Immunity and amyloid beta, total tau and neurofilament light chain: Findings from a community-based cohort study

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Abstract

Introduction: We investigated how components of immunity relate to biomarkers of Alzheimer's disease (AD) in plasma and explored the influence of AD genetic risk factors in the population-based Rotterdam Study.

Methods: In 7397 persons, we calculated the granulocyte-to-lymphocyte ratio (GLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII). In 3615 of these persons, plasma amyloid-beta (Aβ)42 and Aβ40 were measured. Next, we constructed an overall genetic risk score (GRS) based on genome-wide significant variants, both including and excluding APOE ε4.

Results: All innate immunity phenotypes were related to higher Aβ, most strongly with a doubling in GLR leading to a 1.9% higher Aβ42 (95% confidence interval [95% CI] 0.4 to 3.3%) and 3.2% higher Aβ40 (95% CI 2.0 to 4.3%). Higher AD GRS including APOE ε4 was associated with higher immunity markers.

Discussion: Higher levels of immunity markers were associated with higher Aβ in plasma. Participants with a higher genetic predisposition to AD had higher immunity markers, where these effects were mainly driven by APOE ε4.

KEYWORDS
amyloid, cohort study, dementia, immunity, population-based

1 INTRODUCTION

Alzheimer's disease (AD) is characterized pathologically by accumulation of amyloid beta (Aβ) as amyloid plaques and phosphorylated tau as neurofibrillary tangles. This accumulation gave rise to the amyloid hypothesis, posing that Aβ activates a cascade of pathologic changes. As an extension to the amyloid hypothesis, the antimicrobial protection model was recently proposed suggesting that Aβ oligomerization is not intrinsically pathological, but emerges as an innate immune response. Genetic variants identified through genome-wide association studies (GWAS) support this role for immunity. It has also been found that higher activation of the innate immune system and lower activation of the adaptive immune system leads to higher dementia risk. Yet, the link between immunity and AD-related brain pathology is largely unknown.

In the early 1990s, apolipoprotein E (apoE) was found to be a component of amyloid plaques. However, how apoE's function as a lipid-carrier is in itself related to AD, either in relation to Aβ or other factors, is not entirely clear. Lipids serve more than pure nutritional purposes; they also play essential roles in immune regulation. Innate immunity in...
turn affects Aβ and tau pathology buildup. Insoluble tau isolated from postmortem AD brain is shown to be taken up by microglia in vitro and in vivo. This taking in of tau may participate in tau spreading by microglia subsequently releasing some form of tau.

To study further how AD relates to the immune response, we may use serum levels of various blood cells which reflect a systemic immune response and relate them with biomarkers of AD pathology and progression. Serum levels of granulocytes and platelets are known biomarkers of the innate immunity, whereas lymphocyte levels may yield information on the adaptive immunity. Combining these measurements into ratios is thought to reflect the relative balance between innate and adaptive immunity even better, obtaining the granulocyte-to-lymphocyte ratio (GLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII) as phenotypical markers of immunity. Whereas for the diagnosis of AD, biomarkers are grouped into those of β amyloid deposition, pathologic tau, and neurodegeneration (ATN) according to the recent classification by Jack et al., yielding plasma Aβ, total tau, and neurofilament light chain (NFL) as biomarkers of AD-related brain pathology, respectively.

In an earlier study investigating the role of various biological pathways based on a genetic risk score, we found an important role for the immune response pathway in early AD pathology. Relating phenotypical immunity and AD-related markers to each other and additionally to genetic predisposition to AD may help to understand the role of immunity biomarkers in AD pathology and their relation with AD genetic risk, and ultimately identify therapeutic strategies targeting upstream events of altered immune response in amyloidosis and neurodegeneration. We previously found that higher levels of the GLR, PLR, and SII over time reflect higher innate immunity and increased dementia risk. Since we also previously found that low Aβ42 and high NFL plasma levels were associated with risk of AD, we hypothesized that higher innate immunity affects the serum levels of Aβ42 to be lower and NFL to be higher. We tested this hypothesis by investigating whether serum GLR, PLR, and SII were associated with Aβ, total tau, and NFL and how genetic predisposition to AD affected these markers.

2 METHODS

2.1 Study population

The present study is embedded within the Rotterdam Study, a prospective population-based cohort study in Rotterdam, the Netherlands. The Rotterdam Study started in 1989 with 7983 persons (response rate: 78%) aged ≥55 years and residing in the district of Ommoord, a suburb of Rotterdam. This first subcohort (RS-I) was extended with a second subcohort (RS-II) in 2000, consisting of 3011 persons (response rate: 67%), and with a third subcohort (RS-III) in 2006, composed of 3932 persons aged ≥45 years (response rate: 65%). The design of the Rotterdam Study has been described in detail previously. In brief, participants were examined at study entry and follow-up visits every 3 to 5 years. They were interviewed at home by a trained research nurse, followed by two visits at the research facility for additional interviewing and laboratory assessments. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus Medical Center and by the board of the Netherlands Ministry of Health, Welfare, and Sports. Written informed consent was obtained from all participants.

Laboratory tests for granulocytes, platelets, and lymphocytes were introduced from 2002 onwards, corresponding with the following assessment rounds in the Rotterdam Study (baseline for this study): fourth round of RS-I (RS-I-4), second round of RS-II (RS-II-2), and first round of RS-III (RS-III-1). Plasma biomarkers of AD-related brain pathology were only assessed in RS-I-4 and RS-II-2. Blood was drawn from 11,496 participants for genotyping. Of these participants, 7413 underwent granulocyte, platelet, and lymphocyte assessments, while 3627 participants underwent plasma Aβ, total tau, and NFL assessment. We excluded participants with missing APOE genotype and participants with incomplete assessments of serum markers, leaving 7397 participants for analysis with complete blood assessment for immunity markers and 3615 participants with complete plasma Aβ, total tau, and NFL assessment (Figure 1).

2.2 Genotyping

DNA genotyping was performed at the internal genotyping facility of the Erasmus Medical Center, Rotterdam. All samples were genotyped
with the 550, 550, duo or 610 K Illumina arrays. Genotyping quality control criteria include call rate < 95%, Hardy-Weinberg equilibrium of $P < 1.0 \times 10^{-6}$, and minor allele frequency < 1%. Moreover, study samples with excess autosomal heterozygosity, call rate < 97.5%, ethnic outliers, and duplicate or family relationships were excluded during quality control analysis. Genetic variants were imputed from the Haplotype Reference Consortium reference panel (version 1.0),\textsuperscript{18} using the Michigan imputation server.\textsuperscript{19} The server uses SHAPEIT2 (v2.r790)\textsuperscript{20} to phase the genotype data and performs imputation with Minimac 3 software.\textsuperscript{21} For this study, we used genetic variants that had imputation quality ($R^2$) > 0.5. APOE genotype was determined separately by PCR on coded DNA samples in the baseline cohort and with a bi-allelic Taqman assay in the extensions of the Rotterdam Study.\textsuperscript{22}

### 2.3 | Assessment of blood cell counts and their derived ratios

Full blood count measurements were performed using the COULTER® Ac-T diff\textsuperscript{2} Hematology Analyzer (Beckman Coulter, San Diego, California, USA) directly after the blood sample was drawn. Laboratory measurements included absolute granulocyte, platelet, and lymphocyte counts in $10^9$/L. The GLR and PLR were calculated as the ratio of granulocyte count to lymphocyte count, and as the ratio of platelet count to lymphocyte count, respectively. The SII was defined as platelet count times the GLR.\textsuperscript{13} We use the GLR as a proxy measure for the commonly used neutrophil-to-lymphocyte ratio, as granulocytes are the most abundant subtype of neutrophils.

### 2.4 | Assessment of plasma Aβ, total tau, and NfL

Blood was sampled in EDTA-treated containers and centrifuged. Subsequently plasma was aliquoted and frozen at $-80^\circ$C according to standard procedures. Measurements were done in two separate batches. The first batch included in total 2000 samples, obtained from a random selection of 1000 participants from sub-cohort RS-I-4 and 1000 from RS-II-2. The second batch included in total 3094 samples from the remaining participants.

All measurements were performed at Quanterix (Lexington, MA, USA) on a single molecule array (Simoa) HD-1 analyzer platform.\textsuperscript{23} Samples were tested in duplicate. Two quality control (QC) samples were run on each plate for each analyte. NfL was measured with the NF-light advantage kit.\textsuperscript{24} The Simoa Human Neurology 3-Plex A assay (N3PA) was used for measuring the concentration of total tau, Aβ42, and Aβ40. When duplicates or single measurements were missing (the majority of missing samples were due to system failures [n = 279], and few because of insufficient volume [n = 47]) or in the case the concentration coefficient of variation (CV) exceeded 20% (14 to 87 samples) or control samples were out of range (none), participant data were excluded from the analyses.\textsuperscript{16}

### 2.5 | Genetic risk scores

We computed genetic risk scores by selecting late-onset AD-associated single-nucleotide polymorphisms (SNPs) reaching genome-wide significance ($P < 5.0 \times 10^{-8}$). Among common variants, we considered only variants identified by the International Genomics of
Alzheimer’s Project (IGAP) meta-analyses. In addition, we included four rare variants which can be classified under immune response based on their functional role, leading to a total of 28 independent genome-wide significant AD-associated variants. A weighted genetic risk score was constructed using the effect sizes (log of odds ratio) of the genome-wide significant variants from the IGAP meta-analysis as weights and their respective allele dosages from imputed genotype data of our study cohorts. A genetic risk score was constructed as the sum of the products of SNP dosages and their corresponding weights. We constructed genetic risk scores in three ways: (1) combining all 28 selected variants, (2) excluding the APOE ε4 variant to identify the joint independent effect of all other genome-wide significant SNPs, and (3) clustering the variants into the immune response pathway. We classified AD-associated SNPs into immune system pathways based on information on current investigations and reviews. Of the 28 SNPs, 9 could be clustered into the immune response pathway-based genetic risk score (Table S1). All genetic risk scores were standardized to allow direct comparison of results.

2.6 Covariates

Potential confounding factors were chosen on the basis of previous literature. All covariates were measured at the same rounds as the assessments of AD-related brain pathology and immunity markers. Smoking habits were categorized as current versus former and never smoking. Body mass index (BMI) was calculated as weight in kilograms per height in meters squared. Blood pressure was measured twice at the right brachial artery with the participant in sitting position, from which the mean was used. Diabetes mellitus was defined as use of antidiabetic medication, fasting serum glucose level ≥7.1 mmol/L (≥127.9 mg/dL), or random non-fasting serum glucose level ≥11.1 mmol/L (≥200.0 mg/dL). Additional information from the serum samples was collected on high sensitivity C-reactive protein (hs-CRP) and creatinine in a subsample (N = 1342).

2.7 Statistical analysis

As the GLR, PLR, and SII had skewed distributions, analysis was based on the natural logarithmic (Ln) transformation of their values. For the same reason, all plasma biomarkers of AD-related brain pathology, except APOE40 which had a normal distribution, were Ln transformed. We first determined the association between the GLR, PLR, and SII with plasma biomarkers of AD-related brain pathology using linear regression. For this analysis, we conducted two different transformations. First, because we transformed both exposure and outcome (log\(Y_i = \alpha + \beta \log X_i + \epsilon_i\)), we reported mean differences in percentages with 95% confidence intervals (CIs) obtained by exponentiating Ln(2) times the estimated coefficients from the linear regression model to facilitate interpretation of results; these mean differences correspond to the percentage change in plasma A\(\beta\), total tau, or NfL for a doubling of the GLR, PLR, and SII. For this analysis, A\(\beta\)40 was also Ln transformed. Second, we standardized all blood markers to allow direct comparison of the results. We adjusted all models for age, sex, and study cohort (model I). In addition, the following covariates were added to a second model (model II): smoking, diabetes, BMI, systolic blood pressure, and APOE ε4 carrihship. Since plasma A\(\beta\), total tau, and NfL were measured in two batches, we additionally corrected for batch effects in models. We performed additional adjustment for platelet count when analyzing GLR in model II. We assessed effect measure modification by APOE genotype by stratifying for participants having the c2/c2 or c2/c3, c3/c3, and c3/c4 or c4/c4 genotype. We formally tested interaction between APOE genotype and ratios of blood cell counts on the multiplicative scale by adding interaction terms to model II. In addition, we assessed effect measure modification by activity of the innate immune system by stratifying for median granulocyte count (as a pure innate immunity marker rather than studying the effect of the balance between innate and adaptive immunity) when assessing the associations between genetic risk scores and plasma biomarkers of AD-related brain pathology as outcome. We formally tested interaction between genetic risk scores and granulocyte counts on the multiplicative scale by adding interaction terms to the model. As a sensitivity analysis, we also adjusted for creatinine and hs-CRP in a subsample of participants in which these markers were measured. Because hs-CRP is also an important inflammatory biomarker, we also assessed the association between Ln(hs-CRP) as exposure and the standardized plasma biomarkers of AD-related brain pathology as outcome using linear regression. We adjusted all models for age, sex, cohort, smoking, diabetes, BMI, systolic blood pressure, APOE ε4 carrihship, batch effects, platelet count, and creatinine. We then determined associations between all three genetic risk scores per standard deviation (SD) increase as exposure with the standardized immunity markers (Ln[GLR], Ln[PLR] and Ln[SII]) and with the standardized plasma biomarkers of AD-related brain pathology (Ln[A\(\beta\)42], Ln[A\(\beta\)40], Ln[A\(\beta\)42/40 ratio], Ln[total tau] and Ln[NfL]) as separate outcomes, using linear regression. These analyses were all adjusted for age, sex, and study cohort.

Furthermore, we investigated the association between APOE-allele carriership as exposure with serum markers of immunity and plasma biomarkers of AD-related brain pathology as outcomes. For this analysis, we defined APOE-allele carriership as either carrying c2 (c2/c2 or c2/c3 genotype), being homozygous for c3 (c3/c3 genotype) or carrying c4 (c3/c4 or c4/c4 genotype). People with the c2/c4 genotype were excluded from the analyses and c3 homozygosity was considered the reference group. Analyses were adjusted for age, sex, and study cohort. We calculated mean levels of hs-CRP and creatinine levels within the different APOE genotypes.

Missing covariate data (maximum 0.7%) were imputed using 5-fold Multiple Imputation by Chained Equations based on determinant, outcome, and included covariates. All analyses were performed using RStudio version 1.0.153 (R version 3.6.1, RStudio, Inc., Boston, MA). We corrected for multiple testing using Bonferroni adjustment for all analyses using the total population (ie, not stratified or no subgroup analyses): for the associations between serum markers of immunity and plasma biomarkers of AD-related brain pathology, results are...
TABLE 1  Characteristics of study population

| Characteristic                        | Sample with serum markers of immunity (N = 7397) | Sample with plasma biomarkers of AD-related brain pathology (N = 3615) |
|---------------------------------------|--------------------------------------------------|---------------------------------------------------------------------|
| Women                                 | 4208 (56.9%)                                     | 2059 (56.8%)                                                       |
| Age (years)                           | 66.1 ± 10.4                                      | 71.9 ± 7.3                                                        |
| Study cohort                          |                                                  |                                                                    |
| Cohort 1                              | 2656 (35.9%)                                     | 2159 (59.7%)                                                       |
| Cohort 2                              | 1704 (23.0%)                                     | 1456 (40.3%)                                                       |
| Cohort 3                              | 3037 (41.1%)                                     | –                                                                  |
| Current smokers                       | 1427 (19.4%)                                     | 536 (14.9%)                                                        |
| Diabetes mellitus type 2              | 433 (5.9%)                                       | 214 (5.9%)                                                         |
| Body mass index (kg/m²)               | 27.6 ± 4.3                                       | 27.6 ± 4.1                                                         |
| Systolic blood pressure (mmHg)        | 142.8 ± 21.9                                     | 149.2 ± 21.0                                                      |
| APOE genotype                         |                                                  |                                                                    |
| ε2/ε2                                 | 46 (0.6%)                                        | 31 (0.9%)                                                         |
| ε2/ε3                                 | 952 (12.9%)                                      | 490 (13.6%)                                                       |
| ε2/ε4                                 | 202 (2.7%)                                       | 103 (2.8%)                                                        |
| ε3/ε3                                 | 4293 (58.0%)                                     | 2119 (58.6%)                                                      |
| ε3/ε4                                 | 1741 (23.5%)                                     | 810 (22.4%)                                                       |
| ε4/ε4                                 | 163 (2.2%)                                       | 62 (1.7%)                                                         |
| Granulocyte count, × 10³/μL           | 4.0 ± 1.4                                        | 4.0 ± 1.3                                                         |
| Platelet count, × 10³/μL              | 268.2 ± 67.2                                     | 256.4 ± 64.4                                                      |
| Lymphocyte count, × 10³/μL            | 2.3 ± 1.2                                        | 2.2 ± 1.3                                                         |
| Granulocyte-to-lymphocyte ratio       | 1.9 ± 0.9                                        | 2.0 ± 0.9                                                         |
| Platelet-to-lymphocyte ratio          | 128.2 ± 47.3                                      | 130.0 ± 49.8                                                      |
| Systemic immune-inflammation index    | 517.3 ± 280.1                                     | 522.0 ± 290.9                                                     |
| Amyloid-beta 42 (pg/mL)               | –                                                | 10.6 ± 3.0                                                        |
| Amyloid-beta 40 (pg/mL)               | –                                                | 265.6 ± 54.6                                                      |
| Amyloid-beta 42/40 ratio              | –                                                | 0.04 ± 0.009                                                      |
| Total tau (pg/mL)                     | –                                                | 2.6 ± 2.5                                                         |
| Neurofilament light chain (pg/mL)     | –                                                | 15.7 ± 11.6                                                       |

N = number of participants included in study. Data presented as mean (standard deviation) for continuous variables and number (percentages) for categorical variables. Data here are unimputed. Number of missing values for the immunity cohort: 39 (0.5%) for smoking, 19 (0.3%) for diabetes, 20 (0.3%) for BMI, 37 (0.5%) for systolic blood pressure, 0 for APOE carrierhip. Number of missing values for the sample undergoing serum AD measurements are: 25 (0.7%) for smoking, 8 (0.2%) for diabetes, 0 for BMI, 12 (0.3%) for systolic blood pressure and 0 for APOE genotype.

3 RESULTS

Characteristics of study participants are displayed in Table 1. The mean age of participants with serum markers of innate immunity was 66.1 (±10.4) years of which 4208 (57%) were women, and the mean age of the sample undergoing serum AD marker measurements was 71.9 (±7.3) years of which 2059 (57%) were women.

We found that a doubling of SII was associated with a 1.2% higher serum Aβ42, albeit not statistically significant (95% confidence interval [95% CI] = -0.005% to 2.3%, P = .051) and a 1.8% higher serum Aβ40 (95% CI 0.9 to 2.7%, P = < .001). Estimates were even higher for a doubling of GLR, leading to a 1.9% higher serum Aβ42 (95% CI 0.4 to 3.3%,...
TABLE 2  Associations between serum markers of immunity and plasma biomarkers of AD-related brain pathology

| Per doubling in serum markers of immunity | Percentage change in Aβ, total tau or NFL, 95% CI |
|-----------------------------------------|-----------------------------------------------|
|                                         | Aβ42  | P     | Aβ40  | P     | Aβ42/40 ratio | P     | Tau    | P     | NFL   | P    |
| GLR³                                    | 1.6   | 0.025 | 3.2   | 2.1   | 4.3          | <0.001| −1.5   | −2.6  | −0.4  | 0.008 | 0.1   | −1.8  | 2.0   | <0.001| 4.9   | 2.5   | 7.2   | <0.001|
| PLR                                     | −0.7  | (−2.2; 0.8) | 0.351 | −0.2  | (−1.4; 0.9) | 0.688 | −0.5   | −1.6  | 0.7   | <0.001| −4.4  | −6.3  | −2.5  | <0.001| 2.9   | 0.5   | 5.3   | .018 |
| SII                                     | 1.0   | (−0.1; 2.2) | 0.078 | 1.8   | (0.9; 2.7) | <0.001| −0.8   | −1.7  | 0.1   | 0.093 | −0.4  | −1.9  | 1.1   | 0.590 | 3.3   | 1.5   | 5.2   | <0.001|

Model I adjusted for age, sex, study cohort, and batch effects. Model II adjusted for age, sex, study cohort, smoking, diabetes, BMI, platelets, systolic blood pressure, APOE ε4, and batch effects. The GLR, PLR, and SII reflect the balance between innate and adaptive immunity, with higher markers indicating an imbalance towards higher innate immunity. Abbreviations: Aβ40, amyloid-beta 40; Aβ42, amyloid-beta 42; Aβ42/40 ratio, amyloid-beta 42-to-40 ratio; CI, confidence interval; GLR, granulocyte-to-lymphocyte ratio; Ln, natural logarithmic transformation; NFL, neurofilament light chain; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index; Tau, total tau.

³Additional adjustment for platelet count. Results are considered statistically significant if the P-value is below .05/(6×5) = .002.

FIGURE 2  Associations between serum markers of immunity and plasma biomarkers of AD-related brain pathology. Model adjusted for age, sex, study cohort, smoking, diabetes, BMI, platelets, systolic blood pressure, APOE ε4, and batch effects. Additional adjustment for platelet count when analyzing Ln(GLR). All phenotypical markers were Ln(transformed) except for Aβ40. The GLR, PLR, and SII reflect the balance between innate and adaptive immunity, with higher markers indicating an imbalance towards higher innate immunity. Abbreviations: Aβ40, amyloid-beta 40; Aβ42, amyloid-beta 42; Aβ42/40 ratio, amyloid-beta 42-to-40 ratio; CI, confidence interval; c2/2 or c2/c3, apolipoprotein Eε2/ε2 genotype; c3/c3, apolipoprotein Eε3/c3 genotype; c3/c4 or c4/c4, apolipoprotein Eε3/c4 or ε4/ε4 genotype; GLR, granulocyte-to-lymphocyte ratio; Ln, natural logarithmic transformation; NFL, neurofilament light chain; PLR, platelet-to-lymphocyte ratio; SD, standard deviation; SII, systemic immune-inflammation index

P = .010 and 3.2% higher serum Aβ40 (95% CI 2.0 to 4.3%, P = <.001). The Aβ42/40 ratio was lower with a doubling in GLR only (−1.2%, 95% CI −2.3 to −0.1%, P = .028). Only a doubling in PLR was significantly associated with lower total tau (−3.6%, 95% CI −5.5% to −1.7%, P = <.001). NFL was higher with all innate immunity phenotypes, most strongly with a doubling in GLR (4.7%, 95% CI 2.5 to 7.1%, P = .001) (Table 2 and Table S2). Stratification did not reveal statistically significant differences across strata of APOE carriergship, with the effects in the APOE c3/c4 or c4/c4 stratum being only non-significantly stronger than in the other strata, especially for Nfl (Figure 2 and Table S2). In addition, the associations between all genetic risk scores with lower Aβ42/40 ratio became lower in participants with granulocyte counts higher than the median (mean difference = −0.18 [95% CI −0.23 to −0.13, P = <.001] for the overall AD genetic risk score, mean difference = −0.06 [95% CI −0.11 to −0.02, P = .007] for the overall AD genetic risk score excluding APOE, and mean difference = −0.05 [95% CI −0.10 to −0.004, P = .035] for the immune response pathway-based genetic risk score), yet the interaction remained non-significant (Table S3). When additionally adjusting for hs-CRP and creatinine (in a sub-sample, N = 1341), the associations between GLR and SII with higher
Overall AD GRS, including APOE – ε4

Overall AD GRS, excluding APOE – ε4

Immune response GRS

**FIGURE 3** Associations of genetic risk scores reflecting AD including and excluding APOE ε4 and immune response with plasma biomarkers of AD-related brain pathology and serum markers of immunity. Model adjusted for age, sex, and study cohort. GRS are per SD increase. All phenotypical markers were Ln(transformed) except for Aβ40. Gray dotted circle indicates the null with associations becoming increasingly negative towards the center of the circle. Orange indicates the plasma biomarkers of AD-related brain pathology while blue indicates the immunity phenotypes. The GLR, PLR, and SII reflect the balance between innate and adaptive immunity, with higher markers indicating an imbalance towards higher innate immunity. Abbreviations: AD, Alzheimer’s disease; Aβ40, amyloid-beta 40; Aβ42, amyloid-beta 42; Aβ42/40, amyloid-beta 42-to-40 ratio; GLR, granulocyte-to-lymphocyte ratio; GRS, genetic risk score; Ln, natural logarithmic transformation; NFL, neurofilament light chain; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index

**FIGURE 4** Associations of APOE genotypes with plasma biomarkers of AD-related brain pathology and serum markers of immunity. Adjusted for age, sex, and study cohort. All phenotypical markers were Ln(transformed) except for Aβ40. The GLR, PLR, and SII reflect the balance between innate and adaptive immunity, with higher markers indicating an imbalance towards higher innate immunity. Abbreviations: AD, Alzheimer’s disease; APOE, apolipoprotein E; Aβ40, amyloid-beta 40; Aβ42, amyloid-beta 42; Aβ42/40, amyloid-beta 42-to-40 ratio; CI, confidence interval; ε2/ε2 or ε2/ε3, apolipoprotein E ε2/ε2 genotype; ε3/ε3, apolipoprotein E ε3/ε3 genotype; ε3/ε4 or ε4/ε4 genotype; GLR, granulocyte-to-lymphocyte ratio; GRS, genetic risk score; Ln, natural log transformation; NFL, neurofilament light chain; SD, standard deviation; SII, systemic immune inflammation index

Aβ42 and NFL disappeared, the associations between immunity markers with higher Aβ40 were borderline significant, and the associations with lower Aβ42/40 ratio and lower total tau persisted (Table S4). Higher hs-CRP was significantly associated with a higher Aβ42 and higher total tau (Table S5).

Furthermore, we found that a standard deviation (SD) increase in the overall AD genetic risk score including APOE ε4 was associated with higher GLR (mean difference in Ln[GLR] [SD] = 0.024, 95% CI 0.002 to 0.046, P = .032) and significantly with lower Aβ42 and Aβ42/40 ratio (mean difference in Aβ42 [SD] = –0.129, 95% CI –0.162 to –0.095, P = < .001, and Ln[Aβ42/40] [SD] = –0.157, 95% CI –0.190 to –0.124, P = <.001, respectively). These associations were mainly driven by the APOE ε4 variant (Figure 3). The genetic risk score reflecting the immune response showed a suggestive association with lower Aβ42/40 ratio (mean difference = –0.038, 95% CI –0.070 to –0.006, P = .020), but not with the serum markers of innate immunity (Figure 3 and Table S6). We found that APOE ε2 carriers displayed lower serum markers of innate immunity levels compared to ε3/ε3, while these markers were
In this study, we found associations between higher levels of GLR, PLR, and SII reflecting higher innate immunity, with higher Aβ42 and 40, lower Aβ42/40 ratio, lower total tau, and higher NfL in plasma. In APOE ε4 carriers, these associations were even stronger. The overall genetic risk score including APOE ε4 was associated with higher GLR and SII and with lower Aβ42 and Aβ42/40 ratio. These effects were mainly driven by the APOE ε4 variant. The genetic risk score reflecting the immune response was associated with a lower Aβ42/40 ratio, but not with the serum immunity markers. Furthermore, we found that APOE ε2 carriers displayed lower serum markers of innate immunity compared to APOE ε3/ε3, while these markers were elevated in APOE ε4 carriers.

Interestingly, we found that higher innate immunity was associated with higher serum Aβ42, but with even higher Aβ40. The 40-residue peptide represents the most abundant Aβ isoform in the brain, while the 42-residue shows a significant increase with certain forms of AD. Our findings are concordant with the notion that Aβ functions as an antimicrobial peptide (AMP), because the physicochemical and biological properties previously reported for Aβ are similar to those of AMPs. In addition, experiments have shown that Aβ is active against at least eight common and clinically relevant microorganisms. Activity was isoform-specific for six organisms with Aβ42 showing greater potency compared to Aβ40. Taking all evidence together, we propose that APOE ε4 carriers display stronger innate immune responses, and thus produce more/excess Aβ in response to pathogens. Of the two isoforms, Aβ42 will aggregate in the brain (possibly due to its larger size) while Aβ40 will not aggregate (or to a lesser extent) and as a consequence will be higher in the serum. Further study is needed to confirm this hypothesis.

Furthermore, we found that both higher serum markers of innate immunity and the genetic risk score reflecting the immune response associate with the lower serum Aβ42/40 ratio. APOE ε4 carriership, which is the major genetic risk factor for AD, also displays a lower serum Aβ42/40 ratio as well as lower Aβ42. In line with this, a meta-analysis of prospective cohort studies has shown that lower serum Aβ40, and even lower Aβ42 and consequently lower Aβ42/40 ratio, lead to higher AD risk. Previous reports of associations of low plasma Aβ42/40 ratio with increased amyloid brain uptake, as measured by Pittsburgh compound B (PiB) positron emission tomography (PET) scan, support the notion that lower plasma Aβ reflects the aggregation of Aβ in the brain. In this context, our finding that APOE ε4 carriers have higher activity of innate immunity compared to ε3/ε3, while we see the opposite in APOE ε2 carriers suggests that APOE ε4 might have an overactive innate immune response. Our results support experimental studies showing the capacity of APOE to modulate inflammation. Indeed, in healthy humans challenged with intravenous lipopolysacharide (LPS) infusion, ε4 carriers demonstrated significantly higher elevation of body temperature and plasma tumor necrosis factor levels than ε4 non-carriers. In this same study, when whole blood isolates from human subjects were stimulated ex-vivo with Toll-like receptor ligands, increased production of a wide panel of cytokines and chemokines was observed in blood from ε4+ donors compared with ε4− donors. A higher immune response associated with the ε4 allele is also observed in human APOE-targeted replacement mice and in cultured microglia and/or macrophages upon LPS stimulation, which leads to cholesterol accumulation on cell membranes. This mechanism has been proposed to explain the higher immune reactivity associated with APOE ε4, but additional studies are needed to explore this or other potential mechanisms further.

In addition to the role of immune response pathways in Aβ, our data show that higher innate immunity is related to lower plasma total tau. Others assessed the effect of microglial activation on tau pathogenesis, where they found that microglial activation is shown to precede tau pathology in a tauopathy mouse model (Yoshiyama et al. 2007) and administering an immunosuppressant drug FK506 from an early age drastically reduces tau pathology. According to previous studies, higher plasma tau is associated with AD dementia, although correlations were weak and non-linear (J-shape), making the role of immunity in tau elusive. Future studies are warranted with the same crude markers of the immune system in relation to phospho tau.

The associations between immunity markers with higher Aβ42 and NfL disappeared when additionally adjusting for hs-CRP and creatinine. There could be two explanations: (1) This analysis was performed within a subsample of the total population, impeding comparison of the results in the total population and decreasing the power of the analysis, or (2) We found that higher hs-CRP was also associated with higher NfL, albeit not statistically significant, suggesting that hs-CRP could be acting as a mediator. Our result that the immune GRS was not related to the immunity biomarker levels is surprising, especially since several immune genes are key players for innate immunity, including TREM2. TREM2 overexpression is thought to enhance microglial phagocytic capacity, but transcription analyses show mixed microglial activation patterns with suppression of certain disease-associated microglia (DAM) genes, but further activation patterns of other DAM genes, making the role of TREM2 in AD unclear. Further studies are needed to identify the precise gene signatures of microglia that mediate pathology- and neurodegeneration-associated sterile inflammation.

Our study has several limitations. First, ours is a cross-sectional study, limiting our ability to draw causal inferences. Longitudinal cohort studies are required to confirm our findings. Second, we were limited to studying crude markers of the immune system in this population-based setting. These types of measures have been associated with other outcomes such as those related to cancer, pancreatitis, and many other responses. These ratios could be proxies for different systemic
inflammatory responses that affect the mobilization of bone marrow-derived cells and the egress of leukocytes into tissue.\textsuperscript{49} It could also be related to immunosenescence and this might explain the association with NFL because it also increases with age.\textsuperscript{50} Third, we were similarly limited to crude markers of AD-related brain pathology which are not diagnostic of AD, especially since the ATN-classification includes AD biomarkers as measured by PET, structural MRI or CSF and we do not have phospho tau measured. However, plasma and CSF levels of these biomarkers are strongly correlated.\textsuperscript{51-53} Also NFL is a global biomarker and not specific for AD. Fourth, we were unable to categorize the age groups into early and late onset AD, due to the low number of early onset AD patients within our population-based cohort. It would be helpful to categorize the biomarkers according to early and late onset AD in future studies, because Aβ has a better correlation in early onset AD. Early onset AD differs from late onset AD not only with respect to genetic predisposition and pathology but also in relation to the clinical outcome and the natural course. Only 11\% of early AD patients have familial mutations with APP, PSEN1 and PSEN2. For example, patients with early AD have a greater burden in the precuneus and parietal lobes and to a lesser extent in the frontal lobes.\textsuperscript{54} Fifth, we did not offer replication data. We hope that our study provides a stimulus for other cohort studies to replicate our findings. Lastly, our study contains predominantly Caucasians, which limits its external generalizability. Further study in other ethnicities is needed to identify potential ethnic differences.

In summary, in this population-based study we showed that higher innate immunity, as reflected by higher levels of serum GLR, PLR, and SII, were associated with higher plasma Aβ42, 40, and NFL, and with lower Aβ42/40 and total tau. Furthermore, APOE ε2 carriers displayed lower serum markers of innate immunity levels compared to ε3/ε3, while these markers were elevated in APOE ε4. Knowledge of the potential pro-inflammatory role of APOE ε3/ε2 should encourage future studies to find ways to scale down innate immune responses in APOE ε4 carriers to limit AD-related brain pathology in order to prevent AD.

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CONFLICTS OF INTEREST

None.

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All authors have made a substantial intellectual contribution to the conception and design of the study (MKI, MAI, LF, SA), acquisition of data (MKI, LF, MG), analysis and interpretation of data (LF, MKI, MAI), drafting the manuscript (LF), or drafting a significant portion of the manuscript or figures (LF, MAI). All authors approved the final version of the manuscript for publication. MAI had full access to the data in the study and takes responsibility for data integrity and accuracy of data analysis.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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