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Increased plasma macrophage inflammatory protein (MIP)-1α and MIP-1β levels in type 1 Gaucher disease

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Abstract

Pancytopenia, hepatosplenomegaly and skeletal complications are hallmarks of Gaucher disease. Monitoring of the outcome of therapy on skeletal status of Gaucher patients is problematic since currently available imaging techniques are expensive and not widely accessible. The availability of a blood test that relates to skeletal manifestations would be very valuable. We here report that macrophage inflammatory protein (MIP)-1α and MIP-1β, both implicated in skeletal complications in multiple myeloma (MM), are significantly elevated in plasma of Gaucher patients. Plasma MIP-1α of patients (median 78 pg/ml, range 21–550 pg/ml, $n = 48$) is elevated (normal median 9 pg/ml, range 0–208 pg/ml, $n = 39$). Plasma MIP-1β of patients (median 201 pg/ml, range 59–647 pg/ml, $n = 49$) is even more pronouncedly increased (normal median 17 pg/ml, range 1–41 pg/ml, $n = 39$; one outlier: 122 pg/ml). The increase in plasma MIP-1β levels of Gaucher patients is associated with skeletal disease. The plasma levels of both chemokines decrease upon effective therapy. Lack of reduction of plasma MIP-1β below 85 pg/ml during 5 years of therapy was observed in patients with ongoing skeletal disease. In conclusion, MIP-1α and MIP-1β are elevated in plasma of Gaucher patients and remaining high levels of MIP-1β during therapy seem associated with ongoing skeletal disease.

Keywords: Gaucher disease; MIP-1α; MIP-1β; Bone; Skeletal disease

1. Introduction

Type 1 Gaucher disease is the most frequently encountered lysosomal storage disorder. Deficiency in glucocerebrosidase (EC 3.2.1.45) results in massive storage of the glycosphingolipid glucosylceramide in lysosomes of tissue macrophages [1]. The characteristic lipid-laden macrophages, so-called Gaucher cells, secrete various factors involved in local tissue damage and further formation of storage cells [2]. Accumulation of these cells in liver, spleen, and bone marrow leads to pronounced hepatosplenomegaly, pancytopenia and bone manifestations, such as avascular necrosis, pathological fractures, bone pain and bone crises. In addition, radiological evidence of Gaucher related skeletal pathology, such as decreased signal intensity on MRI, reflecting bone marrow infiltration, lytic lesions and osteopenia, is often present [1]. Several plasma abnormalities have been noted in Gaucher patients (see Aerts et al. [2]). The most striking plasma marker reflecting the presence of Gaucher cells was discovered a decade ago [3]. The activity of chitotriosidase (EC 3.2.1.14), a human analogue of chitinases from lower animals, was found to be on average 1000-fold elevated in plasma of symptomatic Gaucher patients. Monitoring of plasma chitotriosidase is now used for decision making regarding initiation and optimization of therapeutic interventions. More recently, a second Gaucher cell marker was identified. CCL18, a member of the human C–C chemokine family, is elevated 10- to 50-fold in plasma of Gaucher patients without overlap between patient and control values [4]. CCL18, also known as PARC (pulmonary and activation-regulated chemokine), was already known to be up-regulated in Gaucher spleen [5]. Chitotriosidase and CCL18 both stem from Gaucher

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cells [4]. The measurement of plasma CCL18, next to TRAP and ACE [2], is a useful alternative to monitor Gaucher patients [4,6–8], especially when dealing with chitotriosidase deficient individuals [9]. The plasma markers chitotriosidase and CCL18 reflect the total body burden of storage cells, but do not correlate with specific clinical symptoms. In particular, the degree of skeletal involvement does not correlate with total Gaucher cell burden. Currently, there are no plasma or urinary biomarkers predicting onset or progression of bone involvement in Gaucher disease. Several studies have indicated that classical osteoporosis markers reflecting osteoclast activity do not correlate well with skeletal disease in Gaucher patients [10,11]. The lumbar spine bone mineral density as assessed by DXA has been found to be on average lower in Gaucher patients as compared with a reference population and to improve upon therapy [12,13].

Two costly therapies are registered for the treatment of type 1 Gaucher disease. First developed was enzyme replacement therapy (ERT), a treatment based on chronic intravenous administration of human placental glucocerebrosidase [14], nowadays recombinant enzyme [15]. ERT results in spectacular improvement of the visceral and hematological problems in Gaucher patients. Another registered therapeutic intervention for type 1 Gaucher disease is substrate reduction therapy (SRT), based on oral administration of *N*-butyldeoxynojirimycin (Zavesca), an inhibitor of glucosylceramide biosynthesis [16]. SRT results in clinical improvements in mildly to moderately affected type 1 Gaucher patients [17]. The efficacy of both ERT and SRT regarding skeletal disease has been, and still remains, topic of debate (see for example Weinreb et al. [18]). Successful treatment is predicated on monitoring response and making appropriate adjustments based on achievement of defined therapeutic goals [19]. However, monitoring the effect of treatment on skeletal manifestations of Gaucher disease is problematical. One advocated approach is to monitor bone mineral density, which may relate to the risk for fractures [11]. In severe skeletal disease its use is however limited to the diffusely involved skeletal parts and a relation with Gaucher related bone complications has so far not been established. A favored approach is the measurement of lumbar bone marrow fat fraction by quantitative chemical shift imaging [20]. A low fat fraction, reflecting massive Gaucher cell infiltration, is associated with a high risk for skeletal complications [20,21]. A limitation of this technique is that it is expensive, time consuming and not widely available.

Given the present limitations, we started a search for a plasma parameter that may relate to skeletal disease in Gaucher patients. For this purpose, we focused on chemokines critical to bone homeostasis. Earlier microarray experiments performed in our laboratory indicated that the mRNAs encoding chemokines macrophage inflammatory protein (MIP)-1α and MIP-1β were up-regulated in the spleen of a symptomatic Gaucher patient 8-fold and 17-fold, respectively, when compared to control spleens. The two chemokines are of special interest since they have recently been implicated in the pathogenesis of skeletal disease in patients suffering from multiple myeloma (MM) [22,23]. In this study we report on the plasma levels of the chemokines MIP-1α and MIP-1β in type 1 Gaucher patients, their relationship with bone complications and their association with ongoing skeletal disease during therapeutic intervention.

2. Patients, materials, and methods

2.1. Gaucher disease patients and controls

Control subjects consisted of 18 male (mean age 39 years, range 24–54) and 21 female healthy volunteers (mean age 38 years, range 23–54). All patients with Gaucher disease type 1 studied (25 males (mean age 39 years, range 16–67) and 24 females (mean age 39 years, range 12–66)) were known by referral to the Academic Medical Center. Of the 49 type I patients, 46 received ERT (alglucerase, imiglucerase, Genzyme, Cambridge, MA, individualized dosing [24]) while 3 patients were not treated. EDTA (ethylenediaminetetraacetic acid) plasma and serum samples were obtained before and during therapeutic intervention. To assess the clinical severity of patients the Severity Score Index (SSI) was used [25,26]. Skeletal disease was defined as having a history of at least one of the following severe bone complications, prior to start of ERT; bone crises, avascular necrosis or pathological fractures. Ongoing skeletal disease after start of therapy was defined as developing a new episode of one of the above mentioned severe skeletal complications and/or experiencing periods of aggravating bone pain, necessitating the use of pain medication, which was in the opinion of the physician related to Gaucher disease. Bone marrow involvement was assessed by measurement of the bone marrow fat fraction using Dixon quantitative shift imaging (QCSI) of the lumbar spine [20,21]. Only a single patient with ongoing skeletal disease received during the entire period of analysis co-medication with bisphosphonates. Two patients, without skeletal medications at start of therapy, received co-medication that may affect bone metabolism during the last 2 and 4 years of analysis, respectively. Approval was obtained from the Ethical Committee. Informed consent was provided according to the Declaration of Helsinki.

2.2. Enzyme-Linked Immunosorbent Assay (ELISA)

Levels of MIP-1α (in EDTA plasma), MIP-1β (in EDTA plasma and spleen extracts) and osteoprotegerin (OPG) (in serum) were measured by sandwich ELISA using commercially available DuoSet ELISA Developmental kits (R&D Systems Inc. Minneapolis, MI), consisting of a capture antibody, a biotinylated detection antibody, recombinant standard, and streptavidin-horseradish peroxidase (HRP) conjugate. Assay conditions were exactly as described by the manufacturer. No diurnal variation or impact of physical activity on MIP-1α or MIP-1β levels was observed in five normal subjects.

Levels of soluble receptor activator of NFκB (sRANKL) in EDTA plasma and serum were measured by ELISA using a commercially available enzyme immunoassay (Biomedica Medizinprodukte GmbH &b Co, Wien, Austria), consisting of a microtiter plate pre-coated with recombinant OPG, a biotinylated anti-sRANKL antibody, recombinant standard, control sample and ready to use streptavidin-HRP conjugate and TMB substrate. Although assay conditions were exactly as described by the manufacturer we could not reliably detect sRANKL in Gaucher plasma or serum samples. The manufacturer warns for complications when lipidemic or hemolyzed samples are used. Even analysis of freshly obtained blood samples from Gaucher patients gave poor results, suggesting that analysis was intrinsically hampered by the known lipidemic and/or hemolytic nature of Gaucher patient materials.

Levels of CCL18 in EDTA plasma were measured by a sandwich ELISA using a commercially available CytoSet (Biosource International, Camarillo, CA), consisting of a capture antibody, a biotinylated detection antibody, recombinant CCL18/PARC standard, and streptavidin-HRP conjugate. Assay conditions were exactly as described by the manufacturer.

2.3. Enzyme activity assays

The standard enzyme activity assay for chitotriosidase with 4 MU-chitotriose (4-methylumbelliferyl β-D-*N,N′,N″*-triacetylchitotriose; Sigma Chemical Company, St. Louis, MI) as substrate was performed at pH 5.2, as previously described [3]. Chitotriosidase values of patients who were heterozygous for the chitotriosidase mutation were multiplied by 2 [3,27].

2.4. Immunohistochemistry

Immunohistochemistry was performed on frozen sections of Gaucher spleen to detect MIP-1β expression patterns. The methodology of immunocytochemical procedures has been described in detail previously [28]. Frozen sections of 6 μm were cut and thaw-mounted on glass slides. Slides were kept overnight at room temperature (RT) in humidified atmosphere. After air-drying the slides for 1 h, they were fixed in fresh acetone containing 0.02% (vol/vol) hydrogen peroxide. Slides were then air-dried for 10 min, washed with phosphate-buffered saline, and incubated with optimally diluted anti-MIP-1β antibody (mouse anti-human MIP-1β; R&D Systems Inc.) overnight at 4 °C in a humidified atmosphere. Incubations with secondary rabbit antimouse–Ig–biotin (Dako, Glostrup, Denmark) and tertiary HRP-labeled avidin–biotin-complex (ABC/HRP; Dako) were performed for 1 h at RT. Between incubation steps slides were washed twice with phosphate-buffered saline. HRP activity was revealed by incubation for 10 min at RT with 3-amino-9-ethyl-carbazole (AEC; Sigma Chemical Company), leading to a bright red precipitate. After washing, sections were counterstained with hematoxylin and embedded with glycerol-gelatin. Primary antibody reagent omission control staining was performed. Photomicrographs were acquired using a Zeiss axioskop microscope equipped with 10 × /0.30 numeric aperture, and 40 × /0.75 numeric aperture Zeiss Plan Neofluar objectives and a Zeiss AxioCam MRc5 digital camera operating with AxioVision AC release 4.5 as acquisition software.

2.5. Preparation of spleen extracts

Spleen extracts were prepared from frozen spleens of 2 control individuals and 4 Gaucher patients. Ten gram of frozen spleen, which had been stored at −80 °C, was minced into little pieces and 30 ml distilled water was added. This suspension was thoroughly homogenized and sonicated 5 times 15 s on/off at 6 μm, MSE. All procedures were performed on ice. Subsequently the extract was centrifuged for 30 min at 12000 rpm (rotor SS34, Sorvall RC-5b Du Pont Instruments, Wilmington, DE) at 4 °C. The supernatant was removed and stored at −20 °C as an aqueous spleen extract.

2.6. Statistical analysis

Results are given as median and range. Mann–Whitney *U* test analysis was used for the following comparisons: Biomarker levels of control subjects and Gaucher patients, Gaucher patients with or without skeletal disease and Gaucher patients with or without splenectomy. To make biomarker level comparisons between patients before and after therapy, data were analyzed using the paired *t* test. Correlations were tested by the rank correlation test (Spearman coefficient, ρ). Differences in the percentage of patients with QCSI and MIP-1β levels above defined thresholds were assessed by Chi-square test. Sensitivity and specificity were determined using two by two table analysis. Results were considered to be statistically significant when two-tailed *P*-values were < 0.05.

3. Results

3.1. Plasma and splenic levels of MIP-1α and MIP-1β in controls and Gaucher patients

MIP-1α levels were determined in plasma from 39 control subjects and 48 symptomatic Gaucher patients prior to therapy or not receiving therapy (Fig. 1A). The median plasma MIP-1α level in Gaucher patients (78 pg/ml, range 21–550 pg/ml) differed significantly ($P < 0.0001$) from that in control subjects (9 pg/ml, range 0–208 pg/ml). Overlap was seen between values in patients and control subjects. MIP-1β levels were measured in plasma from 39 control subjects and 49 symptomatic Gaucher patients prior to therapy or not receiving therapy (Fig. 1B). The median plasma level in Gaucher patients (201 pg/ml, range 59–647 pg/ml) differed significantly ($P < 0.0001$) from that in control subjects (17 pg/ml, range 1–122 pg/ml). Apart from one clear outlier in the control group, showing repeatedly a plasma MIP-1β of 122 ± 5 pg/ml, there was no overlap.

MIP-1β levels in extracts from spleens of 2 control subjects and 4 symptomatic Gaucher patients were determined. MIP-1β levels were on average 2.5-fold elevated in Gaucher spleens. The MIP-1β levels in the two control spleens and the four Gaucher spleens were 1136, 1712, and 2376, 3136, 3912 and 4544 pg/gram spleen tissue, respectively.

3.2. Plasma levels of MIP-1α, MIP-1β and established Gaucher cell markers

The relationship between plasma levels of the chemokines and two well-known biomarkers for Gaucher cells, chitotriosidase and CCL18 was established. Plasma MIP-1α levels correlated weakly with plasma chitotriosidase and very weakly with plasma CCL18 levels (Fig. 2A, B). The correlation of Gaucher cell markers with plasma MIP-1β levels was even poorer (Fig. 2C, D).

3.3. Origin of MIP-1β

We performed immunohistochemistry to establish whether the Gaucher cells and/or the surrounding cells are responsible for the production of MIP-1β. Immunohistochemistry on frozen spleen sections of 2 Gaucher patients revealed that mature Gaucher cells hardly produce MIP-1β. Instead, some cells surrounding the mature storage cells do produce MIP-1β (Fig. 3). This sharply contrasts with the earlier histochemical finding that chitotriosidase and CCL18 are produced by mature storage cells [4]. Thus, MIP-

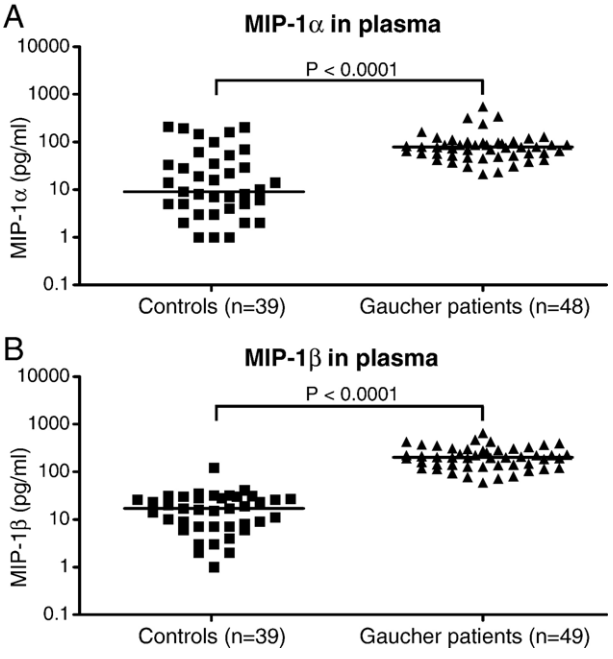

Fig. 1. Plasma levels of MIP-1α and MIP-1β in controls and Gaucher patients. (A) Plasma MIP-1α levels in control subjects ($n = 39$) and Gaucher patients ($n = 48$). (B) Plasma MIP-1β levels in control subjects ($n = 39$) and Gaucher patients ($n = 49$). Chemokine concentrations were determined as described in Patients, materials, and methods. The horizontal line represents the median value in each group. *P*-values (two-tailed Mann–Whitney rank sum test) are indicated when subgroups were statistically different.

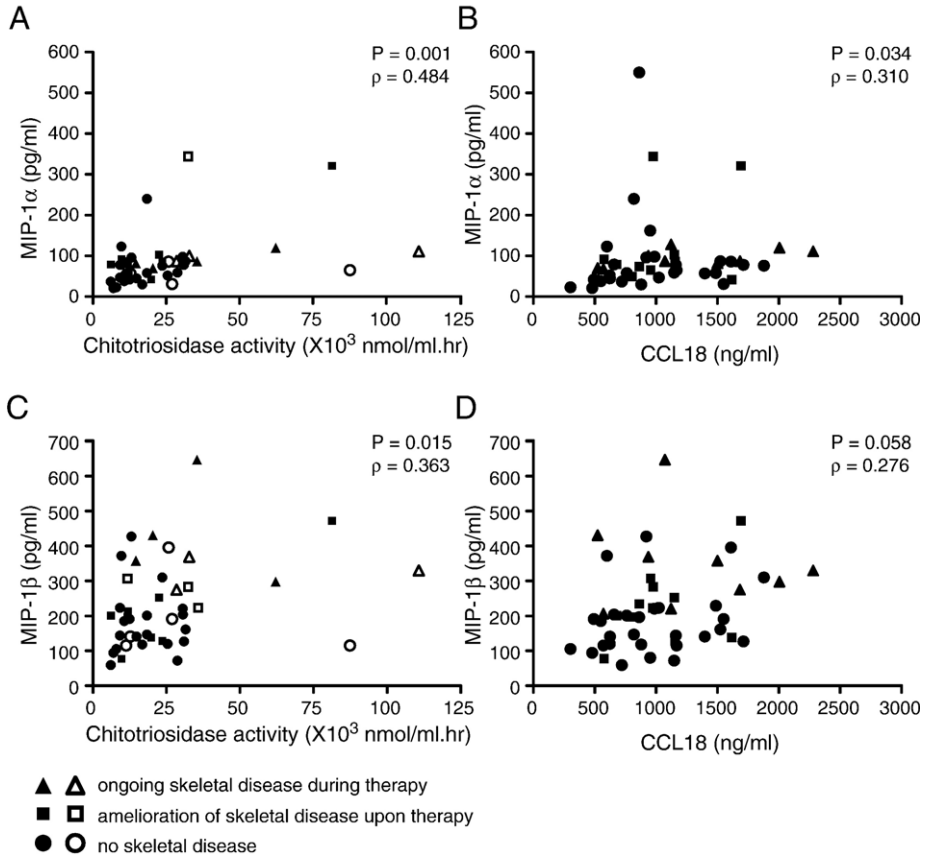

Fig. 2. Relation of plasma levels of MIP-1α and MIP-1β with two established Gaucher cell markers chitotriosidase and CCL18. (A) Plasma MIP-1α versus plasma chitotriosidase. (B) Plasma MIP-1α versus plasma CCL18. (C) Plasma MIP-1β versus plasma chitotriosidase. (D) Plasma MIP-1β versus plasma CCL18. Correlations were tested by the rank correlation test (Spearman coefficient, ρ). Gaucher patients without skeletal disease, amelioration of skeletal disease upon therapy and ongoing skeletal disease during therapy are represented by a circle, a square and a triangle, respectively. Closed symbols represent chitotriosidase wild-type individuals; open symbols, chitotriosidase carrier individuals for which chitotriosidase activity is corrected by doubling the measured activity.

1β seems not to be produced by Gaucher cells but by other cells in the direct environment of the Gaucher cells.

3.4. Effect of treatment on plasma levels of MIP-1α, MIP-1β, chitotriosidase and CCL18

The effect of 3 (± 1) years ERT on plasma levels of MIP-1α and MIP-1β was determined, and compared to changes in the Gaucher cell markers, chitotriosidase and CCL18. The median plasma MIP-1α level in Gaucher patients was 79 pg/ml before therapy (range 23–128 pg/ml, $n = 16$) and 23 pg/ml after therapy (range 0–125 pg/ml, $n = 16$) ($P < 0.0001$). The median plasma MIP-1β level in Gaucher patients was 199 pg/ml before therapy (range 77–330 pg/ml, $n = 16$) and 40 pg/ml after therapy (range 9–182 pg/ml, $n = 16$) ($P < 0.0001$). The median chitotriosidase activity in plasma of Gaucher patients was 14815 nmol/ml

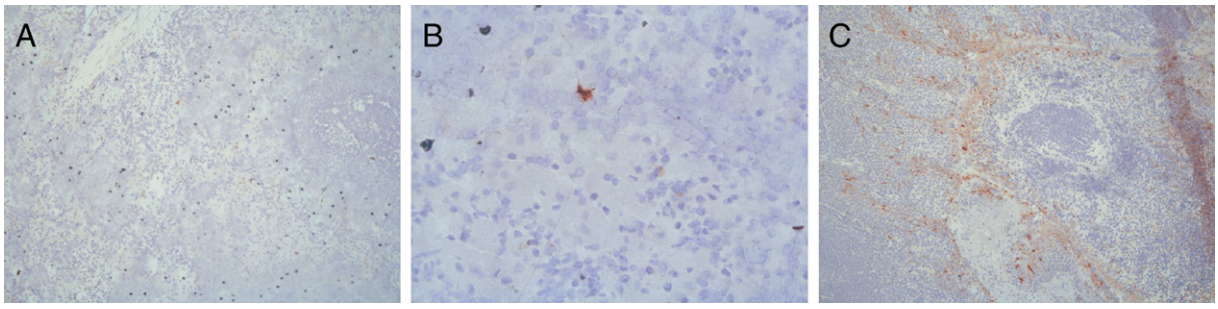

Fig. 3. Detection of MIP-1β protein by immunohistochemistry in Gaucher spleen. Immunohistochemistry, using an antibody against MIP-1β, was performed on frozen sections of Gaucher spleen (A, B) and human tonsil as an internal positive control tissue (C). (A) Overview of Gaucher spleen section. Original magnification × 100. (B) Magnification of the same (Gaucher spleen) section. Original magnification × 400. Stained Gaucher spleen sections show that Gaucher cells (clustered large swollen cells) do not have detectable levels of MIP-1β protein. Some surrounding cells do show labeling. (C) Overview section of human tonsil (control tissue) confirms MIP-1β protein expression as predicted. Original magnification × 100.

h before therapy (range 7917–110754 nmol/ml h, $n = 15$) and 5589 nmol/ml h after therapy (range 428–65244 nmol/ml h, $n = 15$) ($P = 0.0044$). The median plasma CCL18 level in Gaucher patients was 971 ng/ml before therapy (range 576–1713 ng/ml, $n = 10$) and 361 ng/ml after therapy (range 112–1584 ng/ml, $n = 10$) ($P = 0.0007$). Thus, ERT results in reductions in plasma MIP-1α and MIP-1β levels in Gaucher patients.

3.5. Relation of plasma levels of MIP-1α and MIP-1β with clinical symptoms

Plasma levels of MIP-1α or MIP-1β did not correlate with severity of disease as judged by SSI, extent of splenomegaly or hepatomegaly, or hematological abnormalities (as could be deduced from the combination of Spearman coefficient ρ and scatter in the plots of all cases). Plasma MIP-1α levels were not significantly different in plasma of splenectomized Gaucher patients (median 82 pg/ml, range 30–231 pg/ml, $n = 16$) as compared those with a spleen (median 78 pg/ml, range 21–550 pg/ml, $n = 32$). Plasma MIP-1β levels tended to be higher in splenectomized Gaucher patients (median 234 pg/ml, range 115–647 pg/ml, $n = 17$) compared to non-splenectomized Gaucher patients (median 173 pg/ml, range 59–427 pg/ml, $n = 32$).

We examined whether increased plasma MIP-1α and MIP-1β may be associated with skeletal disease in Gaucher patients. For this purpose skeletal disease was strictly defined on the basis of objective criteria such as a history of bone crises, avascular necrosis or pathological fractures. Not included as criteria were subjective bone pains or minor abnormalities in bone mineral density. Fig. 4A shows the plasma MIP-1α levels in untreated Gaucher patients without skeletal disease (median 59 pg/ml, range 21–550 pg/ml, $n = 28$) and untreated Gaucher patients with skeletal disease (median 87 pg/ml, range 42–344 pg/ml, $n = 20$). Plasma MIP-1α levels tended to be higher in patients with skeletal disease compared to patients without skeletal disease ($P = 0.0121$). Fig. 4B shows the plasma MIP-1β levels in untreated Gaucher patients without skeletal disease (median 154 pg/ml, range 59–427 pg/ml, $n = 28$) and those with skeletal disease (median 252 pg/ml, range 77–647 pg/ml, $n = 21$). Plasma MIP-1β levels also tended to be higher in patients with skeletal disease compared to those without skeletal disease ($P = 0.0017$). The increased plasma MIP-1β had a stronger association with the presence of skeletal disease than increased plasma MIP-1α.

3.6. Plasma MIP-1β levels and chitotriosidase in Gaucher patients with or without amelioration of skeletal disease upon therapy

MIP-1β levels were measured in plasma samples taken before and after several years of therapeutic intervention. In patients without skeletal disease plasma MIP-1β decreased in all cases to levels <70 pg/ml. None of these patients developed skeletal complications during therapy. Two additional categories of Gaucher patients were studied: those with skeletal disease before therapy showing marked improvements during therapy ($n = 9$) and those with ongoing skeletal disease during therapy ($n = 9$). Of the 9 patients with ongoing skeletal disease, 5 experienced a severe complication (two bone crisis, two pathological fractures and one avascular necrosis) and 4 suffered from severe pain, as defined in Patients, materials, and methods. Median dose and dosing frequency in patients with ongoing skeletal disease (dose 30 U/kg/4 weeks, range 25–40; frequency 4×/4 weeks, range 2–8) were not significantly different from those in patients without such complications (median 30 U/kg/4 weeks, range 15–120; frequency 4×/4 weeks, range 2–8). Prior to therapy the median plasma MIP-1β levels in the two categories of Gaucher patients were similar. After 1 year of therapy plasma MIP-1β levels were significantly higher in patients with ongoing skeletal disease as compared to those without active skeletal disease ($P = 0.0019$) (Fig. 5A). When analyzed after 1 year of treatment 5 of 9 Gaucher patients without further bone complications during therapy already showed a plasma MIP-1β<85 pg/ml whereas all Gaucher patients with ongoing skeletal disease showed a plasma MIP-1β>85 pg/ml. After 5 years of therapy plasma MIP-1β levels differentiated even better between patients with a reduction of skeletal disease upon therapy and patients with ongoing skeletal disease ($P = 0.0002$) (see also Fig. 6B). When analyzed after 5 years of treatment, all Gaucher patients without further bone complications during therapy showed a plasma MIP-1β<85 pg/

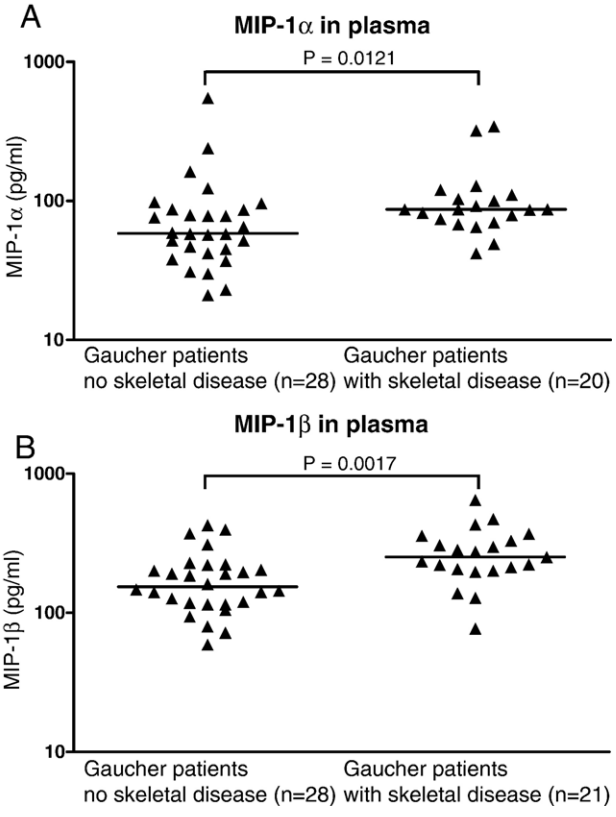

Fig. 4. Plasma levels of MIP-1α and MIP-1β in Gaucher patients with or without skeletal disease. (A) Plasma MIP-1α levels in Gaucher patients with ($n = 20$) or without skeletal disease ($n = 28$). (B) Plasma MIP-1β levels in Gaucher patients with ($n = 21$) or without skeletal disease ($n = 28$). Chemokine concentrations were determined as described in Patients, materials, and methods. The horizontal line represents the median value in each group. *P*-values (two-tailed Mann–Whitney rank sum test) are indicated when subgroups were statistically different.

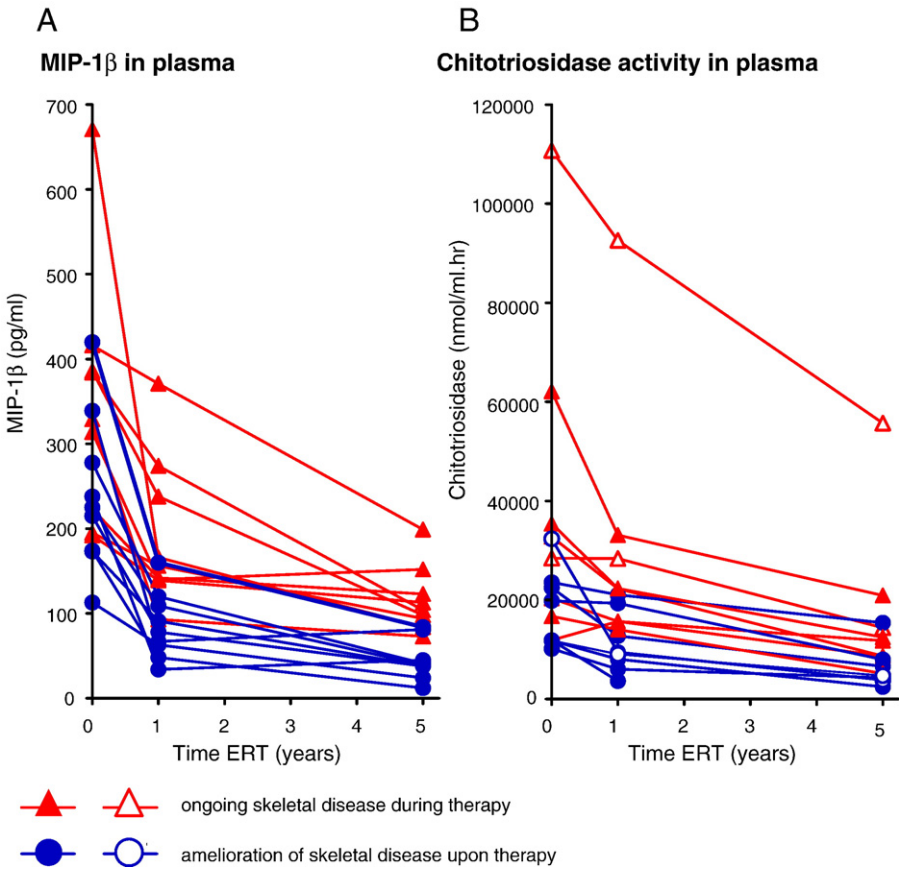

Fig. 5. Effect of treatment (0, 1 and 5 years ERT) on MIP-1β levels and chitotriosidase in plasma of Gaucher patients with or without amelioration of skeletal disease upon therapy. (A) Plasma MIP-1β levels. (B) Plasma chitotriosidase levels. Gaucher patients with ongoing skeletal disease during therapy ($n = 9$) and Gaucher patients with amelioration of skeletal disease upon therapy ($n = 9$) are represented by a red triangle and a blue circle, respectively. Closed symbols represent chitotriosidase wild-type individuals; open symbols, chitotriosidase carrier individuals for which chitotriosidase activity is corrected by doubling the measured activity.

ml whereas 8 of 9 Gaucher patients with ongoing skeletal disease still showed a plasma MIP-1β > 85 pg/ml. A lack in reduction of plasma MIP-1β below a critical threshold during therapy (MIP-1β > 85 pg/ml) seems associated with ongoing skeletal disease.

We investigated whether plasma chitotriosidase could also differentiate between Gaucher patients with and without ongoing skeletal disease during enzyme therapy. Fig. 5B shows that plasma chitotriosidase does not discriminate the two categories of patients as powerful as plasma MIP-1β. After 1 year ($P = 0.0047$) and 5 years of therapy ($P = 0.0205$), chitotriosidase activity also tended to be higher in patients with ongoing skeletal disease as compared with improving patients. However there was far more overlap between the two categories as noted for plasma MIP-1β levels.

3.7. Effect of treatment on the fat fraction of the lumbar spine and plasma MIP-1β levels

Earlier analysis of a large cohort of Gaucher patients has revealed that bone complications occurred primarily in patients with a lumbar marrow fat fraction of less than 23% [20]. Univariate logistic regression analysis indicated that for every decrease of 10% of the fat fraction, the risk of bone complications increased with 85%. Individuals with a fat fraction below 23%, before treatment, are thus considered to be at high risk for bone complications, defined as avascular necrosis, bone crises or pathological fractures [20]. Normal values of fat fraction in healthy volunteers have been determined at 37%±8 [29]. During therapy, an increase of fat fraction occurs [21], but remains below 1SD of the normal value (29%) in a subset of patients. All patients in this study, who experienced ongoing skeletal disease defined as stated above, had a fat fraction that remained below 29%, except for one patient, who reached a fat fraction of 51%. This individual showed persistently elevated plasma MIP-1β, being still 152 pg/ml after 5 years of therapy.

Fig. 6A shows the typical changes during therapy in lumbar fat fractions (L3–5) in a Gaucher patient with ongoing skeletal disease (upper panel) and an individual with a good skeletal response to therapy (lower panel). When we compare Gaucher patients with and without ongoing skeletal disease after a mean period of 5 years of therapy with respect to their values of bone marrow fat fractions and plasma MIP-1β, it becomes clear that most patients with ongoing skeletal disease have a high plasma MIP-1β (>85 pg/ml) and a low fat fraction (Fig. 6B). Gaucher patients without further bone complications during therapy all show relatively low MIP-1β (<85 pg/ml)

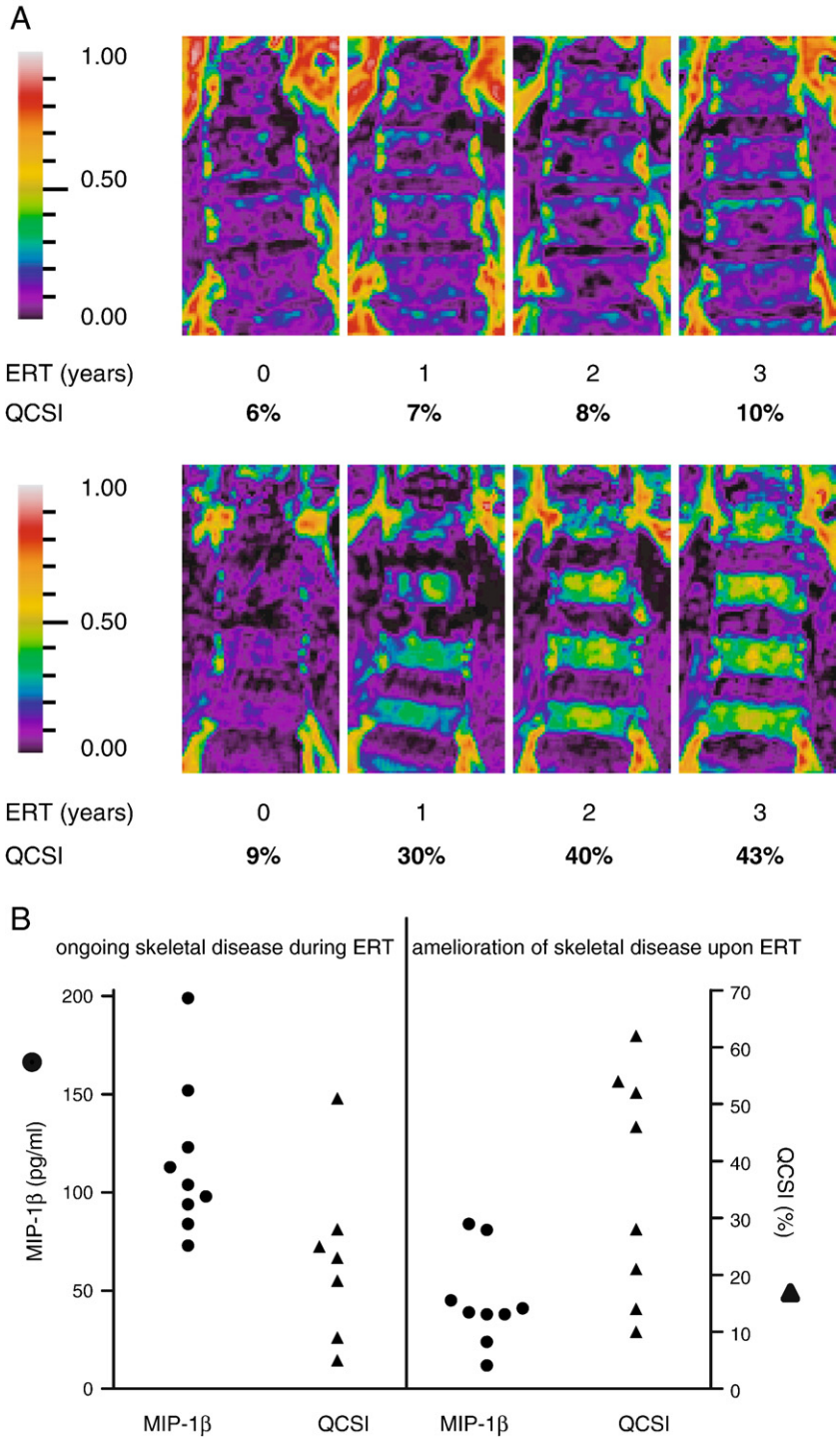

Fig. 6. Effect of treatment on the fat fraction of the lumbar spine and plasma MIP-1β levels. (A) Examples of response in bone marrow fat fraction of the lumbar spine upon therapy as visualized by QCSI. Upper panel: characteristic response in patients showing ongoing skeletal disease. Lower panel: characteristic response in patients without bone complications during therapy. (B) Plasma MIP-1β levels and lumbar bone marrow fat fraction after 5 years of therapy. Plasma MIP-1β levels (circles) and lumbar bone marrow fat fractions (triangles) in patients with ongoing skeletal disease (left panel) and those without (right panel).

and variable fat fractions. Thus it appears that levels of MIP-1β can be of additional value for the assessment of ongoing skeletal disease. For example, when thresholds of 29% for bone marrow fat fraction are combined with MIP-1β levels above 85 pg/ml, the specificity of the combined measurement is greatly enhanced.

4. Discussion

Our investigation revealed that plasma levels of the chemokines MIP-1α and MIP-1β are markedly increased in Gaucher patients. Particularly plasma MIP-1β levels tend to be higher in untreated patients with skeletal disease compared to untreated

patients without skeletal disease. In Gaucher patients the delicate balance between bone resorption and formation is clearly disturbed, favoring bone loss. In postmenopausal osteoporosis, bone loss can be attributed to more generalized 'uncoupled' bone remodeling with enhanced osteolytic resorption by osteoclasts and decreased bone formation by osteoblasts. For Gaucher patients, seemingly conflicting results have been reported on markers of bone formation and resorption [11]. At least, no clear-cut indications for classical osteoporosis have been firmly documented. This suggests that special mechanisms contribute to the skeletal disease in Gaucher patients. It is conceivable that, among other factors, chemokines like MIP-1β play an important role in the disturbed balance of bone resorption and formation in Gaucher patients. Abe et al. [22] showed that MIP-1α and MIP-1β enhance osteoclastic bone resorption in multiple myeloma (MM). They induce local expression of RANKL that after binding to its receptor RANK stimulates osteoclast differentiation and activity. OPG is a decoy receptor for RANKL, inhibiting its biological activity [30]. The local RANKL/OPG ratio is therefore thought to determine the level of osteoclast mediated bone resorption [30–32]. Information on the RANKL/OPG ratio in marrow of Gaucher patients is still lacking. We were unable to detect abnormalities in OPG in Gaucher serum samples. A comparable finding was very recently reported by Magal et al. [33]. sRANKL could not be reliably detected in Gaucher serum samples (see Patients, materials, and methods). It will be of interest to study more closely the presence of MIP-1α and MIP-1β in Gaucher bone marrow as well as the RANK/RANKL/OPG system. It should be noted that the increases in circulating MIP-1α and MIP-1β of Gaucher patients are very pronounced. To the best of our knowledge no data on MIP-1β in serum of MM patients have been published. There are a couple of literature reports on MIP-1α levels in serum of MM patients. In general, a modest increase (about 3-fold) has been reported, although absolute numbers may differ dependent on the analytical method used (see for a review Terpos et al. [34]).

The present data regarding spleen indicate that MIP-1β stems not directly from storage cells, but rather cells surrounding Gaucher cells. An earlier investigation already indicated that lipid-laden Gaucher cells in the spleen are alternatively activated and surrounded by cells expressing macrophage markers [35]. Indeed, MIP-1α and MIP-1β have been proposed as markers of pro-inflammatory macrophages, whilst chitotriosidase and CCL18 are viewed as markers of alternatively activated cells [36]. The observations with Gaucher spleen might not be extrapolated to the bone marrow. It is possible that Gaucher cells in the bone marrow microenvironment have somewhat different characteristics than splenic Gaucher cells. Further research is warranted to establish whether MIP proteins directly underlie disease processes in the bone marrow. The recent availability of suitable Gaucher mouse models should allow such investigations [37]. The present study did not address the relationship between levels of MIP-1α and MIP-1β and localized osteolysis or generalized osteopenia/osteoporosis. Future investigations should address these potential relationships.

Interestingly, analysis of Gaucher patients with ongoing skeletal disease during therapy and those without revealed differences in plasma MIP-1β. After 5 years of therapy plasma MIP-1β levels remained relatively high (>70 pg/ml) in most Gaucher patients showing ongoing skeletal disease. In contrast, all patients showing no skeletal disease upon 5 years therapy had relative low plasma MIP-1β levels (<85 pg/ml). It is of interest to note that high plasma MIP-1β at the start of treatment does not predict the skeletal response to treatment.

At present the most sensitive method to assess the risk for skeletal disease is quantitative chemical shift imaging of lumbar marrow. Fat fractions below 23% in the marrow of L3–5 constitute a high risk for skeletal complications [20]. However, in some Gaucher patients without ongoing skeletal disease during therapy fat fractions nevertheless remain low. All such patients show after 5 years therapy reassuring low plasma MIP-1β levels <85 pg/ml. The specificity of MIP-1β to predict amelioration of bone disease is thus better than QCSI alone. Although additional studies need to prove this, it is our impression that the use of plasma MIP-1β to lumbar marrow fat fraction further improves the assessment of risk for skeletal disease during therapy.

In conclusion, elevated MIP-1α and MIP-1β levels are newly documented plasma abnormalities in Gaucher patients. In particular the increase in MIP-1β seems associated with skeletal disease. Further research with larger groups of well-documented Gaucher patients will have to reveal whether plasma MIP-1β levels can be of additional value in clinical management of Gaucher patients, particularly for the management and prediction of their skeletal disease. Moreover, additional studies are necessary to clarify whether MIP-1β plays a direct role in the pathophysiology of skeletal problems in Gaucher patients via the RANK/RANKL/OPG system.

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