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Relationship between Glucocerebrosidase Activity and clinical response to Enzyme Replacement Therapy in patients with Gaucher Disease type I

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RUNNING TITTLE: Relationship between Glucocerebrosidase activity and clinical response in Gaucher Disease.

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CONFLICT OF INTEREST: Gras-Colomer, Climente-Martí, Fernandez-Zarzoso have received remuneration from Genzyme® and Shire® for educational presentation. They have attended investigational meetings with travel expenses paid by Genzyme®. Giner-Galvañ is consultant for Shire® for some remunerated formative activities. Torralba-Cabeza serves as a consultant for Genzyme® and Shire® and participates on advisory panels and speakers on lysosomal storage diseases, and he has received research support form both companies. Almela-Tejedo, Martínez-Gómez, Marcos-Rodríguez, Rodríguez-Fernández, Merino-Sanjuan declare that there is no conflict of interest.

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Abstract: The quantification of enzyme activity in the patient treated with enzyme replacement therapy (ERT) has been suggested as a tool for dosage individualisation, so we conducted a study to evaluate the relationship between glucocerebrosidase activity and clinical response in patients with Gaucher disease type I (GD1) to ERT. The study included patients diagnosed with GD1, who were being treated with ERT, and healthy individuals. Markers based on glucocerebrosidase activity measurement in patients' leukocytes were studied: enzyme activity at 15 min. post-infusion (Act_{75}) reflects the amount of enzyme that is distributed in the body post ERT infusion, and accumulated glucocerebrosidase activity during ERT infusion ($Act_{75\text{-}0}$) indicates the total drug exposure during infusion. The clinical response was evaluated based on criteria established by Pastores *et al*. and Gaucher Severity Score Index. Statistical analysis included ROC analysis and area-under-curve test. Act_{75} and $Act_{75\text{-}0}$ were found to be moderate predictive markers of an optimal clinical response (area under the ROC of Act_{75} was 0.733 and $Act_{75\text{-}0}$ was 0.817). $Act_{75\text{-}0}$ showed statistical significance in its discriminative capacity ($p<0.05$) for obtaining an optimal response to ERT. The cut-off point was 58% (RR=1.800;CI95% 1.003 to 3.229;$p<0.05$). Moreover, Act_{75} showed a significant and inverse correlation with the Gaucher Severity Score Index, and Act_{75} and $Act_{75\text{-}0}$ presented a significant correlation with residual enzyme activity at diagnosis. Markers based on glucocerebrosidase activity have a good correlation with clinical response to ERT. Therefore, it could provide supporting clinical data for dose management in GD1 patients.

Gaucher disease (GD) is a rare, inherited metabolic disorder caused by a partial or total deficiency of lysosomal beta-glucocerebrosidase (GCase), which leads to a decrease in hydrolysis of glucocerebroside and its subsequent storage in cell lysosomes in the monocyte/macrophage system. Clinical symptomatology of the disease derives from accumulation of glucocerebroside in the lysosomes of these cells (named Gaucher cells) and later on in different tissues, producing a multi-systemic disorder[1]. The most common variant is the non-neuropathic form, Gaucher disease type I (GD1), whose diagnosis is currently based on the presence of clinical signs and symptoms, although the "gold standard" is detection of low GCase activity intra-leukocyte [2-5].

Treatment for GD1 is based on the administration of enzyme replacement therapy (ERT), which improves haematological parameters and leads to a stabilisation or reduction of bone and visceral lesions[6]. The therapeutic enzyme is designed to be internalized by monocyte/macrophage through receptor-mediated endocytosis via mannosa receptors. This receptor is a member of the family type lectin receptor expressed in macrophages/monocytes, dendritic cells and hepatic cells and lymphatic endothelium[7].

There are currently three drugs marketed for ERT: imiglucerase (IMG;Cerezyme® Genzyme, Sanofi Company), velaglucerase (VELA;Vprip® Shire Pharmaceuticals SL) and taliglucerase (TAL; Elelyso®, Pfizer SLU)[8-11]. Nowadays, the selection of the appropriate dose to be used in ERT and other clinical decisions are based on recommendations published by Pastores *et al*., that define an optimal response (OR) if at least five of six therapeutic goals described in the six domains of GD1 (haemoglobin, platelets, splenic and hepatic volume, chitotriosidase and bone pain) are met and a non-optimal response (NOR) when they are not met[12]. Other important score that reflects variations in disease severity induced by treatment is the Gaucher Severity Score Index-type I (GauSSI-I). This score has a maximum of 42 points, distributed over six different domains (skeletal, hematologic, chitotriosidase, visceral, lung and neurological)[13]. Currently, dosage individualisation consists of selecting a maintenance dose at levels ranging between the initial recommended dose

(60 U/kg/14days) and the minimum established effective dose, which is 15 U/kg/14days. Despite an increase in the acceptance of this tendency, the most convenient dosage regimen design for ERT in patients with GD1 remains controversial in several aspects, such as the differential effect of enzymes in patients, the efficacy of a high-dose or a low-dose treatment schedule, the so-called poor responder patients in which increasing doses do not increase the rate of response, or administration of the ERT infusion once every 4 weeks in stable patients [6, 14-17].

Published studies on the pharmacokinetics of ERT[9, 10, 18, 19] have suggested that, after intravenous administration, the activity of circulating enzyme decreases rapidly in plasma because of the principal distribution model of the ERT is through uptake by mannose-6-phosphate (M6P) into peripheral monocytes and their distribution in tissues as macrophages. A direct extravasation to tissues through the vascular endothelium with subsequent uptake by macrophages is theoretically less important or null due to its high molecular weight (70KDa), which causes the tissue distribution of most proteins to be limited to the vascular or interstitial spaces[20].

Consequently, as monocyte/macrophage system, a type of polymorphonuclear leukocyte (monocytes in circulation and macrophages in tissues), are the target cells in GD1, hence the quantification of intra-leukocyte enzyme activity in patients treated with ERT could be used as a tool to monitor ERT. Moreover, intra-leukocyte enzymatic activity seems to show a linear correlation with the low or intermediate doses studied [21-23]. However, studies of high doses have suggested a non-linear pharmacokinetic[9, 10].

Thus, due to the variability in dose and frequency of ERT, the variability of response to ERT and the high cost of these therapies[24], this study was carried out in order to accurately assess the relative efficacy of treatment and to identify tools for ERT individualisation. For this purpose, the relationship between GCase intra-leukocyte activity and clinical response in patients with GD1 treated with ERT was evaluated.

MATERIAL AND METHODS

A prospective follow-up, experimental multicentre study was conducted in four public hospitals from June 2014 to May 2015. The study was carried out with the approval of the Clinical Research Ethics Committee and after obtaining the informed consent of the patients.

Healthy individuals were included as controls to establish the normal cut-off points of GCase activity in peripheral blood leukocytes. Adult patients (>18 years) with GD1 confirmed by enzyme diagnosis who had been receiving stable doses (without changes of doses or frequencies) of ERT treatment for at least 12 months prior to inclusion in the study were included.

The ERT drugs (IMG, VELA and TALI) used in the study were prepared in 0.9% saline solution. Administration was carried out by nursing staff at each health centre for a period of 60 min. according to the specifications of the Product Characteristics Summary[8-10].

Analytical assays

Two blood samples were taken from each patient in a tube with EDTA on two occasions: prior to the start of the infusion with the exogenous enzyme, this was done in order to calculate enzyme activity at time zero (Act_0); and 15-min. post-infusion, to calculate enzyme activity at time 75 min. (Act_{75}). An aliquot of leukocytes was extracted from each blood sample and analytical assay by high performance liquid chromatography (HPLC) with fluorometric detection were conducted: enzymatic reaction between GCase and the substrate 4-methylumbellferyl-β-D-glucoside and sodium taurocholate as detergent with the product formed being 4-methylumbelliferone that is determined by HPLC with fluorimetric detection [25]. This analysis has an incubation time of 60 min. The enzymatic activity was expressed as the number of enzyme units per litre of solution (U/L).

Enzyme activity was measured in leukocytes (instead of monocytes) because activity of Gcase in monocyte represents 96% of total leukocyte approximately [26]. Analysing GCase in total leukocytes simplifies the analytical method for use in routine clinical practice, allows comparing with the activity measure at diagnosis, which carries out in leukocytes [27].

Variables and GCase-based markers

The variables studied were: monthly doses (U/kg/4w); ferritin (ng/mL); chitotriosidase (CT) (nmol/mL.h) or PARC/CCL18 (ng/mL) which are biomarkers secreted by the Gaucher cells and reflect the total body burden of storage cells in patients with GD1. GCase-based markers were: ActD — endogenous enzyme activity measured at diagnosis and expressed as percentage with respect to the healthy individual activity — was quantified in a reference centre at the time of diagnosis, using Raghavan's[28] and Chamoles' [29] analytical methods for peripheral blood leukocytes and dried blood spot samples, respectively. These values were collected from medical history; Act_0 — enzyme activity from the residual exogenous enzyme in peripheral leukocytes after previous infusions plus each patient's own residual endogenous enzyme —, measured prior to ERT and analysed locally at our centre; Act_{75} — enzyme activity measured as the maximum concentration reached in leukocytes after ERT—, measured 15 min. after infusion and analysed locally at our centre; $Act_{75\text{-}0}$ — enzyme activity accumulated during the ERT—, calculated as the difference between Act_{75} and Act_0. All the GCase-based markers were expressed in raw value and in percentage with respect to the healthy individual activity.

Data on clinical variables that support patient follow-up were collected when patients entered the study using the following tools:

The clinical response to ERT was calculated based on criteria established by Pastores *et al.* [12] that define an Optimal Response (OR) if 5/6 clinical criteria are met and a non-optimal response (NOR) when ≤4/6 criteria are met. The 6 parameters considered to evaluate OR/NOR are: Haemoglobin ≥ 11.0g/dL in women and ≥12.0g/dL in men; Platelets $\geq 120 \cdot 10^9$/L; Splenic volume <5 times normal value; Hepatic volume <1.25 times normal value; CT <600 nmol/mL·h; bone involvement, valued as bone pain ≤2 on the EVA scale and without the appearance of bone crises in 6 months.

GauSSI-I is a reliable method for staging the severity of adult GD1, and sensitive method for measuring changes in disease severity caused by therapy. It has a maximum of 42 points, distributed

over six different domains with unequally weighted parameters, skeletal (bone marrow infiltration and bone mineral component subdomains), hematologic and visceral domain represented 76% of points[13]. In this study, we used several tools to measure clinical response, due to the different weight of each of the clinical domains in each one and because they are the most widely used scales in clinical practice for monitoring patients with GD.

Other clinical data collected were: physical and mental quality of life assessment using SF36 [30], the S-MRI scale for infiltration in the bone marrow [31] and the Zimran severity scale (SSI) [32] measured on diagnosis.

Statistical analysis

The statistical analysis was conducted using the SPSS v19 statistical software (IBM SPSS Inc., Chicago, USA). The receiver operating characteristic (ROC) curve was applied and the area under the curve (AUC) was studied according to the OR with the objective of detecting markers related to enzyme activity with the greatest discriminative capacity for obtaining the OR to the ERT. The AUC was used to distinguish whether a marker was non-predictive ($AUC \leq 0.5$), less predictive ($0.5 < AUC < 0.7$), moderately predictive ($0.7 < AUC < 0.9$), highly predictive ($0.9 < AUC < 1$)[33]. Sensitivity (Se), specificity (Sp) and cut-off points for the markers studied were analysed. The Chi-square (χ^2) test and relative risk of the cut-off point of markers was applied.

The mean difference in parameters studied according to the clinical response reached was analysed using non-parametric tests: the Mann-Whitney U-test.

The correlation studies for activity markers with GauSSI-I and other quantitative clinical variables and its cut-off point were conducted using the Spearman Rho correlation coefficient with bilateral signification.

RESULTS

Population and normal values

A total of 19 patients diagnosed with GD1 and 10 healthy individuals were included in the study. Mean total GCase intra-leukocyte activity in healthy patients measured by fluorometric detection was 121.84 U/L (CI95% 101.99 to 141.69; CV 8.2%). Table 1 shows the clinical, biometric and dosage characteristics of the 19 GD1 patients included in the study. Of the 19 GD1 patients, 4 of them had non-optimal response to ERT.

Relationship of GCase-based markers and variables with clinical response

The mean values of the GCase-based markers in percentage with respect to the healthy individual activity and variables studied, and the results of the ROC curve of the patients included in the study are shown in Table 2.

Similar results were observed in analysis with raw values and in analysis with percentage values; AUC were: Act_0 = 0.505(CI95%: 0.254 a 0.757); Act_{75} = 0.763 (CI95%:0.625 a 0.902); Act_{75-0} =0.837 (CI95%: 0.635 a 0.938). The mean values according to optimal response were similar, too: Act_0 was 69.59 (SD 67.07) and 38.18 (SD 50.92) for OR and NOR; Act_{75} was 189.35 (SD 140.21) and 71.47 (SD 75.89) for OR and NOR; Act_{75-0} was 119.76 (SD 103.98) and 33.28 (SD 28.56) for OR and NOR. As analysis with percentage values (table 2), the only marker with statistical significance was Act_{75-0}.

Moderately predictive markers for optimal response (OR) were Act_{75} and Act_{75-0} but statistically significant differences on the AUC ROC curve were only detected for Act_{75-0}. Fig. 1 represents ROC curve for Act_{75-0}.

The association between the cut-off point of 58% of the Act_{75-0} and OR to the treatment presented a Chi-square of 5.630 with statistical significance (p=0.018) and a relative risk of 1.800 (IC95% 1.003 to 3.229) representing a probability of 64% of obtaining an OR to ERT.

Table 2 also presents the average values and the dispersion (SD) of the variables and markers studied according to the OR/NOR of the patients and their statistical significance. Also, the marker Act_{75-0} presented significant differences ($p<0.05$) between the mean values of patients with OR and NOR.

Relationship of GCase-based markers with clinical variables

The correlation between the clinical variables and Act_{75} and Act_{75-0} (Table 3) was studied. Act_{75} and Act_{75-0} presented significant and positive correlation with ActD. GauSSI-I showed negative and significant correlation with Act_{75}. The rest of the clinical variables presented non-significant negative linear correlation with Act_{75} and Act_{75-0}. Table 3 also shows the mean values of the individual clinical variables according to the cut-off point of 58% Act_{75-0}. ActD showed significant differences.

DISCUSSION

This is the first study to evaluate the relationship between intra-leukocyte enzymatic activity and clinical response in patients with GD1. It shows that GCase intra-leukocyte activity has correlation with clinical response: on the one hand, Act_{75}, the amount of enzyme inside the leukocyte 15-min. post ERT infusion, has an inverse and significant relationship with GauSS-I; thus, patients with low enzyme concentrations inside leukocyte post ERT infusion, have more severe Gaucher disease. Moreover, Act75 is a moderately predictive marker for optimal response based on criteria by Pastores *et al*. On the other hand, Act75-0, representing the total drug exposure during infusion, is also a moderately predictive marker for optimal response showing statistically significant difference.

ERT for GD1 aims to replace deficient endogenous enzyme activity in patients. Therefore, it should be expected that ERT would achieve the resolution of clinical symptoms of the disease in all patients. However, there is evidence of high inter-individual variability in clinical response and patient evolution. It has been postulated that this is due to residual Gaucher cells that remain in the body

and are associated with a highest risk of long-term complications[34, 35]. Results reported in this study could contribute to explain, at least in part, the non-optimal response to ERT observed in some patients, since due to the exogenous enzyme does not enter into the monocyte-macrophage system in sufficient concentrations and therefore does not reach the target organs and does not reverse the symptoms. A number of causes have been postulated for this low penetration of the exogenous enzyme into monocyte-macrophage of GD1 patients. These include different macrophage immunophenotypes[10], the high variability of the M6P receptor[36], the saturation of the mannose receptor at high doses[9] or that the enzyme is eliminated by peptidases or outside of the vascular endothelium at a faster rate than the rate of uptake by the monocyte[18, 37].

GCase activity measurement could give an answer to these questions because if the amount of enzyme inside the leukocyte post ERT infusion (endogenous plus exogenous enzyme) is known and that is distributed throughout the body by monocyte-macrophages system, it could predict the patient's clinical response. Results reported in this study suggest that Act_{75} could act as this marker. Moreover, $Act_{75\text{-}0}$ calculated as the difference in GCase activity measured prior to ERT and 15 min. post-infusion, reflects the amount of exogenous enzyme uptake by the leukocyte could be a tool to detect non-responder patients to ERT, because exogenous enzymes do not penetrate in leukocytes.

The mean values of $Act_{75\text{-}0}$ and Act_{75} in patients who have OR to ERT have 98% (SD 85%) and 154% (SD 115%) of the enzyme activity of healthy individuals, while non-responding patients have much lower values as 27% (SD 23%) and 58% (SD 62%), respectively.

High between subjects variability in the maximum activity achieved after the infusion into the leukocytes, which is later distributed throughout the organism (Act_{75}) could be due to the patients have different values of endogenous enzyme and because the variability in the degree of exogenous enzyme uptake [18]. Therefore, the amount of enzyme that comes from the ERT ($Act_{75\text{-}0}$) in patients with OR is virtually the same amount as healthy individuals have of endogenous enzyme. These data back the postulated hypothesis that is based on a low response rate can be explained because

patients' leukocytes take up less exogenous enzyme; thus, this marker could detect and clarify non-responding patients.

The rising ROC curve reflects the trade-off existing between sensitivity and specificity and statistical significance was only observed with the marker Act_{75-0} (fig. 1) with a cut-off point of 58%. This result indicates that this marker is useful to detect patients who are poor responders although with a relatively low sensitivity. Thus, 44% of the patients who have been defined as non-responders (<58%) will have OR and will, therefore, be false negatives. However, the relative risk of this marker with the OR is 1.8; thus, patients with Act_{75-0} over 58% have an increased probability of 64% of obtaining an OR to ERT.

Furthermore, we analysed raw values and in percentage relative to the normal one in order to check the accuracy of data. Results are similar because the variability of enzyme in healthy individuals was very small in our study (CV 8%) and when compared with other methods [(Raghavan CV=12,0%[28]; Peters CV=18,8%[25]; Beutler CV=21,4%[4]; Chamoles median 3.54U (min. 2.16 and max. 5.29) [29]].

The current gold standard marker for diagnosis of GD1 is an ActD enzyme activity value in leukocytes under 30% of the mean value in leukocytes obtained from healthy individuals. This study shows that patients with accumulated activities in leukocytes that are greater than 58% have a higher probability of an optimal response to ERT. These results match, because GCase deficiency under 30% and an exogenous enzyme replacement of 58% give values close to those of a healthy individual.

In regard to the relationship between activity markers and the clinical variables, Act_{75} showed a significant and negative correlation with GauSS-I, that is, low Act_{75} values are correlated with greatest Gaucher Severity Score Index. However, patients included in this study have no severe GD with mean GauSS-I of 4.6 of 42 points in the score (table 3).

Furthermore, Act_{75} and Act_{75-0} showed a significant and positive correlation with ActD. Similarly, Torralba *et al*. [38] recently defined a new criterion for the prognosis of the disease in addition to the diagnosis based on ActD: low ActD values are correlated with the greatest severity of GD1.

Results reported in this study indicate that ActD also shows correlation with the OR, although no statistical significance was obtained (Table 2), and there is a positive and linear correlation with $Act_{75\text{-}0}$ and Act_{75} (Table 3), so that patients with low ActD also have low $Act_{75\text{-}0}$ and Act_{75}. In this sense, patients with an OR and NOR in our study have mean values of ActD of 12% and 4%, respectively. While these values do not reach statistical differentiation, probably due to the variability of the data and small population, it could be attributed that the patients with non-optimal response have much lower residual enzyme activities and a lower response to the ERT. Based on the results of this study, if patients have low ActD even though high doses of ERT have been administered, $Act_{75\text{-}0}$ and Act_{75} values will not increase sufficiently in patients, and therefore, they will not achieve an OR to ERT.

Nevertheless, in this study, there are three limitations, which must be taken into account; firstly, a small population, as is typical in rare diseases was included in the study, and all of them with a low to moderate severity of the disease, showing low score in GauSS-I. Secondly, the small number of NOR patients because the patients studied are being treated for a long time (mean 16 years) with ERT dose adjustments based on clinical guidelines. However, and despite of these limitations, reported results are the first and novel approach in this field and may have important clinical implications in ERT individualization in GD patients; nowadays, time to optimal response could be delayed until 2 years with the economic cost that the treatment involves. These markers can lead to anticipate these decisions. Finally, a pharmacokinetic and pharmacodynamic modelling approach would allow a better understanding of the distribution of ERT in patients and confirm GCase enzymatic activity as a marker for therapeutic individualization.

In conclusion, the results of this study demonstrate that GCase-based activity markers ($Act_{75\text{-}0}$ and Act_{75}) have a good correlation with clinical response to ERT, and therefore it is possible to hypothesize that it could provide supporting clinical data for dose management in GD1 patients.

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TABLE1. Baseline characteristics of patients receiving treatment with enzyme replacement therapy

Patient nº	Sex	Genotype	Weight (kg)	Age at diagnosis (years)	Current age (years)	ActD (%)	Dose/kg (U/kg)	Dosing interval (days)	Time (years) in ERT	CT (nmol/ml.h)	ERT	GauSSI-I (0-44)	Response (items reached of 6)
1	M	N370S/G195W	55	6	26	2.94	17.00	28	20	1003	IMG	5	NOR (4/6)
2	W	N370S/L444P	67	15	33	0.14	23.88	14	19	641	IMG	6	OR (5/6)
3	W	N370S/L444P	75	21	52	2.00	32.00	14	16	380	IMG	7	NOR (4/6)
4	W	N370S/L444P	49	27	33	3.00	24.49	14	5	363	IMG	3	OR (6/6)
5	W	N370S/N370S	51	28	49	18.75	15.69	14	22	614	IMG	2	OR (5/6)
6	M	N370S/N370S	80	28	52	15.63	30.00	14	19	323	IMG	0	OR (6/6)
7	W	N370S/N370S	51	28	49	18.75	15.69	14	22	359	IMG	2	OR (6/6)
8	M	N370S/DELTA 55	90	8	20	15.00	26.67	14	13	321	IMG	2	OR (6/6)
9	W	N370S/L444P	63	20	49	6.25	31.75	14	14	29	VELA	4	OR (6/6)
10	W	N370S/unknown	53	21	64	27.00	67.92	14	12	1005	VELA	4	OR (5/6)
11	M	N370S/L444P	107	25	54	33.00	29.91	14	17	47	VELA	7	OR (5/6)
12	M	N370S/N370S	84	27	52	16.00	33.33	14	8	431	IMG	8	OR (5/6)
13	W	N370S/N188S	38	39	56	6.88	11.00	28	19	157	IMG	4	OR (5/6)
14	M	N370S/N188S	104	15	53	6.88	35.29	14	19	771	IMG	6	OR (5/6)
15	M	LEU375Arg/N370S	75	49	57	10.00	42.67	14	10	274	IMG	8	NOR (4/6)
16	W	L444P/Gly416Ser	77	27	71	2.50	20.78	14	18	40	IMG	9	NOR (4/6)
17	M	N370S/p.Arg159Trp	80	10	43	5.00	15.00	28	14	135	IMG	2	OR (5/6)
18	W	N370S/p.Arg159Trp	46	13	38	3.75	34.78	14	21	72	IMG	3	OR (6/6)
19	M	N370S/L444P	75	29	36	6.88	60.00	14	7	265	VELA	6	OR (6/6)

ERT: Enzyme replacement therapy; W:women; M: men; IMG: Imiglucerase; VELA: Velaglucerase; CT: chitotriosidase; GauSSI-I: the Gaucher Severity Score Index-type I; OR: optimal response; NOR: non-optimal response

TABLE 2. Results of biomarkers studied according to the optimal response. Mean values and the dispersion of the variables and markers studied by fluorimetric detection according to the optimal or non-optimal response of the patients and its statistical significance

Parameter	Mean (SD)	Mean values according to Optimal Response			ROC CURVE			
		ERT Response	Mean (SD)	p-value	AUC (IC95%)	Cut off	Se (%)	Spe (%)
Clinical parameters								
Ferritin (ng/ml)	395.22 (410.37)	Optimal	405.79 (408.45)	0.845	0.536 (0.290-0.769)	270.00	57	75
		Non-optimal	358.25 (478.52)					
ActD (%)	10.54 (9.07)	Optimal	12.19 (9.43)	0.057	0.807 (0.575-0.954)	2.97	93	75
		Non-optimal	4.36 (3.78)					
CT (nmol/ml.h)	380.56 (310.91)	Optimal	368.87 (283.67)	0.733	0.483 (0.252- 0.719)	522.00	27	75
		Non-optimal	424.00 (502.26)					
Therapy parameters								
Monthly doses (U/kg/4w)	57.51 (32.25)	Optimal	58.99 (33.78)	0.920	0.518 (0.190 -0.846)	45.00	73	50
		Non-optimal	51.97 (29.38)					
Act_0 (%)†	51.69 (52.58)	Optimal	57.12 (55.04)	0.230	0.700 (0.402-0.998)	22.00	73	75
		Non-optimal	31.34 (41.79)					
Act_{75} (%)‡	135.04 (112.20)	Optimal	154.41 (115.07)	0.162	0.733 (0.484-0.906)	30.02	87	50
		Non-optimal	58.65 (62.29)					
Act_{75-0}(%)§	83.35 (81.48)	Optimal	98.29 (85.34)	0.047*	0.817 (0.575-0.954)	57.98	67	100
		Non-optimal	27.32 (23.44)					

enzyme activity measured prior to ERT infusion; ‡ enzyme activity measured as the maximum concentration reached in leukocytes after ERT, measured 15 min after infusion; § enzyme activity accumulated during the ERT, calculated as the difference between Act_{75} and Act_0. SD: Standard deviation; ROC receiver operating curve; AUC: area under curve; Se: sensitivity: Sp: specificity. * $p<0.05$ represents statistical significance of 5%.

TABLE 3.

The correlation of the quantitative clinical variables and the Act_{75}(%) and the Act_{75-0}(%) and the averages of the individual clinical variables according to the cut-off point of 58% Act_{75-0}.

Variable	Mean (SD)	Correlation with		Mean according Act_{75-0}		
		Act_{75}, r (p-value)	**Act_{75-0}, r (p-value)**	**≥58%**	**<58%**	**p-value**
ActD	10.54 (9.07)	0.748 (0.0002)*	0.806 (0.00003)*	15.35	5.20	0.006*
ZimranDiag	7.45 (3.64)	-0.060 (0.861)	-0.060 (0.861)	7.83	7.00	0.662
Escala EVA	2.18 (2.46)	-0.104 (0.692)	-0.104 (0.692)	2.11	2.25	0.963
%SF36FIS	48.12 (8.47)	-0.248 (0.338)	-0.248 (0.338)	45.68	50.88	0.167
%SF36Ment	57.07 (8.70)	-0.103 (0.694)	-0.103 (0.694)	50.19	52.06	0.743
SMRI	6.16 (6.87)	-0.196 (0.421)	-0.266 (0.271)	5.60	6.78	0.400
GauSSI-I	4.63 (2.52)	-0.461 (0.044)*	-0.280 (0.246)	5.20	4.33	0.720

ZimDiag: Zimran severity scale (SSI) to the diagnosis; %SF36FIS y %SF36Ment are physical and mental quality of life assessment using SF36; GauSSI-I: the Gaucher Severity Score Index-type I; S-MRI: scale for infiltration in the bone marrow; r: correlation coefficient. (* $p<0.05$ represents statistical significance of 5%).

FIGURE.1

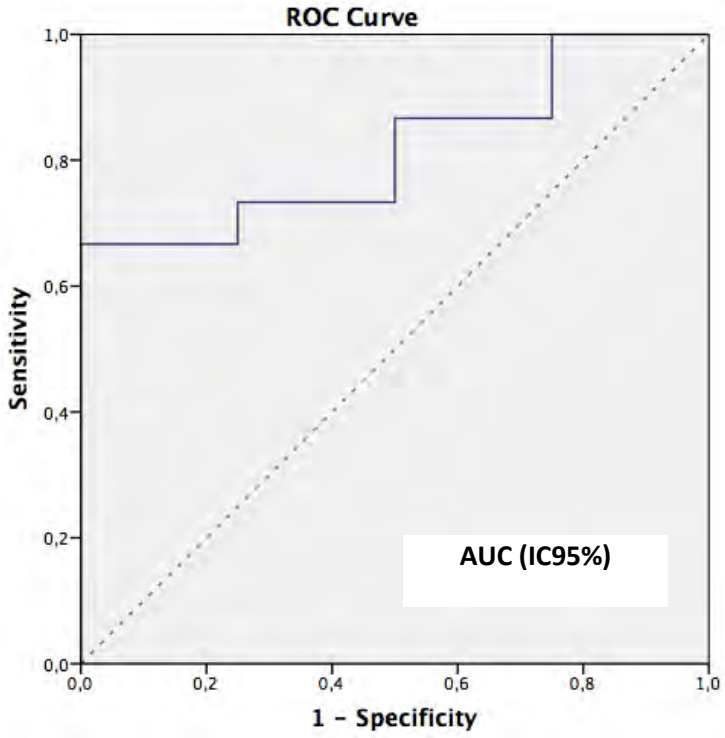

FIGURE CAPTIONS

FIGURE.1 Receiver operating characteristic (ROC) curve for the $Act_{75\text{-}0}$ (in percentage value) with optimal response (shown by the solid lines). Diagonal reference line is shown by the dotted lines.