

Can erythrocyte proteins be used as dementia biomarkers?

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Aims

To explore and identify erythrocyte proteins that can be used as blood biomarkers for dementia, by measuring changes in erythrocyte membrane protein levels in demented and control subjects.

Introduction

➤ **Alzheimer's Disease (AD), Vascular dementia (VaD) and Dementia with Lewy bodies (DLB)** are the leading causes of dementia in the older people. Improving current diagnostic methods to facilitate early dementia treatments remains an optimal goal. Recent research primarily focusses on plasma proteins as dementia peripheral (blood) biomarkers, whereas blood cellular components, including **erythrocytes (ERC)**, are poorly investigated.

➤ ERC's shape and morphology relies on the internal structures of cytoskeletal membrane⁽¹⁾. ERC proteins such as **Ubiquitin** and **IgG** play an important role in membrane integrity and stability⁽²⁾ (Figure 1).

➤ **Ubiquitin** is important for protein-degradation by interacting with ERC proteasomes. Failure to activate ubiquitination of cytoskeletal proteins may result to pathological changes in ERC morphology that results in AD⁽²⁾.

➤ Research has shown an increase in **IgG** levels in erythrocytes membranes in patients with neurodegenerative-related diseases such as Parkinson's disease, AD and schizophrenia. IgG interactions with other ERC proteins may explain for these changes resulting in dementia pathology⁽³⁾.

Protein	kDa	Associated Pathology
Ubiquitin	8.5	AD, Sickle cell disease
IgG	23 / 53	AD, Parkinson's Disease, Schizophrenia

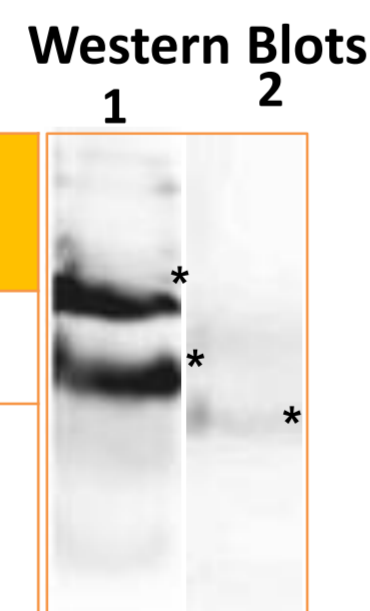


Figure 1: ERC proteins and dementia pathology. Western blot refers to a control ERC sample at pH 5.7. 1, IgG, 2, ubiquitin. (* indicates location of target protein size)

➤ ERC morphology is altered in dementia⁽⁴⁾: ERCs are elongated and less flexible, and associated with neuronal damage in hypoxic areas in the brain⁽⁵⁾. Modification of ERC membrane and cytoplasmic proteins may be linked to progression of dementia pathology.

Materials and Methods

- Blood samples (n=72) came from: 28 controls, 17 AD, 20 VaD and 7 DLB subjects.
- All participants had an extensive clinical assessment for their cognitive and behavioural functioning⁽⁶⁾.
- Blood samples were processed to obtain plasma, platelets and ERC fractions (Figure 2).
- Novel **ELISA immunoassays** were developed and used to measure Ubiquitin and IgG using commercially available immunoprobes.

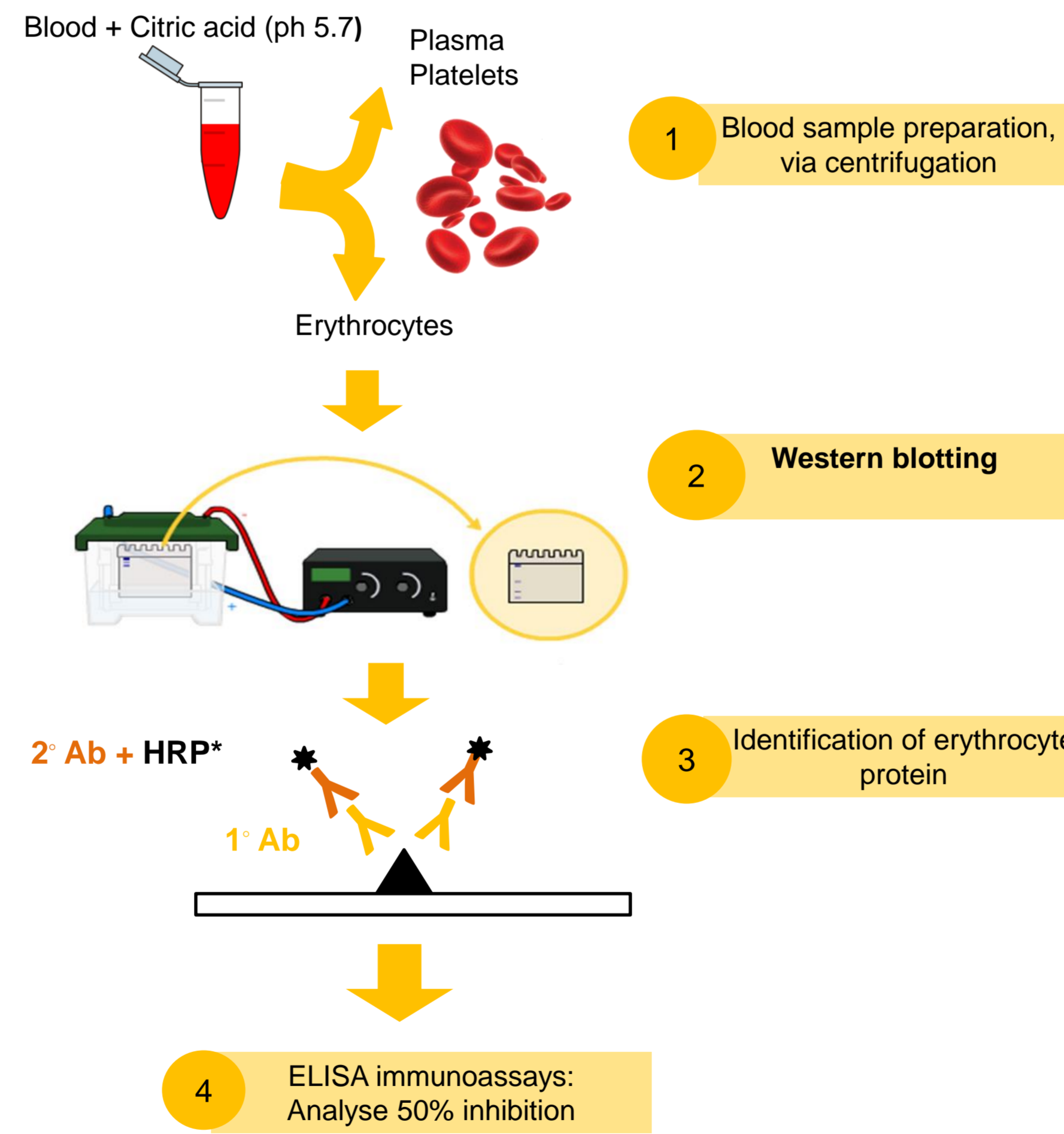


Figure 2: ERC extraction (pH5.7) and use in Western blot and ELISAs.

Reference

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2. Pasini, et al. *Blood* 2006, 108(3):791-801;
3. Bosman, G.J. *Cell Mol Biol* 2004, 50(1): 81-6.
4. Mohanty et al. *Adv Exp Med Biol* 2008, 614:29-35.
5. Jayakumar et al, *Biochim Biophys Acta* 2008, 1622(1): 20-8;
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Results

Mean protein concentration for Ubiquitin and IgG for each subjects

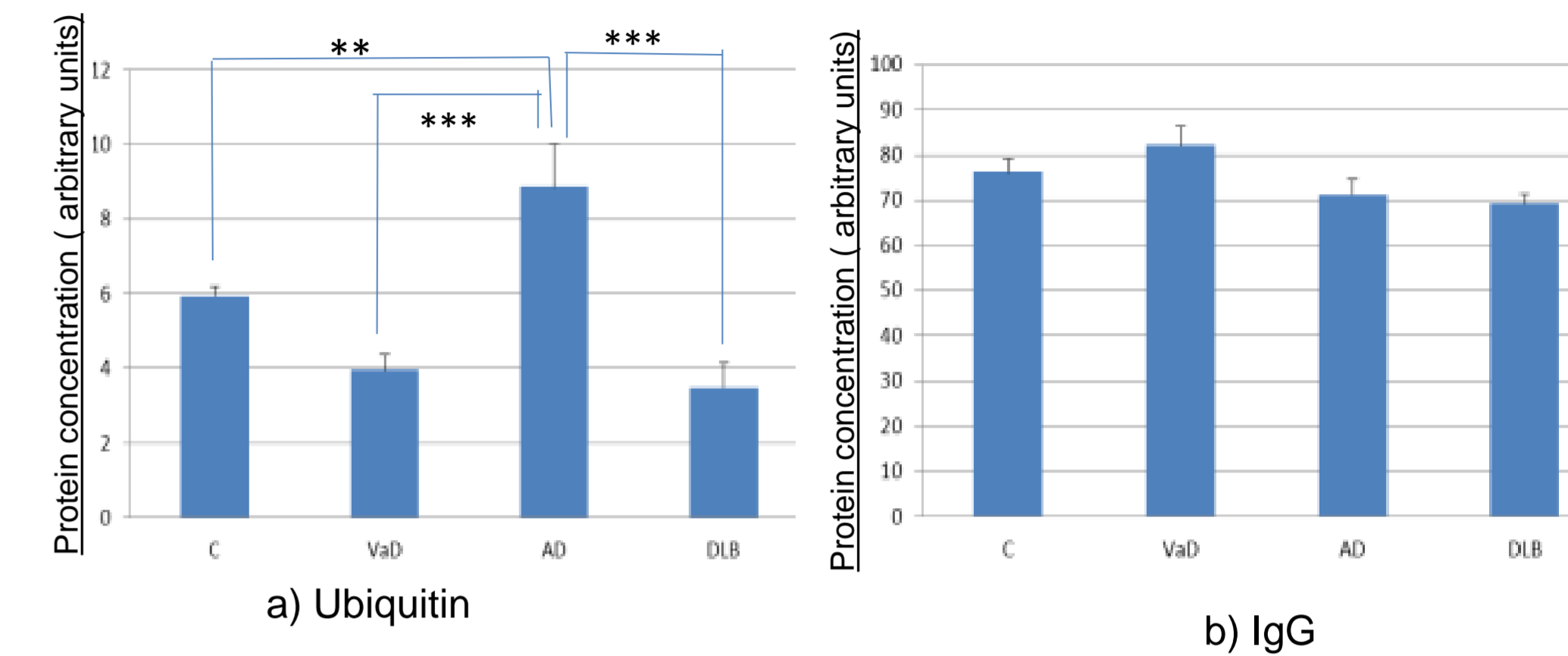


Figure 3: Ubiquitin (a) and IgG (b) ERC content in control and dementia subjects. Y-axis values represent Relative Values (%). **, p<0.01; ***, p<0.0001.

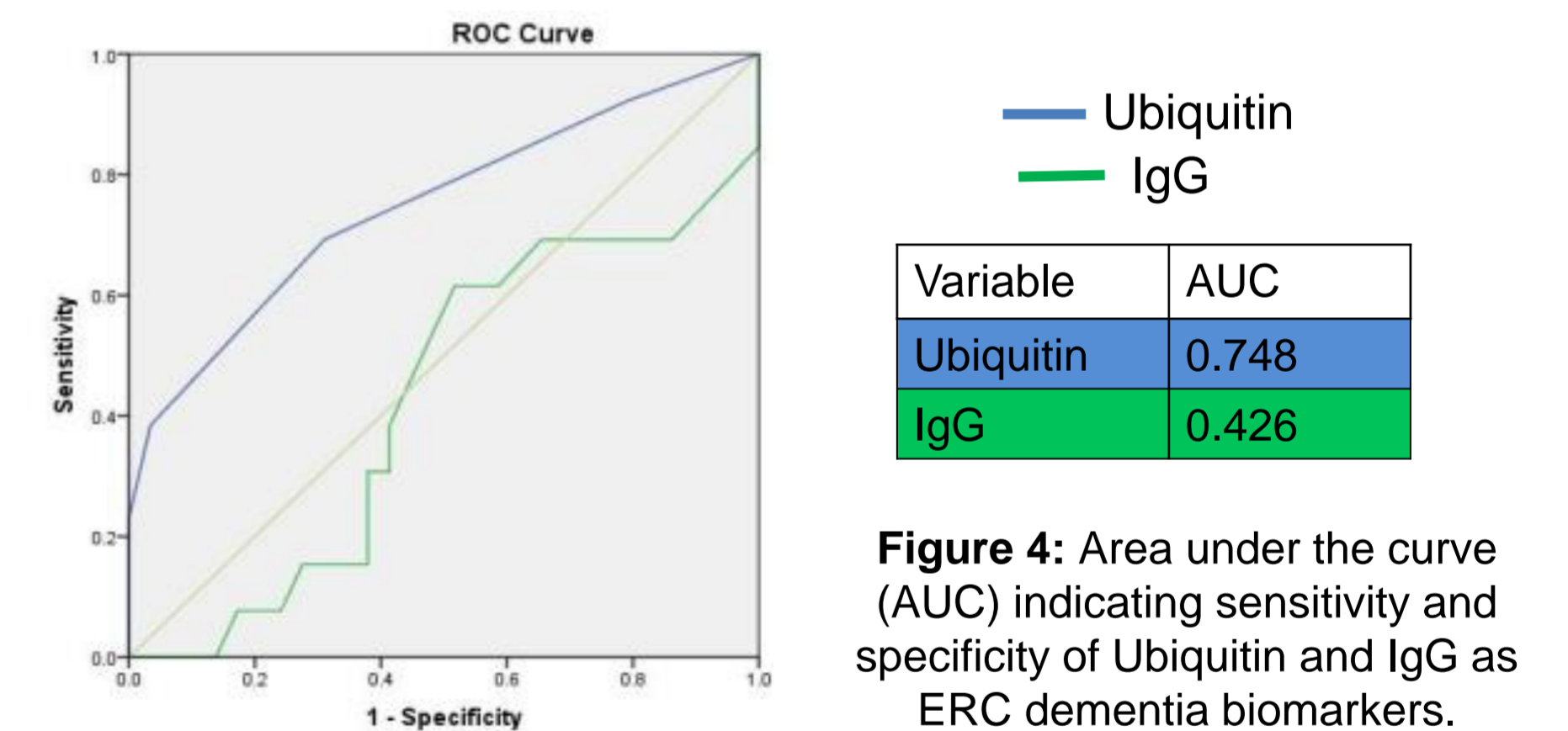


Figure 4: Area under the curve (AUC) indicating sensitivity and specificity of Ubiquitin and IgG as ERC dementia biomarkers.

Discussion

- This is the first study to explore the clinical utility of ERC protein content for diagnosis of dementia.
- ERC ubiquitin levels were highest in AD subjects. Control and dementia subjects had similar ERC IgG showing no significant difference (Figure 3).
- The ubiquitin ERC measures had high accuracy to distinguish dementia from cognitively intact subjects (74.8%) (Figure 4).

Conclusion

Further work is needed to validate the ERC ubiquitin assay in a larger clinical sample with various extent of cognitive impairment and aetiologies so that it can be used in routine clinical setting for dementia diagnosis.