Complement regulator factor H as a serum biomarker of multiple sclerosis disease state

Gillian Ingram,1,* Svetlana Hakobyan,2,* Claire L. Hirst,1 Claire L. Harris,2 Trevor P. Pickersgill,3 Mark D. Cosburn,1 Sam Loveless,2 Neil P. Robertson1,* and Bryan Paul Morgan2,*

1 Department of Neurosciences, Cardiff University, Cardiff CF14 4XN, UK
2 Department of Infection, Immunity and Biochemistry, Cardiff University, Cardiff CF14 4XN, UK
3 Department of Neurology, Helen Durham Neuro-inflammatory Unit, University Hospital of Wales, Heath Park, Cardiff CF14 4XW, UK

*These authors contributed equally to this work.

Correspondence to: Bryan Paul Morgan,
Department of Infection,
Immunity and Biochemistry,
School of Medicine, Cardiff University,
Heath Park, Cardiff, CF14 4XN, UK
E-mail: morganbp@cardiff.ac.uk

Multiple sclerosis has a variable phenotypic presentation and subsequent disease course that, although unpredictable at disease onset, is of crucial importance in guiding interventions. Effective and accessible biomarkers are required in order to stratify patients and inform treatment. We examined whether the complement regulator factor H and its Tyr402His polymorphism, recently implicated as biomarkers in other chronic inflammatory central nervous system conditions, might identify or predict specific pathological processes and outcomes in multiple sclerosis. Employing novel assays, we measured factor H and its His402 variant in serum from 350 patients with multiple sclerosis classified according to disease course and relapse status. Serum factor H levels were significantly higher in progressive disease (P < 0.001) compared to controls and relapsing patients, after controlling for variables including disease duration, age, gender, disability and treatment. Serum factor H levels were capable of distinguishing secondary progressive from relapsing remitting disease (excluding patients in clinical relapse) with a sensitivity of 89.41%, specificity of 69.47% and a positive predictive value of 72.38%. Acute relapse was also associated with transiently increased factor H levels (P = 0.009) compared to stable relapsing disease. In clinically stable patients, factor H levels remained constant over 1 year (coefficient of variation percentage = 6.8), however, in patients in transition from relapsing to progressive disease, factor H levels significantly increased over a period of 2 years (P = 0.007). Concentration of the His402 variant in heterozygotes was significantly higher in secondary progressive (P < 0.01) and primary progressive (P < 0.05) disease, suggesting altered expression or consumption of variants when factor H is upregulated. Serum factor H may be an effective indicator of progression and a practical and accessible biomarker and stratifying tool in determining disease course, providing objective evidence to help guide therapeutic decisions.

Keywords: factor H; complement; multiple sclerosis; biomarker; alternative pathway

Abbreviations: EDSS = expanded disability status scale; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis

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Introduction

Multiple sclerosis, the most common cause of chronic neurological disability in young adults, has a variable phenotypic presentation and subsequent disease course that cannot be predicted at disease onset. Current therapeutic interventions may control the early inflammatory component of disease, but have limited efficacy in progressive disease. Patient-specific disease phase is difficult to determine clinically and, as a consequence, some treatments can be applied inappropriately. A combination of phenotypic subtyping and laboratory investigations of serological and genetic biomarkers has already led to improved diagnosis and targeted therapy for some subtypes of CNS demyelinating disease previously encompassed within the syndromic diagnosis of multiple sclerosis, including neuromyelitis optica (Lennon et al., 2004; Wingerchuk et al., 2006) and Leber’s hereditary optic neuropathy (Taylor et al., 2003). In addition, several potential biomarkers have been suggested as aids for determining disease course or response to treatment in multiple sclerosis, but their clinical usefulness has not yet been clearly established (Berger and Reindl, 2007; Lutterotti et al., 2007), and currently there are no readily accessible clinical or biological markers sensitive or specific enough to guide patient management reliably.

Pathological and functional studies have established the role of complement in the pathology of multiple sclerosis (Ingram et al., 2009) and as a result, a number of complement proteins have been investigated as potential biomarkers of disease activity. The majority of these studies have been limited to examination of individual components (C3, C4, C9) or activation products (terminal complement complex) in multiple sclerosis serum and CSF, and so far results have been conflicting (Link, 1972; Jans et al., 1984; Morgan et al., 1984; Compston et al., 1986; Sanders et al., 1986; Mollnes et al., 1987; Sellebjerg et al., 1998; Jongen et al., 2000; Barnum and Szalai, 2005). The reasons for the inconsistent results in these studies are unclear but may have been a consequence of assay variation, sample collection, low patient number or lack of comprehensive clinical phenotypic data. This last factor is likely to be important in multiple sclerosis, where clinical status will have considerable effects on complement levels. More recently, proteomic analysis has identified changes in levels of specific complement proteins (factor I, C3, clusterin) in CSF samples from patients with multiple sclerosis compared to controls (Rithidech et al., 2009; Stoop et al., 2009). One such study identified significant reduction in CSF levels of an unspecified isoform of factor B and factor H-like protein 1 (an alternative splice product of the factor H gene) (Finehout et al., 2005), and suggested that further detailed examination of these complement protein isoforms could be informative in the understanding of the pathological process of multiple sclerosis.

Complement factor H is a single chain serum glycoprotein that regulates the formation and function of complement C3 and C5 convertase enzymes. Regulatory activity is attributed to its ability to recognize and bind C3b fragments (Zipfel et al., 1999). Factor H is synthesized in the liver and has a normal serum concentration of about 250 mg/l; of note, many papers quote higher serum levels of factor H in normal individuals; we have previously described the optimization of the assays and standards for factor H on which our normal range is based (Hakobyan et al., 2008). Changes in plasma factor H levels have been linked to atherosclerosis (Oksjoki et al., 2003; Goverdhan et al., 2006) and Alzheimer’s disease (Honda et al., 2000; Hye et al., 2006). In Alzheimer’s disease, plasma factor H levels, assessed semi-quantitatively, were elevated in disease and shown to reflect risk of disease progression in early disease (Thambisetty et al., 2008). The most common polymorphism in factor H, Tyr402His (allele frequency 62:38 in Caucasians (HapMap)), has been associated with increased risk for age-related macular degeneration, homozygosity for the His allele conferring a 6-fold increased risk (Edwards et al., 2005; Sjoberg et al., 2007). In Alzheimer’s disease, one large study found no association with this polymorphism (Hamiton et al., 2007), while another found an association of the His402 allele with disease, but only in those also carrying the ApoE4 risk allele (Zetterberg et al., 2008). Factor H appears to discriminate self from non-self by recognizing polyanionic structures on self cells, such as sialic acid and the glycosaminoglycan chains of proteoglycans (e.g. heparin sulphate and dermatan sulphate), thus inhibiting complement activation on host surfaces (Kazatchkine et al., 1979; Carreno et al., 1989). The Tyr402His polymorphism located within short consensus repeat (SCR) 7, a putative binding site for glycosaminoglycans (Skerka et al., 2007), may influence surface binding. Factor H and its polymorphic variants have not been measured in multiple sclerosis serum or CSF samples to date.

We set out to test the hypothesis that, as in some other inflammatory and degenerative CNS diseases, serum factor H levels would reflect chronic inflammation in multiple sclerosis and therefore help define disease state; we also speculated that differential expression and/or consumption of the factor H Tyr402His polymorphic variants would further inform risk of disease progression and other disease characteristics in multiple sclerosis. To achieve this, we have developed optimized methods to interrogate factor H levels and polymorphisms using novel monoclonal antibodies and unique enzyme-linked immunosorbent assays (ELISAs) that enable the quantification of total factor H and the Tyr402His polymorphic variants in plasma, serum or CSF (Hakobyan et al., 2008). Here, we have employed these unique ELISAs to measure factor H and the Tyr402His polymorphic variants in subgroups of patients with multiple sclerosis with comprehensive clinical phenotypic data.

Methods

Subjects

Serum samples were prospectively obtained between 2006 and 2008, from 350 patients with clinically definite multiple sclerosis (McDonald et al., 2001), including 212 patients with relapsing remitting multiple sclerosis (RRMS) (97 with no clinically evident relapse for at least 12 months and 115 in acute relapse), 85 patients with secondary progressive multiple sclerosis (SPMS) and 53 with primary progressive multiple sclerosis (PPMS). Serial samples were available on 11 patients with clinically stable relapsing remitting disease and 12 patients who were in transition from relapsing remitting to secondary progressive disease. Patients with stable relapsing remitting disease were sampled over a limited period of 1 year so as to ensure disease stability, whereas...
transitional patients were sampled over 2 years so that samples were available in both relapsing remitting and secondary progressive disease phases. Parallel information was recorded consisting of disease course, relapse status, disability [measured by the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983)], co-morbidity, inter-current infection and medications. The Multiple Sclerosis Severity Score was calculated from EDSS and disease duration (Roxburgh et al., 2005). The control group comprised 86 non-related age-matched subjects with no personal or family history of neurological disease. A replication study was performed on an additional 105 patients including 70 patients with RRMS (35 of whom were in acute relapse) and 35 with SPMS, and 40 controls.

Demographic details of cases and controls are outlined in Table 1 and are broadly representative of the population-based characteristics recently reported for this region of the UK (Hirst et al., 2008). No patient reported significant co-morbidity. Mean time to SPMS from disease onset was 10.14 years (SD 9.40), and mean duration of progressive phase disease in patients with SPMS was 8.79 years (SD 6.84). Of the 115 patients in acute relapse (defined according to Schumaker et al., 1965), 72.2% were treated with glucocorticosteroids, according to the clinical judgement of the assessing neurologist, after blood was sampled. No patients were on glucocorticosteroid treatment at the time of sampling and there were no coincidental infections at the time of relapse. Of the patients, 27.2% were on some form of disease modifying treatment including 45.4% in the RRMS-remission group, 23.4% of RRMS-relapse group and 25.9% of patients with SPMS. Disease modifying treatments included Rebif (5.3%), Betaferon (5.3%), Avonex (5.0%), Copaxone (0.8%), alemtuzumab (6.1%) and mitoxantrone (4.7%).

CSF was obtained with paired serum samples from 44 patients who had been admitted for investigation of suspected or known multiple sclerosis. Of these, 22 patients were subsequently found to have symptoms not related to demyelinating disease; these samples were used as controls. Subsequent neurological diagnosis within the control group included cervical myelopathy in five patients, cerebrovascular disease in two patients, trigeminal neuralgia in two patients, fibromyalgia in five patients with RRMS, four patients with RRMS in acute relapse and three patients with progressive disease.

Ethical approval was gained from South East Wales Ethics committee (ref no.05/WSE03/112 and ref no. 09/MRE09/35).

Samples and analysis

Serum samples were separated (2000 g/10 min) within 3 h of collection and stored in aliquots at −80°C until analysed. CSF was taken in line with guidelines from Teunissen et al. (2009), atraumatically from vertebral body L3–5. Bloody samples were discarded and samples were centrifuged (2000 g/10 min) within 30 min of collection before being aliquoted and frozen at −80°C until use.

We have recently described assays for quantification of total factor H and the factor H-His402 polymorphic variant (Hakobyan et al., 2008). In brief, microtitre plates (96-well Nunc MaxiSorp, Life Technologies, Paisley, UK) were coated with affinity-purified rabbit anti-factor H IgG diluted in bicarbonate coating buffer (pH 9.6) at 5 μg/well and incubated. After blocking with 1% bovine serum albumin in phosphate buffered saline, standards, serum (1:6000 in phosphate buffered saline/bovine serum albumin) or CSF (1:10 in phosphate buffered saline/bovine serum albumin) samples were added in triplicate and incubated. Plates were washed three times in phosphate buffered saline/0.1% Tween-20 (PBS/T) before either horseradish peroxidase-labelled affinity-purified rabbit anti-human factor H (100 μl; 1 mg/l) or horseradish peroxidase-labelled MBI-7 anti-factor H-His402 (100 μl; 1 mg/l) was used to measure total factor H or factor H-His402, respectively. After incubation and washing (three times with PBS/T with 1 min incubation on the last wash), bound antibody was detected using orthophenylenediamine (AbD Serotec, Martinsried, Germany). Development was stopped by the addition of 10% sulphuric acid, and absorbance at 492 nm was measured. All incubation steps were performed motionless for 1 h at 37°C. Purified factor H-His402 and an equimolar mixture of both variants were used as standards for estimation of serum and CSF factor H-His402 and total factor H, respectively. Concentrations of total factor H and factor H-His402 in serum and CSF were calculated by reference to the appropriate calibration curve prepared from the standards and expressed as mg/l of serum or CSF. Concentration of factor H-Tyr402 polymorphic variant was calculated by subtraction of factor H-His402 from total factor H concentration. The calculated detection limit of the assay was 0.007 mg/l and the working range 0.01–0.2 mg/l. The assay performance was assessed by taking multiple measures from independently diluted aliquots of the same plasma samples, either within the same assay or in separate assays. The within-assay precision ranged from 4.1 to 7.0% with an average of 5.5% for total factor H measurement and from 7.7% to 12.8% with an average of 11.0% for factor H-H402 measurement. Between-assay precision ranged from 4.9% to 10.1% with an average of 8.0% for total factor H measurement and from 10.1% to 15.8% with an average of 12.5% for factor H-H402 measurement. Serial dilutions of standard, serum and CSF were measured to demonstrate parallelism (Supplementary Fig. 1).

Table 1 Demographic details and serum factor H concentration of multiple sclerosis disease subgroups compared to healthy control subjects

<table>
<thead>
<tr>
<th>Number</th>
<th>Age Mean (SD)</th>
<th>Gender</th>
<th>Disease duration Mean (SD)</th>
<th>EDSS Mean (SD)</th>
<th>Factor H concentration (mg/l) Mean (SD)</th>
<th><em>P</em></th>
<th>P**</th>
<th>%</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RRMS</td>
<td>86</td>
<td>42.26</td>
<td>16.50</td>
<td>62.9</td>
<td></td>
<td>209.54</td>
<td>61.29</td>
<td>NA</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Remission</td>
<td>97</td>
<td>39.11</td>
<td>8.95</td>
<td>72.16</td>
<td>9.79 (7.16)</td>
<td>2.97</td>
<td>1.38</td>
<td>225.29 (57.96)</td>
<td>0.091</td>
</tr>
<tr>
<td>Relapse</td>
<td>115</td>
<td>36.27</td>
<td>8.44</td>
<td>80.00</td>
<td>7.97 (6.63)</td>
<td>4.55</td>
<td>1.64</td>
<td>248.19 (68.34)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SPMS</td>
<td>85</td>
<td>49.99</td>
<td>10.13</td>
<td>62.35</td>
<td>18.74 (11.18)</td>
<td>6.26</td>
<td>1.27</td>
<td>295.71 (51.17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PPM</td>
<td>53</td>
<td>53.51</td>
<td>11.34</td>
<td>50.94</td>
<td>10.25 (7.38)</td>
<td>5.74</td>
<td>1.70</td>
<td>280.58 (74.83)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*P = comparison with controls. **P = comparison with ‘RRMS relapse’. Factor H test is the percentage of patients with factor H levels above the arbitrary cut-off taken as the upper 95% CI of the RRMS-remission group. PPMs = primary progressive disease course; Remission = no relapses reported for at least 12 months prior to sampling; Relapse = patients in acute relapse at time of sampling before treatment with glucocorticosteroids. Of note, age, disease duration and EDSS are, as expected, higher in progressive patients; however, when accounted for as covariates, did not significantly alter the differences in factor H between disease subgroups.
CSF and paired serum samples were analysed in the routine laboratory for IgG, albumin and oligoclonal bands. IgG and albumin were measured on the BN 11 nephelometer and oligoclonal bands on the Sebia Hydrasys using Hydrogel 9 CSF iso-electric focusing. Reference ranges for normal values were: serum IgG 5.2–15.5 g/l, CSF IgG <58 mg/l, serum albumin 35–50 g/l, CSF albumin 160–360 mg/l, albumin/IgG index 0.3–0.8.

Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences version 16 (SPSS inc., Chicago, IL, USA). A pilot study (Supplementary Table 1) demonstrated that a sample size of 35 gave a calculated power of 0.997 with 5% precision; pilot data were included in the main study. All data were normally distributed and mean serum factor H levels were compared in disease subgroups using one-way ANOVA. The effect of demographic and clinical variables (age, disease duration, EDSS, Multiple Sclerosis Severity Score) on factor H levels were determined individually using Pearson’s correlation coefficient and then modelled using multivariate regression analysis to assess any dominant effect (data were normally distributed). The effect of gender and treatment with disease modifying drugs was examined in individual subgroups using a Students t-test. The effect of disease course on factor H was re-analysed accounting for variables using a univariate linear model. Where sample sizes are limited we present median values, interquartile ranges and use non-parametric analysis.

Results

Serum factor H levels reflect disease course in multiple sclerosis

Serum factor H levels were significantly elevated in both primary and secondary progressive disease compared to controls and patients with RRMS (P < 0.001 for each) (Fig. 1). Within the RRMS group, factor H levels in remission were higher than controls although this was not significant (P = 0.09). Relapse was associated with a small but significant increase in factor H concentration compared to controls (P < 0.001) and stable patients with relapsing disease (P = 0.009). Analysis of the data demonstrated that serum factor H concentration had value as a surrogate marker of disease course in patients with multiple sclerosis, in particular in distinguishing SPMS from RRMS; sensitivity for this distinction was 89.41%, with specificity of 69.47% and a positive predictive value of 72.38% (test cut-off value >237 mg/l) (Table 1). When patients in clinical relapse were included, sensitivity and specificity were reduced to 71.18% and 62.40%, respectively, with a positive predictive value of 59.48%. Serum factor H concentration was less helpful in distinguishing stable RRMS from patients in relapse, with a sensitivity of 67.37%; specificity 48.00% and positive predictive value 49.61% (cut-off value <233 mg/l).

To test whether factor H levels fluctuated independent of disease state, concentrations of factor H were measured in 33 samples from 11 clinically stable patients (from the RRMS group) venuexcised sequentially over a 12 month period, during which time they were deemed by the assessing neurologist to have clinically stable disease based on lack of reported relapses and lack of change in EDSS. These sequential samples showed little variation in factor H concentrations with an average coefficient of variation (SD/mean) of 6.8% (similar to the documented inter-assay coefficient of variation or 8%), suggesting both reproducibility of the assay and stable serum factor H concentration over a protracted time course in patients with clinically stable disease (median factor H levels initially were 199.35 mg/l and after 1 year were 193.42 mg/l) (Fig. 2).
An in-house replication study was performed in an additional 105 patients and 40 controls which confirmed a significant difference between controls and SPMS, mean difference 131.58 mg/l (95% confidence interval 100.28–162.88; \( P < 0.001 \)); and between RRMS remission and SPMS, mean difference 80.90 mg/l (95% confidence interval 48.57–113.23; \( P < 0.001 \)) (Supplementary Table 2).

Serum factor H levels increase with disease progression in multiple sclerosis patients

To investigate the dynamic changes in serum factor H levels during the transition from relapsing to progressive phase disease further, 12 patients were selected in whom three serum samples were available over a 2-year period from onset of progression. In this small cohort, factor H levels increased over the two year period in 10 of the 12 patients by a median of 59.76 mg/l, with median factor H concentrations of 206.64 mg/l (interquartile range 181.3–220.2) in the first year, 232.89 mg/l (interquartile range 207.4–262.1) in the second year and 266.70 mg/l (interquartile range 227.4–312.0) in the final year for the whole group (Fig. 2). Non-parametric testing showed that these changes in factor H levels over the 2-year period were highly significant \( (P = 0.007) \) and factor H levels at the endpoint were significantly higher compared to the RRMS group from the main study \( (P = 0.01) \). Of note, using the test cut-off for SPMS suggested previously (test cut-off value >237 mg/l), 9 of the 12 patients had a positive test at Year 2. Of the 12 patients, 9 were on disease modifying treatments throughout the assessed time; this did not affect the outcome.

Serum factor H levels do not reflect the nature of the lesion in multiple sclerosis relapse

We tested whether relapse clinical characteristics influenced factor H levels. Of the 115 patients in relapse, 29 had a brainstem event, 56 had a spinal or cortical event, 7 had optic neuritis and 23 had a pure sensory event as judged by history, examination and clinical criteria; factor H levels were similar in these four relapse groups [mean factor H 246.51 mg/l (SD 76.51) brainstem, 247.50 mg/l (SD 65.38) spinal or cortical, 256.61 mg/l (SD 81.06) optic neuritis, 236.94 mg/l (SD 71.90) sensory, \( P = 0.807 \)]. In the whole relapse group, mean number of relapses over a 4-year period was 3.53 (SD 2.16) and the mean interval between relapses
was 8.75 months (SD 6.09); neither number of relapses nor relapse interval correlated with factor H levels (data not shown). Mean EDSS in the relapse group was 4.55 (SD 1.64) and Multiple Sclerosis Severity Score was 6.59 (SD 2.31); neither of these parameters correlated with factor H levels (data not shown).

**Serum factor H levels reflect disease activity independent of other patient factors**

Correlation of factor H concentrations with other phenotypic characteristics in the population was examined and revealed positive correlation with age ($r=0.31$, $P<0.001$), disease duration ($r=0.16$, $P=0.003$), EDSS ($r=0.20$, $P<0.001$) and Multiple Sclerosis Severity Score ($r=0.14$, $P=0.010$); however, in a multivariate regression model, only age remained significant. Disease modifying treatment had no effect on factor H levels in RRMS relapse or SPMS groups; however, in the RRMS-remission group, patients on treatment had significantly lower factor H levels than those off treatment ($P<0.001$) (Supplementary Table 3). We re-analysed the effect of disease course on factor H when adjusted for the covariates above, with no significant changes to the results except patients in RRMS who, when accounting for treatment, were not different from patients in relapse (Supplementary Table 4). No independent effect from gender was observed (mean difference in factor H between males and females was 1.75 mg/l). Serum factor H concentrations failed to show any additional correlation in the SPMS group with time from disease onset to start of progression ($r=0.08$, $P=0.47$) or duration of progressive disease ($r=0.12$, $P=0.32$). These latter findings demonstrate that serum factor H levels predominantly reflect the prevailing disease course at the time of sampling.

**CSF factor H levels correlate with serum factor H and blood–CSF barrier breakdown**

It was possible to measure factor H in CSF using the assays described, although concentrations were <1% of serum; demographic details of patients and controls are shown in Table 2. CSF factor H levels were higher in patients with multiple sclerosis compared to controls; however, these differences did not reach significance (Table 3). Of note, the CSF:serum factor H ratio was significantly higher in multiple sclerosis patients when compared to controls (Table 3) suggesting that CSF factor H levels were increased in multiple sclerosis as a result either of blood–CSF barrier leak or intra-thecal synthesis. CSF:serum albumin ratio has been suggested as the most appropriate variable to establish the presence of blood–CSF barrier breakdown (Link and Tibbling, 1977; Tibbling et al., 1977; Eeg-Olofsson et al., 1981). In disease cases the CSF:serum factor H ratio was strongly correlated with CSF:serum albumin ratio (Pearson’s correlation = 0.83, $P<0.001$). Patients with blood–CSF barrier leak (demonstrated in five cases with a raised CSF:serum albumin ratio) had significantly higher factor H levels than patients with a normal CSF:serum albumin ratio (median factor H 1.17 mg/l compared to 0.68 mg/l, $P=0.02$). An abnormally high IgG:albumin index has been shown to reflect intra-thecal IgG synthesis (Link and Tibbling, 1977; Eeg-Olofsson et al., 1981) and in our patients, normal values were exceeded in 17 cases including 4 of the 5 relapse cases. However, CSF factor H levels were not significantly raised in these cases (median factor H 0.80 mg/l versus 0.65 mg/l, $P=0.337$) and there was no related evidence of intra-thecal factor H synthesis measured by a factor H:albumin Index ($P=0.151$) (Table 3). These data indicate that raised factor H CSF concentrations are predominantly due to influx of peripheral factor H at times of blood–CSF barrier breakdown, rather than central synthesis.

**Tyr402His allele frequency is identical in patients with multiple sclerosis and controls**

The Tyr402His polymorphic status was determined for each sample based on the quantification of total factor H and the factor H-His402 variant. Out of 350 patients with multiple sclerosis, 43 were homozygous for the His allele of the Tyr402His polymorphism, 175 were heterozygous and 132 were homozygous for Tyr allele, giving a His allele frequency of 37.28%, which was not significantly different from frequencies observed in the control population (37.21%). Of note, similar analyses in age-related macular degeneration populations readily detect at the protein level the over-representation of the His allele anticipated from genetic studies (Hakobyan et al., 2008). The Tyr402His allelic status also had no effect on factor H levels [mean serum factor H 248.40 mg/l (SD 68.55) in factor H-His402 homozygotes, 250.74 mg/l (SD 73.31) in factor H-Tyr402His heterozygotes and 243.84 mg/l (SD 67.04) in factor H-Tyr402His homozygotes], in patients with multiple sclerosis or controls. An unexpected finding in post hoc analysis of the factor H-Tyr402His heterozygote group was that higher concentrations of the factor H-His402 variant compared to factor H-Tyr402 were found in both progressive and relapsing multiple sclerosis but not in controls or multiple sclerosis.

**Table 2 Demographic details and routine CSF results in patients with multiple sclerosis and controls**

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Age Mean (SD)</th>
<th>Gender Female (%)</th>
<th>Disease duration Mean (SD)</th>
<th>EDSS Mean (SD)</th>
<th>OCB positive (%)</th>
<th>Albumin ratio Mean (SD)</th>
<th>Ig index Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22</td>
<td>50.09 (14.11)</td>
<td>77.27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>22</td>
<td>41.68 (12.68)</td>
<td>68.18</td>
<td>7.55 (9.34)</td>
<td>2.6 (2.3)</td>
<td>100</td>
<td>5.12 (2.83)</td>
<td>0.49 (0.05)</td>
</tr>
</tbody>
</table>

OCB = oligoclonal bands; Albumin ratio = CSF albumin (mg/l)/serum albumin (g/l) ratio; Ig index is derived from CSF:serum ratios of IgG and albumin concentrations.
sclerosis in remission, with significantly higher levels in the SPMS (P < 0.01) and primary progressive multiple sclerosis (PPMS; 156.55 mg/l (SD 57.58) versus 132.21 mg/l (SD 45.14); P = 0.05) with higher concentrations also seen in relapsing patients, although this did not reach significance [135.36 mg/l (SD 59.48) versus 109.13 mg/l (SD 58.54); P = 0.06]. There was no difference in variant concentrations observed in the control [120.35 mg/l (SD 58.54)] or RRMS [107.65 mg/l (SD 43.15)] versus 111.44 mg/l (SD 34.15) groups. Data are shown as a dot plot with a horizontal line to denote the mean.

Discussion

The use of serum or plasma as a source of biomarkers in CNS disorders is not novel; indeed, the well-known serum marker, oligoclonal IgG, reflects systemic immune disturbance and is associated with elevated intra-thecal IgG synthesis in multiple sclerosis (Zeman et al., 1996). More recently, serum antibody titre against Epstein-Barr virus nuclear antigen-1 has been suggested to be associated with disease activity and presented as a possible biomarker in multiple sclerosis (Farrell et al., 2009). Nevertheless, it is clear that there is still no disease-specific and/or diagnostic serum biomarker available for multiple sclerosis (Harris and Sadiq, 2009), and most studies search for biomarkers within the CSF with the view that this is more likely to reflect CNS disease. However, CSF has become increasingly difficult to obtain as the necessity for lumbar puncture to make a diagnosis has reduced, and serum markers may exist in multiple sclerosis if there is a systemic component to the disease, or if peripheral changes mimic central disease. In this study, we have shown, in a large cohort of comprehensively clinically characterized patients, that raised serum levels of the complement regulator factor H strongly correlate with disease course, independently of other phenotypic parameters such as age, disability or disease duration. Serum factor H measurement may therefore be a useful non-invasive and simple clinical test for patient stratification, particularly to distinguish SPMS from RRMS, where it has a sensitivity of 89.41% and specificity of 69.47%. With the advent of more aggressive treatments...
Factor H, a serum biomarker for multiple sclerosis

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useful only in the relapsing disease phase, an informative and readily measurable biomarker to distinguish disease subtypes is likely to be useful in guiding patient management.

It may also be that, with further refinements, measurement of factor H levels in patients with multiple sclerosis can be used to predict disease course. Clinical details of patients with outlying factor H levels were reviewed to identify obvious causes or atypical features. Of 29 patients in the stable RRMS group with factor H levels above the 95% CI, 1-year follow-up revealed that eight had a relapse shortly after initial evaluation, and four developed SPMS within a year of evaluation; no other patients in the stable RRMS group underwent relapse or developed SPMS within a year of sampling. Further to this, we have demonstrated that patients with stable relapsing remitting disease have constant serum factor H levels, in contrast to patients developing progressive disease in whom serum factor H levels increased. These data, though preliminary, suggest that elevated factor H levels in clinically perceived stable RRMS may predict risk of relapse and progression and therefore may also be useful in recognizing or predicting breakthrough disease in patients on disease modifying treatments.

Serum factor H is the major fluid-phase regulator of the alternative pathway of complement, increasingly recognized as a critical player in many diseases (Oksjoki et al., 2003; Edwards et al., 2005; Pickering and Cook, 2008; Thambisetty et al., 2008). Changes in factor H levels, as well as being a marker for disease course, also imply altered regulation of activation of the alternative pathway of complement throughout the course of disease and in relation to acute inflammatory events. At initiation of the pathological process, multiple sclerosis is driven by the adaptive immune response, with antigen primed T cells migrating into the CSF where T helper 1 and 17 cells release pro-inflammatory cytokines to mount an inflammatory attack (Gocke et al., 2007; Kebir et al., 2007; McFarland and Martin, 2007). At times of relapse, there is extensive inflammatory infiltration in which both the adaptive and innate immune system are upregulated causing demyelination and oligodendrocyte damage. Increased levels of factor H at times of relapse therefore may be a response to increased complement activation. It has been suggested that as patients move through their disease course into the progressive phase, the innate immune system (including macrophages, dendritic cells, mast cells and complement) plays a more significant role (Weiner, 2008). The alternative pathway is largely responsible for the propagation of inflammation in a range of inflammatory disorders (Brodeur et al., 1991; Oksjoki et al., 2003; Thurman and Holers, 2006; Scholl et al., 2008); and in animal models of multiple sclerosis (experimental autoimmune encephalomyelitis), the alternative pathway contributes to disease process, whereas the roles of the classical and terminal pathways are less clear (Ingram et al., 2009). These lines of evidence lead us to speculate that increased factor H levels in progressive disease reflect chronic activation of the alternative pathway contributing to disease.

Although factor H is present in CSF at levels quantifiable in our assays, the low concentrations and low patient numbers made correlation of concentrations to disease parameters non-informative. Due to ethical restrictions in collection of control specimens, we were not able to collect CSF from non-neurological controls; as a result, the CSF control patients may have had some other forms of neurological disease, such as vascular pathology, which has also been linked to activation of complement. Nevertheless, we have demonstrated raised factor H CSF concentrations in multiple sclerosis corresponding with breakdown of the blood–brain barrier (measured by albumin CSF:serum ratios), thus likely to reflect movement of peripheral factor H into the CNS at times of disease activity.

Analysis of the frequencies of the factor H Tyr402His variants, implicated in other inflammatory diseases, showed no difference in multiple sclerosis patients and controls, indicating that this polymorphism was not linked to disease in the population studied. However, measurement of the individual polymorphic variants in serum from Tyr402His heterozygotes has revealed differences in levels of the factor H-Tyr402 and factor H-His402 variants, with higher levels of the His402 variant in some subgroups of disease. This apparent allelic imbalance was seen only in those patient groups where total factor H levels were also elevated, progressive and relapsing disease, suggesting either that there is selective upregulation of the His402 variant or selective consumption of the Tyr402 variant in disease. The biological significance of this finding is uncertain.

In summary, our work has demonstrated that measurement of serum factor H is a simple, robust, cost-effective and transferable test for distinguishing disease sub-groups in multiple sclerosis which should have important applications in clinical practice. There is now a pressing need for this work to be validated in other cohorts. Elevation of factor H levels in serum and CSF might imply that systemic complement activation is in part responsible for driving pathology in progressive and relapsing disease. To explore this it will be necessary to measure other complement components, activation products and regulators in multiple sclerosis serum or plasma and identify associations with changes in factor H. Complement deposition in multiple sclerosis plaques was described over 30 years ago (Woyciechowska and Brzosko, 1977; Compston et al., 1989) and has recently been confirmed by Lassmann and co-workers (1996, 2000) who have shown that abundant deposition of antibody and complement in a subset of multiple sclerosis patients. Characterization of alternative pathway products, particularly factor H and its isoforms, in multiple sclerosis tissue will clarify the role of the alternative pathway of complement in multiple sclerosis as well as the relationship of factor H to demyelination and axonal damage. Work in other inflammatory disorders has implicated dysregulation of the alternative pathway, linked to genetic polymorphisms in complement components and regulators (Scholl et al., 2008; Hecker et al., 2010). A thorough molecular analysis of the complement system in multiple sclerosis may, in addition to providing new biomarkers, identify contributions of complement dysregulation to specific aspects of the pathology.

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Supplementary material

Supplementary material is available at Brain online.
References


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