Protective associations of HDL with blood-brain barrier injury in multiple sclerosis patients

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Abstract The purpose of this work was to investigate the associations of serum cholesterol and apolipoproteins with measures of blood-brain barrier (BBB) permeability and CNS inflammation following the first clinical demyelinating event. This study included 154 patients [67% female; age, 29.5 ± 8.2 years (mean ± SD)] enrolled in a multi-center study of interferon β-1a treatment following the first demyelinating event. Blood and cerebrospinal fluid (CSF) were obtained at screening prior to treatment. A comprehensive serum lipid profile and multiple surrogate markers of BBB breakdown and CNS immune activity were obtained. Higher levels of serum HDL cholesterol (HDL-C) and ApoA-I were associated with lower CSF total protein level, CSF albumin quotient, and CSF IgG level (all $P < 0.001$ for HDL-C and all $P < 0.01$ for ApoA-I). HDL-C was also associated with CSF CD80+ (P < 0.001) and with CSF CD80+CD19+ (P = 0.007) cell frequencies. Higher serum HDL is associated with lower levels of BBB injury and decreased CD80+ and CD80+CD19+ cell extravasation into the CSF. HDL may potentially inhibit the initiation and/or maintenance of pathogenic BBB injury following the first demyelinating event.—Fellows, K., T. Uher, R. W. Browne, B. Weinstock-Guttman, D. Horakova, H. Posova, M. Vaneckova, Z. Seidl, J. Krasensky, M. Tyblova, E. Havrdova, R. Zivadinov, and M. Ramanathan. Protective associations of HDL with blood-brain barrier injury in multiple sclerosis patients, J. Lipid Res. 2015. 56: 2010–2018.

Supplementary key words high density lipoprotein • cholesterol • apolipoproteins • clinically isolated syndrome

Blood-brain barrier (BBB) injury creates a permissive environment for inflammation and the extravasation of immune cells (1) in multiple sclerosis (MS), a chronic inflammatory and neurodegenerative disease of the CNS (2). Compromised BBB structural integrity is necessary for the formation of contrast-enhancing lesions (CELs), which are frequently found on brain MRI from MS patients, even in the absence of clinical relapses. CELs represent focal areas with compromised BBB structural integrity and are associated with parenchymal and meningeal inflammation (3). Increased BBB water permeability precedes the appearance of CELs and is pervasive in normal-appearing white matter (4).

In CNS regions with loss of BBB structural integrity, there is increased permeability to macromolecules such as proteins, albumin, and immunoglobulins, that are normally

Abbreviations: BBB, blood-brain barrier; CEL, contrast-enhancing lesion; CRP, C-reactive protein; CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; LV, lesion volume; MS, multiple sclerosis; OCB, oligoclonal band; PON1, paraoxonase-1; TC, total cholesterol; SET, Study of Early Interferon β-1-a Treatment.

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excluded by the BBB. These macromolecules enter more readily into the CNS and can be detected in cerebrospinal fluid (CSF). CSF levels of proteins produced exclusively outside the CNS, such as albumin, provide useful surrogate measures of the pathological injury to BBB structural integrity. The presence of T and B cells and immunoglobulins in CSF resulting from extravasation through the BBB provides surrogate measures of immune activity in the CNS. The presence of immunoglobulin oligoclonal bands (OCBs) in CSF is used as a paraclinical diagnostic criterion in MS (5).

Chronic hypercholesterolemia can promote exaggerated immune responses, stronger leukocyte-vascular endothelial cell adhesion, and immune cell extravasation in the microvasculature (6, 7). In this research, we leverage a range of CSF measures to understand the contributions of lipid profile variables to pathophysiological increases in BBB permeability and to immune activity in CSF. We focused on serum cholesterol profiles because there is an emerging body of data suggesting associations between serum cholesterol profiles and MRI measures of lesional and neurodegenerative injury in MS patients (8–13). In our previous work, we found an adverse association between greater serum cholesterol and MS disease progression. Higher LDL cholesterol (LDL-C) and total cholesterol (TC) levels were positively associated with worsening disability measured on the Expanded Disability Status Scale (EDSS) and MS Severity Scale (10, 13). Higher HDL levels were associated with fewer CELs (9, 13). We also investigated the role of cholesterol profiles in patients following the first demyelinating event, prior to disease-modifying treatment, and found that greater LDL-C, TC, and ApoB levels were associated with a greater number of T2 lesions after 2 years (12, 14). The main goals of this study are to investigate the associations of cholesterol and apolipoprotein levels with CSF-derived measures of increased BBB permeability and cellular and humoral immune activity.

**METHODS**

**Study population**

**Study setting.** This was a multi-center, prospective, longitudinal, and observational study.

**Informed consent.** The Medical Ethics Committees of the General University Hospital and First Faculty of Medicine of Charles University, Prague, Czech Republic, approved the study protocol and the informed consent procedure. Additionally, approvals were obtained from local medical ethics committees of all other participating centers. Written informed consent was obtained from all patients at enrollment.

**Clinical study design.** The Observational Study of Early Interferon β-1a Treatment (SET) in High Risk Subjects after Clinically Isolated Syndrome (SET study, clin.gov #NCT01592474) and its design have been previously described (15, 16). The study was coordinated by Charles University in Prague, Czech Republic. The study screened 259 patients and enrolled 220 patients from eight Czech Republic MS centers.

**Inclusion criteria.** Patients included in the study had the following characteristics: 18–55 years of age, enrolled within 4 months from the clinical event, presence of two or more T2-hypointense lesions on diagnostic MRI and presence of two or more OCBs in CSF obtained prior to corticosteroid treatment, and EDSS ≤3.5.

This sub-study included 154 patients for whom lipid profiles and CSF-derived measures of increased BBB permeability were available.

**Treatments.** All patients were treated with 3–5 g of methylprednisolone for the first symptom and baseline MRI was performed ≥30 days after steroid administration.

All patients were started on a 30 μg once weekly intramuscular interferon β-1a (AVONEX®) treatment at baseline.

**MRI acquisition and analysis**

This sub-study was limited to MRI measures obtained at baseline. MRI methods are summarized in the supplementary Methods. We investigated the associations of the number of CELs and T2-lesion volume (LV) at baseline with CSF measures of BBB permeability in statistical analyses.

**Serum lipids and apolipoproteins**

Serum for lipid and apolipoprotein analyses was obtained in the nonfasted state at the screening visit prior to the start of corticosteroid or interferon. The methods for lipid profile and apolipoprotein analyses have been previously described (14), but are succinctly recapitulated here.

Immunoturbidimetric diagnostic kits (Kamiya Biomedical, Thousand Oaks, CA) were used for the apolipoprotein (ApoA-I, ApoA-II, ApoB, and ApoE), lipoprotein(a), and high sensitivity C-reactive protein (CRP) assays. Diagnostic reagent kits (Sekisui Diagnostics, Lexington, MA) were used to measure serum TC, HDL cholesterol (HDL-C), phospholipids, and triglycerides. These assays were conducted on an automated chemistry analyzer (ABX Pentra 400; Horiba Instruments, Irvine, CA). The coefficient of variation of these assays is <5%. LDL-C was obtained from the Friedewald equation (17).

Specific probes for both single nucleotide polymorphisms, rs7412 and rs429358 (OpenArray; Applied Biosystems, Life Technologies, Foster City, CA), were used to genotype APOE gene variants ε2, ε3, and ε4.

The arylesterase and paraoxonase activities of the human serum paraoxonase-1 (PON1) enzyme were measured using phenyl acetate (arylsterase activity) and paraoxon (paraoxonase activity) as substrates, respectively. The assay coefficient of variation was 0.6–1.4%. The PON1 Q192R polymorphism was obtained from the paraoxonase and arylesterase activities, as previously described (18).

Clinical data collected included height and weight for BMI calculations, and history of statin use.

**CSF assays**

**Lumbar punctures.** All lumbar punctures were performed prior to treatment with corticosteroids at the study-coordinating center during the morning hours. CSF was drawn from L5-5, L4-5, or L3-4 inter-space with the patient sitting upright using a standard sterile preparation and 20 gauge Sprotte atraumatic needle. A total of 20–25 ml of CSF and a 5 ml volume of blood were obtained.

**Biochemical, immunological, and cellular assays.** Total protein in CSF was determined photometrically using the pyrogallol red-molybdate reaction method (Synchon LX 20, Beckman Coulter analyzer). Albumin, IgG, and IgM concentrations were quantified.
RESULTS

Demographic and clinical characteristics

The clinical, demographic, and MRI characteristics of the study sample at baseline and the CSF measures and lipid profile variables at screening are summarized in Table 1.

The mean time ± SD between disease onset and lumbar puncture was 28.2 ± 23.2 days (median = 20.0 days, interquartile range = 34 days). None of the subjects were on statins.

The clinical and demographic characteristics of subjects with lipid profile and CSF measures that were included in the study were similar to the SET study sample that was not included (data not shown).

Associations of CSF variables with lipid profile variables

Table 2 summarizes the associations of CSF variables with the serum cholesterol variables (HDL-C, LDL-C, and TC), serum apolipoprotein variables (ApoA-I, ApoA-II, ApoB, and ApoE), and CRP.

Greater HDL-C and TC levels were associated with lower CSF total protein level, CSF albumin level, albumin quotient, and CSF IgG level (Table 2). Additionally, TC was negatively associated with alkaline OCBs (23) (P = 0.003, q = 0.007). The CSF variables that were negatively associated

### Table 1. Demographic and clinical characteristics at baseline, lipid profile and CSF variable values at disease onset

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample size (n)</td>
<td>154</td>
<td>—</td>
</tr>
<tr>
<td>Female (%)</td>
<td>67</td>
<td>—</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.5 ± 8.2</td>
<td>—</td>
</tr>
<tr>
<td>EDSS</td>
<td>1.71 (0.67)</td>
<td>—</td>
</tr>
<tr>
<td><strong>MRI Characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2-LV (cm³)</td>
<td>5.15 ± 6.44</td>
<td>791 ± 47</td>
</tr>
<tr>
<td>Brain volume (cm³)</td>
<td>1.505 ± 72</td>
<td>—</td>
</tr>
<tr>
<td>Gray matter volume (cm³)</td>
<td>791 ± 47</td>
<td>—</td>
</tr>
<tr>
<td><strong>Lipid profile characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>70.3 ± 19.0</td>
<td>40–83</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>134 ± 40.1</td>
<td>57–189</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>204 ± 52.9</td>
<td>133–234</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>110 ± 50.9</td>
<td>37–281</td>
</tr>
<tr>
<td>ApoA-I (mg/dl)</td>
<td>158 ± 40.6</td>
<td>115–224</td>
</tr>
<tr>
<td>ApoA-II (mg/dl)</td>
<td>37.3 ± 8.65</td>
<td>25–35</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>73.9 ± 23.5</td>
<td>60–130</td>
</tr>
<tr>
<td>ApoE (mg/dl)</td>
<td>3.28 ± 1.05</td>
<td>3.3–6.1</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>2.77 ± 4.40</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td><strong>CSF variable characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF-leukocytes (in 3 mm³)</td>
<td>26.5 ± 34.1</td>
<td>&lt;12</td>
</tr>
<tr>
<td>CSF-total protein (mg/l)</td>
<td>345 ± 119</td>
<td>150–450</td>
</tr>
<tr>
<td>CSF-albumin (mg/l)</td>
<td>217 ± 86</td>
<td>120–300</td>
</tr>
<tr>
<td>Albumin quotient</td>
<td>4.75 ± 1.84</td>
<td>≪6.5 if age &lt;40 years, ≪8.0 if age ≥40 years</td>
</tr>
<tr>
<td>CSF-Restricted total OCBs</td>
<td>12.1 ± 5.1</td>
<td>Not available</td>
</tr>
<tr>
<td>CSF-Restricted total alkaline OCBs</td>
<td>8.33 ± 3.7</td>
<td>Not present</td>
</tr>
</tbody>
</table>

*All continuous variables (age, disease duration, T2-LV, and T1-LV) are mean ± SD. For the ordinal EDSS, the median (interquartile range) is given.

Reference range provided by diagnostic reagent kit manufacturers or clinical laboratory.
The associations of lipid profile variables (HDL-C, LDL-C, TC, ApoA-I, ApoB and ApoE) with CSF variables (CSF total protein, CSF albumin, CSF IgG, IgM, IgG index, Albumin quotient, IgG quotient, IgM quotient, IgM index, Total OCBs, Alkaline OCBs, CSF leukocytes, CD80, CD80 CD19, CD4, CR5, CXCR3) were assessed. The Wald $\chi^2$ and $P$ values from the regression analyses and the ApoA-I, ApoB, ApoE, HDL-C, LDL-C and TC variables (HDL-C, ApoA-I, ApoB, ApoE, HDL-C, LDL-C and TC) were included in the model. The associations of CSF total protein, CSF albumin, CSF IgG, IgM, IgG index, Albumin quotient, IgG quotient, IgM quotient, IgM index, Total OCBs, Alkaline OCBs, CSF leukocytes, CD80, CD80 CD19, CD4, CR5, CXCR3 with HDL-C, LDL-C, TC, ApoA-I, ApoB, ApoE were assessed. The Wald $\chi^2$ and $P$ values from the regression analyses and the ApoA-I, ApoB, ApoE, HDL-C, LDL-C and TC variables (HDL-C, ApoA-I, ApoB, ApoE, HDL-C, LDL-C and TC) were included in the model.

**Associations of lipid profile variables with CSF variables**

<table>
<thead>
<tr>
<th>CSF Variable</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>TC</th>
<th>ApoA-I</th>
<th>ApoB</th>
<th>ApoE</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C</td>
<td>0.11</td>
<td>0.01</td>
<td>0.09</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.09</td>
<td>0.001</td>
<td>0.01</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>TC</td>
<td>0.09</td>
<td>0.01</td>
<td>0.02</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>0.09</td>
<td>0.01</td>
<td>0.02</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.09</td>
<td>0.01</td>
<td>0.02</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>ApoE</td>
<td>0.09</td>
<td>0.01</td>
<td>0.02</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>CRP</td>
<td>0.09</td>
<td>0.01</td>
<td>0.02</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Partial correlation ($r$) and $P$-value from linear regression are shown.**

**Negative binomial regression was used and Wald $\chi^2$ (x$^2$) values are provided instead of partial correlation.**
CEL number was not associated with IgG index. The associations of albumin quotient and CSF leukocytes with CEL number are summarized in Fig. 4A and Fig. 4C, respectively.

Only a subset of the CSF variables associated with CEL number were associated with T2-LV. CSF total protein level \((P = 0.016, q = 0.048)\), albumin level \((P = 0.014, q = 0.056)\), albumin quotient \((P = 0.006, q = 0.072)\), CSF IgG level \((P = 0.017, q = 0.041)\), and IgG quotient \((P = 0.012, q = 0.072)\) were associated with T2-LV. The associations of albumin quotient and CSF leukocytes with T2-LV are summarized in Fig. 4B and Fig. 4D, respectively. We did not find evidence for associations for CSF IgM level, IgM quotient, IgM index, total OCBs, alkaline OCBs, and leukocytes with T2-LV.

These results are consistent with a role for increased BBB permeability (as assessed by albumin quotient) and CSF humoral immunity (as assessed by OCBs and IgG) in CEL formation following the first demyelinating event.

**DISCUSSION**

We investigated the role of cholesterol and apolipoproteins in BBB breakdown following the first demyelinating event suggestive of MS. Greater HDL-C was associated with less BBB permeability, as assessed by several CSF measures including CSF albumin quotient. High levels of HDL-C and ApoA-I were associated with lower CSF frequencies of CD80+ and CD80+CD19+ cells. To our knowledge, the associations of cholesterol and cholesterol biomarkers with BBB breakdown have not been extensively investigated.
Although our results demonstrate protective associations between greater HDL-C levels and lower BBB permeability, the strengths and limitations of our study design merit discussion. The SET study was a well-controlled longitudinal treatment study that accrued a wealth of clinical MRI and blood biomarkers over a 4 year period. However, a potential criticism of the study design is the lack of a placebo-controlled group. A 4 year placebo-controlled trial would clearly be ethically infeasible given that proven disease-modifying therapies are now available for MS.

Fig. 2. Associations of ApoA-I quartiles with albumin quotient (A), IgG index (B), IgM index (C), and CSF leukocytes (D). The ApoA quartile boundaries were: the lowest quartile corresponds to ApoA-I \( \leq 128.3 \text{ mg/dl} \), \( 128.3 \text{ mg/dl} < \text{quartile 2 (Q2)} \leq 151.1 \text{ mg/dl} \), \( 151.1 \text{ mg/dl} < \text{quartile 3 (Q3)} \leq 175.8 \text{ mg/dl} \), and the highest quartile > 175.8 mg/dl. The bars compare mean values of the CSF measures shown on the y axis to the quartiles of ApoA-I shown on the x axis. The error bars indicate the standard error of the mean.

Fig. 3. Associations of mean CSF CD80+ (A–C) and CSF CD80+CD19+ (D–F) cell frequencies in percent with HDL-C quartiles, ApoA-I quartiles, and ApoB quartiles. A, D: The HDL-C quartile boundaries were: the lowest quartile corresponds to HDL-C \( \leq 56.27 \text{ mg/dl} \), \( 56.27 \text{ mg/dl} < \text{quartile 2 (Q2)} \leq 66.25 \text{ mg/dl} \), \( 66.25 \text{ mg/dl} < \text{quartile 3 (Q3)} \leq 80.69 \text{ mg/dl} \), and the highest quartile > 80.69 mg/dl. B, E: The ApoA-I quartile boundaries were: the lowest quartile corresponds to ApoA-I \( \leq 128.3 \text{ mg/dl} \), \( 128.3 \text{ mg/dl} < \text{quartile 2 (Q2)} \leq 151.1 \text{ mg/dl} \), \( 151.1 \text{ mg/dl} < \text{quartile 3 (Q3)} \leq 175.8 \text{ mg/dl} \), and the highest quartile > 175.8 mg/dl. C, F: The ApoB quartile boundaries were: the lowest quartile corresponds to ApoB \( \leq 54.0 \text{ mg/dl} \), \( 54.0 \text{ mg/dl} < \text{quartile 2 (Q2)} \leq 70.75 \text{ mg/dl} \), \( 70.75 \text{ mg/dl} < \text{quartile 3 (Q3)} \leq 87.0 \text{ mg/dl} \), and the highest quartile > 87.0 mg/dl. The bars compare mean values of the CSF cell frequencies in percent shown on the y axis to the quartiles of HDL-C, ApoA-I, or ApoB on the x axis. The error bars indicate the standard error of the mean.
However, it would have been useful to compare interferon treatment in the SET trial to a different treatment, such as glatiramer acetate, to establish whether our results were generalizable to other MS disease-modifying treatments. Our study was also limited because the lipid profile and CSF measures were obtained only at screening, making this analysis cross-sectional in nature. Although our statistical results are consistent with a protective role for high HDL levels against BBB injury after the first demyelinating event, these associations are not proof of causation.

Our study would be further strengthened if we had also obtained cholesterol and lipoprotein profiles in the CSF. The CSF from SET study patients at the screening visit was primarily used to assess OCB status of patients for meeting inclusion criteria. Additionally, measures of BBB breakdown and immune cell extravasation were also obtained. Although the CNS and peripheral cholesterol compartments are relatively segregated by the BBB, there is evidence for regulatory interactions and homeostatic mechanisms. Glial cells play a critical role in cholesterol production and homeostasis in the brain where an HDL-like particle containing ApoE mediates cholesterol transport. The ApoE that comprises this HDL-like particle is secreted by astrocytes and microglia. Although ApoA-I is not produced in the brain, recent evidence suggests that circulating ApoA-I can enter the CSF through the choroid plexus (24). ApoA-I entering the brain is likely derived from scavenger receptor class B member 1-mediated uptake of circulating discoidal HDL particles into CSF at the choroid plexus (24, 25). ApoA-I and ApoE measurements in CSF would have yielded information regarding these interactions between the CNS and blood compartments. It would also be interesting to know whether HDL particle size, particularly small discoidal preβ-HDL, is more predictive of BBB integrity than total HDL-C alone.

MS is associated with significant cerebral vascular endothelial cell dysfunction (26–29). In atherosclerosis, HDL-C plays an important protective role in the immune cell-vascular endothelial interactions that mediate lesion formation. HDL can modulate immune cell phenotype by altering cellular cholesterol because it stimulates cholesterol removal from macrophages and downregulates foam cell production. Plasma HDL-C has been found to be negatively associated with baseline monocyte counts (30). HDL-C-mediated cholesterol transport also preserves active eNOS dimer levels that maintain endothelial cell function (31).

The mechanisms by which these protective processes occur in MS remain unknown. Meyers et al. (32) reported that ApoA-I levels were lower in the MS patients compared with healthy controls and the primary progressive MS group had lower levels than relapsing-remitting and secondary-progressive MS groups. Interestingly, in a small study of 36 Alzheimer’s disease patients, low HDL was associated with increased BBB breakdown, as assessed by albumin quotient (33). This provides independent evidence, albeit from a different neurological disease, that HDL and lipid profiles can modulate BBB breakdown. However, Alzheimer’s disease is prevalent in the elderly, and 47% of the study group in (33) had metabolic dyslipidemia and 22% were on statins. In contrast, our patient group was younger and none were on statins. Results in the induced experimental allergic encephalomyelitis animal model also provide supporting evidence. In experimental allergic encephalomyelitis-induced ApoA-I-deficient mice, there was increased T cell penetration into the CSF that led to an increase in demyelination (32).

We used a diverse range of CSF-derived measures of altered BBB permeability and immune activity. For example, we used the albumin quotient ($Q_{Alb}$), a calculated measure that normalizes CSF albumin levels to serum albumin. Because serum albumin is synthesized in the liver, any albumin present in CSF enters via CNS regions of increased BBB permeability. In contrast, immunoglobulins can be synthetized in the CSF by extravasating B cells in addition to entering the CSF from blood at regions where BBB integrity is compromised. CD80+ is a costimulatory molecule for T cell activation that is expressed on activated B cells and monocytes, whereas CD19+ is found on B cells; CCR5 and CXCR3 are chemokine receptors that are expressed on T cells, particularly pro-inflammatory Th1 cells. Studies have

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\text{Fig. 4. } \text{Associations of albumin quotient (A, B) and CSF leukocytes (C, D) with CEL number and T2-LV MRI measures. The bars compare mean values of the MRI measures shown on the y axis for the quartiles of the CSF variable shown on the x axis. A, B: The albumin quotient quartile boundaries were: the lowest quartile corresponds to albumin quotient} \leq 3.36 \text{ mg/g}; 3.36 \text{ mg/g} < \text{quartile 2 } (Q2) \leq 4.25 \text{ mg/g}; 4.25 \text{ mg/g} < \text{quartile 3 } (Q3) \leq 5.58 \text{ mg/g}; \text{and the highest quartile} > 5.58 \text{ mg/g. C, D: The CSF leukocytes quartile boundaries were: the lowest quartile corresponds to CSF leukocytes} \leq 0.0/3 \mu l; 0.0/3 \mu l < \text{quartile 2} \leq 17.0/3 \mu l; 17.0/3 \mu l < \text{quartile 3} \leq 33.5/3 \mu l; \text{and the highest quartile} > 33.5/3 \mu l. \text{The error bars indicate the standard error of the mean.}
\]
demonstrated that CD80+ cells and CD19+ cells are increased in peripheral blood mononuclear cells in MS (34, 35). CXCR3- and CCR5-expressing T cells are found in MS lesions and are higher in the peripheral blood mononuclear cells of progressive MS patients (36).

Interestingly, increased HDL-C and ApoA-I were associated with lower IgG quotient; no associations were found for IgG index, IgM quotient, or IgM index. We attribute the differences between IgG and IgM to the approximately 5-fold higher molecular mass of IgM (approximately 970 kDa vs. 150 kDa for IgG) and lower prevalence of intrathecal synthesis of IgM in MS patients.

Weinstock-Guttman et al. (13) found that higher HDL-C levels were associated with decreased CEL activity in a large group of MS patients, whereas Giubilei et al. (8) found a similar association in clinically isolated syndrome patients. The protective associations of the serum HDL compartment with CSF measures of BBB integrity were confirmed via three different HDL biomarkers, namely, HDL-C, ApoA-I, and PON1 arylesterase activity. ApoA-I is the characteristic protein of HDL-C that mediates its important interactions with other lipoproteins and cells. ApoA-I was associated with all of the same BBB permeability measures and CSF immune cell subset frequencies as HDL-C. In contrast to ApoA-I, ApoA-II has only secondary supporting roles in HDL functionality (37). ApoA-II is not as anti-atherogenic as ApoA-I and its associations with the risk of cardiovascular disease are considered weaker and more controversial than ApoA-I (38). These physiological and clinical findings related to ApoA-II provide the context for understanding the lack of ApoA-II associations with CSF measures. Taken together, the concordance of our HDL-C and ApoA-I results provide support for potentially protective roles for the HDL compartment on the CSF measures of altered BBB permeability (e.g., albumin quotient) and immune activity (e.g., CD80+ and CD80+CD19+ cell frequencies in the CSF). The TC associations with CSF measures are the result of key contributions from HDL.

In additional analyses (data not shown), we found that $Q_{AB}$ was associated with clinical disability progression and with brain MRI measures of lesoinal injury and neurodegeneration. Higher baseline $Q_{AB}$ was associated with greater EDSS over the 4 years following the initial demyelinating event. These findings suggest that CSF measures may have prognostic importance in MS (39).

Multiple groups have independently reported that high LDL-C and TC levels are associated with increased disability and T2 lesion burden (8, 10–13). ApoB, the characteristic protein of LDL-C, was associated with greater CSF CD80+CD19+ cell frequency, suggesting a possible role for the LDL compartment in promoting extravasation, proliferation, or survival of CD80+CD19+ cells in the CSF milieu. We surmise that while the HDL compartment is important for protecting against increased BBB permeability at the earliest stages of MS, the LDL-C and TC compartments are more important to the subsequent processes that promote T2 lesion burden.

Our results indicate a protective role for HDL-C in the pathophysiological BBB injury that precedes the formation of MS lesions. The findings are consistent with the intriguing possibility that loss of BBB structural integrity is nucleated in membrane subdomains actively involved in cholesterol homeostasis or at pathophysiologically dyslipidemic tissue regions.

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REFERENCES


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