Is there a role for serum cystatin C as a biomarker of multiple sclerosis?

Maria Antonietta Isgrò[1], **Carmelo Caldarella**[2], **Donata Scribano**[1], **Luigi Colacicco**[1], **Angela Giannace**[1], **Cecilia Zuppi**[1], **Teresa De Michele**[1]

[1]Laboratorio Analisi I, Department of Laboratory Medicine, and [2]Institute of Nuclear Medicine, Department of Radiological Sciences, Catholic University of the Sacred Heart, Rome

ABSTRACT

Multiple sclerosis (MS) is the most common chronic demyelinating disorder of the central nervous system (CNS). No single clinical feature or diagnostic test is sufficient for the diagnosis of MS and the clinical assessment is very difficult, mainly at the early disease stages. Considering MS as a disorder confined to CNS compartment and related to CNS specific pathogenetic pathways, several studies selectively investigated cerebrospinal fluid (CSF) components to detect predictive/prognostic MS markers. Several molecules, such as CSF 14-3-3 protein, tau protein and cystatin C, have been found dysregulated, even though with discordant results. We analyzed serum and CSF cystatin C concentrations of MS patients, comparing them with results obtained from individuals affected by other neurological diseases. We found no statistical differences between groups in CSF cystatin C, cystatin C difference (Δ_{CystC} = CSF - serum cystatin C) and ratio ($CystC_{ratio}$ = CSF/serum cystatin C). Interestingly, serum cystatin C concentrations of MS patients resulted significantly lower than in control population [0.71 (interquartile range, 0.64-0.84) mg/L vs. 0.80 (0.67-0.93) mg/L, P=0.008], with no gender-related differences. The pathophysiologic explanation of this finding is unclear, although it cannot be excluded that pathologic mechanisms that lead to MS may involve not only the CNS compartment, but also systemic pathogenetic pathways.

INTRODUCTION

The accurate identification of patients with multiple sclerosis (MS), the prototypical inflammatory demyelinating disorder of the central nervous system (CNS), can be challenging, mainly at the time of disease onset. Even with the aid of magnetic resonance imaging (MRI), visual evoked potentials (VEP) and cerebrospinal fluid (CSF) analysis, the diagnosis is still based on clinical criteria. Although recent studies have focused on a likely dysimmune etiology for MS, no single antigen has been specifically associated with the disease, posing difficulties in discovery and validation of reliable serological tests. Moreover, the availability of effective immunomodulatory therapy makes it important to identify biological markers that reliably distinguish MS from other neurological disorders (OND) and detect MS in early disease stages.

Multiple immune cells, neuroglia and neurons have complex interactions with each other in physiologic conditions, but in MS these interactions can vary over time. Thus, the clinical course of MS is variable and unpredictable, and biomarker discovery for this disease poses unique challenges.

Analysis of CSF has several advantages over serum for biomarker discovery in neurological diseases. CSF better reflects local events in the brain as compared with serum; on the other hand, high-abundance serum proteins may mask the low-abundant, low MW proteins that are the likely biomarker candidates. For these reasons, CSF components have mainly been investigated for new MS biomarker discovery. Several molecules have been identified as significantly dysregulated in patients with MS or clinically isolated syndromes, such as CSF 14-3-3 protein, tau protein, cystatin C, a cleavage product of cystatin C and free κ light chains (1-8).

Cystatin C is a nonglycosylated molecule of 120 amino acids formed after removal of a 26-amino acid signal peptide (9). It is produced by all nucleated cells, highly expressed by choroidal and leptomeningeal cells and localized in glial and neuronal cells. Cystatin C is a main physiologic, negative regulator of cysteine peptidases, including cathepsin B, H, K, L and S (10, 11). Thus, any altered activity or concentrations of cystatin C would also result in dysregulation of cathepsin function,

Correspondence to: Maria Antonietta Isgrò, Laboratorio Analisi I, Department of Laboratory Medicine, Catholic University of the Sacred Heart, Largo Agostino Gemelli 8, 00168 Roma. Tel. +390630154222, Fax +390630156783, E-mail mariantoniettaisgro@yahoo.it

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which have been implicated in a variety of effects, including degranulation of cytotoxic lymphocytes (12), and in processing of major histocompatibility class II antigen in monocytes (13). Increased activity of cathepsin B, which has been implicated in the physiologic degradation of myelin basic protein, was shown in MS brain (14).

Cystatin C is present in all human body fluids, but is most abundant in CSF (15). In healthy adults, the concentration of cystatin C is 5 to 6 times higher in CSF than in plasma; thus, cystatin C is classified as a predominantly brain-derived protein in CSF, with a negligible blood-derived fraction (16). An initial study showed that CSF cystatin C concentrations did not significantly differ between MS and non-MS patients, demonstrating that evidently cystatin C measurement could not be used to distinguish MS cases from controls (16). More recently, Hansson et al. (17) found similar results. Other studies consistently demonstrated reduced cystatin C concentrations in CSF samples of MS and other immune-mediated neurological diseases, which probably resulted from consumption by inflammation (18). Similarly, Nakashima et al. found that CSF cystatin C concentrations tended to be lower in patients with MS and neuromyelitis optica (19).

As literature findings about CSF cystatin C in MS patients are contrasting and studies concerning the possible role and association of altered concentrations of cystatin C in serum of individuals affected by MS are lacking, the aim of our study was to investigate the behavior of this molecule not only in CSF, but also in serum samples from MS patients.

MATERIALS AND METHODS

All CSF and serum samples used in this study were obtained from patients undergoing a lumbar puncture and venous withdrawals as part of their diagnostic evaluation, after a written informed consent. The protocol was conducted according to the Helsinki Declaration of 1975, as revised in 1996. We enrolled individuals with definite MS and a remitting-relapsing clinical pattern (n=28, 11 men) diagnosed according to current criteria (20-23). They presented dissemination of lesions in both time and space detected by MRI, oligoclonal IgG bands and/or an elevated IgG index on CSF analysis, abnormal (delayed, but with well-preserved wave form) VEP and clinical findings suggestive for MS. CSF and serum samples from patients with various OND (n=63, 31 men) were used as controls. A diagnosis in each of those patients was defined according to individual disease criteria and included: 11 cases with aspecific findings, 9 polyneuropathies, 6 encephalitis, 5 cervical/thoracic/lumbar herniated discs, 5 myelopathies, 4 cerebrovascular diseases, 4 CNS tumours, 3 mononeuropathies, two degenerative nervous diseases, two cerebellar ataxia, one sarcoidosis, inclusion body myositis, ischemic stroke, paraneoplastic syndrome, miastenia gravis, headache, pernicious vomiting, Wilson disease, hydrocephalus, Creutzfeldt-Jacob disease, polymyositis and psychoneurotic disorder. We excluded patients with acute and chronic renal failure, systemic diseases, cardiovascular disorders, MS patients treated with corticosteroids and β-interferon up to 3 months before, patients aged <18 and >65 years and suffering from conditions predisposing to hemodilution.

All CSF and serum samples were centrifuged immediately after collection, aliquoted and stored at -80 °C. CSF and serum cystatin C were determined with a nephelometric method (N Latex Cystatin C Test kit) on BN II system (Siemens Healthcare Diagnostics).

To verify a possible association with MS of CSF cystatin C values normalized to serum cystatin C ones, cystatin C difference (Δ_{CystC}) and ratio ($CystC_{ratio}$) were calculated for both MS patients and controls according to the following formulas:

Δ_{CystC} = CSF cystatin C – serum cystatin C;

$CystC_{ratio}$ = CSF cystatin C/serum cystatin C.

The presence and degree of blood-brain barrier impairment were assessed in both MS patients and control subjects by calculating the CSF/serum albumin ratio according to the formula: (CSF albumin/serum albumin) x 1000. Values >5.5 suggested the presence of blood-brain barrier disruption.

Statistical analysis was performed using SPSS software (SPSS Inc.). Statistical comparisons were performed using the Mann-Whitney *U* test, since Shapiro-Wilk test demonstrated that data were not normally distributed. The significance threshold was set up at P <0.05.

RESULTS

CSF and serum cystatin C concentrations were first compared in all patients: cystatin C resulted ~3.5-fold higher in CSF than in serum [median 2.39 (interquartile range, 1.79-3.64) mg/L vs. 0.74 (0.66-0.89) mg/L; P <0.001]. CSF and serum cystatin C concentrations, Δ_{CystC} and $CystC_{ratio}$ were then compared in MS patients and OND individuals (Table 1). No statistical differences were found between CSF cystatin C concentrations of MS patients and control subjects. Δ_{CystC} and $CystC_{ratio}$ were also similar in MS patients and controls. Interestingly, serum cystatin C concentrations in MS patients resulted significantly lower than those found in OND subjects [0.71 (0.64-0.84) mg/L vs. 0.80 (0.67-0.93) mg/L; P=0.008], with no evidence of gender-related differences in MS subjects [0.72 (0.64-0.90) mg/L in men vs. 0.71 (0.64-0.83) mg/L in women, respectively; P=0.173]. These data were not affected by altered permeability of the blood-brain barrier, as serum cystatin C concentrations [0.74 (0.60-0.83) mg/L vs. 0.68 (0.64-0.85) mg/L, respectively; P=0.839] were similar in MS patients with (n=9) or without (n=19) blood-brain barrier disruption [relative CSF/serum albumin ratios of 6.54 (6.05-8.44) and 3.80 (3.36-4.75), respectively].

DISCUSSION

MS is the most common chronic demyelinating

Table 1
Cystatin C in multiple sclerosis (MS) patients vs. patients with other neurological disorders (OND)

Parameter	MS median (interquartile range)	OND median (interquartile range)	P value
Serum cystatin C, mg/L	0.71 (0.64-0.84)	0.80 (0.67-0.93)	0.008
CSF cystatin C, mg/L	2.30 (1.81-3.11)	2.51 (1.71-3.79)	0.292
Δ_{CystC}, mg/L	1.60 (1.15-2.37)	1.70 (0.92-2.82)	0.624
$CystC_{ratio}$	3.79 (2.58-4.28)	3.01 (2.33-4.33)	0.565

CSF, cerebrospinal fluid; Δ_{CystC}, CSF-serum cystatin C; $CystC_{ratio}$, CSF/serum cystatin C.

disorder of the CNS. Although many advances have been made in the comprehension of its pathogenesis, the etiology is still unknown. Because no single clinical feature or diagnostic test is sufficient for the diagnosis of MS, diagnostic criteria have included a combination of both clinical and paraclinical evidences. MRI can demonstrate dissemination of lesions in both time and space; abnormality on CSF analysis can provide supportive evidence of the immune and inflammatory nature of lesions, which may be helpful when imaging criteria fall short, when they lack specificity, as in the older patients, or when the clinical presentation is atypical. On the other hand, CSF analysis cannot provide information about dissemination of lesions or events in time or space (20-23).

The role of cystatin C in the pathogenesis of MS is not fully understood. Earlier reports of either undetectable or low concentrations of cystatin C in the CSF of MS patients led to the speculation that the regulation of cysteine protease is impaired in MS and that enhanced activity of cysteine proteases leads to the breakdown of myelin (24). Considering the possible role of cystatin C in the pathogenesis of MS, we tried to investigate the behavior of this molecule also in the serum of these patients as a marker of demyelinating disorder. In our study, we found that CSF cystatin C was similar in MS patients and OND subjects; also Δ_{CystC} and $CystC_{ratio}$ resulted similar in both populations. The lack of concordant results about the possible association of increased/decreased CSF cystatin C concentrations in MS patients in previous studies could arise from the selection of heterogeneous groups of patients (MS patients at different stages of disorder) and controls (individuals affected by OND or, more infrequently, healthy subjects) (5-7, 16, 19). Also the use of different methods (ELISA, nephelometry, mass spectrometry) and/or the lack of preanalytical and analytical harmonization could affect the results, by detecting different parts or cleavage products of the cystatin C molecule (25).

Interestingly, in our study we found significantly decreased concentrations of serum cystatin C in the MS group. The physiopathologic explanation of this observation is unclear, but, on the basis of several literature findings (5, 6, 14, 26, 27), we can formulate two hypotheses: 1) cystatin C could be involved in MS-related systemic pathogenetic pathways leading to a downregulation of synthesis and/or release of the molecule in the circulation; 2) in presence of an ongoing demyelinating process, serum cystatin C could pass through blood-brain barrier to limit consequences of increased activity of cathepsins and its serum concentrations would be consequently reduced.

In conclusion, because of the complex pathogenesis and clinical course of MS, it cannot be excluded that MS physiopathology may involve not only CNS compartment, but it may be the expression of more systemic pathogenetic pathways. In this context, further studies focused on the investigation of new MS biomarkers in both serum and CSF could provide new insights into early pathologic mechanisms affecting the onset and the clinical course of the disease.

CONFLICTS OF INTEREST

None.

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