Blood-brain barrier breakdown is linked to small vessel disease in the aged human brain

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Background

- Alzheimer's disease (AD) is characterised by the presence of 2 proteins ('pathologies') in the brain; amyloid beta (Aβ) and hyperphosphorylated tau (HPτ)
- 'Small Vessel Disease' (SVD) refers to degenerative changes to the brain's blood vessels, particularly in the 'white matter' which consists of the axons that connect all brain cells.
- Cerebral white matter lesions (WMLs) occur when this white matter becomes damaged and can occur in both AD-affected and normal ageing brains. They are typically assumed to be caused by SVD but may also be linked to AD-related damage.
- The blood brain barrier (BBB) normally restricts the entry of harmful substances from the blood into brain tissue. Previous research has shown that BBB breakdown is a consequence of SVD and is indicated by plasma protein leakage into the brain matter.

Aims

The aim of this project was to use a blood plasma protein (fibrinogen) to determine the amount of blood brain barrier breakdown in both Alzheimer's disease affected and normal ageing brains. To do this, I used antibodies to stain the brain tissue and used quantitative analysis to measure the percentage coverage of fibrinogen in both normal and damaged white matter.

The study cohort consisted of 26 post-mortem brains; 12 AD and 14 Control. Brain tissue was obtained at autopsy and stored within the NBTR in accordance with Newcastle University Ethics Board.

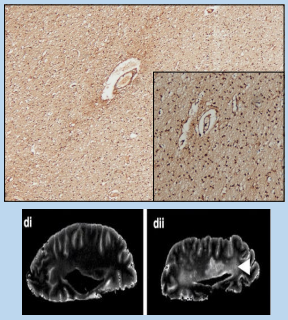

Figure 1. Image (top) shows example of fibrinogen leakage. Image (below) shows a WML as seen on an MRI scan.

Methods

Tissue preparation

Sections from the parietal lobe were used for this project as it contains large amounts of white matter. Fixed brain tissue sections were stained for antibodies against fibrinogen then cleared of wax and mounted onto glass slides.

Image analysis

All image analysis was performed blinded to diagnosis.
3x3 images were taken at different locations in the tissue and regions of interest were set to exclude the vessels and any artefacts. Thresholds to determine the 'binary area fraction' were set to a level that reached immunopositive fibrinogen. The mean area fraction ('binary area fraction') was determined for both the WML (white matter lesion) and NAWM (normal appearing white matter) in each sample case.

Statistical analysis

The Statistical Package for Social Sciences software (SPSS ver. 21) was used for statistical evaluation. The data was found to not be normally distributed. Differences in the WML and normal appearing white matter (NAWM) fibrinogen burden between AD and control cases were assessed using the non-parametric Wilcoxon test. Spearman's correlation coefficients (two tailed) were used to assess associations between SVD scores and WML and NAWM fibrinogen.
A significance level below 0.05 is significant and unlikely to be due to chance.

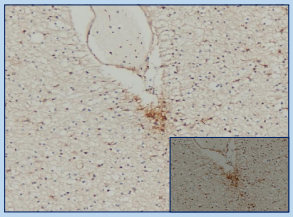

Figure 2. Fibrinogen leakage was identified by observing staining (immunopositivity) surrounding the blood vessels within the tissue.

Results

As we would expect, AD cases had significantly higher levels of AD change (HPτ and Aβ) compared with the controls (figure 2) but no significant differences were observed in age or gender between AD and control groups.

Relationship between fibrinogen immunoreactivity and dementia status

It was found that there was an overall increase in fibrinogen in the NAWM compared with WML in control cases. The significance of this increase was found to be 0.093 which is very close to being statistically significant. For the AD cases the significance was 0.263 and so can be concluded to be due to chance.

Correlations between SVD severity and fibrinogen immunoreactivity in normal and damaged white matter

Across all cases it was found that there was a positive correlation between the WML fibrinogen and parietal lobe SVD score. This positive correlation (0.562) was found to be statistically significant at 0.012 (the p value). There was no significant correlation between NAWM fibrinogen and SVD score (p = 0.129).

Table 1 shows the findings for the control cases.

Correlation between...	Correlation coefficient	Significance value	Conclusion
WML fibrinogen and parietal SVD score	0.773	0.005	**Significant**
NAWM fibrinogen and parietal SVD score	0.236	0.417	**Not significant**

Table 2 shows the findings for the AD cases.

Correlation between...	Correlation coefficient	Significance value	Conclusion
WML fibrinogen and parietal SVD score	0.238	0.570	**Not significant**
NAWM fibrinogen and parietal SVD score	0.519	0.102	**Not significant**

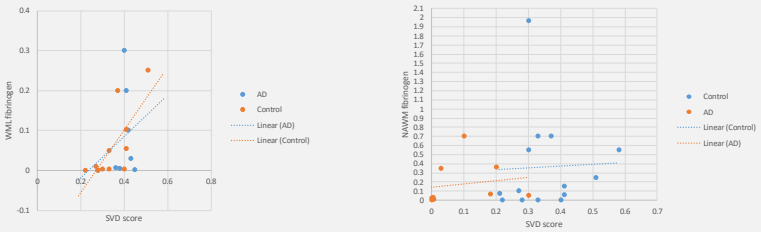

Figure 3. Scatter graphs to show the correlation between parietal SVD score and NAWM/WML fibrinogen burden. A steep upward line indicates a strong positive correlation.

Discussion

- There was an significant increase in fibrinogen in the NAWM compared with WML seen in the controls but not in the AD cases
- There was a positive correlation found between SVD score and WML fibrinogen in controls but no correlation between the two in AD.
- An increase in fibrinogen in controls, and not AD cases, suggests that plasma protein leakage is not related to typical AD pathology and therefore BBB breakdown in aged brains is likely due to an independent mechanism. This agrees with previous research suggesting SVD are BBB dysfunction are linked.
- We can assume the link between fibrinogen leakage and WML must due to SVD in the control cases, but in AD the WML are more likely due to AD-related mechanisms and not SVD directly.

What next?

Future work would involve correlating the fibrinogen burden in NAWM and WML with the distinct AD pathologies and uncover the mechanism linking AD pathology to plasma protein leakage.

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