

Plasma Chitotriosidase Activity in Patients with β -thalassemia

Submitted 12/04/98; revised 12/28/98

(communicated by Ernest Beutler, M.D., 12/29/98)

Rita Barone¹, Felicia Di Gregorio², Maria A. Romeo², Gino Schilirò³, Lorenzo Pavone¹

ABSTRACT: Chitotriosidase, a macrophage marker, which is extremely increased in plasma of Gaucher patients, was measured in patients with β -thalassemia, an haematological disorder characterized by the genetic defect of β -globin chains synthesis resulting in unproductive erythropoiesis and enormous expansion of the reticuloendothelial system. Plasma chitotriosidase was increased to a variable extent in 13 of 70 patients with β -thalassemia major treated with the intense transfusion regimen and iron chelation therapy. It was normal in 22 and slightly elevated in 3 subjects with β -thalassemia intermedia which were not transfused.

The highest levels of plasma chitotriosidase, as high as in Gaucher patients, were found in 7 (10%) of the β -thalassemia major patients which also had the highest degree of iron overload as judged by their serum ferritin level (> 3000 ng/ml), high SGPT level and elevated urinary iron excretion.

To our knowledge, β -thalassemia is hitherto the only disorder in which an increase of plasma chitotriosidase, comparable to that seen in Gaucher disease, may occur. The increase of plasma chitotriosidase activity in β -thalassemia patients with high iron overload, could be related to an iron mediated damage to the lysosomal apparatus. In addition, similarly to Gaucher disease, the increased chitotriosidase production in β -thalassemia might reflect macrophage activation probably related to the intracellular iron overload, storage of erythrocytes membrane break-down products and oxidation of excess alpha-hemoglobin subunits. Further studies are required to define the role of chitotriosidase evaluation to assess the efficacy of chelation therapy in reducing the macrophage activation due to intracellular iron overload in β -thalassemia.

© 1998 Academic Press

Keywords: β -thalassemia, chitotriosidase, Gaucher disease

INTRODUCTION

Chitotriosidase is a functional chitinase, with high homology to chitinases belonging to family 18 of glycosylhydrolases and present in different species. Predominantly it is a secretory protein

but it is in part processed and sorted into lysosomes. Chitotriosidase mRNA is expressed only at a late stage of differentiation of monocytes to activated macrophages in culture indicating that the enzyme has a strongly regulated expression. It appears that

¹ Division of Pediatric Neurology, Institute of Pediatrics, V.le A. Doria, 6, 95125 Catania-Italy;

² Center for Thalassemia, Institute of Pediatrics, V.le A. Doria, 6, 95125 Catania-Italy;

³ Division of Pediatric Hematology and Oncology, Institute of Pediatrics, University of Catania, Institute of Pediatrics, V.le A. Doria, 6, 95125 Catania-Italy.

Reprint request to: Rita Barone, M.D., Division of Pediatric Neurology, Institute of Pediatrics, V.le A. Doria, 6, 95125 Catania-Italy, phone 39 095256407, fax 39 095222532, e-mail: r.barone@tau.it

chitotriosidase might play a role in the degradation of chitin-containing pathogens and also could be used as a macrophage marker (1-4).

A marked increase of plasma chitotriosidase, has been reported in patients with Gaucher disease, a lysosomal storage disorder due to the deficiency of β -glucocerebrosidase (5).

Gaucher disease is characterized by the accumulation of glucocerebroside, a major component of the erythrocyte membrane, in the cells of the reticulo-endothelial system. The progressive storage of Gaucher-cells, lipid-laden macrophages, underlies the clinical manifestation of the disease including hepatosplenomegaly, haematological abnormalities and skeletal changes (6).

In Gaucher disease chitotriosidase can serve as a new diagnostic hallmark and it may be useful for monitoring the efficacy of therapeutic intervention (5). Enzyme replacement therapy with alglucerase results in a rapid decline but not in a correction of plasma chitotriosidase levels (5,7) whereas successful bone marrow transplantation of Gaucher patients leads to a nearly complete correction of chitotriosidase activity in plasma (8).

Evidence is accumulating that in GD plasma chitotriosidase levels might actually reflect the burden of Gaucher-cells: it is normal or only slightly elevated in plasma of asymptomatic Gaucher patients (5); isolated splenic Gaucher cells are rich in chitotriosidase and *in situ* hybridization studies have shown that Gaucher cells in spleen, liver and bone marrow produce high level of chitotriosidase mRNA (9).

The occurrence of the so-called "pseudo-Gaucher" cells has been reported in β -thalassemia (10) and in different haematological disorders characterized by an activation of the reticuloendothelial system with high cell turnover and storage of membrane glycolipids in the macrophages (11-12).

In β -thalassemia major the genetic defect of β -globin chains synthesis results in unproductive erythropoiesis due to intramedullary hemolysis

and extreme peripheral anaemia. The patients need assiduous and regular transfusions leading to a progressive iron overload, while the enormous expansion of the reticuloendothelial system results in an elevated blood consumption (13). The possibility of monitoring macrophage functions through plasma chitotriosidase activity might be useful in the clinical management of thalassemia patients.

The aim of this study was to assess plasma chitotriosidase levels in β -thalassemia patients with respect to clinical and laboratory parameters reflecting the status of the disease.

PATIENTS AND METHODS

Seventy-Two Sicilian patients with β -thalassemia major and 25 with β -thalassemia intermedia, were studied.

The median age of β -thalassemia major patients is 17 yrs (range 4-29) and they are all regularly transfused in the Thalassemic Center of Catania University in order to maintain their baseline Hb level above 9.5 g/dl. The patients are treated with subcutaneous desferrioxamine infusion (40-50 mg/Kg, 3-6 days a week) according to the ad hoc Italian protocol (14). Splenectomy was performed in 14 subjects.

Patients with β -thalassemia intermedia have a median age of 9 yrs (range 4-37) and are only occasionally transfused, when their Hb level falls below 7 g/dl. They never received desferrioxamine therapy, but a supplement of folic acid for monthly long period. Five were splenectomized.

Data regarding age, splenectomy, yearly blood consumption, pretransfusional Hb, HbF level, serum ferritin, serum transaminases (SGPT) and urinary iron excretion were obtained from the clinical case sheet, at the Thalassemic Center.

Fresh EDTA blood was obtained from β -thalassemia major patients preceding blood transfusion. Chitotriosidase activity was measured by incubating 5 μ l plasma with 100 μ l 22 μ mol/L 4-methylumbelliferyl- β -d-N,N',N''-triacetylchitotriose (Sigma Chemical

CO.) in citrate-phosphate buffer pH 5.2 for 15 min at 37C (5). The reaction was stopped by the addition of 2 ml 0.5 mol/l Na₂CO₃-NaHCO₃ buffer, pH 10.7, and the fluorescence was read on a Perkin Elmer fluorimeter, excitation 365 nm, emission 450 nm. Chitotriosidase activity was measured as nanomoles of substrate hydrolysed per minute per ml (mU/ml). Samples with a chitotriosidase activity > 1.8 mU/ml were reassayed after a dilution of 10-fold or 50-fold with distilled water.

A control group was also included and consisted of 85 healthy subjects with age and sex distribution as the patients. Moreover chitotriosidase was measured in plasma of 8 Sicilian subjects with Gaucher disease type I aged 5-64 years. According to the modified Severity Score Index (15) the clinical manifestations of the disease were mild in three patients, moderate and severe in two and three patients respectively. Enzyme replacement

therapy with alglucerase was performed in four subjects. In these latter, chitotriosidase was measured before starting the therapy.

STATISTICAL ANALYSES

The results are expressed as median and range or mean ± standard deviation (SD). Sample variances were compared by the F-test ($p < 0.05$), whereas sample means were compared by the Student t-test ($p < 0.05$).

RESULTS

In Table 1 the clinical and laboratory features of β-thalassemia major and β-thalassemia intermedia patients are reported. In patients with β-thalassemia major, comparison between serum ferritin and SGPT values and between serum ferritin and urinary iron excretion revealed no correlation.

Table 1. Clinical and laboratory features of β-thalassemia major and β-thalassemia intermedia patients.

	Thalassemia Major (n = 70)	Thalassemia Intermedia (n = 25)
Age (yrs)	17 (4-29)	9 (4-37)
Sex (M/F)	27/43	10/15
Splenectomy	14/70	5/25
Blood consumption (ml/Kg/year)	141±33.7	-
Pre-transfusional Hb level (gr/dl)	10±0.5	8.05±1.11
Hb F (%)	3 (0.88-2.83)	37 (5-81)
Serum ferritin (ng/ml) (Normal range 22-322)	1397 (319-3487)	122 (43-1378)
Serum GPT (mU/ml) (Normal range 5-50)	23 (10-203)	33 (17-50)
Urinary iron excretion (mg/day)	12 (3-59)	-

ranges in parenthesis

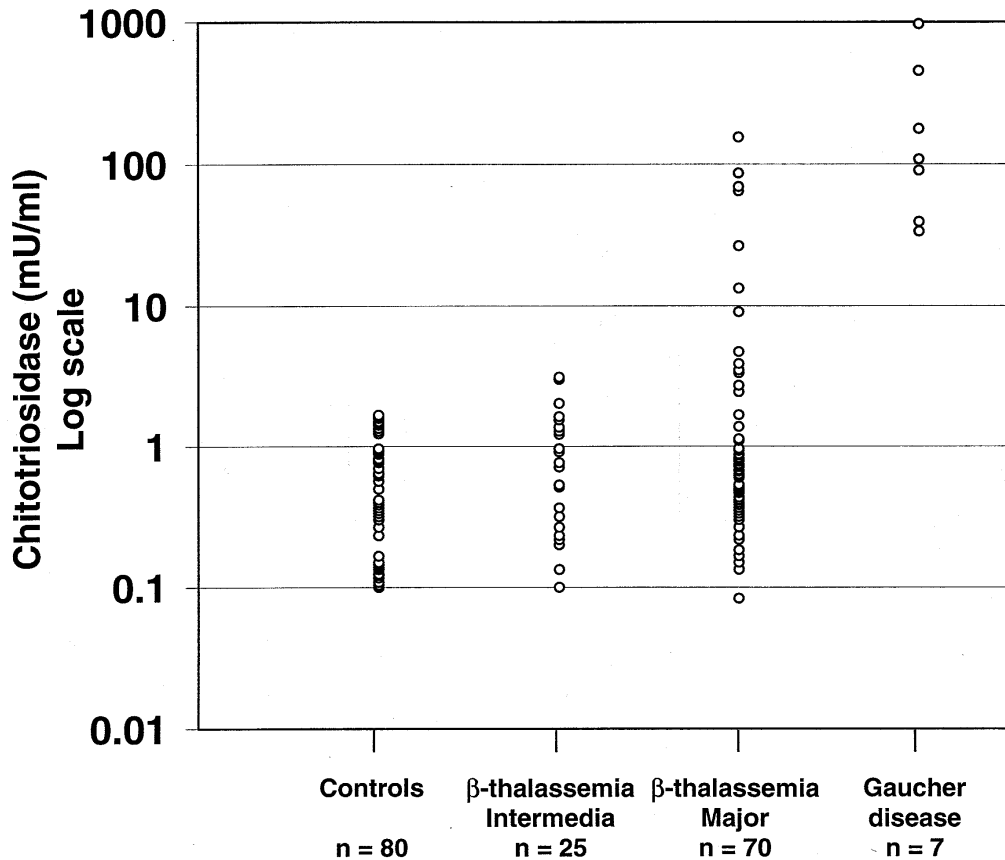


Figure 1. Values of chitotriosidase in β -thalassemia major and in β -thalassemia intermedia patients compared with values obtained in patients with Gaucher disease and in control subjects.

Mean value of plasma chitotriosidase levels in control individuals was 0.7 ± 0.5 mU/ml. Among controls, 5/85 (6%) had almost undetectable plasma chitotriosidase activity (chitotriosidase deficiency). In the group of patients with β -thalassemia major, 2/72 (3%) had chitotriosidase deficiency whereas no chitotriosidase deficient subjects were found among patients with β -thalassemia intermedia. Patients with chitotriosidase deficiency will not be mentioned below as they probably belong to a separate group.

Chitotriosidase activity was regarded as elevated if it was greater than the highest reference value (1.7 mU/ml). Chitotriosidase plasma levels in β -thalassemia patients, Gaucher patients and control subjects are shown in Figure 1.

An increase of plasma chitotriosidase levels was found in 13 of 70 (18%) patients with β -thalassemia major. On a whole, 3 groups of β -thalassemia major patients might be detected according to chitotriosidase activity: group A, (n. 57), has chitotriosidase activity in the same range as the control group (median 0.5 mU/ml; range 0.1-1.8). In group B (n. 6) chitotriosidase activity is comprised between 1.8 and 8.3 mU/ml (median 2.7; range: 1.8-8.0). Group C patients (n. 7) have chitotriosidase activity > 8.3 mU/ml (median: 60; range 13-154).

Group C patients, having chitotriosidase levels > 8.3 mU/ml showed a significantly higher mean serum ferritin level (> 3000 ng/ml), higher serum GPT level and higher urinary iron excretion, compared to β -thalassemia major patients with normal or moderately increased

chitotriosidase activity (Figure 2).

No significant differences were observed between the above groups regarding the age, number of splenectomized patients, mean blood yearly consumption and pretransfusional Hb levels.

All of β -thalassemia intermedia patients have plasma chitotriosidase activity in the normal range with exception of three patients in whom chitotriosidase is 3, 3.1 and 11.5 mU/ml respectively.

Chitotriosidase levels are extremely elevated in plasma from 7/8 Gaucher patients, the median being > 100-fold than in the control subjects (median: 108 mU/ml; range 33-966). One Gaucher patient, a 45 years old male with mild phenotype (SSI 5) had chitotriosidase deficiency (0.008 mU/ml).

No correlation was found between plasma chitotriosidase and serum ferritin levels in Gaucher patients ($r < 0.15$).

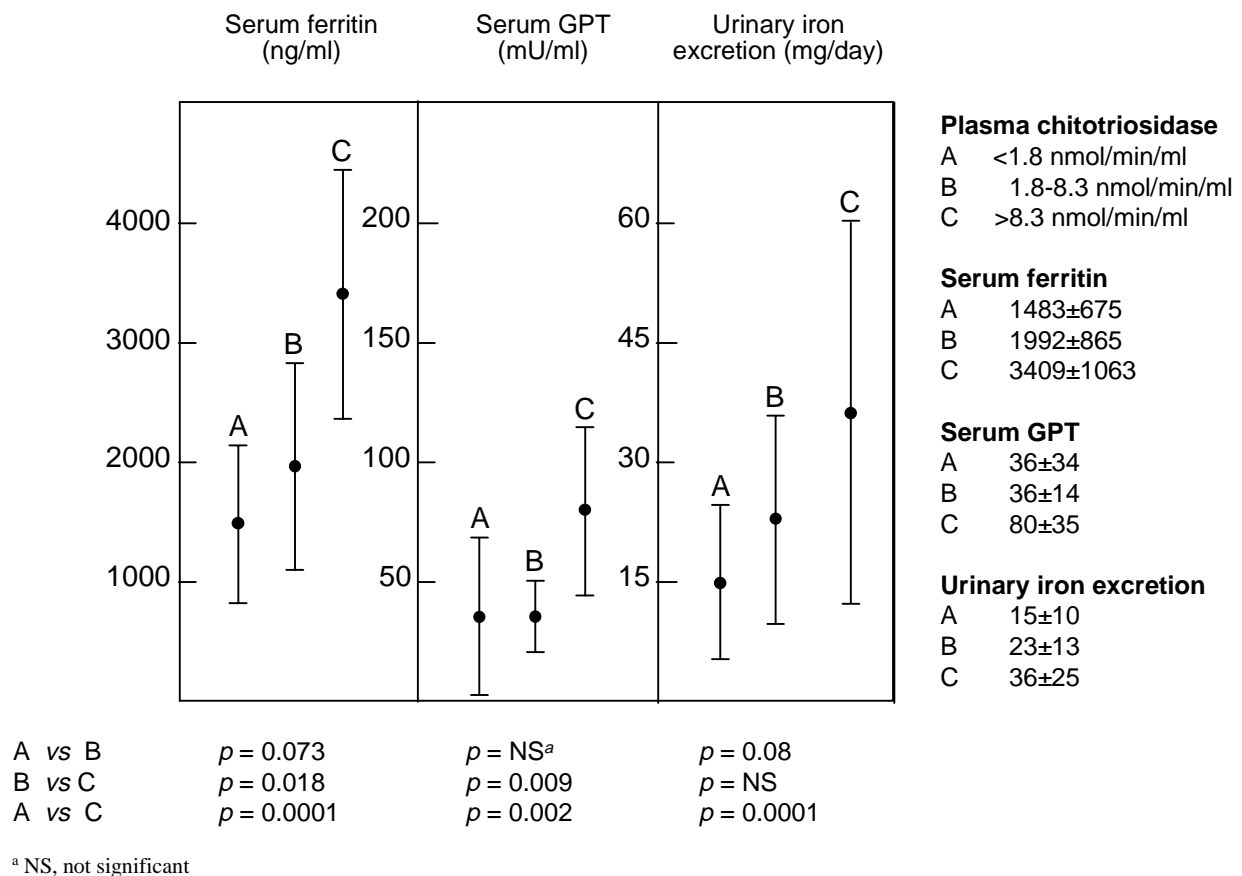


Figure 2. Mean values of serum ferritin (ng/ml), serum GPT (mU/ml) and urinary iron excretion (mg/day) in three separated groups (A-C) of β -thalassemia major patients according to their chitotriosidase activity.

DISCUSSION

After the initial description of a striking increase in plasma of symptomatic Gaucher patients, chitotriosidase has been studied in various lysosomal storage disorders (16-17) and different pathological conditions including infectious and immunological diseases (5,17). Among lysosomal diseases, slight to moderate elevations of plasma chitotriosidase activity have been reported in a restricted number of patients with Wolman disease, GM1-gangliosidosis, Krabbe disease and Niemann-Pick disease type A,B,C. Chitotriosidase was normal in plasma from patients with Wegener's granulomatosis, tuberculosis, leprosy, infectious diseases with hepatomegaly and histiocytosis whereas it was elevated in subjects with sarcoidosis and leishmaniasis (5,16-17). It has been suggested that elevated levels of chitotriosidase activity in plasma from patients with unexplained diseases may be indicative for a lysosomal involvement (16).

In this study we found an elevation of chitotriosidase in 13 of 70 patients with β -thalassemia major and in 3 of 25 patients with β -thalassemia intermedia. The highest levels of plasma chitotriosidase, as high as in Gaucher patients, were found in 7 (10%) of the β -thalassemic major patients treated with the intense transfusion regimen, which also had the highest degree of iron overload as judged by their serum ferritin level (> 3000 ng/ml), high SGPT level and elevated urinary iron excretion.

Interestingly the increase of chitotriosidase was not so high in the three patients with β -thalassemia intermedia, which are not transfused and have normal or only slightly increased serum ferritin level (< 1000 ng/ml). It has to be mentioned that the β -thalassemia intermedia patient with more pronounced increase of chitotriosidase (11.5 mU/ml) also had an extreme medullary and extramedullary erythropoiesis expansion.

To our knowledge, β -thalassemia is hitherto the only disorder in which an increase of plasma

chitotriosidase, comparable to that seen in Gaucher disease, may occur.

The possible role of chitotriosidase in iron metabolism is as yet unknown. Experimentally induced iron overload in different rat tissues (liver, spleen, kidney) leads to an increase of chitotriosidase activity which is different amongst various tissues and it is not directly related to the extent of iron accumulation, reflecting a different ability of each tissue in the synthesis and/or secretion of chitotriosidase (18).

It is known that iron accumulation results in lysosomal instability due to mechanical injury as well as to iron mediated peroxidative injury of lysosomal membranes (19-21). Increased urinary levels of the lysosomal enzyme N-acetyl- β -D-glucosaminidase is considered to be an harbinger of kidney damage due to high degree of iron overload in patients with β -thalassemia major (22). Similarly it may not be excluded that the increase of plasma chitotriosidase activity in our β -thalassemia major patients with high iron overload, could reflect an iron mediated damage to the lysosomal apparatus.

It has to be noted that in addition to the increase of plasma chitotriosidase, symptomatic Gaucher patients have high serum ferritin levels and Gaucher cells do contain high concentration of iron and ferritin as well (9). However, as in our own experience, a direct correlation between plasma chitotriosidase and serum ferritin levels in patients with Gaucher disease, has not been demonstrated.

Such findings suggest that the iron storage alone is not a sufficient condition for the elevation of chitotriosidase and most probably the activation of other pathways plays an important role in bringing about the changes seen in Gaucher disease and in β -thalassemia major.

It is interesting to note that thalassemic patients and Gaucher patients share other biochemical abnormalities that address to an important role of activated macrophages into the physiopathology of both these disorders: an increased consumption of Vitamin E reflecting

the existence of activated macrophages and excess of free oxygen radicals production has been documented for a very long time (23). More recently, high levels of serum macrophage colony stimulating factor (M-CSF), which controls and enhances several monocyte-macrophage functions, has been shown in β -thalassemia (24) and Gaucher disease (9). Therefore, it is probable that similarly to Gaucher disease, the increased chitotriosidase production in β -thalassemia might reflect macrophage activation probably related to intracellular iron overload, storage of erythrocytes membrane break-down products and oxidation of excess alpha-hemoglobin subunits.

If the use of the high transfusion regimen in patients with β -thalassemia major is effective in preventing peripheral hypoxia as well as medullary and extramedullary erythropoiesis (25), on the other hand, the continuous blood transfusions lead to a large iron accumulation so that thalassemia major is currently considered a disease of chronic iron overload (13, 25-26). Up to date the management of thalassemia patients with the appropriate use of iron chelators aims to obtain the most correct balance between iron intake and output (26). If these data will be confirmed plasma chitotriosidase evaluation could represent an additional biochemical parameter to assess the efficacy of chelation therapy in reducing the macrophage activation due to intracellular iron overload.

Further studies are required including the investigation of chitotriosidase mRNA expression and the determination of chitotriosidase activity in different tissues to elucidate the meaning of chitotriosidase increase in β -thalassemia and in lysosomal storage diseases.

ACKNOWLEDGMENTS

We gratefully acknowledge the help of Prof. S. Musumeci (Department of Paediatrics, University of Sassari-Italy) for statistical analyses of the data and his useful suggestions during the preparation of the manuscript.

REFERENCES

1. Renkema GH, Boot RG, Muijsers AO, Donker-Koopman WE, Aerts JM. Purification and characterization of human chitotriosidase, a novel member of the chitinase family of proteins. *J Biol Chem* 270:2198-2202, 1995.
2. Boot RG, Renkema GH, Strijland A, van Zonneveld AJ, Aerts JM. Cloning of a cDNA encoding chitotriosidase, a human chitinase produced by macrophages. *J Biol Chem* 270:26252-26256, 1995.
3. Renkema GH, Boot RG, Strijland A et al. Synthesis, sorting and processing into distinct isoforms of human macrophage chitotriosidase. *Eur J Biochem* 244:279-285, 1997.
4. Renkema GH, Boot RG, Au F et al. Chitotriosidase, a chitinase, and the 39-kDa human cartilage glycoprotein, a chitin-binding lectin, are homologues of family 18 glycosyl hydrolases secreted by human macrophages. *Eur J Biochem* 251:504-509, 1998.
5. Hollak CE, van Weely S, van Oers MHJ, Aerts JMFG. Marked elevation of plasma chitotriosidase activity. A novel hallmark of Gaucher disease. *J Clin Invest* 93:1288-1292, 1994.
6. Beutler E, Grabowski GA. Glucosylceramide lipidoses: Gaucher disease. In: Scriver CR, Beaudet AL, Sly W, Valle D, eds. *The Metabolic Basis of Inherited Disease*. New York: McGraw-Hill, pp. 2641-2670, 1995.
7. Den Tandt WR, van Hoof F. Plasma methylumbelliferyl-tetra-N-acetyl- β -D-chitotetraoside hydrolase as a parameter during treatment of Gaucher patients. *Bioch Mol Med*. 1996; 57:71-72.
8. Young E, Chatterton C, Vellodi A, Winchester B. Plasma chitotriosidase activity in Gaucher disease patients who have been treated either by bone marrow transplantation or by enzyme replacement therapy with alglucerase. *J Inher Metab Dis* 20:595-602, 1997.
9. Aerts JMFG, Hollak CEM. Plasma and metabolic abnormalities in Gaucher's disease. In: Zimran A, Guest ed. *Gaucher's Disease*. Baillière's Clinical Haematology. Baillière's Tindall, vol. 10(4), pp. 691-711, 1997.
10. Zaino EC, Rossi MB, Pham TD, Azar HA. Gaucher's cells in thalassemia. *Blood* 38:457-462, 1971.
11. Scullin DC Jr, Shelburne JD, Cohen HJ. Pseudo-Gaucher cells in multiple myeloma. *Am J Med* 67:347-352, 1979.
12. Zidar BL, Hartsock RJ, Lee RE et al. Pseudo-Gaucher cells in the bone marrow of a patient with Hodgkin's disease. *Am J Clin Pathol* 87:533-536, 1987.

13. Piomelli S. The management of patients with Cooley's anemia: transfusions and splenectomy. *Semin Hematol* 32:262-268, 1995.
14. Cao A, Gabutti V, Masera G, Modell B, Sirchia G, Vullo C. Raccomandazioni per il trattamento della β -thalassemia. Saronno, Ciba-Geigy, 1990.
15. Zimran A, Kay AC, Gelbart T, et al. The natural history of adult type Gaucher disease: clinical, laboratory, radiological and genetic features of 53 patients. *Medicine* (Baltimore) 71:337-353, 1992.
16. Guo Y, He W, Boer AM, et al. Elevated plasma chitotriosidase activity in various lysosomal storage disorders. *J Inher Metab Dis* 18:717-722, 1995.
17. Den Tandt WR, Van Hoof F. Marked increase of methylumbelliferyl-tetra-N-acetylchitotrioside hydrolase activity in plasma from Gaucher disease patients. *J Inher Metab Dis* 19:344-350, 1996.
18. Meraitou M, Dimitriou E, Michelakakis H. Iron load and chitotriosidase activity in rat tissues (Abstract). Meeting of the Second Working Group of Gaucher Disease, Maastricht, May 1-3, 1997.
19. Seymour SA, Peters JL. Organelle pathology in primary and secondary Haemochromatosis with special reference to lysosomal changes. *Br J Haematol* 40:239-253, 1978.
20. Solecki R, Zolinicki T von, Muller HM, et al. Iron overload of spleen, liver and kidney as a consequence of hemolytic anemia. *Exp Pathol* 23:227-235, 1983.
21. Tong Mak I, Weglicki EB. Characterization of iron-mediated peroxidative injury in isolated hepatic lysosomes. *J Clin Invest* 75:58-63, 1985.
22. Michelakakis H, Dimitriou E, Georgakis H, et al. Iron overload and urinary lysosomal enzyme levels in β -thalassemia major. *Eur J Pediatr* 156:602-604, 1997.
23. Rachmilewitz EA, Kornberg A, Acker M. Vitamin E deficiency due to increased consumption in β -thalassemia and in Gaucher disease. *Ann NY Acad Sci* 336-346, 1982.
24. Wiener E, Wanachiwanawin W, Chinprasertsuk S et al. Increased serum levels of macrophage colony-stimulating factor (M-CSF) in α - and β -thalassemia syndromes: correlation with anemia and monocyte activation. *Eur J Haematol* 57:364-369, 1996.
25. Gabutti V, Borgna-Pignatti C. Clinical manifestations and therapy of transfusional haemosiderosis. In: Baillière's Clinical Haematology, Baillières Tindall vol.7(4), pp.919-940, 1994.
26. Giardina PJ, Grady RW. Chelation therapy in β -thalassemia: the benefits and limitations of Desferrioxamine. *Semin Hematol* 32:304-312, 1995.