Gaucher disease type I: associated morbidities and long term efficacy of enzyme replacement therapy

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The plasma level of the macrophage-derived soluble CD163 is increased and positively correlates with severity in Gaucher disease

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Abstract

Recently, soluble CD163 (sCD163) has been identified as a macrophage/monocyte specific plasma protein and increased concentrations have been measured in patients with infection and myeloid leukaemia. In the present study we investigated the levels of sCD163 in patients with Gaucher disease, an inherited lysosomal storage disorder characterized by hepato- and splenomegaly due to excessive accumulation of macrophages. The sCD163 plasma levels, median (25-75 percentiles), were far above the levels in normal subjects (7.1 mg/l (4.8 – 10.3) vs. 1.9 mg/l (1.5 – 2.4), p< 0.0001). After initiation of enzyme supplementation therapy, the sCD163 levels were significantly reduced (4.7 mg/l (3.2 – 6.6), p=0.0004). sCD163 correlated with disease severity (rho=0.43, p<0.0061) and Chitotriosidase activity (rho=0.71, p>0.0001). This study further establishes that sCD163 may be a valuable laboratory parameter in monitoring disease with increased macrophage activity.
Introduction

The recently identified macrophage haemoglobin-scavenger receptor CD163 is restricted exclusively to cells of the monocyte-macrophage lineage\(^1,2\). CD163 is a member of the group B scavenger receptor cysteine-rich (SRCR) family and consists of nine extra-cellular SRCR-domains, a transmembrane segment and a short cytoplasmic tail\(^3,4\). The expression of CD163 is down-regulated by proinflammatory mediators (lipopolysaccharide, interferon-\(\gamma\), tumour necrosis factor-\(\alpha\)), whereas interleukin-6, dexamethasone and the anti-inflammatory interleukin-10 strongly upregulate CD163\(^5,7\). We have recently shown that a soluble form of CD163 (sCD163) is present in plasma, and that subgroups of haematological patients have highly elevated levels as compared to normal subjects\(^8\). The protein is probably shed from the cell surface by metallo-proteinase activity\(^9,10\), and the lineage specific nature of CD163 suggests that sCD163 may be an excellent clinical marker molecule for macrophage activity and proliferation.

Gaucher disease is one of the most frequently encountered inherited lysosomal storage disorders and is characterized by an excessive accumulation of macrophages throughout the body. Resulting from a deficiency in lysosomal glucocerebrosidase activity, glucocerebroside (glucosylceramide) accumulate in so-called Gaucher cells in spleen, liver and bone marrow (characteristic lipid-laden macrophages), leading to pronounced hepatomegaly, splenomegaly, pancytopenia and skeletal deterioration\(^11,12\). Gaucher patients can be treated successfully – however extremely costly – by chronic intravenous administration of glucocerebrosidase, and early biochemical indicators of response to treatment are therefore of great interest\(^13\). In the present study, we have measured the concentrations of sCD163 in Gaucher disease in order to evaluate the use of this new marker in monitoring of diseases with excessive macrophage activity.

Materials and Methods

Patient samples

A total of 25 Gaucher patients, diagnosed on the basis of clinical signs, demonstration of deficient glucocerebrosidase activity and genotyping, were examined. Twenty-two of the patients were also studied 2-9 years after initiation of therapy with intravenous enzyme supplementation therapy (recombinant glucocerebrosidase (Cerezyme, imiglucerase injection, Genzyme, MA)), and two patients were studied for three years after start of oral substrate deprivation therapy (Zavesca, OGT918, Oxford Glycosciences, UK). Patients had type 1 or 3 Gaucher disease as classified according to the criteria described\(^11\) and classification was confirmed by determining the glucocerebrosidase genotype\(^14\). The clinical manifestations of the patients were classified using the modified severity scoring index (SSI), which is based on an assessment of the extent of liver, spleen and bone involvement and the severity of
pancytopenia\textsuperscript{15}. None of the patients received either glucocorticoids and/or chemotherapy or other immuno-modulating drugs. A control population of 130 blood donors were formerly used for establishment of a sCD163 reference interval\textsuperscript{16}. In addition, 5 healthy volunteers, all of whom were found to have normal glucocerebrosidase activity and genotype, were also included.

**Determination of the concentration of sCD163 in serum**

sCD163 was measured in ELISA as described in detail\textsuperscript{17}. In brief, rabbit anti-CD163, 4 mg/l was coated onto micro-titer wells. After wash, 100 μl of sample (diluted 1:50 in PBS with albumin, pH 7.2) was added and incubated for 1 h. The wells were washed and 100 μl of monoclonal anti-CD163 (GHI/61, diluted 1:500) was added and incubated for 1 h. After wash, 100 μl of peroxidase-labeled antibody (goat anti-mouse immunoglobulins, DAKO P447, diluted 1:4000) was added and incubated for 1 h. The wells were washed, and 100 μl of a H2O2/1,2-phenylenediamine dihydrochloride substrate solution was added. After 15 min 50 μL of 1 mol/l H2SO4 was added, and the plates were read at 492/620 nm. Control-samples and standards of purified CD163 were co-analysed in each run.

**Precipitation and identification of CD163 in solubilised spleen tissue**

Five samples of spleen tissue (3 Gaucher, 2 controls) were obtained after surgery and immediately frozen. Control spleens were from patients with immune thrombocytopenia who were splenectomized because they were refractory to medication. These patients did not use glucocortocoids but were pre-treated before splenectomy with intravenous immunoglobulins. Tissues were homogenized and solubilised in 50 mM potassium phosphate buffer (pH 6.5) containing 0.25 % v/v Triton X-100. CD163 protein was detected by non-reducing immunoblotting\textsuperscript{8}.

**Enzyme assays**

Chitotriosidase activity was measured as described before\textsuperscript{18}. The assay mixture contained 0.027 mM 4-methylumbelliferyl-tri-N-acetylglucosaminide (Sigma, St. Louis, MO), 0.1% BSA and 0.1/0.2 M citrate/phosphate buffer (pH 5.2). β-Hexosaminidase activity was measured with 1.6 mM 4-methylumbelliferyl-β-N-acetylglucosaminide (Sigma, St. Louis, MO) as substrate in 0.05/0.1 M citrate/phosphate buffer (pH 4.0). Tartrate-resistant acid phosphatase activity was measured using 4-methyl-umbelliferyl phosphate as substrate in the presence of 3 M mercaptoethanol as described by Chambers et al\textsuperscript{18,19}. Angiotensin-converting enzyme activity was measured using hippuryl-L-histidyl-L-leucine as substrate.

**Statistics**

Mann-Whitney test and Wilcoxon signed rank test was used to compare Gaucher patients with normal controls and to compare Gaucher patients before and after
enzyme supplementation therapy respectively. Spearman’s correlation (rho) was used for determination of the relationship between sCD163 and other biochemical markers.

Results

Sera from 25 patients with Gaucher disease were analysed for sCD163 before initiation of enzyme supplementation therapy. The levels in the Gaucher group, median (25-75 percentiles), were far above the levels as measured in the control population (7.1 mg/l (4.8 – 10.3) vs. 1.9 mg/l (1.5 – 2.4), p<0.0001. The median level of sCD163 in 5 healthy subjects, all of whom were found to have normal glucocerebrosidase activity and genotype, was 1.4 mg/l with a range from 1.2 – 2.9 mg/l (figure 1) [16]. sCD163 correlated with the modified severity scoring index (rho=0.43, p<0.0061) (figure 2), and there was a significant correlation between sCD163 and other biochemical markers for Gaucher disease (chitotriosidase, β-Hexosaminidase, and ACE (see table 1).

![Figure 1](image1.png)

**Figure 1.** Determination of sCD163 in serum by sandwich ELISA in patients with Gaucher disease (n=25), subjects with normal glucocerebrosidase activity (n=5), and blood donors (n=130).

![Figure 2](image2.png)

**Figure 2.** Correlation between modified severity scoring index (SSI) and sCD163 in patients with Gaucher disease (n=39).
In 24 of the patients, sCD163 was also measured after initiation of enzyme supplementation therapy, and sCD163 levels were significantly reduced (median 4.7 mg/l (3.2 – 6.6), p=0.0004) (Figure 3). There was no correlation between sCD163 and peripheral blood leucocyte or monocyte counts (see table 1) or with C-reactive protein (not shown). CD163 was easily detectable in homogenized spleen biopsies solubilised for measurement of membrane bound CD163 by western blotting. However, the staining intensity in the Gaucher patients was only slightly more pronounced as compared to the controls (Figure 4).

### Table 1. Correlation between sCD163 and biochemical parameters. Abbreviations: TRAP, tartrate-resistant acid phosphatase; ACE, Angiotensin-converting enzyme.

<table>
<thead>
<tr>
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<th>Rho</th>
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<tbody>
<tr>
<td>Chitotriosidase</td>
<td>0.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>β-hexosaminidase</td>
<td>0.47</td>
<td>0.0006</td>
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<td>TRAP</td>
<td>0.07</td>
<td>0.81</td>
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<tr>
<td>ACE</td>
<td>0.56</td>
<td>0.0004</td>
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<tr>
<td>Blood-WBC</td>
<td>-0.05</td>
<td>0.71</td>
</tr>
<tr>
<td>Blood-monocytes</td>
<td>0.16</td>
<td>0.29</td>
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Discussion

CD163 is expressed exclusively in monocytes and macrophages, and a soluble fragment of CD163 has been detected in sera of healthy individuals. Due to the high macrophage load in Gaucher patients, we intended to evaluate if the levels of sCD163 might reflect the non-inflammatory proliferation of macrophages in this well characterized patient group. sCD163 was highly increased in the Gaucher-patients, with almost complete separation between (untreated) patients and controls, and the sCD163 level correlated with the clinical manifestations as determined using the modified severity scoring index. sCD163 might therefore be used for assessment of the disease course. The sCD163 levels were reduced in 21 of 24 patients after medical treatment, but did not reach normal levels in all, which is in parallel with observations for chitotriosidase.

The diagnosis of Gaucher disease is established on the basis of the demonstration of deficient glucocerebrosidase activity. Biochemical markers, however, are valuable in a first screening for disease, for confirmation, and for monitoring the disease course and effect of medical therapy. Of the markers often employed, chitotriosidase shows by far the most pronounced increase, whereas increases in β-hexosaminidase, TRAP and ACE are neither universal nor pronounced. Six % of all Gaucher patients, however, completely lack serum chitotriosidase activity as a result of a defect in the chitotriosidase gene, and sCD163 might therefore add supplementary diagnostic information, because a normal level of sCD163 seems to rule out disease with a high certainty.

There was no correlation between plasma sCD163 and peripheral blood leucocyte or monocyte counts which indicated that the soluble CD163 in the Gaucher patients originated from accumulated macrophages in the tissues. CD163 was easily detectable in homogenized spleen biopsies, however, the CD163 band in the Gaucher patients had only an about 1.5-2 fold higher intensity as compared to the healthy controls, and the level of sCD163 in splenectomised patients were generally not lower than in non-splenectomised patients (not shown). Therefore, the vast increase in lipid-laden macrophages in various body locations other than spleen most likely is the dominating source of sCD163. The levels of sCD163 in plasma, however, most likely do not simply reflect the number of Gaucher cells in the tissues. In vitro, sCD163 is known to be shed from the surface of monocytes/macrophages by metalloproteinase activity after an inflammatory stimulus, and LPS induces a steep increase in sCD163 in vivo. A general high degree of proteinase activity in Gaucher serum might therefore contribute to the shedding of sCD163, thereby leading to increased plasma levels. Furthermore, there is increasing evidence for the presence of a sustained inflammatory reaction in Gaucher disease, with release of inflammatory cytokines that can potentially modulate the expression of CD163 in Gaucher cells.

In conclusion, the present data show sCD163 as a novel supplementary parameter for diagnosing and monitoring patients with Gaucher disease and support that sCD163 might be a valuable marker for the clinical assessment of macrophage proliferation and activity.
Future clinical studies may further evaluate the usefulness of sCD163 in monitoring Gaucher disease.

Reference List


