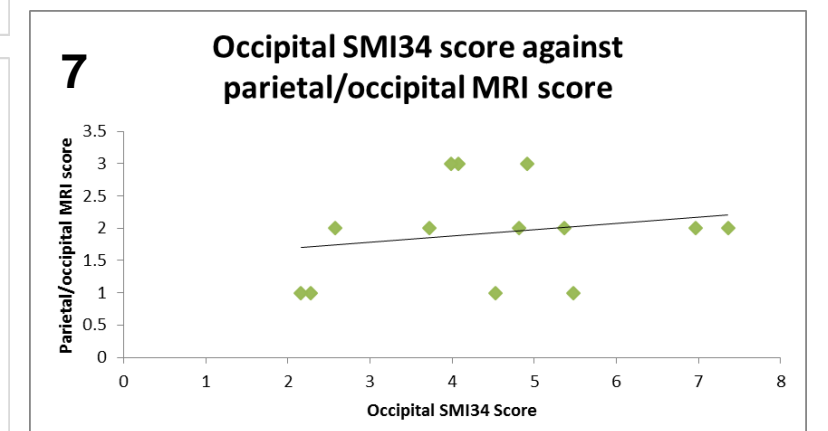
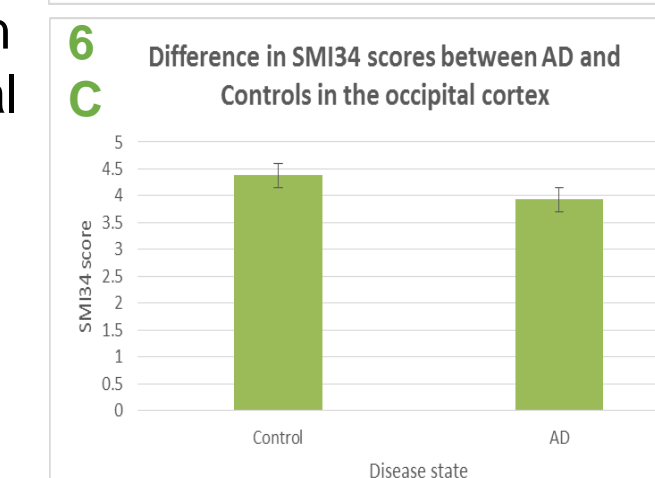
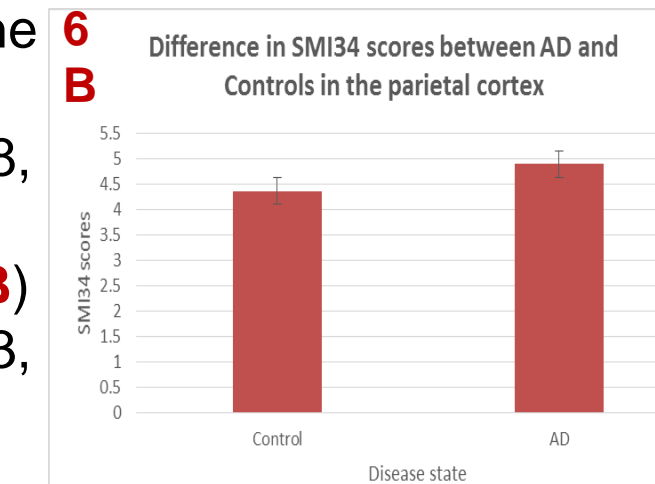


Figure 7: Correlation was found between SMI34 and SI but only in the occipital white matter ($Rho=0.519$, $p=0.042$ *) .

Discussion

- The significant correlation found between MRI scores, quantified tau pathology and SI scores in the frontal and parietal cortex agrees with work done by McAleese *et al* and other scientific literature (6). This indicates that the mechanism behind WMH is related to both blood vessel damage and Alzheimer's pathology in the grey matter.
- The non-significant result of the Mann Whitney u test in all 3 of the lobes studied suggests that **SMI34 is not a reliable marker of degenerative axonal loss in AD**. The relevance of this result is that SMI34 cannot determine between control and AD groups and therefore should not be used as a marker in similar future studies.
- Surprisingly correlation was found between SMI34 and SI but only in the occipital lobe. Research into relevant literature indicates that this effect could be attributed to the vascular diseases of the brain such as cerebral amyloid angiopathy (CAA). Previous studies have shown that this disease primarily affects the occipital lobe, however further investigations are needed to decipher the relationship between these 2 variables (7).

References

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