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Evaluation of high density lipoprotein as a circulating biomarker of Gaucher disease activity

Philip Stein

Department of Pediatrics, National Gaucher Disease Treatment Center, Yale University School of Medicine, 333 Cedar Street, LMP 4093, New Haven, CT 06562, USA philip.stein@yale.edu

Ruhua Yang

Department of Internal Medicine, National Gaucher Disease Treatment Center, Yale University School of Medicine, 333 Cedar Street, LMP 4093, New Haven, CT 06562, USA ruhua.yang@yale.edu

Jun Liu

Department of Internal Medicine, National Gaucher Disease Treatment Center, Yale University School of Medicine, 333 Cedar Street, LMP 4093, New Haven, CT 06562, USA jun.liu@yale.edu

Gregory M. Pastores

NYU Department of Neurology, Neurogenetics Unit, 403 East 34th Street, New York, NY 10016, USA Gregory.pastores@nyumc.org

Pramod K. Mistry

Department of Pediatrics, National Gaucher Disease Treatment Center, Yale University School of Medicine, 333 Cedar Street, LMP 4093, New Haven, CT 06562, USA

Department of Internal Medicine, National Gaucher Disease Treatment Center, Yale University School of Medicine, 333 Cedar Street, LMP 4093, New Haven, CT 06562, USA

Pediatric Gastroenterology and Hepatology, Yale University School of Medicine, PO Box 208064, 333 Cedar Street; LMP 4093, New Haven, CT 06520, USA

Abstract

Circulating biomarkers are important surrogates for monitoring disease activity in type I Gaucher disease (GD1). We and others have reported low high-density lipoprotein (HDL) in GD1. We assessed HDL cholesterol as a biomarker of GD1, with respect to its correlation with indicators of disease severity and its response to imiglucerase enzyme replacement therapy (ERT). In 278 consecutively evaluated GD1 patients, we correlated HDL cholesterol, chitotriosidase, and angiotensin-converting enzyme (ACE) with indicators of disease severity. Additionally, we measured the response of these biomarkers to ERT. HDL cholesterol was negatively correlated with spleen volume, liver volume, and GD severity score index; the magnitude of this association of disease severity with HDL cholesterol was similar to that for ACE and for chitotriosidase. Within individual patients monitored over many years, there was a strikingly strong correlation of HDL with liver and spleen volumes; there was a similarly strong correlation of chitotriosidase and ACE with disease severity in individual patients monitored serially over many years (chitotriosidase $r=0.96$ to 0.98 , ACE $r=0.88$ to 0.94 , and HDL $r=-0.84$ to -0.94 , $p<0.001$). ERT

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pramod.mistry@yale.edu .

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for 3 years resulted in a striking increase of HDL while serum levels of chitotriosidase and ACE decreased. Our results reveal markedly low HDL cholesterol in untreated GD1, a correlation with indicators of disease severity in GD1, and a rise towards normal after ERT. These findings suggest HDL cholesterol merits inclusion within the “biomarker basket” for monitoring of patients with GD1.

Introduction

Deficiency of lysosomal glucocerebrosidase (GCase) due to mutations in the *GBA1* gene results in systemic accumulation of macrophages (Gaucher cells) laden with glucosylceramide-engorged lysosomes (Mistry et al. 2011; Grabowski et al. 2010). This cellular metabolic abnormality leads to chronic systemic inflammation and a heterogeneous, multi-systemic phenotype involving variable combinations of hepatomegaly, splenomegaly, diverse forms of skeletal disease, bone marrow infiltration, and rarely pulmonary and lymphatic involvement. In addition to chronic systemic inflammation, there are extensive metabolic abnormalities that include insulin resistance, low HDL cholesterol, and increased risk of cholesterol gallstones (Langeveld et al. 2008; Taddei et al. 2010). There is an extraordinary variability in disease severity and patterns of organ involvement in GD1 even among patients harboring the same *GBA1* genotypes and affected sib-pairs (Sidransky 2004; Grabowski 2008). Given this complexity of phenotypic expressions, there is an important need for validated circulating biomarkers of the systemic burden of GD to help monitor patients longitudinally and to assess response to therapy. Current biomarkers in widespread use include serum chitotriosidase and CCL18 (Hollak et al. 1994; Boot et al. 2004), which have replaced the first generation biomarkers, ACE and TRAP (Tuchman et al. 1956; Lieberman and Beutler 1976), all secretory products of glucosylceramide-laden macrophages. Although GD is primarily an intracellular lipidosis, plasma levels of glucosylceramide are elevated where it circulates bound to serum lipoproteins (Clarke 1981). Both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) appear to be enriched in glucosylceramide (Clarke 1981). Associated with this altered lipoprotein composition, there is profound hypolipidemia due to decreased number of lipoprotein particles, attributed to increased rates of catabolism (Ginsberg et al. 1984; Le et al. 1988; De Fost et al. 2009; Lorberboym et al. 1997). Moreover, cell types other than macrophages are involved in the development of GD, underscoring the necessity to develop a “biomarker basket” to monitor diverse aspects of GD beyond the macrophage system (Mistry et al. 2010).

Enzyme replacement therapy with macrophage-directed, recombinant GCase reverses and/or prevents multiple manifestations of GD1 (Weinreb et al. 2008; Mistry et al. 2009). New recombinant enzyme preparations and small molecule therapies are currently in advanced stages of development (Cox 2010; Grabowski 2008). Validated biomarkers are needed to compare relative efficacies of these emerging therapies (Aerts et al. 2005). Additionally, qualified biomarkers may have utility in determining optimal timing of initiation of therapy, optimal dosing, and maintenance dosing regimens as well as monitoring of compliance. Moreover, stochastic events, such as avascular necrosis (AVN) occur against a background of chronic disease. Biomarker monitoring promises to reveal changing patterns that may trigger a change in therapy to prevent these devastating complications. Finally, availability of validated biomarkers may enable novel approaches for discovery of modifier genes as exemplified recently with chitinase 3-like 1 and asthma (Chupp et al. 2007; Ober et al. 2008).

Currently, chitotriosidase and CCL18 are the most widely used circulating biomarkers for monitoring GD status (Aerts et al. 2005). Angiotensin-converting enzyme (ACE) was the first biomarker of GD ever described (Lieberman and Beutler 1976) that correlates with

disease severity (Deegan et al. 2005). However, its use as a biomarker suffers from significant overlap with controls and rapid normalization upon ERT long before the systemic burden of the disease is reversed. Chitotriosidase and CCL18 do not appear to suffer from this limitation (Aerts et al. 2005). We report strikingly low HDL in GD1 in keeping with previous studies reported by us and others (Ginsberg et al. 1984; Le et al. 1988; De Fost et al. 2009; Taddei et al. 2010). We performed a retrospective study in a large cohort of patients that revealed an impressive correlation of HDL cholesterol with severity of GD1 and response to ERT.

Methods

We performed a retrospective analysis of 278 consecutively evaluated patients followed since 1991 by P.K.M. and G.M.P. at the Gaucher Disease Treatment Centers of Yale School of Medicine and NYU School of Medicine, respectively, to assess the candidacy of HDL cholesterol as a biomarker of GD1. Patients followed at both centers underwent an identical comprehensive evaluation that include confirmation of diagnosis of GD by measurement of leukocyte acid β -glucosidase activity, *GBA1* gene mutation analysis, blood counts, skeletal series, MRI of the abdomen for volumetric liver and spleen measurement, MRI of femurs for assessment of burden of marrow disease, and biomarker measurements (Taddei et al. 2009). Patients were evaluated at baseline and serially every 6–12 months.

Initially, we analyzed the correlation of HDL cholesterol and other biomarkers with GD1 overall disease severity and with severity of involvement of individual disease compartments (i.e., hepatomegaly and splenomegaly). Biomarker measurements included chitotriosidase, ACE, ferritin, immunoglobulins, HDL cholesterol, LDL cholesterol, triglycerides, total cholesterol, LDL cholesterol/HDL cholesterol ratios; more recently we have added CCL18 to our biomarker basket. Levels of biomarkers at baseline prior to initiation of ERT were correlated with the extent of splenomegaly and hepatomegaly as well as with overall severity score index (SSI) (Zimran et al. 1992). We compared results of correlations of HDL cholesterol and GD1 severity with parallel analyses of chitotriosidase and ACE. A subset of 107 patients was examined in whom we could perform genotyping of *CHIT1* gene for common polymorphisms to adjust for this major determinant of circulating chitotriosidase activity (Boot et al. 1998). There is likely a major interindividual heterogeneity in biomarker metabolism and regional tissue burden of Gaucher cells as well as molecular phenotype of Gaucher cells, for example alternatively activated vs. classically activated polarization of macrophages (Boven et al. 2004). Since these aspects of the disease are not adequately reflected by standard measures of disease severity, we examined individual patients for correlations of their serial biomarker levels with corresponding liver and spleen volume measurements over many years of ERT. (Representative data of one patient followed for >10 years including pre-ERT and multiple post-ERT data points is depicted below in Fig. 3.) Finally we examined the biomarker response to imiglucerase ERT. The dataset for this analysis was limited to patients that had the same biomarker measured pre-ERT and 2.5–4 years post-ERT. Results of paired samples were compared pre- and post-ERT. Percent change in biomarker level from baseline was compared with percentage change in spleen and liver volume over the same time course. Data were stratified for spleen status (intact spleen vs. splenectomy). Analyses of LDL and HDL cholesterol levels were referenced to gender-adjusted 5% and 95% percentiles from the Framingham Heart Study.

Patient demographics are summarized in Table 1. Means are described as $\pm$ standard error of the mean (SEM). Liver and spleen volumes were determined by volumetric MRI and converted to fold-enlargement compared to normal ($\times$ N). For these calculations, we used a normal liver volume of 2.5% of the total body weight and a normal spleen volume of 0.2%

body weight (Barton et al. 1991). Baseline CBC, MRI, SSI, and Hermann score were determined before ERT. Assay of serum chitotriosidase activity was performed using 4-methylumbelliferyl-(4-deoxy) chitobiose as a substrate as described previously (Aguilera et al. 2003). Genotyping for chitotriosidase (*CHIT1*) was performed as described previously (Boot et al. 1998). Patients homo-zygous for *CHIT1* null allele were excluded from analysis since they had undetectable serum chitotriosidase activity. Patients that were heterozygous for the null 24 bp insertion polymorphism in *CHIT1* gene (99/75 bp PCR fragment) had their chitotriosidase activity normalized to wild-type homo-zygous levels (75/75 bp PCR fragment) by multiplying their chitotriosidase activity by a factor of 2 (Bussink et al. 2006). Cholesterol and ACE levels were measured by accredited hospital laboratories.

Statistical analysis

Differences in means among the subgroups of patients were determined by an independent sample two-tailed *T*-test for equality of means. The *F*-test (Levine's test) for equality of variances was run first. Based on the results of the *F*-test, equal variances were either assumed or not assumed in the determination of the significance of the *T*-test. Two-tailed Pearson correlation tests were run to investigate correlations between biomarkers and liver volume, spleen volume and SSI. A paired *T*-test was performed to determine differences in biomarkers pre- and post- ERT.

The study was approved by the Human Investigation Committee of the Yale University School of Medicine and IRB of NYU School of Medicine.

Results

The study cohort comprises 278 patients with GD1 followed for up to 20 years at our centers. The mean age of patients at baseline evaluation was 38.4 ± 1.1 (range 2–86 years of age) [Table 1]. There was a wide range of GD1 severity. The mean splenic volume was $9.7 \times N \pm 0.75$, however this ranged from a normal spleen volume to $61.83 \times N$. Mean liver volume at baseline in the study population was $1.43 \times N \pm 0.04$, range $0.29 - 4 \times N$. Mean HDL cholesterol at baseline was 30.2 mg/dl (<5th percentile for the normal population as depicted in Fig. 1), ACE 178.1 U/ml (normal <52), and mean chitotriosidase activity $8,864 \text{ nmol ml}^{-1} \text{ h}^{-1} \pm 856$ (normal <50). There was no overlap of chitotriosidase activities in GD1 patients compared to the controls. HDL cholesterol appeared consistently below the 5th percentile of the normal population (Fig. 1). In contrast, LDL cholesterol, while low, remained at or slightly above the 5th percentile.

HDL cholesterol, serum chitotriosidase, and serum ACE were all significantly correlated with liver and spleen volume as well as with SSI (Table 2). Serum levels of all three biomarkers correlated with SSI, but the strength of correlation was less compared with accurately measured liver and spleen volumes (Table 2). This likely reflects the ability of SSI to capture a significant burden of severe irreversible disease (i.e., AVN, fragility fractures) that is nevertheless inactive. Of note these biomarkers all significantly correlate with each other. HDL correlates with chitotriosidase ($r = -0.49$, $p < 0.001$) and with ACE ($r = -0.39$, $p < 0.001$).

Next, we correlated serial biomarker levels with corresponding liver and spleen volumes in individual patients followed serially over many years after commencement of ERT. We performed this analysis to adjust for the likely major confounding effect of interindividual variations in metabolism of biomarkers and body burden of Gaucher cells not adequately reflected in standard measures of disease severity. While correlations of biomarkers with disease severity were significant in our patient population as a whole (Fig. 2), the extent of correlation of circulating biomarkers was striking within individual patients monitored

serially over many years. Representative data of one patient are depicted in Fig. 3: HDL cholesterol correlated with ACE ($r=-0.992$, $p=0.001$) and chitotriosidase correlated with ACE ($r=-0.94$, $p=0.001$)

Biomarker response to 2.5–4 years of ERT is depicted in Table 3. HDL cholesterol rose strikingly; there was an increase in LDL cholesterol as well. However the latter was less consistent in analysis of all datasets. Moreover, LDL cholesterol:HDL cholesterol ratio decreased on ERT, in keeping with the notion that HDL cholesterol is the more prominent abnormality in dyslipidemia of GD. ERT resulted in commensurate reduction of serum chitotriosidase and ACE (Table 3). ERT did not cause a change in serum triglycerides. The largest absolute and percent reduction was noted for chitotriosidase at 1 year of ERT compared to percent reduction of spleen/liver volumes or reversal of thrombocytopenia. After stratification for age, at baseline patients under 18 years of age had a lower mean HDL cholesterol (21 ± 1.7 mg/dl) compared to patients older than 18 years (29 ± 1.4 mg/dl; $p=0.05$). In the pediatric subset of patients, there was a significant response to ERT, with an increase to 35.1 ± 3.8 from 20.1 ± 2.8 mg/dl ($p<0.001$) after a mean of 4.4 years. In comparison, HDL cholesterol response to ERT in adults was an increase to 38 ± 1.5 from 23.5 ± 2.1 mg/dl ($p<0.001$) after a mean of 4.5 years.

To adjust for the confounding effect of splenectomy, we compared biomarker levels in patients with intact spleens with those that had undergone prior splenectomy at baseline before initiation of ERT (Supplementary Table 1). Compared to patients with intact spleen, patients who had undergone prior splenectomy had a higher SSI, worse hepatomegaly, and worse bone disease score (Hermann score). This greater disease severity in asplenic patients was reflected in higher serum ACE (243 vs. 157 U/ml, $p<0.001$), higher total cholesterol (164 vs. 146 mg/dl, $p=0.002$), higher LDL cholesterol (96.7 vs. 85.7 mg/dl, $p=0.043$), and higher triglycerides (178.46 vs. 148.5 mg/dl, $p=0.008$). In contrast, HDL cholesterol and serum chitotriosidase activity were similar in patients with intact spleens compared to asplenic patients.

Discussion

Our study reveals that HDL cholesterol has attractive attributes as a biomarker to monitor the activity of Gaucher disease. Levels of HDL cholesterol correlate negatively with severity of hepatomegaly and splenomegaly as well as with overall severity score index. Moreover HDL cholesterol levels demonstrate robust rise upon ERT. There is a remarkably strong correlation of serial HDL cholesterol with liver/spleen volume measurements within individual patients followed longitudinally for many years. This underscores its ability to reflect changes in disease severity in patients followed longitudinally. Additionally, HDL cholesterol demonstrates a robust negative correlation with chitotriosidase and ACE, two classic secretory products of glucosylceramide-laden macrophages (Hollak et al. 1994; Lieberman and Beutler 1976). While we found a significant correlation of disease severity with HDL cholesterol and other biomarkers in our large cohort at baseline, we found the most impressive correlations of HDL cholesterol and other biomarkers with disease severity within individual patients monitored longitudinally over many years. Taken together, HDL cholesterol appears to be a strong indicator of GD activity and therefore, we believe it merits inclusion in the biomarker basket for monitoring patients in the clinic. Moreover, our study suggests that greatest utility of HDL cholesterol and other biomarkers in clinical practice is likely to be in monitoring individual patients serially over time.

A few studies have reported hypocholesterolemia in GD1, which has been attributed to markedly reduced levels of LDL and low HDL. Ginsberg et al. (1984) and Le et al. (1988) demonstrated that the reduction of LDL cholesterol and HDL cholesterol in GD1 was

associated with reduced levels of apolipoprotein B100 and apolipoprotein A1, respectively, consistent with reduced numbers of these lipoprotein particles. Proteomic analysis of GD1 sera also point to marked reduction of plasma apolipoprotein AI in GD that increases after ERT (Vissers et al. 2007). In contrast, apolipoprotein E level was found to be elevated, attributed to increased production by glucosylceramide-laden macrophages, likely reflecting enhanced reverse cholesterol transport (Ginsberg et al. 1984; Le et al. 1988). Experimental lipid loading of macrophages is known to increase synthesis and secretion of apolipoprotein E, which in turn increases cholesterol efflux (von Eckardstein et al. 2001). In a study of in vivo turnover of radiolabelled LDL and HDL, decreased levels of these lipoproteins were found to be due to enhanced fractional catabolism of apo B and apo A1, respectively (Le et al. 1988). Taken together with the increased level of apo E, available data suggest a central role of the pathological glucosylceramide-laden macrophages in aberrant lipoprotein metabolism in GD. Consistent with this notion, imaging for sites of lipoprotein uptake in vivo in GD revealed increased uptake of LDL primarily in the spleen and bone marrow, sites of major accumulation of glucosylceramide-laden macrophages in GD (Lorberboym et al. 1997). However, the site(s) of increased HDL uptake in vivo in GD is not known. We reported recently an increased incidence of cholesterol gallstones in GD1 associated with low HDL, presumably reflecting increased reverse cholesterol transport (Taddei et al. 2010). The latter may underlie lack of atherosclerotic vascular disease in GD1 despite very low levels of HDL cholesterol (de Fost et al. 2009). In this study, the carotid artery intima thickness of GD1 patients with low HDL cholesterol was similar to non-GD1 patients with normal HDL cholesterol.

It should be noted that although LDL and HDL particle numbers as well as cholesterol content appear to be reduced in GD, there is increased level of plasma glucosylceramide and ganglioside GM3 (for which glucosylceramide is a precursor) (Ghauharali-van der Vlugt et al. 2008; Groener et al. 2008). Therefore it follows that the absolute amount of glucosylceramide and GM3 per lipoprotein particle may be dramatically increased and moreover their uptake and catabolism in vivo are also markedly increased. These perturbations likely result in massive glucosylceramide and GM3 flux via receptor-mediated lipoprotein metabolism and could therefore represent a major contributor to lysosomal glycolipid accumulation in the presence of a *GBA1* deficiency. This should be an important area for future investigation. The cell surface receptors that mediate increased uptake of LDL and HDL in GD are not known, but scavenger receptors are attractive candidates (Nguyen et al. 2006; Suzuki et al. 1997).

There are a number of limitations of our study. Our study was retrospective. We used HDL cholesterol as a surrogate marker of high-density lipoprotein. We did not measure apolipoprotein A1 or E. Moreover, we did not measure glucosylceramide or GM3 levels. Our studies underscore the importance of addressing these aspects of lipoprotein metabolism due to their obvious relevance in pathophysiology of GD. These studies are now possible since several animal models of GD have been developed (Xu et al. 2003; Mistry et al. 2010)

As chitotriosidase activity levels in GD have no overlap with normal controls and they exhibit the greatest fold-increase compared to controls, chitotriosidase has become the biomarker of choice (Boot et al. 2009). Our study and others (Le et al. 1988; Ginsberg et al. 1984; de Fost et al. 2009; Cenarro et al. 1999) show marked perturbation of HDL in GD. However, HDL has not been examined as a biomarker previously. All three markers examined in our study have utility in monitoring GD activity. Chitotriosidase and ACE represent secretory products of glucosylceramide-laden macrophages in GD. Therefore these biomarkers could be viewed as directly reflecting total body burden of these pathological macrophages and their state of activation. In contrast, the connection between body burden of glucosylceramide-laden macrophages and perturbation of HDL is not so readily evident.

Could it be that apo E secreted by Gaucher cells results in apo E-rich HDL, which accelerates reverse cholesterol transport as was suggested previously (Le et al. 1988)? Alternatively, altered composition of HDL with respect to lipid and glycosphingolipid content may alter its ability to bind to hepatocyte cell surface receptors such as SR B1 and ultimately lead to enhanced biliary cholesterol secretion (Lund-Katz and Phillips 2010).

Recently broader biological functions of HDL have been recognized in the form of regulation of stem cell proliferation in the bone marrow (Yvan-Charvet et al. 2010) that may be relevant in GD since failure of hematopoiesis is being recognized as a contributor to organomegaly and cytopenia (Berger et al. 2010; Mistry et al. 2010). It should also be kept in mind that *GBA1* locus has been proposed to be a major determinant of low HDL cholesterol levels after observations that obligate heterozygote carriers of GD have low HDL cholesterol levels (Pocovi et al. 1998)

In addition to the potential of HDL to reflect aspects of GD beyond the macrophage, there are several attractive features of its potential utility as a biomarker of disease activity. First, HDL cholesterol as well as apolipoprotein A1 testing is readily available at all hospitals and does not require specialized laboratories, circumventing the relatively poor access to biomarker testing experienced with chitotriosidase and CCL18. Second, there are no drastic polymorphisms that impact on HDL levels as is the case with the highly prevalent exon 10 polymorphism in the *CHIT1* gene (Bussink et al. 2006). A number of severe inherited disorders, e.g., familial hypoalphalipoproteinemia, LCAT deficiency, and Tangiers disease, result in marked hypoalphalipoproteinemia, but all of these are extremely rare and most unlikely to confound interpretation in GD. A relatively common inherited disorder that is known to be associated with low HDL cholesterol is heterozygous familial hypercholesterolemia (FH). The vast majority of GD patients in our cohort have markedly low levels of LDL cholesterol, suggesting that coincidence of GD and heterozygous FH is indeed a very rare occurrence. In fact we have encountered only one such patient in our combined experience of >600 GD1 patients (P.K.M., unpublished observations). Taken together, our results suggest that HDL may play an important role in pathophysiology of GD and demonstrate an excellent correlation with disease severity that is commensurate to that observed with other bio-markers in routine clinical use. Therefore, we suggest inclusion of HDL in the biomarker basket for GD monitoring.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

HDL and LDL cholesterol levels at baseline in the female study population compared to the 5th and 95th percentile for the normal population (from the Framingham Heart Study) stratified for gender. The figure depicts data for females. Similar results were obtained for males (data not shown)

Fig. 2.

Correlation of biomarkers at baseline (pre-ERT) in the entire cohort of patients. Two sample correlation graphs are shown. HDL is correlated with spleen volume, and chitotriosidase is correlated with liver volume. Table 2 displays the remaining correlation results

Fig. 3.

Representative association of biomarkers with disease severity in an individual patient currently 43 years old with N370S/84GG. Patient started ERT in 1992 at age 26, and data over a 17-year period are shown with first data points prior to initiation of ERT. Initial liver volume was $2.7 \times N$, and spleen volume was $45.6 \times N$. Patient was heterozygous for *chit 1* polymorphism; all chitotriosidase values were multiplied by a factor of 2 to adjust for this effect. Chitotriosidase activity in $\text{nmol ml}^{-1} \text{h}^{-1}$; ACE in U ml^{-1} ; HDL concentration in mg dl^{-1}

Table 1

Patient demographic

Category	Value	Range
Number of patients	278	
Age (years)	38.44±1.1	2–86
Male gender (%)	50.7%	
Liver volume (× N)	1.43±0.04	0.292–4.01
Spleen volume (× N)	9.7±0.8	0.972–61.83 (65 asplenic pts)
Hemoglobin (g/dL)	12.3±0.1	62–16.8
Platelet count (× 10 ⁹ /L)	138.4±5.3	172–500
Hermann score	2.4±0.09	12–5
Total cholesterol (mg/dl)	150.4±2.6	612–417
Triglycerides (mg/dl)	155.1±4.7	332–575
LDL (mg/dl)	88.27±2.3	4.52–307
HDL (mg/dl)	30.2±0.9	22–105
LDL:HDL	3.37±0.1	0.382–10
SSI	6.8±0.2	12–19
ACE (U/ml)	178.1±7.5	42–712
Chitotriosidase (nmol ml ⁻¹ h ⁻¹)	8,864±856	1762–31,740

Unless otherwise stated, values are mean ± SEM

Table 2

Correlation of biomarkers and lipoprotein cholesterol with GD severity

	Spleen volume (× N)	P value	Liver volume (× N)	P value	SSI	P value
Total cholesterol	-0.48	<0.001	-0.30	<0.001	-0.11	0.085
TG	0.023	0.768	0.13	0.087	0.13	0.038
HDL	-0.46	<0.001	-0.35	<0.001	-0.25	<0.001
LDL	-0.37	<0.001	-0.19	0.005	-0.10	0.113
LDL:HDL	0.23	0.003	0.69	<0.001	0.18	0.006
Chitotriosidase	0.48	0.001	0.62	<0.001	0.34	0.005
ACE	0.57	<0.001	0.53	<0.001	0.26	<0.001

Two-tailed Pearson correlation with spleen, volume, liver volume, and SSI. Correlation of biomarker level with spleen volume was performed in patient with intact spleens at the time of evaluation

Table 3

Response to ERT. Baseline pre-ERT were compared with values 2.5–4 years post-ERT by paired *t*-test

	Pre-ERT	3 years post-ERT	<i>P</i> value
HDL (mg/dl)	23.9±1.4	34.8±2.3	<0.001
LDL (mg/dl)	73±5	88±4.9	0.003
Chitotriosidase (nmol ml ⁻¹ h ⁻¹)	13,406±2,167	4,159±612	<0.001
ACE (U/ml)	212±10.4	106±7.1	<0.001

The total cholesterol pre-ERT was 138±5.4 mg/dl, which increased to 156±5.2 mg/dl post-ERT ($p<0.001$). The LDL:HDL cholesterol ratio decreased from 3.5±0.38 to 2.8±0.2 ($p=0.09$). There was no significant change in serum triglycerides: 98±16 vs .76±12 mg/dl ($p=0.3$)