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# The Effect of an Aloe Polymannose Multinutrient Complex on Cognitive and Immune Functioning in Alzheimer's Disease

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**Abstract**. Alzheimer's disease (AD) is a leading killer of Americans, imparts a significant toll on the quality of life of the patient and primary caregiver, and results in inordinate costs in an already overburdened medical system. Prior studies on cholinesterase inhibitors among AD patients have shown minimal amelioration of disease symptoms and/or restoration of lost cognitive functioning. The effect of improved nutrition, particularly with dietary supplements, on cognitive functioning may offer an alternative strategy compared to standard treatment. The present pilot study investigated the effect of an aloe polymannose multinutrient complex (APMC) formula on cognitive and immune functioning over 12 months among adults diagnosed with AD. Subjects participated in an open-label trial and consumed 4 teaspoons per day of the APMC. The ADAS-cog, MMSE, ADCS-ADL, and SIB were administered at baseline and 3, 6, 9, and 12 months follow-up. Cytokines and lymphocyte and monocyte subsets were assessed at baseline and 12 months. The mean ADAS-cog cognition score significantly improved at 9 and 12 months from baseline, and 46% of our sample showed clinically-significant improvement ($\geq$4-point change) from baseline to 12 months. Participants showed significant decreases in tumor necrosis factor-α, vascular endothelial growth factor, and interleukins-2 and -4. CD90+, CD95+CD3+, CD95+CD34+, CD95+CD90+, CD14+CD34+, CD14+CD90+, and CD14+CD95+ decreased significantly, whereas CD14+ significantly increased. Participants tolerated the APMC supplement with few, temporary adverse reactions. Our results showed improvements in both clinical and physiological outcomes for a disease that otherwise has no standard ameliorative remedy.

Keywords: Aloe, Alzheimer's disease, B-lymphocyte subsets, cognition, cytokines, dietary supplementation, growth factors, oligosaccharides, T-cell subsets

## INTRODUCTION

In the U.S. today, roughly 5.5 million people suffer from Alzheimer's disease (AD), which accounts for about 70% of the total cases of dementia [1]. The incidence of dementia doubles every five years after age 60 from 1% of those ages 60 to 64 to up to 50% of those over age 85, and dementia is the leading cause of institutionalization among the elderly [2]. AD is the sixth-leading cause of death in the U.S. and is the only one among the top 10 killers of Americans that has no cure or preventive measure, nor can be delayed [1]. The current combined paid-for-care

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(formal) and unpaid care (informal caregiving) costs associated with dementia and AD in the U.S. are estimated to be more than $410 billion per year [1]. Based on the current costs and future estimates, any intervention that can ameliorate the effects of AD would not only improve the quality of life of the individual suffering from the disease (and the patient's caregivers), but could also potentially save the American health care system an inordinate sum of money. Randomized clinical trials for pharmacological agents (i.e., the cholinesterase inhibitors and memantine) for efficacy, safety, and treatment of AD have had no to moderate success [3], and the effect of using compounds to act against abnormal amyloid-β (Aβ) proteins within the brain is unknown at this time [4]. Unfortunately, current strategies have not been shown to prevent the onset of dementia or cognitive decline and do not halt the progression of disease [1, 5].

In light of the inability of pharmaceutical agents to prevent AD or sufficiently restore the functioning of AD-related symptoms, researchers and consumers have turned to nutrient and herbal formulae to determine their possible efficacy on cognitive functioning. Several studies have investigated the effects of antioxidants, particularly vitamins E and C. One study received particular attention showing that vitamin E (α-tocopherol) delayed the time to several outcomes (e.g., death, institutionalization, and loss of functioning) for moderate-severity AD patients [6]. However, the results of the majority of trials suggest limited to no beneficial effect of both antioxidants, due in part to the types of agents studied (e.g., synthetic, unnatural) on the primary prevention of cognitive decline or ameliorating the symptoms of AD patients [7, 8]. In fact, the level of vitamin E studied (>400 IU) in many dementia or AD trials has now been shown to be related to an increased risk of all-cause death [7].

Fatty acids (particularly omega 3) have recently been studied for their effects on cognitive functioning as well. Using different types of omega 3 fatty acids, two recent studies showed improvement in cognitive functioning in people with mild cognitive impairment, but not AD [9, 10]. Another study showed no effect of docosahexaenoic acid (DHA) in persons with mild to moderate AD, but a subgroup of subjects who were normal according to the Mini-Mental State Examination (MMSE) had improved delayed recall, and attention and cognitive function appeared to stabilize [11]. Ginkgo biloba is another nutrient that has been studied by several investigators for its possible effects on cognitive functioning [12–16]. A meta-analysis of studies of AD patients in 3 to 6 month treatment periods with 120 to 240 mg of ginkgo revealed a small though significant effect size of 0.40 ($p<0.01$) on objective measures of cognitive function [17].

The emerging field of glycobiology, which is the study of different forms of saccharides and their biosynthetic activity [18], may offer a novel nutritional approach for AD and its symptoms. Glycosylation is the most common form of protein and lipid modification, where saccharides are attached to proteins and lipids through a complex, but ordered, process in the ribosome, endoplasmic reticulum, and Golgi of the cell to enable intracellular functioning and cell-to-cell communication [19]. Glycolipids, glycoproteins, and proteoglycans are critical components of the cell surface recognition process throughout all organ systems [20].

Following the intake of aloe polymannose (an oligosaccharide), large CD14+ monocytes were noted in peripheral blood [21]. It was recognized in 2002 that CD14+ monocytes had pleuripotent adult stem cell capacities [22]. CD14+ cells purified in culture, when cytokines and growth factors are added to the medium, can be predictably transformed into neurons, among other cell types [22]. In addition, a patent has been issued for the increased production of adult stem cells by the use of complex carbohydrates originating from the cell wall of blue green algae and by complex fucans (monosaccharides) of seaweed origin [23]. Thus, the principle for the induction of adult stem cells by ingesting complex carbohydrates of plant origin has been well-established [24].

Supplementing with concentrated amounts of dietary oligosaccharides has demonstrated benefit for the following: cancer [25], HIV/AIDS [26, 27], immune system functioning [28], hyperlipidemia [29, 30], atherogenesis [31], chronic fatigue syndrome [32], and attention deficit hyperactivity disorder [33]. Additionally, a novel oligosaccharide-based, multinutrient formula improved quality of life in an open-label, pilot study of 48 AD patients [34]. Subjects were assessed with a symptom severity measure adapted from the Alzheimer's Association [35]. At the end of 6 months, 25 subjects (52.1%) improved their AD severity score by an average of 28.9%. The 23 non-responders deteriorated in their severity score by an average of 34%. None of the subjects reported untoward side effects.

Given the rising prevalence and cost of AD, treatment options are limited, standard drugs and certain nutrients have not been effective in restoring functionality and quality of life, dietary supplement use is highly prevalent among the elderly population [36,

Table 1
Sociodemographic characteristics of the sample

| Variable | Category | Baseline assessment ($n=34$) |
|---|---|---|
| Age | – | M = 79.9 (SD = 8.4; $R$ = 60, 98) |
| Gender | Male | 6 (17.6%) |
| | Female | 28 (82.4%) |
| Race/ethnicity | White, non-hispanic | 10 (29.4%) |
| | Black, non-hispanic | 3 (8.8%) |
| | Hispanic | 21 (61.7%) |
| Education | Up to high school | 23 (67.6%) |
| | Some post high school training | 3 (8.8%) |
| | College graduate | 4 (11.8%) |
| | Master's degree or higher | 4 (11.8%) |
| Marital status | Never married | 2 (5.9%) |
| | Married | 15 (44.1%) |
| | Widowed | 13 (38.2%) |
| | Divorced | 4 (11.7%) |
| Years diagnosed with Alzheimer's disease | – | M = 3.2 (SD = 2.0; $R$ = 1, 11) |

*M*, mean; *SD*, standard deviation; *R*, range.

37], and the initial success of the aforementioned pilot study, additional examination of the oligosaccharide-based formula is justified. Thus, we investigated the effect of a 12 month course of an oligosaccharide-based multinutrient formula on cognitive and immune functioning in a sample of persons with AD. Because of the known links between chronic brain and systemic inflammation and the neuropathology of AD (i.e., cognitive impairment due to cytokine-mediated interactions between neurons and glial cells) [38–43], we evaluated a panel of cytokines and lymphocyte and monocyte subsets in response to our intervention.

## MATERIALS AND METHODS

### *Study participants*

Participants ($n=34$) were recruited from referrals to the Miami Jewish Health Systems outpatient facility from 2008 to 2011. The study was conducted with the approval of the Stein Gerontological Institute Institutional Review Board for human subjects research, which operates within the standards set forth by the Helsinki Declaration of 1975, and each subject (and/or the primary caregiver) signed informed consent before participating in the study. The sample comprised of 82% females ($n=28$) and 18% males ($n=6$) with a mean age of 79.9 years (SD = 8.4; range = 60–98 years). The racial/ethnic distribution of the subjects was as follows: 62% Hispanic ($n=21$), 29% white, non-Hispanic ($n=10$), and 9% black, non-Hispanic ($n=3$). See Table 1 for all sociodemographic characteristics of the sample. Subjects were not required to stop or change their medication regimen for entry into the study and continued taking their drugs as ordered by the treating physician. Additionally, subjects had to be diagnosed with moderate-to-severe AD for at least 1 year prior to entering the study. Our participants were typically not eligible for other trials due to the severity of their condition and/or other co-morbid conditions. Each participant was evaluated by the study psychiatrist prior to enrollment in the study to verify the diagnosis of AD.

### *Intervention*

The oligosaccharide-based multinutrient formula used in this study is a nutritional supplement that has been sold by several commercial entities for over 15 years. The formula used in the study is an aloe polymannose multi-nutrient complex (APMC) composed of the following constituents in a fixed combination by weight, including: aloe powder containing more than 15% acetylated polymannose (BiAloe®), stabilized rice bran, larch tree fiber, larch tree soluble extract, cysteine, soy lecithin, UltraTerra® calcium alumino silicate, cherry tart powder, inositol hexaphosphate, dioscorea (yam) powder, omega 3 spherules, citric acid, and glucosamine. The final product is a powder, packaged in 300 gram containers, which dissolves readily in any liquid. All participants consumed 1 teaspoon orally of the APMC four times per day (with 3 meals and before bedtime). The primary caregiver was shown how to administer the APMC at the baseline assessment, and the first dose was given to the participant at our facility to ensure compliance with

the method and to monitor for any complications or adverse effects.

*Outcomes and assessments*

Each participant and caregiver completed a basic demographics and medical history questionnaire at baseline. In addition to a neuropsychological battery to measure changes in cognitive functioning, activities of daily living, and quality of life, a standard assessment at each follow-up (3, 6, 9, and 12 months) was conducted to monitor: (a) adverse reactions and compliance to the intervention, (b) basic medical and health status, and (c) current medications. A blood sample was drawn at baseline and 12 months to assess changes in cytokines and lymphocyte and monocyte subsets. Criteria used to select the assessment included: (a) appropriateness and sensitivity, (b) ease of administration and scoring, (c) adequate psychometric properties, (d) sufficient content coverage, while not becoming too much of a response burden for this sample, (e) experience administering these measures, and (f) employment of measures involving a multi-method (i.e., self-report and observational tests and biological measures) approach to enhance the validity of the overall assessment.

The neuropsychological battery consisted of four measures to assess changes in disease severity, overall cognitive functioning, and activities of daily living. The Alzheimer's Disease Assessment Scale-cognitive score (ADAS-cog) [44] is a sensitive and reliable psychometric scale and is considered the benchmark measure to assess cognitive functioning in dementia studies [45]. It has 11 subscales that evaluate memory, orientation, attention, language, reasoning, and constructional and ideational praxis that are summed to create a total cognition score [46]. The total score can range from zero (no impairment) to 70 (severe impairment). Different, counterbalanced word lists were used at the follow-up visits to ensure that practice and carry-over effects would not confound our results. The ADAS-cog assessment included an additional concentration score with values ranging from zero (no impairment) to 5 (severe impairment). The MMSE [47] is one of the most widely utilized and popular brief cognitive assessments, providing a rapid screen of orientation, registration, attention and calculation, recall, and language domains. The score can range from zero to 30 (25+ is normal) and can indicate severe ($\leq$9 points), moderate (10–20 points), or mild (21–24 points) cognitive impairment [48]. The modified 19-item Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL) [49] is a structured measure originally designed to assess functional capacity over a wide range of dementia severity. Each statement includes a series of hierarchical questions designed to determine the patient's ability to perform one of the activities of daily living, ranging from total independence to total inability. A total score of 54 signifies optimal performance, and lower scores indicate worse performance. Caregivers were asked to assess a patient's activities during the preceding four-week interval. The Severe Impairment Battery (SIB) [50, 51] is a 40-item questionnaire designed to assess the severity of cognitive dysfunction in AD and is divided into nine domains: memory, language, orientation, attention, praxis, visuospatial, construction, orientation to name, and social interaction. The total score on the SIB ranges from zero (greatest impairment) to 100 (no impairment).

*Sample collection and processing*

Venous blood was obtained at two different time points (baseline and 12 months) from all participants. Blood samples were collected in EDTA tubes and delivered to the laboratory within 2 hours of collection. All specimens were subjected to complete blood cell counts and auto 5-part differential count determinations by a fully-automated Coulter AcT5 hematology analyzer (Beckman Coulter, Fullerton, CA). Flow cytometric enumeration of T, B, and NK cell subsets were performed on a 4-color flow cytometer, FACS Calibur (BD Biosciences, San Jose, CA), and the different cell populations were analyzed using Cell Quest Pro software (version 5.2, BD Biosciences, San Jose, CA).

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque gradient centrifugation. PBMC were recovered from the gradient interface and washed in phosphate buffered saline. Blood was diluted with 1 : 1 RPMI 1640 (Gibco, Grand Island, NY), layered over Ficoll-Hypaque solution (Pharmacia, Piscataway, NJ), and centrifuged for 30 minutes at 1,500 rpm at ambient temperature. The PBMC were collected, washed with RPMI 1640, and counted and assessed for viability in trypan blue dye. Plasma for cytokine detection was separated and stored at $-80°C$ until used.

*Multiplex cytokine and growth factor testing*

Due to their central role as signaling compounds in the immune system, cytokines and growth factors are

involved in a variety of immunological, inflammatory, and infectious diseases. New microarray-based biochip cytokine technologies combine the latest technological advances with innovative system design to present a fully-automated system for rapid multiplex testing. It enables up to 12 cytokines and growth factors to be detected simultaneously in a single sample, providing valuable information related to each molecule being tested and possible associations between them in each sample. This system saves time, costs, and resources and also provides high-quality, reliable results.

Cytokine and growth factor levels in plasma specimens were measured using a biochip array system, Evidence Investigator™ (Randox Laboratories Ltd., Crumlin, UK) as reported previously [52]. The testing platform consists of biochips secured in the base of a well placed in a carrier holding nine biochips in a 3×3 format. Each biochip is coated with the capture antibodies specific for each of the 12 cytokines and growth factors (interleukin [IL]-2, IL-4, IL-6, IL-8, IL-10, IL-1α, IL-1β, interferon [IFN]-γ, tumor necrosis factor [TNF]-α, monocyte chemotactic protein [MCP]-1, vascular endothelial growth factor [VEGF], and epidermal growth factor [EGF]) on a particular test region. A sandwich chemiluminescent assay was performed with 100 µl plasma using reagents (including the calibrators and controls) and protocols supplied by the same manufacturer. The light signal generated from each of the test regions on the biochip was detected using a charge-coupled detector camera and imaging system and compared with a calibration curve generated with known standards during the same run. All specimens were run in duplicate, and the concentration of each cytokine present in each plasma specimen was calculated from the standard curve and reported in pg/ml.

### *Statistical analysis*

Data were analyzed using SPSS 19 (IBM Inc., Chicago, IL) for Windows. Frequency and descriptive statistics were calculated on all variables. We utilized linear mixed modeling (LMM) to assess the fixed effect of time on changes in our outcome variables from baseline to follow-up. If the type III test of the fixed effect of time was significant, then we evaluated the parameter estimate between baseline and 12 month follow-up. If that parameter estimate was significant, then we used pairwise comparisons to determine the unique differences between baseline and follow-up at 3, 6, 9, and 12 months for the cognitive assessment and between baseline and 12 months for the physiological variables. LMM with heterogeneous compound symmetry covariance allowed us to account for subject attrition, inter-correlated responses between time points, and non-constant variability. Given that the ADAS-cog is widely recognized as the primary neuropsychological measure to determine cognitive functioning in AD trials, we categorized subjects at each follow-up assessment as improved ($\leq -4$), worse ($\geq 4$), and no change ($-3$ to 3) according to other methods [45], as an additional measure of assessing the efficacy of the intervention [53–56]. We examined the relationships between the cognitive assessments and the physiological outcomes at baseline and 12 months follow-up with Pearson product-moment correlations. The criterion for statistical significance was $\alpha = 0.05$.

## RESULTS

### *Safety and tolerability*

During the 12 month study period, one subject's caregiver reported an initial 3-day period of loose stool that was remedied by halving the amount of supplement given per day and then increased to the 4 teaspoon/day amount in one week. A second subject's caregiver reported elevations in blood pressure and pulse, which were remedied by reducing the daily amount to 1 teaspoon/day and increased by 1 teaspoon/day/week until achieving the desired dose of 4 teaspoons/day. No other adverse events were reported in this study. Three participants died during the course of the intervention, which were deemed unrelated to the study: one male due to myocardial infarction and two females due to stroke. Five other participants dropped out of the study due to non-compliance with the protocol according to the caregivers (e.g., the participant was unwilling to take the APMC 4 times per day), leaving 26 subjects who completed the 12 month intervention.

Table 2 shows the descriptive values of the ADAS-cog cognition and concentration scores, MMSE, ADCS-ADL, and SIB at baseline and 3, 6, 9 and 12 months follow-up. For the ADAS-cog cognition score, a significant fixed effect was found for time ($F[4, 71.5] = 3.2$, $p < 0.05$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[74.4] = 2.0$, $p < 0.05$). Pairwise comparisons revealed that ADAS-cog cognition score significantly improved at 9 months (mean difference = 3.7; SE = 1.9; 95% CI: −0.02, 7.4; $p = 0.05$) and at 12 months (mean difference = 3.8; SE = 1.8; 95% CI: 0.1, 7.5; $p < 0.05$) compared to baseline. For the ADAS-cog concentration score, a non-significant

Table 2
Descriptives for the ADAS-cog, MMSE, ADCS-ADL, and SIB

| Variable | Baseline | 3 Months | 6 Months | 9 Months | 12 Months |
|---|---|---|---|---|---|
| ADAS-cog cognition score* | 42.0 ± 14.0 (13, 69) | 43.8 ± 15.5 (11, 69) | 41.7 ± 15.2 (13, 69) | 37.8 ± 14.5 (9, 68) | 37.8 ± 12.9 (8, 70) |
| ADAS-cog concentration score | 1.56 ± 1.65 (0, 5) | 1.38 ± 1.70 (0, 5) | 1.39 ± 1.78 (0, 5) | 1.30 ± 1.82 (0, 5) | 1.23 ± 1.63 (0, 5) |
| MMSE | 10.7 ± 6.5 (0, 23) | 10.1 ± 8.2 (0, 28) | 9.0 ± 7.8 (0, 25) | 9.5 ± 8.7 (0, 28) | 9.2 ± 8.0 (0, 26) |
| ADCS-ADL* | 17.7 ± 12.6 (0, 47) | 18.4 ± 13.4 (0, 53) | 15.0 ± 11.0 (0, 50) | 14.1 ± 9.9 (0, 43) | 13.2 ± 10.9 (0, 49) |
| SIB* | 58.1 ± 31.4 (0, 96) | 57.5 ± 32.2 (0, 95) | 53.6 ± 32.5 (0, 93) | 49.5 ± 34.1 (0, 95) | 48.9 ± 35.3 (0, 97) |

*Values are significantly different ($p < 0.05$) from Baseline to 12 Months, mean ± standard deviation (minimum, maximum), and higher scores indicate better performance on the MMSE, ADCS-ADL, and SIB, otherwise lower scores indicate improvement on the ADAS-cog cognition and concentration scores.

Table 3
Cytokines and growth factors at baseline and 12 months follow-up

| Variable | Baseline | 12 Months |
|---|---|---|
| IL-2 (pg/mL)* | 6.4 ± 4.6 (0, 19.1) | 4.3 ± 6.7 (0, 29.8) |
| IL-4 (pg/mL)* | 0.94 ± 1.42 (0, 4.3) | 0.25 ± 0.89 (0, 3.8) |
| IL-6 (pg/mL) | 5.2 ± 7.6 (0, 37.7) | 5.1 ± 11.6 (0, 56.5) |
| IL-8 (pg/mL) | 7.4 ± 11.3 (0, 62.9) | 11.5 ± 34.3 (0, 174.0) |
| IL-10 (pg/mL) | 0.34 ± 0.62 (0, 2.4) | 0.97 ± 3.65 (0, 18.4) |
| IL-1α (pg/mL) | 0.21 ± 0.31 (0, 0.75) | 0.13 ± 0.60 (0, 3.0) |
| IL-1β (pg/mL) | 1.8 ± 2.3 (0, 8.6) | 4.0 ± 10.8 (0, 44.0) |
| IFN-γ (pg/mL) | 0.91 ± 1.79 (0, 7.4) | 0.47 ± 1.21 (0, 5.0) |
| TNF-α (pg/mL)* | 2.8 ± 1.6 (0, 5.0) | 1.7 ± 1.4 (0, 4.0) |
| MCP-1 (pg/mL) | 127.3 ± 62.8 (45.6, 302.5) | 122.1 ± 44.7 (47.6, 212.6) |
| VEGF (pg/mL)* | 50.4 ± 31.6 (12.8, 150.1) | 31.2 ± 22.6 (0, 79.9) |
| EGF (pg/mL) | 10.1 ± 14.0 (0, 53.7) | 10.1 ± 15.0 (0, 68.6) |

*Values are significantly different ($p < 0.05$) from Baseline to 12 Months; mean ± standard deviation (minimum, maximum).

fixed effect was found for time ($F[4, 63.5] = 0.6$, $p = 0.70$). For the MMSE, a non-significant fixed effect was found for time ($F[4, 65.3] = 2.2$, $p < 0.10$). For the ADCS-ADL, a significant fixed effect was found for time ($F[4, 69.7] = 5.1$, $p < 0.01$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[75.7] = 3.2$, $p < 0.01$). Pairwise comparisons revealed that the ADCS-ADL worsened from baseline to 9 months (mean difference = 3.1; SE = 1.3; 95% CI: 0.5, 5.7; $p < 0.05$) and 12 months (mean difference = 4.3; SE = 1.3; 95% CI: 1.6, 6.9; $p < 0.01$). The score at 3 months improved non-significantly (mean difference = 1.9; SE = 1.5; 95% CI: −1.1, 4.9; $p = 0.20$) above the baseline value. For the SIB, a significant fixed effect was found for time ($F[4, 75.6] = 5.6$, $p < 0.01$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[81.8] = 3.7$, $p < 0.01$). Pairwise comparisons revealed that the SIB score worsened at 6 months (mean difference = 3.8; SE = 1.9; 95% CI: 0.01, 7.5; $p = 0.05$), 9 months (mean difference = 8.1; SE = 2.1; 95% CI: 3.9, 12.4; $p < 0.01$), and 12 months (mean difference = 8.0; SE = 2.2; 95% CI: 3.6, 12.4; $p < 0.01$) compared to baseline. The score at 3 months was unchanged from baseline (mean difference = 1.6; SE = 2.0; 95% CI: −2.2, 5.5; $p = 0.40$).

According to the change in ADAS-cog cognition score from baseline to 3 months, 16.7% of the subjects improved, 46.7% did not change, and 36.7% worsened. From baseline to 6 months, 29.0% of the subjects improved, 38.7% did not change, and 32.3% worsened. From baseline to 9 months, 47.8% of the subjects improved, 26.1% did not change, and 26.1% worsened. From baseline to 12 months, 46.2% of the subjects improved, 23.1% did not change, and 30.8% worsened.

Table 3 shows the descriptive values for all 12 cytokines and growth factors at baseline and 12 months follow-up. For IL-2, a significant fixed effect was found for time ($F[1, 24.1] = 7.9$, $p = 0.01$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[24.1] = 2.8$, $p = 0.01$). Pairwise comparisons revealed that IL-2 declined from baseline to 12 months (mean difference = 2.5; SE = 0.9; 95%

Table 4
T cell subsets at baseline and 12 months follow-up

| Variable | Baseline | 12 Months |
|---|---|---|
| WBC (Cells/μL) | 6,891.2 ± 2,168.1 (2,700, 11,600) | 6,652.0 ± 2089.9 (3,300, 10,800) |
| Lymphs (%) | 28.3 ± 7.6 (14.3, 41.4) | 29.9 ± 8.0 (18.7, 48.1) |
| CD45+ (Cells/μL) | 1,897.2 ± 709.3 (840, 3,828) | 1,936.2 ± 625.6 (780, 3,402) |
| CD3+ (%) | 71.3 ± 9.5 (46, 86) | 71.8 ± 8.6 (54, 88) |
| CD3+ (Cells/μL) | 1,342 ± 503.7 (551, 2,666) | 1,381.4 ± 470.3 (507, 2,888) |
| CD3+ CD4+ (%) | 46.6 ± 9.8 (26.3, 68) | 46.6 ± 9.7 (31, 71) |
| CD3+ CD4+ (Cells/μL) | 861.7 ± 293.3 (337, 1,514) | 881.1 ± 291.9 (398, 1,592) |
| CD3+ CD8+ (%) | 24.4 ± 9.8 (7, 43) | 24.7 ± 9.9 (2, 44) |
| CD3+ CD8+ (Cells/μL) | 476.2 ± 292.6 (97, 1,418) | 489.4 ± 279 (32, 1,238) |
| B Cells CD19+ (%) | 9.4 ± 5.7 (0.9, 30) | 9.2 ± 6.4 (1, 28) |
| B Cells CD19+ (Cells/μL) | 193.4 ± 191.4 (14, 1,093) | 197.7 ± 170.5 (16, 772) |
| NK Cells CD16 + 56 (%) | 18.5 ± 9.1 (5.5, 46) | 17.8 ± 7.3 (4, 31) |
| NK Cells CD16 + 56 (Cells/μL) | 346.2 ± 203.8 (92, 838) | 338.4 ± 167.2 (71, 676) |
| CD3 + CD4 +/CD3 + CD8 + Ratio* | 2.5 ± 2.0 (0.6, 9.7) | 3.4 ± 6.8 (0.7, 35.5) |

*Values are significantly different ($p < 0.05$) from Baseline to 12 Months; Values are mean ± standard deviation (minimum, maximum).

Table 5
CD14, CD34, CD90, and CD95 subsets at baseline and 12 month follow-up

| Variable | Baseline | 12 Months |
|---|---|---|
| CD34+ (%) | 24.5 ± 20.5 (0.3, 66.3) | 13.1 ± 18.2 (0.5, 56.9) |
| CD34+ (Cells/μL) | 441.6 ± 378.8 (5, 1,251) | 231.1 ± 322.3 (6, 990) |
| CD90+ (%)* | 9.3 ± 15.1 (0.9, 48.1) | 1.2 ± 1.7 (0.1, 6.1) |
| CD90+ (Cells/μL)* | 154.4 ± 251.0 (17, 775) | 23.5 ± 38.0 (2, 133) |
| CD95+ CD3+ (%)* | 52.5 ± 19.9 (7.1, 85.8) | 15.6 ± 16.6 (1.1, 65.5) |
| CD95+ CD3+ (Cells/μL)* | 937.7 ± 396.9 (134, 1,586) | 305.7 ± 361.8 (15.0, 1,427) |
| CD95+ CD34+ (%)* | 24.9 ± 12.6 (5.7, 47.7) | 4.3 ± 10.3 (0.1, 39.5) |
| CD95+ CD34+ (Cells/μL)* | 427.3 ± 198.8 (127, 796) | 87.3 ± 222.7 (2, 861) |
| CD95+ CD90+ (%)* | 7.5 ± 12.0 (0.8, 40.9) | 1.7 ± 2.7 (0, 9.4) |
| CD95+ CD90+ (Cells/μL) | 125.3 ± 196.0 (13, 659) | 31.1 ± 44.2 (0, 129) |
| CD14+ (%)* | 10.3 ± 5.0 (5.5, 17.6) | 39.8 ± 22.6 (5.2, 80.0) |
| CD14+ CD34+ (%) | 7.5 ± 14.3 (0, 38.4) | 3.4 ± 4.9 (0.3, 18.5) |
| CD14+ CD90+ (%)* | 18.0 ± 16.4 (1.9, 61.3) | 2.4 ± 3.6 (0.1, 14.3) |
| CD14+ CD95+ (%)* | 77.5 ± 19.0 (23.7, 97.3) | 26.2 ± 19.2 (4.9, 82.7) |

*Values are significantly different ($p < 0.05$) from Baseline to 12 Months; Values are mean ± standard deviation (minimum, maximum).

CI: 0.7, 4.4; $p = 0.01$). For IL-4, a significant fixed effect was found for time ($F[1, 32.6] = 5.2$, $p < 0.05$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[32.6] = 2.3$, $p < 0.05$). Pairwise comparisons revealed that IL-4 declined from baseline to 12 months (mean difference = 0.69; SE = 0.30; 95% CI: 0.07, 1.30; $p < 0.05$). For TNF-α, a significant fixed effect was found for time ($F[1, 34.3] = 7.3$, $p < 0.05$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[34.3] = 2.7$, $p < 0.05$). Pairwise comparisons revealed that TNF-α declined from baseline to 12 months (mean difference = 1.13; SE = 0.42; 95% CI: 0.28, 1.98; $p < 0.05$). For VEGF, a significant fixed effect was found for time ($F[1, 29.2] = 13.5$, $p < 0.01$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[29.2] = 3.7$, $p < 0.01$). Pairwise comparisons revealed that VEGF declined from baseline to 12 months (mean difference = 19.5; SE = 5.3; 95% CI: 8.6, 30.3; $p < 0.01$). All other cytokines and growth factors showed non-significant changes from baseline to 12 months.

Table 4 shows the descriptive values of the T cell subsets, including CD45+, CD3+, CD3+CD4+, CD3+CD8+, CD19+, and CD16+56+, none of which significantly changed from baseline to 12 months follow-up. For the CD3+CD4+/CD3+CD8+ratio, a significant fixed effect was found for time ($F[1, 520.7] = 4.0$, $p < 0.05$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[520.7] = 2.0$, $p < 0.05$). Pairwise comparisons revealed that the CD3+CD4+/CD3+CD8+ratio increased from baseline to 12 months (mean difference = 2.7; SE = 1.4; 95% CI: 0.04, 5.4; $p < 0.05$).

Table 5 shows the descriptive values of the CD14+, CD34+, CD90+, and CD95+protein subsets, which revealed significant changes other than in the CD34+cells. For CD90+ (%), a significant fixed effect was found for time ($F[1, 286.6] = 4.2, p < 0.05$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[286.6] = 2.1$, $p < 0.05$). Pairwise comparisons revealed that the CD90+ (%) decreased from baseline to 12 months (mean difference = 8.9; SE = 4.3; 95% CI: 0.39, 17.4; $p < 0.05$). For CD95+CD3+ (%), a significant fixed effect was found for time ($F[1, 29.0] = 26.4, p < 0.01$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[29.0] = 5.1$, $p < 0.01$). Pairwise comparisons revealed that the CD95+CD3+ (%) decreased from baseline to 12 months (mean difference = 36.8; SE = 7.2; 95% CI: 22.1, 51.4; $p < 0.01$). For CD95+CD34+ (%), a significant fixed effect was found for time ($F[1, 29.8] = 25.3$, $p < 0.01$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[29.8] = 5.0$, $p < 0.01$). Pairwise comparisons revealed that the CD95+CD34+ (%) decreased from baseline to 12 months (mean difference = 22.1; SE = 4.4; 95% CI: 13.1, 31.0; $p < 0.01$). For CD95+CD90+ (%), a significant fixed effect was found for time ($F[1, 41.6] = 10.2$, $p < 0.01$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[41.6] = 3.2, p < 0.01$). Pairwise comparisons revealed that the CD95+CD90+ (%) decreased from baseline to 12 months (mean difference = 9.0; SE = 2.8; 95% CI: 3.3, 14.6; $p < 0.01$). For CD14+ (%), a significant fixed effect was found for time ($F[1, 50520.3] = 42.8, p < 0.01$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[50520.3] = 6.5, p < 0.01$). Pairwise comparisons revealed that the CD14+ (%) increased from baseline to 12 months (mean difference = 25.4; SE = 3.9; 95% CI: 17.8, 33.0; $p < 0.01$). For CD14+CD90+ (%), a significant fixed effect was found for time ($F[1, 119.3] = 10.9$, $p < 0.01$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[119.3] = 3.3$, $p < 0.01$). Pairwise comparisons revealed that the CD14+CD90+ (%) decreased from baseline to 12 months (mean difference = 15.9; SE = 4.8; 95% CI: 6.3, 25.4; $p < 0.01$). For CD14+CD95+ (%), a significant fixed effect was found for time ($F[1, 30.2] = 47.7$, $p < 0.01$), and the parameter estimate between baseline and 12 month follow-up was also significant ($t[30.2] = 6.9$, $p < 0.01$). Pairwise comparisons revealed that the CD14+CD95+ (%) decreased from baseline to 12 months (mean difference = 50.7; SE = 7.3; 95% CI: 35.7, 65.7; $p < 0.01$).

At baseline, the ADAS-cog cognition score was inversely related to VEGF ($r = -0.35$, $p < 0.05$), CD90+ (%; $r = -0.57, p = 0.05$ and cells/uL; $r = -0.58$, $p = 0.05$), and CD95+CD90+ (%; $r = -0.60$, $p < 0.05$ and cells/uL; $r = -0.62$, $p < 0.05$). The MMSE was linearly related to CD90+ (%; $r = 0.57$, $p = 0.05$ and cells/uL; $r = 0.57$, $p = 0.05$) and CD95+CD90+ (%; $r = 0.61$, $p < 0.05$ and cells/uL; $r = 0.61$, $p < 0.05$). The ADAS-cog concentration score was inversely related to CD14+ (%; $r = -0.59$, $p = 0.05$).

At 12 months follow-up, the ADCS-ADL was linearly related to IL-4 ($r = 0.44, p < 0.05$). The ADAS-cog concentration score was linearly related to IL-2 ($r = 0.45$, $p < 0.05$), IL-6 ($r = 0.49$, $p < 0.05$), IL-10 ($r = 0.58$, $p < 0.01$), IL-1α ($r = 0.57$, $p < 0.01$), IL-1β ($r = 0.44$, $p < 0.05$), and IFN-γ ($r = 0.54$, $p < 0.01$). The ADAS-cog cognition score was inversely related to CD19+ (%; $r = -0.49$, $p < 0.05$ and cells/uL; $r = -0.46$, $p < 0.05$) and linearly related to the CD3+CD4+/CD3+CD8+ratio ($r = 0.51, p < 0.01$). The ADAS-cog concentration score was linearly related to CD3+CD4+ (%; $r = -0.47$, $p < 0.05$) and the CD3+CD4+/CD3+CD8+ ratio ($r = 0.58, p < 0.01$) and was inversely related to CD3+CD8+ (%; $r = -0.45$, $p < 0.05$ and cells/uL; $r = -0.46, p < 0.05$).

## DISCUSSION

The loss, agony, and frustration of the victims of AD and their caregivers are collectively immeasurable and call science to continued and urgent action to counteract this debilitating disease. Additionally, the prevalence of AD and its associated financial costs are a significant drain on an already overburdened U.S. health system and are getting worse. This cause of death shows signs of spiraling out of control with an aging U.S. population and no options for prevention or treatment of the disease. Thus, any intervention that demonstrates promise for improving the condition of the AD patient is urgently needed.

In the current study, we have demonstrated in an open-label trial that cognitive functioning *improved* in AD victims over a 12 month period according to the ADAS-cog cognition score, the most widely utilized tool of its type for dementia research [57]. While cognitive functioning briefly worsened at the 3-month assessment, we showed improvement in our participants from 6 to 12 months in response to taking the APMC dietary supplement. Almost half (46%) of our

sample showed clinically-significant improvement at 12 months according to the change ($\leq -4$ points) on the ADAS-cog cognition score [45].

Given that cognitive functioning worsens over time in the typical AD patient regardless of treatment [1], our finding that cognitive functioning improved on the ADAS-cog cognition score is promising. The MMSE showed essentially no difference in score over the course of the intervention, while the average score at baseline was consistent with an indication of severe impairment in this sample. Although our subjects appeared to decline according to the SIB and the ADCS-ADL, we are encouraged by the findings in the ADAS-cog cognition score. Given the dissimilarities between the ADAS-cog cognition score and the SIB and the ADCS-ADL scores, we can only speculate that discrepancies in our battery occurred because these assessments have different items and scales (and units of measure) and are evaluating different domains. Additionally, we are uncertain about the clinical meaningfulness of the change in SIB (9-point drop at 12 months) and ADCS-ADL (4-point drop at 12 months) scores, unlike the ADAS-cog, which has been cited consistently as the most important cognitive assessment in research for AD participants [45, 53–56].

Our study may also be one of the first of its kind to assess a panel of 12 cytokines (pro- and anti-inflammatory) and growth factors before and after dietary supplementation in AD. To our knowledge, cytokines have been assessed (e.g., IL-1β, TNF-α, and IL-6) in AD patients and compared to controls or other disease groups, such as vascular dementia or cerebrovascular disease, but typically these are cross-sectional or observational studies, not clinical trials [43, 58]. Thus, the scope of our assessment contributes to a broader understanding of the links (or lack thereof) between selected markers of immune functioning and AD in response to improved nutritional status with APMC. Additionally, we did not observe changes over time in some of our markers (e.g., IL-1β, IL-6, and IL-10), even though they have previously been associated with AD in some mechanistic fashion [42, 43].

In the present study, we showed that IL-2 decreased in response to APMC, which is consistent with the normal decline in IL-2 production and expression over time found among the elderly [59]. Other investigators have noted that while weakened immune functioning in AD is also a hallmark of aging generally, low IL-2 production in this population may determine their increased susceptibility to infections [60], although no change in co-morbid infections was noted in our study. We also showed a non-significant decrease in IFN-γ that paralleled the reduction in IL-2 (values were significantly correlated at baseline [$r = 0.50$, $p < 0.01$] and 12 months [$r = 0.68$, $p < 0.01$]), which is consistent with other findings that IL-2 induces interferons [61]. Thus, our results may suggest the presence of altered Th-1 clones (secretors of IL-2 and IFN-γ) in our sample of moderate-to-severe AD participants in response to APMC [62].

We also found a significant decrease in the anti-inflammatory IL-4, which is posited as capable of reducing neuroinflammation by blunting the pro-inflammatory activity of IL-1β [39]. Given the non-significant rise in IL-1β, our results are consistent with that hypothesis. Although IL-4 decreased in our sample, the 12 month level was still higher than that found in another study of AD participants [63]. Thus, as with most cytokines, a clinically-determined level that is directly related to AD symptomatology and functioning is essentially unknown at this time. TNF-α significantly declined in response to supplementation with APMC, perhaps offering a promising means to reduce the chronic inflammatory load consistent with AD, neurodegeneration, and neuroinflammation. TNF-α has many recognized physiological functions, but it has been historically linked to a variety of human diseases and in particular is now related to neuroinflammation, neurodegeneration, and an etiology of cognitive dysfunction and AD through several mechanisms [64–66]. In fact, TNF-α and other cytokines have been shown to be elevated in the cerebrospinal fluid and plasma of persons with AD compared to controls [43, 67, 68], but the findings are not unequivocal as other studies show no differences [69, 70]. Nonetheless, anti-TNF-α therapy has been posited as way to prevent or decrease the effects of neuroinflammation and perhaps cognitive disorders, but that position is controversial [65, 66]. Our results suggest that APMC has an anti-TNF-α effect.

We also found a substantial drop in VEGF levels at the 12 month follow-up assessment. Others have suggested that VEGF might be linked to the progression of AD through abnormal endothelial activation, resulting in neuronal loss and Aβ deposits [42]. Another group identified associations between well-known VEGF genotypes and specific genotype combinations and the risk for development of AD [71]. Our higher VEGF concentration at baseline may also support the hypothesis that VEGF lacks a neuroprotective effect among neurodegenerative disorders [71, 72].

We studied an extensive panel of T and B cell (lymphocyte and monocyte) subsets, which may also be a unique contribution to the AD literature in response

to a dietary supplement intervention. Several similar lymphocytes were studied in response to methylprednisolone in multiple sclerosis patients [73], which has an obvious similar neurodegeneration etiology to AD. We found no relative or absolute changes in T cell (CD3+, CD3+CD4+, and CD3+CD8+), B cell (CD19+), or natural killer cell (CD16+56+) subsets. The CD3+CD4+/CD3+CD8+ ratio increased at follow-up, perhaps suggestive of a positive response to supplementing with APMC, as this ratio classically has been shown to decline with age [74] and/or in the presence of immunodeficiency, such as HIV [75]. Thus, our study might be the first to demonstrate an increase in this ratio in a sample of persons with AD. Conversely, relative and absolute decreases were noted in lymphocyte regions (CD90+, CD95+CD3+, CD95+CD34+, and CD95+CD90+) and relative decreases in monocyte regions (CD14+CD90+ and CD14+CD95+). Only the relative CD14+ monocytes increased at follow-up. We noted inverse correlations at baseline between the ADAS-cog cognition score with CD90+ and CD95+CD90+ and with CD19+ at 12 months follow-up, thus providing some evidence for clinical performance being observed in parallel with decreases in T and B cell activity.

In support of other prior sub-clinical work mentioned below, our study showed an increase in CD14+ (%) in response to the intervention, and we also found that the ADAS-cog concentration score was inversely related to the CD14+ level at the baseline assessment. CD14+ is known as the monocyte receptor for Gram-negative bacterial lipopolysaccharide (LPS) [76]. Monocytes express many pro-inflammatory cytokines, when stimulated with LPS through the CD14+ receptor [77]. CD14+ expression is greater in microglia in an AD-mouse model, and microglia from CD14-insufficient mice showed reduced activation of Aβ peptide, signifying that CD14+ is necessary for Aβ-induced microglia activation [78]. However, an AD case-control human study found no relationship between the CD14+ (−260) polymorphism, several pro-inflammatory cytokine genes, and AD [77].

In summary, neuroinflammation is suspected of being causative in the pathogenesis of neurodegenerative diseases [79], and many studies have demonstrated mechanistic links among multiple inflammatory pathways in AD [39]. Nonetheless, due to the inconsistency in the prior findings of these mechanisms and immune markers [43, 80], the field is incapable of making absolute treatment or diagnostic recommendations for those suffering from the disease. However, few studies have showed changes in biomarkers of neuroinflammation in neurodegenerative diseases, particularly in AD after 12 months of intervention. In our study, we have showed multi-directional immunomodulation in response to APMC in the profile of this sample of AD patients. We showed statistically significant changes in the values of IL-2, IL-4, TNF-α, and VEGF, and multiple lymphocyte and monocyte regions, along with several correlations in these markers with cognitive functioning according to the ADAS-cog cognition score.

*Limitations*

We note several limitations of the current investigation. In this study, we did not assess dietary intake, physical activity level, depression, anxiety, or caregiver support, so we are unsure how these variables could have affected the results of the study. Our neuropsychologist was not blinded to the study participants, but she assessed subjects for all studies occurring simultaneously at our center, so her influence on this study should have been no different than on any other. It was not possible to objectively determine compliance with the protocol, as the APMC formula does not readily lend itself to metabolite analysis. The findings of our study are limited by a small sample size. A larger sample size could result in even more significant findings for cognitive functioning, cytokines, and lymphocyte and monocyte subsets. A larger sample could have also helped to resolve the discrepancy in our findings between the ADAS-cog cognition score and the SIB and ADCS-ADL. The results of our cytokine and growth factor assays could have been unduly influenced by the combination of our participants' medications, although that position is not definitive [80]. As the purpose of our study was to evaluate the effect of improved nutritional status with APMC on cognitive and immune functioning, we did not restrict or change the use of medications by our participants, given the ethical considerations associated with such decision. For our blood sampling procedures, we utilized all leukocyte cell populations (e.g., lymphocytes, macrophages, and monocytes), which also could have affected the results of our immune functioning markers [80]. Nonetheless, the Ficoll-Hypaque gradient is easily replicated clinically, is simple, does not alter the function of isolated cells, and thus is routinely used in many settings. Using this methodology, 60–70% of the cells are lymphocytes, while the remaining cells are monocytes and macrophages. Although we found significant changes in several key cytokines and T and B cell subsets from baseline to 12 months follow-up,

we are uncertain if these markers demonstrate linear or quadratic responses during that time. Because of the complexity in the cytokine network involving bi-directional feedback, pleiotropism makes it complicated to surmise the exact mechanisms of individual markers. Additionally, these cells have been noted to drift between neuroprotection and neuroinflammation, so even within the same marker lies complex answers to questions about how the immune system works (i.e., the role of IL-6 in AD etiology) [39]. Thus, having more frequent assessments of these markers might help to better elucidate their responses to the APMC formula and its ability to modulate immune system function over an extended period of time.

## CONCLUSIONS

AD is an escalating burden for patients and their families. In addition, with the global population of aged individuals increasing exponentially, AD represents a significant economic drain on society. The development of an effective approach for the treatment of AD is thus of major importance, as current strategies are limited to agents that attempt to attenuate disease symptomatology without addressing the causes of disease. A considerable need exists for the development of an effective therapy to prevent, or at least delay, the progression of AD.

The APMC formula used in the current study was well-tolerated among all subjects. The product showed a significant improvement in the ADAS-cog cognition score and demonstrated sound immunomodulator activity with noteworthy responses in cytokines and several lymphocyte and monocyte subsets. Several correlations were found between the cognitive assessments and the physiological outcomes at baseline and 12 months follow-up. Our results are consistent with prior work by other investigators using similar oligosaccharide-based formulae, who also demonstrated improvements in various indices of quality of life and functioning in other disease states. At this time, the mechanism by which APMC influences cognitive functioning in AD is unclear. The amelioration of cognitive functioning may be associated with some modulation of host immune activity, but additional immune functioning data are required to understand with more certainty. However, what is clear is that our results compel further study, especially in the investigation of an AD-type neurodegeneration model that may eventually enable elucidation of the mechanism(s) at work. Utilizing an AD-type neurodegeneration model would allow us to gain deeper, if not novel, insights into the pathophysiology of a disease that is the source of much human suffering.

Thus, our study shows that a high-quality, concentrated dietary supplement may offer an alternative option for persons with AD. This APMC formula may not only facilitate cognitive improvement, but may also improve the inflammatory and immune functioning profile as well, thereby enhancing host recovery and improving overall quality of life.

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