Effect of Blueberry Supplementation on Alzheimer's Biomarkers

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Scientific Rationale

Worldwide, some 40 million adults have Alzheimer's disease (AD) and several more million may be at elevated risk for AD by virtue of mild cognitive impairment (MCI) and/or silent buildup of cortical AD pathology (1). The failure of multiple therapeutic strategies in AD dementia highlights the need to develop risk reduction strategies in the earlier at risk stages.

Blood biomarkers have emerged to the forefront of AD research (2-13) spurred by the development of reliable ultra-sensitive "single-molecule array" assays with 100-1000-fold great sensitivity over traditional platforms (Table 1). Neurofilament light (NfL) is a marker of neuronal dysfunction whose elevation and rate of change are associated with cognitive decline and hippocampal atrophy (2-6). Studies in a variety of neurodegenerative disorders have also shown that elevated blood NfL levels reduce or normalize following therapy, sometimes within 10 weeks (2-6). A promising marker of glial activation is GFAP (glial fibrillary acidic protein). There is increased expression of GFAP in reactive glial cells surrounding amyloid plaques and blood GFAP is elevated in AD (7). Other blood markers (Aß42, Aß40, t-au, p-tau) reflect neuropathology (8-15). For example, Schindler et al showed that plasma Aß42/Aß40 ratio predicts current and future brain amyloidosis (15). Park et al found plasma t-tau/ Aß42 predicted an AD pattern of neurofibrillary tangle deposition (8) as well as longitudinal changes in cerebral amyloid deposition, brain glucose metabolism, and hippocampal atrophy (8). Overall, the evidence makes a strong case for biomarker guided pilot trials in AD and MCI.

Practical, well tolerated and non-invasive lifestyle interventions are highly desirable for AD prevention in at-risk middle aged and older adults (16). Preclinical studies have reported that blueberry bioactives, such as anthrocyanins, may impact many pathways involved in brain function or dementia risk - such as reduction of oxidative stress, improvement of inflammatory response, reversal of age-related decrements in cognition, increased cerebral blood flow, enhance microglial clearance of Aß, inhibits aggregation of Aß1–42, suppresses microglial activation and protect against Aß-induced neurotoxicity (reviewed in 16, 21-23). Despite this promising body of evidence, to our knowledge, no study has evaluated the effects of blueberry supplementation on pathological and neurodegeneration biomarkers in MCI.

Specific Objectives

The proposed pilot, open trial will examine the effect of treatment of blueberries on neuronal, glial, and pathology biomarkers in subjects with MCI. The blood markers to be measured are Neurofilament light (NfL), glial fibrillary acidic protein (GFAP), Aß42 and p-tau181 using an ultra-sensitive state-of-the-art digital immunoassay.

Primary Aim:
To generate pilot data on the effect of 3-months of blueberry consumption on blood biomarkers in MCI in order to calculate sample size estimates for future trials.
Hypothesis: Treatment with blueberry supplements will improve biomarker profiles in MCI.

Study Design
12-week open pilot trial. Following IRB approval all subjects will provide written informed consent. We will enroll 10 participants with MCI. The study duration is based upon the minimal time needed to expect a change in cognition and biomarkers. Eligible participants will be asked to abstain from berry fruits and other anthocyanin-rich foods during a 2-week washout period and for the duration of the trial.

Key Inclusion Criteria:
Age 55-85 years; both genders; stable medically; meets criteria for amnestic mild cognitive impairment (impaired delayed verbal recall, normal or near normal overall cognition and function). MCI diagnosis will be operationalized using criteria used widely (e.g. ADNI).

Key Exclusion Criteria:
Dementia , significant confounding active neurological/psychiatric disease; participation in an experimental investigational drug trial in the past 30 days; unwilling to restrict consumption of anthocyanin-rich foods; inability to complete cognitive testing (e.g. significant visual or hearing impairment); allergy or intolerance to blueberries; significant gastrointestinal disorders or surgery that influences digestion and absorption; history of frequent urinary tract or Clostridium difficile infections; presence of unstable, acutely symptomatic, or life-limiting illness.

After washout, participants will be receive blueberry supplement. Blood biomarkers (primary outcome) will be measured at zero and 12 weeks (as well as vital signs). Nutrient and berry intake function will be assessed at 0, 4, 8, and 12 weeks.

The blueberry dose of 36 grams per day in a split dose consumed with meals is based on 1) a 33% increase in dose over that previously used in a longer (6-month) trial; 2) delivering the most effective dose of blueberry bioactives; and 3) reduced likelihood of any gastrointestinal symptoms. Subjects will consume lyophilized blueberry powder, mixed with water (18 grams, equivalent to 3/4ths cup of blueberries) with 2 daily meals (36 g/d blueberry powder total; approx. 1.5 servings/d). Blueberry powders will be packaged in sealed single-serving packets (18 g/packet) to prevent exposure to light and moisture. Participants will be instructed to store them in home refrigerator to avoid degradation of blueberry bioactives. Participants will be instructed on storage, rehydration, dosing, and logging of supplement consumption as well as any inadvertent anthocyanin intake. Adherence will be measured during interim safety calls and return of empty packets.

Biomarker Assay
Venous blood will be collected at baseline and exit, and banked in -80 freezer for specified NfL, Aβ42, p-tau and GFAP biomarker analyses. Participants will be counseled to consume a standardized flavonoid-free meal the night before the blood sample collection. Plasma NfL, Aβ42, p-tau, GFAP are measured per Table 1.

Safety: AEs monitored via visits/phone calls (Table 2). SAEs will be reported to the IRB as appropriate. Study physician will oversee this. Possible risks are minor including anxiety or fatigue from testing and phlebotomy risks. The possible risks of blueberry consumption are minimal and well-studied. Participant information is kept confidential. Subjects will not be informed of their biomarker results due to their experimental nature and this will be pre-specified in the consent.

Data Analysis of Outcomes

As such this is a pilot study designed to generate power estimates for a larger future trial. Summary statistics will evaluate the change from baseline to endpoint in biomarker outcomes. Drop-out rates are expected to be low due to short duration and relatively benign intervention.

Possible Outcomes

The main possible outcomes in this study are a better understanding of the effects of blueberry on markers of neuronal dysfunction (reduction in neurofilament light levels), glial activation (reduction in GFAP) and plaque/tangle pathology (amyloid-beta, tau). This data will provide a strong scientific rationale for a larger controlled trial of blueberries in MCI or AD prevention.

Timetable

We plan to complete the study within 12 months from the receipt of funds/blueberry packets and IRB approval. We anticipate it will take about 30 days for staff training, purchase study materials such as biomarker assay kits, and finalize CRFs/database. We expect to finish enrolment by month 7 and complete the study by month 10. Biomarker assays and statistical analyses will be done by month 12. A final study report will be submitted.

Revised Budget

Budget and budget justification are presented in Table 2.

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Table 1: Quanterix Simoa Ultrasensitive Biomarker Assay
Measurements in this study will be done using Quanterix SR-X instrument, a new, state-of-the-art digital immunoassay platform is used for the biomarker assays in our study. The Simoa technology at the heart of this platform enables the detection and quantification of biomarkers previously difficult or impossible to measure, opening up new applications to address significant unmet needs in life science research. The ultrasensitivity of Simoa assays sets it apart from all other immunoassays available today, offering PCR-like limits of detection with both existing and novel protein biomarkers. A robust menu of ultrasensitive assays are available for proteins important in brain health, aging, inflammation, metabolism, and cardiovascular disease. This system and assays are available as a shared resource at Duke laboratory on a cost basis. The SR-X is designed for multiplex detection of up to six analytes per well, with low volume requirements to increase productivity and throughput, while conserving your precious samples.

A menu of over 70 Simoa assay kits is available to measure critical biomarkers with substantially higher sensitivity than standard immunoassay methods, enabling detection of both normal and acute levels with high precision across a range of sample types.

Sensitivity of the platform is 100-1000-fold higher than traditional ELISAs and chemiluminescence and electroluminescence (Luminex, Mesoscale) platforms. The instrument is being used for a number of ongoing NIH studies of brain dysfunction and the laboratory staff are fully trained in its use. For each participant, peripheral venous blood are drawn after overnight fasting in EDTA-coated tubes. Within 30 minutes after collection, blood is centrifuged to obtain plasma, aliquoted and stored at -80°C. The concentration of plasma Aß40, Aß42, tau and NFL are measured. Neurology 4-Plex E (included Aß40, Aß42, NfL, GFAP) and pTau181 kits are used according to the kit protocols with minor modifications. To eliminate inter-assay variability as a confounding factor, all samples belonging to the same subject were run in duplicate on the same day with the same standard. The few samples with intra-assay coefficients of variation >20% are measured again. All measurements were performed by professionally trained technicians blinded to participant's state and clinical data. The platform can also measure GFAP (LOD 0.26 pg/ml, range 0-40000 pg/ml).

A list of over 400 publications and posters validating the technology and it's utility in neurological disorders can be found here (https://www.quanterix.com/resources/publications-posters/neurology).

Analyte	LoD (pg/mL)	LoQ (pg/mL)	Dynamic Range (pg/mL)	Median Endogenous (pg/mL)	Sample Volume	Sample Type*
NF-Light	0.072	0.343	0-1,800	7.34	25 µL	E, S
Aβ40	0.196	0.675	0-800	209	25 µL	E
Aβ42	0.045	0.142	0-400	11.1		
Tau	0.019	0.063	0-400	1.43		