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## Platelet Biomarkers for the Early Detection of Alzheimer's Disease

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**Purpose:** Alzheimer's disease (AD) is a common and fatal neurodegenerative disease affecting 5.4 million people in the US and many more worldwide. Despite this, diagnostic biomarkers for the early detection of AD are limited to costly, invasive procedures and may be unreliable. In this pilot study, platelet activity in response to agonists was profiled in early-stage AD and healthy control subjects in order to determine whether the prothrombotic state of AD can be used to formulate early diagnostic outcomes and biomarkers for AD.

**Methods:** Subjects with early-stage AD (n=12) and aged healthy controls (n=17) were recruited from the practice of Zaparackas and Knepper, Ltd. Diagnoses of early-stage AD were confirmed by neuropsychological testing, previous neuroimaging, and medical histories. After IRB approval and written consent, blood samples from each subject were used to isolate platelets and measure outcomes of platelet activity by flow cytometry and rheometry. Specifically, flow cytometry was used to measure levels of procoagulant platelet subtypes superactivated platelets (SAPs) and transglutaminase-active platelets (TAPs) in response to PAR1/4 and GPVI agonists thrombin and convulxin. SAPs were defined as platelets with inactivated glycoprotein IIb/IIIa and high levels of fibrinogen binding after 5 minutes. TAPs were defined as SAPs with high levels of the transglutaminase-dependent protein α2-antiplasmin after 1 hour. Cone-plate rheometry was used to measure platelet-rich plasma viscosity in response to graded amounts of thrombin at a low fixed shear rate of 50 $s^{-1}$. The lowest dose of thrombin (in NIH units) to yield an increase in viscosity > 10 centipoises was termed the thrombin index and stood as a relative measure of the sensitivity or responsiveness of an individual's platelets.

**Results:** All outcomes of platelet activity were significantly altered in early-stage AD subjects. Both SAP (49.5 ± 5.7% vs. 33.1 ± 6.8%, P<0.0001) and TAP (31.8 ± 3.2% vs. 23.5 ± 3.9%) levels were significantly increased in early-stage AD subjects compared with healthy controls. Furthermore, the thrombin index was significantly lower in early-stage AD subjects compared with healthy controls (0.02 ± 0.03 vs. 0.05 ± 0.02, P=0.02), indicating increased platelet sensitivity to thrombin.

**Conclusions:** Early-stage AD subjects exhibited a prothrombotic state consisting of high levels of procoagulant platelet subtypes and increased platelet responsiveness. These results suggest that outcomes of platelet activity may be reliable biomarkers for early-stage AD. Significantly, accessible vascular biomarkers such as platelets could be a new step forward for the early diagnosis of AD and monitoring of progression. Furthermore, these vascular outcomes may represent novel therapeutic targets. Notably, in a separate parallel study examining the effect of Toll-like receptor 4 inhibitors resveratrol, curcumin, and quercetin, two early-stage AD subjects who have completed at least 16 months of follow-up have remained stable in terms of neuropsychological testing, platelet activity outcomes examined in this study, and other emerging ocular biomarkers for AD including retinal beta-amyloid load and macular vessel density.

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