RESEARCH PAPER

Lipid profiles are associated with lesion formation over 24 months in interferon-β treated patients following the first demyelinating event

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ABSTRACT

Objectives To investigate the associations of serum lipid profile with disease progression in high-risk clinically isolated syndromes (CIS) after the first demyelinating event.

Methods High density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) were obtained in pretreatment serum from 135 high risk patients with CIS (>2 brain MRI lesions and ≥2 oligoclonal bands) enrolled in the Observational Study of Early Interferon β-1a Treatment in High Risk Subjects after CIS study (SET study), which prospectively evaluated the effect of intramuscular interferon β-1a treatment following the first demyelinating event. Thyroid stimulating hormone, free thyroxine, 25-hydroxy vitamin D3, active smoking status and body mass index were also obtained. Clinical and MRI assessments were obtained within 4 months of the initial demyelinating event and at 6, 12 and 24 months.

Results The time to first relapse and number of relapses were not associated with any of the lipid profile variables. Higher LDL-C (p=0.006) and TC (p=0.001) levels were associated with increased cumulative number of new T2 lesions over 2 years. Higher free thyroxine levels were associated with lower cumulative number of contrast-enhancing lesions (p=0.008). Higher TC was associated as a trend with lower baseline whole brain volume (p=0.020). Higher high density lipoprotein was associated with higher deseasonalised 1,25-dihydroxy vitamin D3 (p=0.003) levels and a trend was found for deseasonalised 25-hydroxy vitamin D3 (p=0.014).

Conclusions In early multiple sclerosis, lipid profile variables particularly LDL-C and TC levels are associated with inflammatory MRI activity measures.

INTRODUCTION AND BACKGROUND

Although serum cholesterol and its metabolism are unlikely to be causative factors in multiple sclerosis (MS), there is now emerging evidence indicating that dyslipidaemia is associated with MS disease progression.

Comorbidities linked to dyslipidaemia were reported to be associated with increased risk for MS disability progression in a retrospective analysis of the North American Research Committee on Multiple Sclerosis (NARCOMS) registry.1 High total cholesterol (TC) was associated with worsening disability changes and decreased brain parenchymal fraction whereas higher high density lipoprotein cholesterol (HDL-C) levels were associated with lower contrast-enhancing lesion (CEL) volume.2 Increased TC was associated with increases in the number of CELs in clinically isolated syndrome (CIS) following the first clinical event.3 Patients with MS were found to have decreased paraoxonase-1 (an antioxidant enzyme associated exclusively with HDL) activity during relapses.4

Chronic hypercholesterolaemia can promote exaggerated immune responses, stronger leukocyte-vascular endothelial cell adhesion and immune cell extravasation in the microvasculature in addition to altering blood flow and vessel wall elasticity.5 6 Oxidised low-density lipoprotein cholesterol (LDL-C) has proinflammatory properties and can be a target of autoimmune antibodies. HDL, beyond its reverse cholesterol transport functions, has anti-inflammatory and antioxidant properties.

The present research investigates the role of lipid profile in clinical disease progression and in the inflammatory and neurodegenerative pathophysiological processes that cause brain injury in CIS, which represents an early stage in the development of MS. The study was conducted in patients enrolled in a prospective, multicentre longitudinal observational trial of CIS called the Observational Study of Early Interferon β-1a Treatment in High Risk Subjects after CIS (SET study).

METHODS

Study population

Prospective, longitudinal observational trial. The study protocol was approved by the ethical committee at the Charles University in Prague and by ethical committees at each of the eight participating sites.

Clinical study design

The objective of the SET study, coordinated by the Charles University at Prague and involving eight Czech Republic MS centres, was to determine clinical and MRI predictors of response to interferon-β-1a treatment following the first demyelinating event in high risk CIS patients.

The SET study protocol was approved by the ethical committee at the Charles University in Prague and by the ethical committees at each of the eight participating sites.
β-1a therapy in patients with CIS. All patients were treated with 30 μg, intramuscular interferon β-1a.  

The SET study trial design includes clinical visits every 3 months for 4 years with 6 years of observational follow-up. Its clinical outcome measures include time to clinically definite MS (CDMS) according to Poser criteria,8 time to 1-point Expanded Disability Status Scale (EDSS) progression and quality of life measures.

This report is based on the findings at the end of 2 years of follow-up. Quantitative measures of T2, CEL, as well as atrophy at 2 years were computed from MRI scans acquired at baseline, 6, 12 and 24 months of follow-up.

**Study population**

Patients with CIS with the following characteristics were included: 18–55 years of age, enrolled within 4 months from the clinical event, EDSS ≤3.5, presence of ≥two T2-hyperintense lesions on diagnostic MRI obtained prior to steroid treatment and presence of ≥two oligoclonal bands in cerebrospinal fluid (CSF) obtained prior to steroid treatment.

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

This sub-study only included patients enrolled at the 1st Faculty of Medicine of Charles University, Prague, site who had received lipid profile analysis at the screening visit prior to any treatment (122/135 subjects, 90%) or at the baseline visit >30 days after corticosteroid treatment and prior to interferon β-1a treatment (13/135 subjects, 10%).

The data collected included demographic and clinical information, statin and thyroid medication use history, height and weight and non-fasting lipid profile laboratory values: HDL-C, LDL-C, triglycerides, TC, free thyroxine (FT4), thyroid stimulating hormone (TSH). All lipid profile, FT4 and TSH analyses were obtained from a single laboratory (Institute of Biochemistry, Prague). None of the patients were on statins and only 3/135 (2.2%) patients were on oral thyroid supplementation therapy for hypothyroidism. The individuals conducting the lipid profile, thyroid, vitamin D (VD) and active smoking biomarkers were blinded to the patients’ clinical and MRI characteristics.

**Environmental factors**

All of the following analyses were conducted in serum samples obtained at the screening visit prior to corticosteroid or interferon β-1a treatment.

**Smoking status**

Cotinine levels were measured using a validated liquid chromatography-mass spectrometry method. A cotinine level threshold of 10 ng/ml was used to categorise subjects as active smokers.9 10

**Vitamin D levels**

The VD metabolites 25-hydroxy vitamin D3 (25(OH)VD3) and 1,25-dihydroxy vitamin D3 (1,25(OH)2VD3) were measured using liquid chromatography-tandem mass spectrometry methods as published.11 Levels were available for 113 patients. The remaining samples could not be assessed because of insufficient serum.

The raw 25(OH)VD3 and 1,25(OH)2VD3 levels were deseasonalised using sinusoidal regression (see online supplementary data).

**MRI acquisition and analysis**

**Image acquisition**

MRI was performed using a 1.5 T magnet (Philips Gyroscan NT 15, Best, The Netherlands). Details of the acquisition protocol are in the online supplementary data.

**Image analysis**

The MRI scans acquired at baseline, 6, 12 and 24 months were collected centrally at the Department of Radiology at Charles University (Prague, Czech Republic). Scans were transferred to, and analysed by the Buffalo Neuroimaging Analysis Center, State University of New York (Buffalo, USA). The MRI analysts were blinded to the patients’ clinical and lipid profile status.

**Lesion measures**: Numbers of T2 and CEL, and lesion volumes were measured on fluid attenuated inversion recovery (FLAIR) and on T1 postcontrast images, respectively, using techniques previously published.12

**Global and tissue-specific atrophy measures**: Per cent changes in whole brain volume (WBV), grey matter volume (GMV) and white matter volume were measured as previously described.13

Details of the MRI analysis are provided in the online supplementary data.

**Data analysis**

SPSS V19.0 (SPSS Inc, Chicago, Illinois, USA) statistical program was used for statistical analyses. A conservative p value of ≤0.01 was used to assess significance given that the multiple clinical and MRI endpoints tested; p values ≤0.05 were considered trends. Statistical analyses methods are described in the online supplementary data.

**RESULTS**

**Characteristics of the study cohort**

Tables 1 and 2 show the demographic, clinical and MRI features of the study cohort. The cohort was representative of the larger SET study cohort on demographic, clinical and MRI characteristics except for a statistical difference (p=0.003, Mann-Whitney test) in baseline EDSS (median EDSS±IQR=1.5±1.0 in the excluded group vs 1.5±0.5 in the included group).

The associations of lipid profile with demographic and other factors such as body mass index (BMI) and smoking are summarised in the online supplementary data.

**Interdependence of lipids with vitamin D biomarkers**

VD biomarkers were included because the VD and cholesterol biochemical pathways are connected via 7-dehydrocholesterol. The mean±SD value of deseasonalised 25(OH)VD3 levels were 15.4±8 ng/ml whereas those for deseasonalised 1,25 (OH)2VD3 were 0.029±0.021 nmol/l. The frequency of VD3 deficiency (defined as 25(OH)VD3 levels <20 ng/ml) was 87/113 (77%).

In linear regression analyses, higher deseasonalised 25(OH)VD3 levels were associated with trends with higher HDL-C (r=0.24, p=0.014) and with HDL-C >60 mg/dl status (partial correlation r=0.20, p=0.033). Similarly, higher deseasonalised 1,25 (OH)2VD3 levels were associated with higher HDL-C (r=0.28, p=0.003) and with HDL-C >60 mg/dl status (r=0.25, p=0.008).

**Interdependence of lipids with thyroid biomarkers**

We included thyroid biomarkers because thyroid abnormalities can affect the lipid profile. None of the patients had abnormal FT4 (range: 10.2–21.5 pmol/l, laboratory normal range: 9.8–23.1 pmol/l). Three of 135 patients (2.2%) had high TSH (range:
Multiple sclerosis

In regression analysis that corrected for age and BMI, male gender was associated with higher FT4 (16.8 pM in males vs 15.6 pM in females; \( r_p = 0.28, p = 0.001 \)).

In linear regression analyses, FT4 levels were not associated with HDL-C (\( p = 0.62 \)), LDL-C (\( p = 0.28 \)) or TC (\( p = 0.70 \)). Likewise, TSH levels were not associated with HDL-C (\( p = 0.98 \)), LDL-C (\( p = 0.85 \)) or TC (\( p = 0.59 \)).

Associations of lipid profile with clinical progression

There was no evidence for significant associations of time to first relapse, risk of developing CDMS and number of relapses with HDL-C, LDL-C and TC levels. There was also no evidence for associations between any of these dependent variables with HDL-C, LDL-C and TC status indicator variables.

Associations of lipid profile with MRI outcomes

New and newly enlarging T2 lesions

The number of new and newly enlarging T2 lesions over 2-years dependent variable was analysed with negative binomial regression. Higher LDL-C levels were associated with a higher cumulative number of new T2 lesions (\( p = 0.006 \)) and also with a higher cumulative number of new/enlarging T2 lesions (\( p = 0.008 \)). A trend was found for LDL-C >100 mg/dl status with the greater cumulative number of new T2 lesions (\( p = 0.047 \)).

Higher TC levels were associated with a greater cumulative number of new T2 lesions over 2 years (\( p = 0.001 \)) and with the cumulative number of new/enlarging T2 lesions over 2 years (\( p = 0.003 \)); there was no evidence for associations with the cumulative number of newly enlarging T2 lesions (\( p = 0.34 \)). TC that showed a >200 mg/dl status was also associated with a higher cumulative number of new T2 lesions over 2 years (\( p < 0.001 \)) and with a higher cumulative number of new/enlarging lesions over 2 years (\( p < 0.001 \)). The marginal mean number of new T2 lesions in the TC >200 mg/dl group was 5.17±SE 1.2 lesions compared with 1.86±SE 0.25 lesions in the TC ≤200 mg/dl group.

Contrast-enhancing lesions

The cumulative number of CEL over 2 years was analysed with negative binomial regression. The cumulative number of CEL over 2 years was negatively associated with higher FT4 levels (\( p = 0.008 \)). No significant association was found with TC (\( p = 0.075 \)) or LDL-C (\( p = 0.11 \)). Figure 1 summarises the dependence of cumulative CEL on FT4 level. Because all of the patients had normal FT4, the data were dichotomised for graphical summary based on the highest quartile of FT4.

Table 1  Demographic and clinical characteristics of the cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females:males (% female)</td>
<td>85.50 (63%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>28.2±7.8</td>
</tr>
<tr>
<td>Monosymptomatic onset</td>
<td>118/135 (87%)</td>
</tr>
<tr>
<td>Median EDSS (IQR) at baseline</td>
<td>1.50 (0.50)</td>
</tr>
<tr>
<td>Number of CEL at baseline</td>
<td>0.77±2.3</td>
</tr>
<tr>
<td>Number of T2-lesions at baseline</td>
<td>11.7±8.4</td>
</tr>
<tr>
<td>T2-lesions volume at baseline, cm³</td>
<td>4.9±5.6</td>
</tr>
<tr>
<td>Time between lipid profile and baseline visit, days</td>
<td>−49±19</td>
</tr>
<tr>
<td>Time between onset date and lipid profile, days</td>
<td>32±25</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.2±3.7</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>55.1±15</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>107±27</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>182±36</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>108±58</td>
</tr>
<tr>
<td>Total cholesterol to HDL ratio</td>
<td>3.46±0.84</td>
</tr>
<tr>
<td>Free thyroxine, pM</td>
<td>16.0±2.1</td>
</tr>
<tr>
<td>TSH, mIU/l</td>
<td>2.20±1.4</td>
</tr>
</tbody>
</table>

The continuous variables expressed as mean±SD and the categorical variable as frequency (%).

CEL, contrast-enhancing lesion; HDL, high density lipoprotein; LDL, low density lipoprotein; TSH, thyroid stimulating hormone.

Table 2  Clinical and MRI outcomes at baseline and at 2 years

<table>
<thead>
<tr>
<th>Clinical or MRI characteristic</th>
<th>Baseline</th>
<th>2 years</th>
<th>( p ) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median EDSS (IQR)</td>
<td>1.50 (0.50)</td>
<td>1.50 (1.0)</td>
<td>0.012</td>
</tr>
<tr>
<td>Clinically definite MS</td>
<td></td>
<td>57/135 (42%)</td>
<td></td>
</tr>
<tr>
<td>Number of subjects with ≥1 relapses</td>
<td>–</td>
<td>57/135 (42%)</td>
<td></td>
</tr>
<tr>
<td>Total number of relapses over 2 years</td>
<td>–</td>
<td>0.92±1.4</td>
<td></td>
</tr>
<tr>
<td>Annual relapse rate</td>
<td>–</td>
<td>0.46±0.67</td>
<td></td>
</tr>
<tr>
<td>Median time to first relapse, months</td>
<td>–</td>
<td>5.7±7.9</td>
<td></td>
</tr>
<tr>
<td>Contrast-enhancing lesion number</td>
<td>0.77±2.3</td>
<td>0.70±3.9</td>
<td>0.21</td>
</tr>
<tr>
<td>Contrast-enhancing lesion volume, cm³</td>
<td>0.068±0.24</td>
<td>0.080±0.53</td>
<td>0.27</td>
</tr>
<tr>
<td>T2-LV, cm³</td>
<td>4.9±5.6</td>
<td>4.9±7.6</td>
<td>0.022</td>
</tr>
<tr>
<td>Normalised WBV, cm³</td>
<td>1504±71</td>
<td>1486±71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normalised WMV, cm³</td>
<td>712±37</td>
<td>706±37</td>
<td>0.004</td>
</tr>
<tr>
<td>Normalised GMV, cm³</td>
<td>792±47</td>
<td>779±48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cumulative contrast-enhancing lesion number</td>
<td>–</td>
<td>11.4±4.7</td>
<td></td>
</tr>
<tr>
<td>Cumulative number of new T2 lesions</td>
<td>–</td>
<td>3.8±9.0</td>
<td></td>
</tr>
<tr>
<td>Cumulative number of new/enlarging lesions</td>
<td>–</td>
<td>5.3±13</td>
<td></td>
</tr>
<tr>
<td>Change in brain volume, %</td>
<td>–</td>
<td>−1.26±1.3</td>
<td></td>
</tr>
<tr>
<td>Change in grey matter volume, %</td>
<td>–</td>
<td>−1.59±2.2</td>
<td></td>
</tr>
</tbody>
</table>

The MRI outcomes are shown as mean±SD.

*Non-parametric Wilcoxon test.

1For the subset with ≥1 relapses.

GMV, grey matter volume; LV, lesion volume; MS, multiple sclerosis; WBV, whole brain volume; WMV, white matter volume.

A small proportion of patients developed disability during the CDMS in the treatment arm compared with placebo. Even 2 years despite decreases in relapse rate and conversion to associated as trends with higher LDL-C (LDL-C >100 mg/dl status). Likewise, in interferon β-treatment trials of CIS, only a small proportion of patients developed disability during the first 2 years despite decreases in relapse rate and conversion to CDMS in the treatment arm compared with placebo. Even after 5 years, relatively few patients had developed significant disability compared to the interferon β-1a therapy. In our earlier report, higher TC and LDL-C were associated with lower WBV (p=−0.15, p=0.033 for TC; trend for LDL-C p=−0.11, p=0.14) in patients with MS. Our results here for baseline WBV are concordant with adverse associations of higher TC and of LDL-C.

Notably, we did not find significant MRI associations with HDL-C. However, there is heterogeneity within the HDL compartment. The smallest HDL diameter particles are less mature and less capable of facilitating the reverse cholesterol transport and of supporting the antioxidant activity of paraoxonases. The cholesterol efflux, antioxidant and anti-inflammatory properties of HDL are interdependent and influenced by the maturation and structure of HDL. The smallest diameter HDL particles increase cardiovascular disease risk whereas the larger HDL particles decrease risk. A more detailed characterisation of the HDL lipoproteins and HDL particles may provide a perspective on their role in MS progression.

In a small study of 18 patients with CIS, Giubilei et al found associations between TC levels and CEL in CIS. Interestingly Morra et al18 who longitudinally followed 253 patients with MS treated for 1 year with different interferon-β products found a modest but significant decrease in TC at 1 year. We did not assess follow-up lipid profile in this study. Small studies have suggested that interferon-β treatment can potentially change triglyceride levels and lipoprotein A1 levels. We did not include triglyceride levels in our analyses because our lipid profiles were obtained under non-fasting conditions.

The possibility that non-MS lesions, particularly ischaemic lesions, may accumulate in patients with hypercholesterolaemia cannot be formally excluded. The considerations that reduce the likelihood of lesions of ischaemic aetiology are: (1) the young age of patients and (2) absence of comorbidities, for example, hypertension, heart disease and diabetes, that are risk factors for ischaemic lesions. In general, conventional MRI techniques are not able to distinguish between demyelinating and ischaemic lesions, and to the best of our knowledge no studies have reported prevalence of ischaemic lesions to be location-related in patients with MS. Non-conventional MRI techniques, like diffusion-weighted imaging and tensor imaging would be more appropriate for investigating underlying pathological substrate differences. For example, Tallantyre et al24 showed that high-resolution images from ultra high-field (7 Tesla) MRI may be helpful for distinguishing MS lesions from non-MS lesions. Periventricular location was characteristic of MS and CIS lesions—>80% of MS lesions, but not non-MS white matter lesions, had a central vein. Periventricular location was more predictive than subcortical and periventricular lesion location at distinguishing MS versus non-MS lesions.

We included FT4 and TSH in our study because thyroid dysfunction is associated with dyslipidaemic features and hypothyroidism is frequent in patients with MS. However, only 3/135 (2.2%) of our patients were on thyroid supplementation for hypothyroidism; all three had normal FT4 and TSH levels. We were surprised to find associations between FT4 levels and MRI measures of lesion formation. Evidence from the rat cuprizone-induced demyelination model29 and the mar-moset model of experimental allergic encephalomyelitis30 indicates that thyroid hormone can promote remyelination. Thyroid hormone receptor and triiodothyronine have been implicated in facilitating the differentiation of progenitor cells to oligodendrocytes. The implications of these interesting findings for therapy are not clear but require further study.

We included VD because of the known immunomodulatory effects of its metabolites and because VD sufficiency is associated with interferon effect on relapse rate. Biochemically, the cholesterol and VD pathways are linked at 7-dehydrocholesterol, which is the key precursor for endogenous VD production in the skin. Multiple epidemiological studies have found associations between serum cholesterol levels and VD levels in different populations and in vitro studies suggest that higher VD levels can decrease cholesterol synthesis.
In this report, we also confirmed and extended to patients with CIS, our previous findings from patients with MS, that low HDL cholesterol levels are associated with low 25(OH)VD₃ levels. We also found that HDL cholesterol levels were associated with the active 1,25(OH)₂VD₃ metabolite. A limitation is that VD deficiency was frequent in our sample.

None of our patients were on statins, a potent class of drugs that inhibit cholesterol biosynthesis. Statin treatment trials in MS have yielded mixed results. In a study of 30 patients with MS, statin treatment significantly decreased CEL on monthly MRI. The SIMCOMBIN trial and post hoc analysis of the interferon β treated control arm of the SENTINEL study did not support a statin effect in patients with MS on interferon β. The STAYCIS trial of atorvastatin in CIS did not meet its primary endpoint. A mechanistic understanding of the cholesterol pathway may be relevant for evaluating other lipid-modulating drugs for MS in the future.

The primary weakness of the study is its limited sample size. We could not use all of the subjects from the SET study because lipid profiles were not obtained during the screening or baseline visit at the other centers. However, we expect that the positive findings from this study could provide the rationale and impetus for detailed mechanistic studies of the cholesterol pathway in CIS and for systematic evaluation of archived samples from completed randomised, placebo-controlled drug trials in CIS. Such studies in other populations and with other approved treatments for CIS can help evaluate the generalisability of the findings.

In conclusion, we have demonstrated that the lipid profile can adversely affect MRI lesion activity in relatively young, high-risk patients with CIS who are experiencing their first demyelinating event. Early intervention with dietary, exercise and lifestyle modifications shown to reduce cholesterol may be useful for managing MS progression but require further study.

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Contributors
DH and EH—Study concept and design, manuscript preparation. RZ—MRI data analysis, manuscript preparation. BW-G—Data interpretation, manuscript preparation. GS, EL and KD—Data analysis. JO, MT-B, DB, MT, NB, SH, LW, JK, MV, and ZS—Data acquisition. MR—Study concept and design, data analysis, manuscript preparation.

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Competing interests
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REFERENCES
Multiple sclerosis


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