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RARE DISEASE WORKSHOP SERIES
Improving the Clinical Development Process

Chitotriosidase

surrogate endpoint in Gaucher Disease

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November 8, 2011



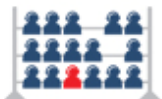
About Amdix

- Founded mid 2010 as spin-off from Academic Medical Center Amsterdam; AMC only shareholder;
- Exclusive licenses to relevant AMC patents;
- Expertise in product development and sales of IVD's;
- Qualified contract manufacturing partners and regulatory consultants;



Gaucher Disease

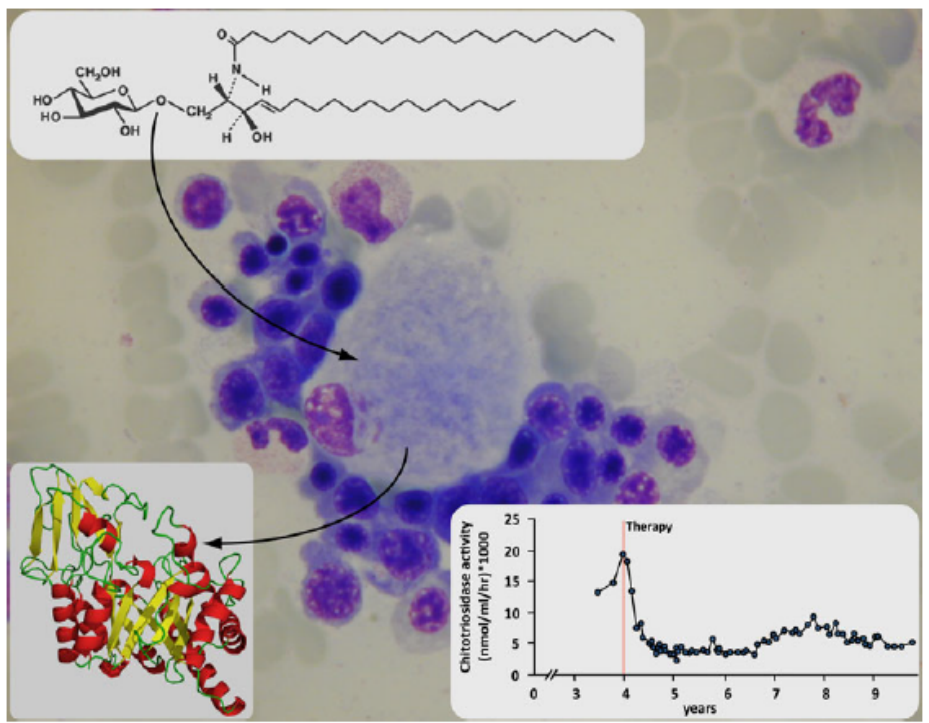
- Deficiency of enzyme glucocerebrosidase due to autosomal recessive inheritance (1 in 200.000);
- Lysosomal hydrolase responsible for the breakdown of glucocerebroside (glucosylceramide);
- Massive accumulation of glucosylceramide-laden macrophages in the spleen, liver and bone marrow;
- 2 ERT and 1 SRT products on the market; 1 MA pending
 - treatment costs approximately \$200,000 per year per pt (for life)
- More products in clinical trial;
- Official clinical endpoints for GD: spleen and liver size, hemoglobin levels, and platelet levels;



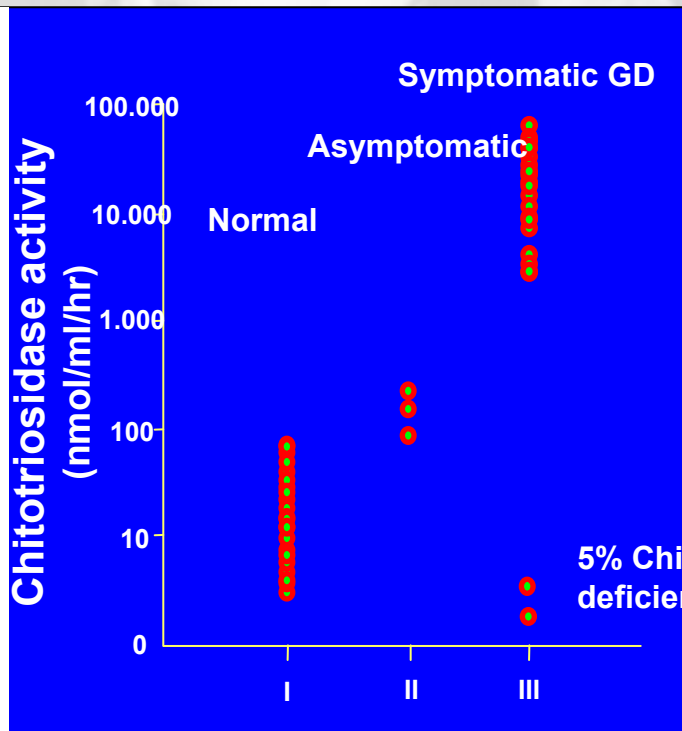
The marker Chitotriosidase (CT)

Discovered 17 years ago in plasma of Gaucher patients but
CT is also secreted by **activated macrophages** in other inflammatory diseases;

Fig. 1 Gaucher cell accumulating the glycosphingolipid glucosylceramide and specifically secreting the biomarker chitotriosidase that can be detected in plasma. Example of corrections in plasma chitotriosidase in Gaucher disease patients receiving enzyme replacement therapy



J. Inher. Metab. 2010, Aerts et al



J. Clin. Inv. 1994 Hollak et al

(CT measured with 4MU-chitobiose substrate)



About Chitinases

- Belong to endoglycosidase family;
- Hydrolyse chitin, present in fungi and exoskeleton;
- Two types of chitinases discovered in humans
 - **Chitotriosidase** (LSD, CAD, MS, RA, sarcoidosis,..)
 - Acidic Mammalian Chitinase (COPD, asthma)
- Detectable in plasma, DBS, CSF, BALF specimen;
- ~5% of population is CT deficient due to homozygous 24 bp duplication (0 activity); most pts genotyped;
- Normal CT activity level between 0-100 nmol/ml/hour (depending on age);



Direct effect in CT by ERT

Z. Šumarac et al / Clinical Biochemistry 44 (2011) 950–954

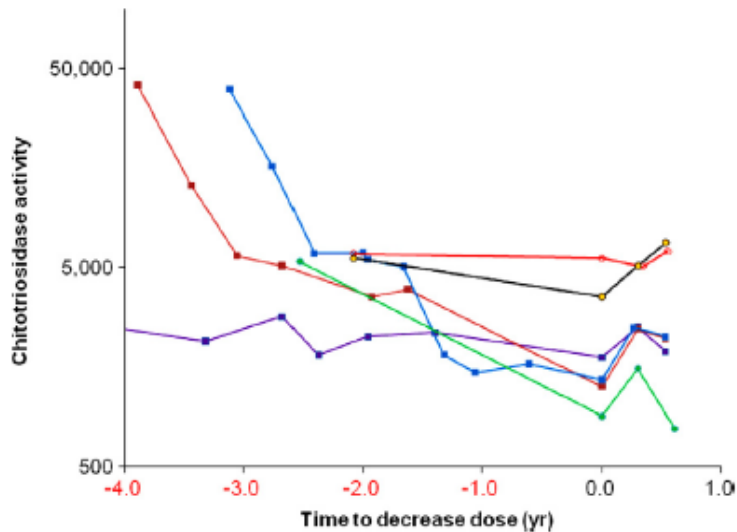
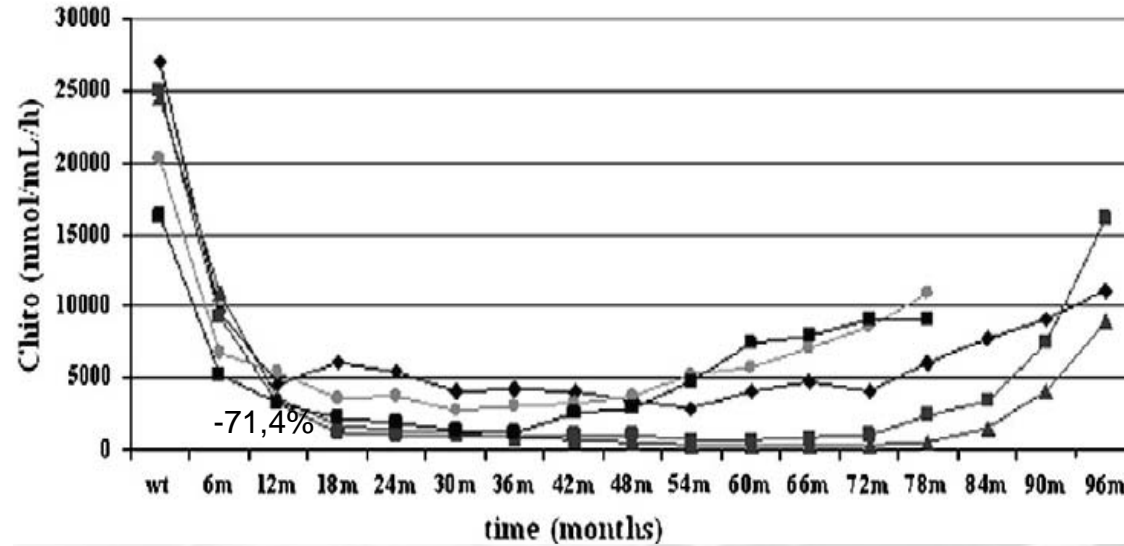


Fig. 1. Change of chitotriosidase before and after dose reduction.



(CT measured with 4MU-chitobiose substrate)

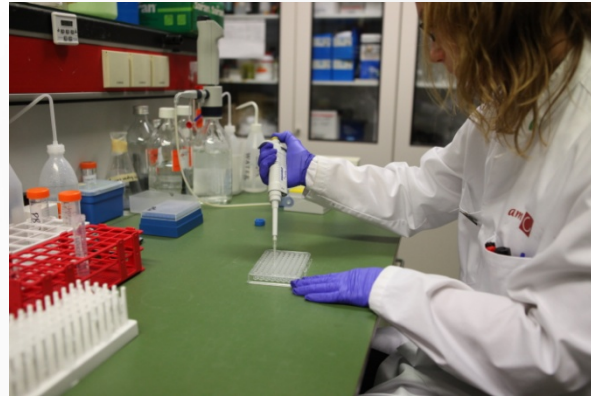


Chitotriosidase

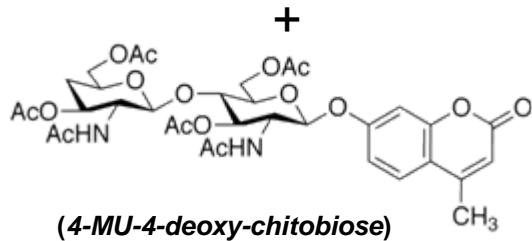
- Meets most requirements for a surrogate
 - ✓ Must accurately represent a **direct** effect on a clinical outcome, not a parallel pathway to disease mechanism
 - ✓ Clinical evidence that intervention is not adverse
 - ✓ Must fully capture net effect on clinical endpoint
 - ✓ Must be strong and durable effect
 - ? Specific to a drug MOA and specific indication
 - Meets all biomarker criteria (sensitive, represent. sampling site)
 - So.. CT is an ideal biomarker for disease and treatment monitoring in GD..
- But**....conventional chitinase assay was not standardized and inaccurate due to transglycosylation effect.



Chitinase Assay



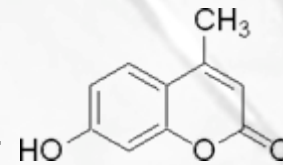
Chitinase/plasma sample



20 min 37°C
pH=5,2



4-deoxy-chitobiose +



(4-MU)

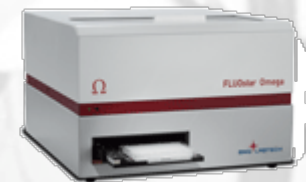
+ GlycineNaOH
pH=10,6



$\lambda_{ex}:360nm$



$\lambda_{ex}:450nm$

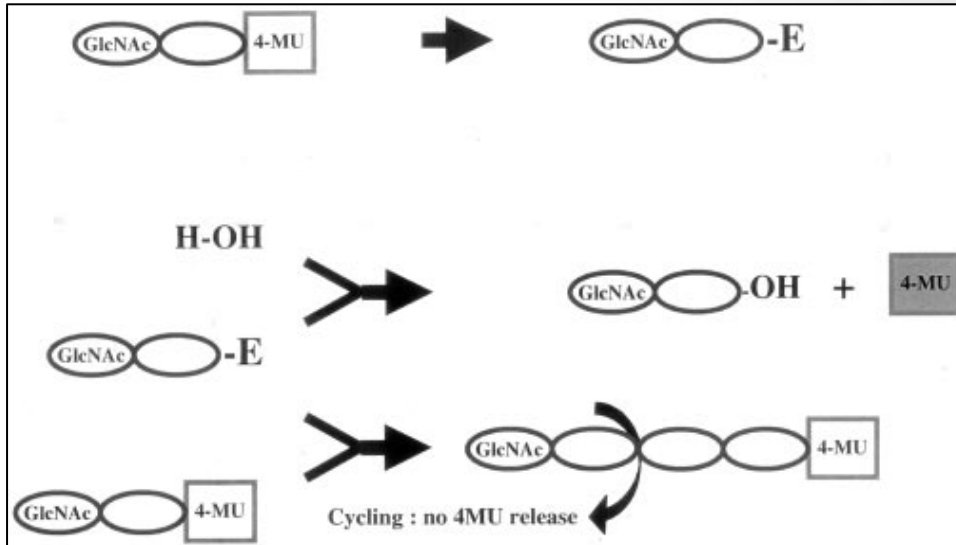


$$\text{Units/ml} = \frac{(\text{FLU}_{\text{sample}} - \text{FLU}_{\text{blank}}) \times 1.9 \times 0.3 \times \text{DF}}{\text{FLU}_{\text{standard}} \times \text{time} \times V_{\text{enz}}}$$

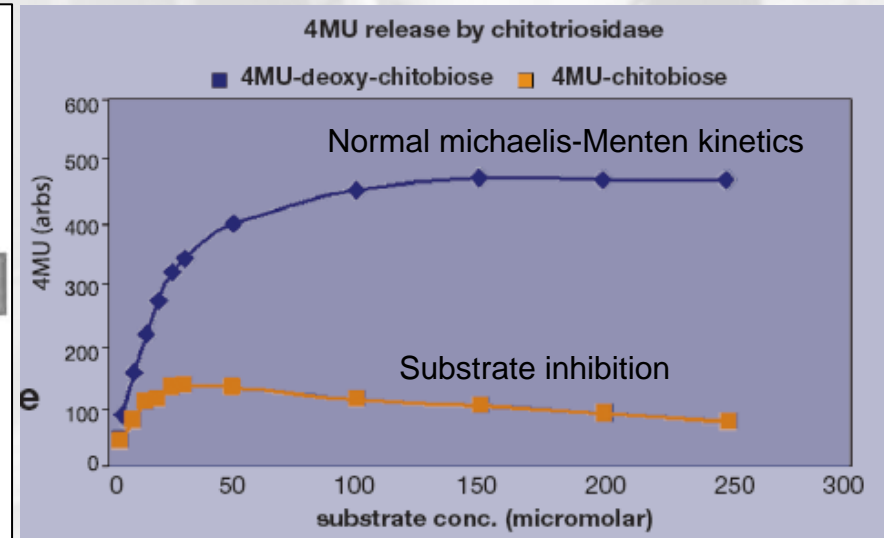


Transglycosylation

Deoxy-substrate prevents transglycosylation



Common (~37%) G102S substitution also has a transglycosylation effect (30% or 60% reduced)



A significant additional advantage is the 5-fold increase in sensitivity.



Development of a *kit*

Standardized, quality, simplified assay needed because..

- Novel substrate broadens market for Chit assay
Major clinical labs are unfamiliar with the protocol
- Qualification of CT as surrogate endpoint requires standardized robust assay method;
 - Now validated to be robust and accurate assay;
 - Chito Reference Standard available;
 - Ready-made solutions;
- to pursue FDA approval for clinical use of IVD.



Chito-kit



- Launched April 2011;
- Content: protocol, 4MU standard for calibration, stopbuffer, fluorogenic substrate solution;
- Patented substrate 4MU-*deoxy*- β -D-chitobiose;
- Reference Standard (spiked plasma) available;
- GMP manufactured;
- For use in any type of fluorometer;
- FDA IVD approval pending;
- Stability > 2 years when stored at 4°C;



Benefits

• *Improved accuracy*

- Linear
- Highly robust and precise (CV<15%);
- A *standardized* assay method for all users;
- Qualified surrogate assay>> accelerated FDA approval for IND's

• *High Sensitivity*

5-fold increased due to longer dynamic range;

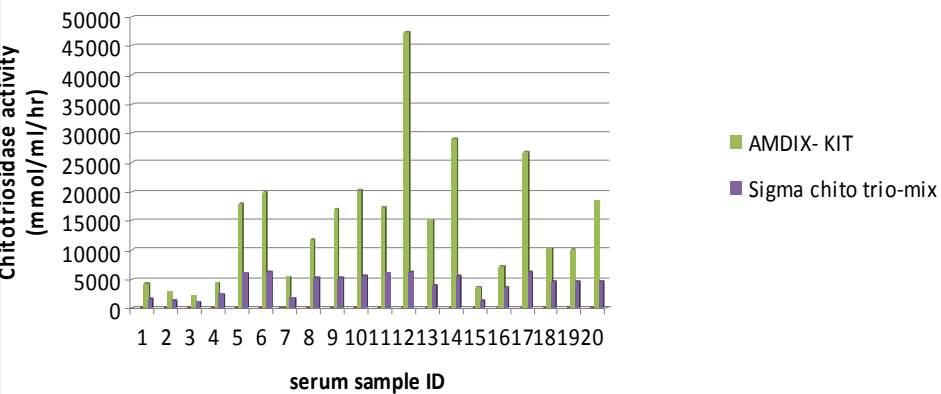
• *Greater applicability*

- Also applicable in diseases with 2-20 fold elevated Chit levels;
- Also accurate in ~37% of people with Chit polymorphism G102S;



Improved accuracy

Comparison of substrate use in chito assay



Spearman correlation between clinical parameters and CT activity

Substrates/Groups	4MU-Chitotriose	4MU-Chitobiose	4MU-Deoxychitobiose
Liver volume	0.453** (n=91)	0.388** (n=81)	0.513** (n=88)
Spleen volume	0.354** (n=79)	0.385** (n=71)	0.451** (n=76)
Spleen and liver	0.445** (n=91)	0.420** (n=81)	0.525** (n=88)
Hemoglobin	-0.037 (n=91)	-0.046 (n=81)	-0.136 (n=88)
Platelet	-0.217* (n=91)	-0.302** (n=81)	-0.271* (n=88)
Age	-0.264* (n=91)	-0.205 (n=81)	-0.166 (n=88)
SSI	0.302** (n=83)	0.336** (n=74)	0.359** (n=80)

Significance: * $p < 0.05$, ** $p < 0.01$; n = number of included patients. (activity of CT-carriers doubled).

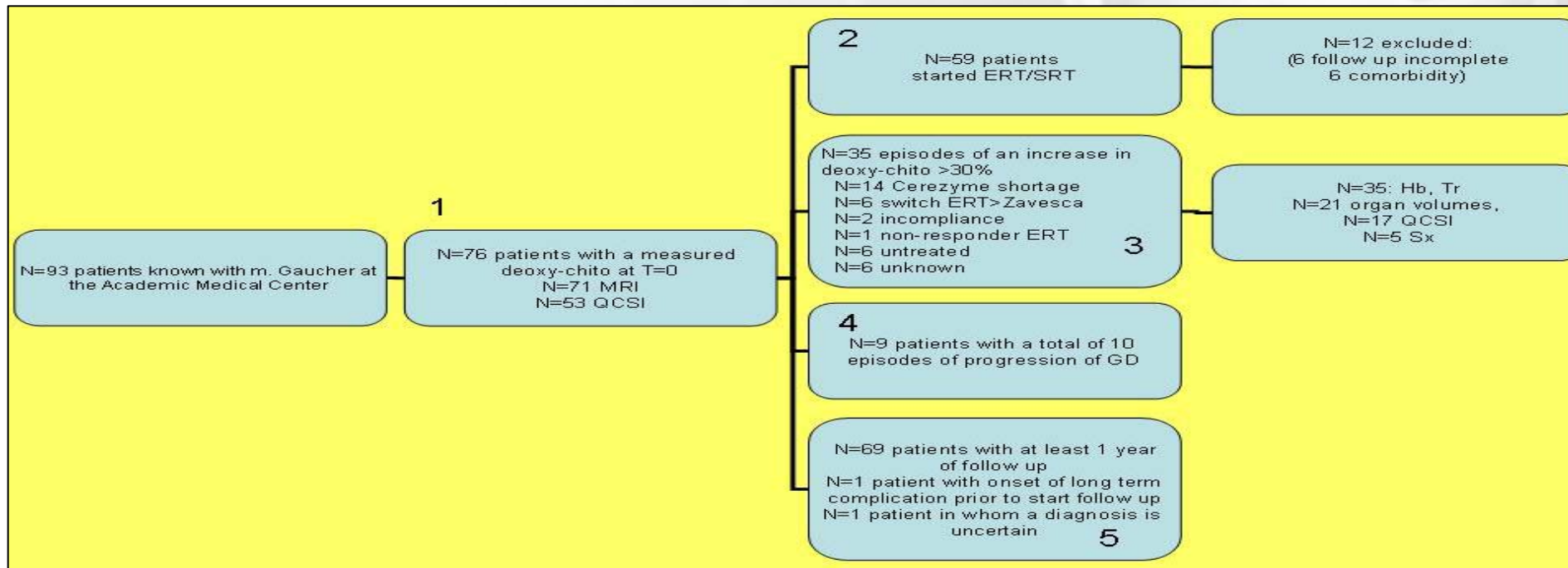
Clinica Chimica Acta 381 (2007) 136–139

Chitotriosidase activity is a surrogate marker of Gaucher disease: a qualification study

L van Dussen¹, EJ Hendriks², JEM Groener², EM Akkerman³, M Maas³, CEM Hollak¹, JMFG Aerts²

Amsterdam Lysosomal Center Sphinx. ¹Department of Internal Medicine, Division of Endocrinology and Metabolism; ²Medical Biochemistry; ³Department of Radiology. Academic Medical Center, University of Amsterdam, The Netherlands

Retrospective cohort study, presented at the ESGLD meeting sep 2011, Finland.



Criteria for progression of disease

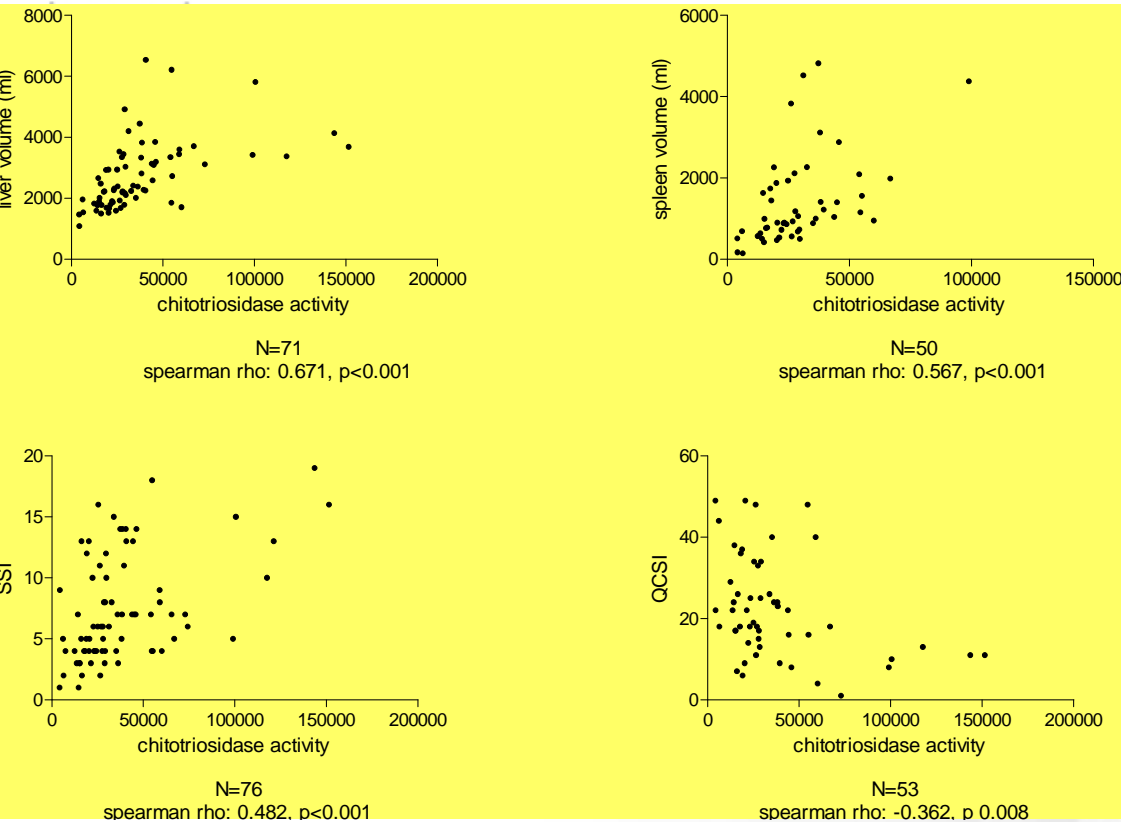
At least 2 of the following criteria:

- Increase in liver- and/or spleenvolume $\geq 10\%$
- Decrease in QCSI $\leq 23\%$ or relative decrease of 20% combined with an absolute decrease of $> 5\%$
- Decrease in Hb < 8 mmol/l (σ) or $< 7,5$ mmol/l (ϱ) and with > 1 mmol/L
- Decrease in platelet count $\leq 150 \times 10^9/l$ and $> 20 \times 10^9/l^{**}$

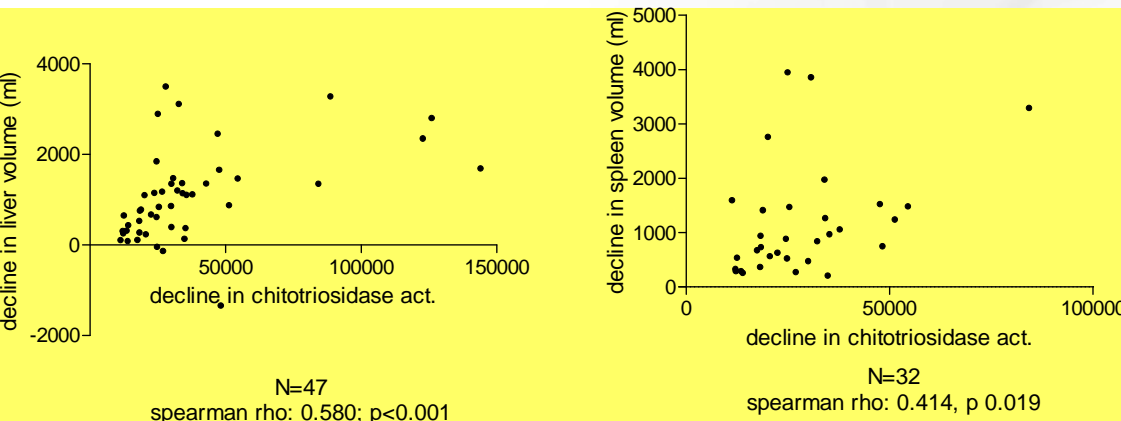
**persisting = in at least 2 succeeding measurements

In splenectomized patients only the last criterion suffices

Results



(CT measured with deoxy substrate)



- Baseline CT showed **significant correlations** with organ volumes, SSI scores and QCSI measurements;

- The absolute decline in CT activity in response to treatment **correlated** with the decrease in organ volumes;

- In 20 patients showing an increase in CT of >30%, the absolute increase **correlated** with the increase in spleen volume (rho 0.681; p 0.004);

- In 8 of 10 episodes of a clinically significant deterioration, CT increased >30%;



Other GD plasma biomarkers

- CCL-18
- MIP-1
- Tartrate-resistant acid phosphatase (TRAP)
- angiotensin-converting enzyme (ACE)

Show lower correlation according to literature;

Other parameters:

- Bone MRI
- QSCI and SSI



CT testing in LSD

Gaucher disease (type I)

- ~1000x fold elevated;
- Confirmation of genetic diagnostic test;
- Regular monitoring of all diagnosed patients;
- Tx monitoring every 3-6 mo; dose adjustments;
- Clinical trials of investigational drugs;
- (possible future) neonatal screening in EU + other countries;
- (CT deficient need to be monitored by CCL18)

Chito in Fabry
Vedder et al 2006 Mol Gen Metab



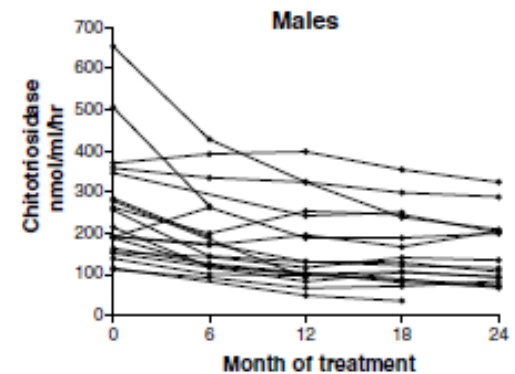
CT in LSD other than GD

Table 2 Plasma chitotriosidase activity in 24 lysosomal disorders^a

Disease	Plasma chitotriosidase activity	
	Abnormal/total	Elevated activity (nmol/h per ml)
Aspartyl glucosaminuria	0/3	
Fabry	0/8	
Gaucher type I ^{a,b}	20/21	5580–51 800
Glycogen storage disease II	2/8	360; 420
GM1-gangliosidosis ^b	7/13	380; 720–1420
GM2-gangliosidosis	0/11	
Krabbe disease ^b	7/11	610–1670
α -Mannosidosis	1/3	300
β -Mannosidosis	0/2	
Metachromatic leukodystrophy	1/29	550
Mucopolysaccharidoses (I; II; IIIA; B, C; IVA, B)	2/63	600; 400
Mucopolipidosis II/III	0/3	
α -NAGA deficiency	0/2	
Niemann-Pick A/B ^b	13/15	250; 602–2800
Niemann-Pick C ^b	6/11	263; 304–940
Sialic acid storage disorders	0/1	
Sialidosis	0/1	
Total	58/205	(28%)

^aThe patients in this table were diagnosed in the authors' laboratories (Rotterdam, Nijmegen and Leiden) and reflect the relative frequencies of the various diseases. The number of Gaucher patients is therefore different from the high number in Table 1

^bDiseases where majority of patients have elevated chitotriosidase activity



CT decline in Fabry treatment.



Inflammatory diseases

2-100x elevated *chitotriosidase* (or *AMCase**)

- Atherosclerosis
- Sarcoidosis, Asthma*
- Rheumatoid arthritis
- Thalassemia
- Visceral leishmaniasis
- Multiple sclerosis



Pulmonary diseases

SARCOIDOSIS, ASTHMA, COPD

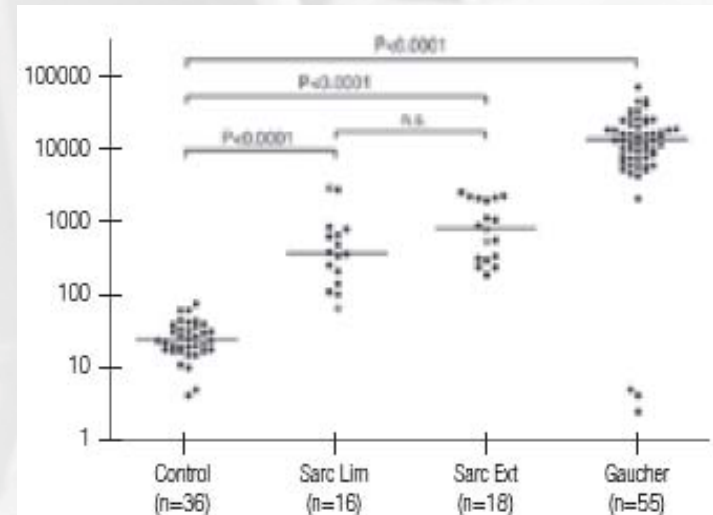
In cases of sarcoidosis where high Chitotriosidase activities are found, this enzyme could serve as a useful marker supporting the diagnosis of sarcoidosis when following the effects of treatment and in and surveillance for recurrence of the disease.

Chitotriosidase activity in sarcoidosis and some other pulmonary diseases (Scand. J Clin & Lab Inv 2009 Vol. 69, No. 5, 575–578).

Tercelj et al, University Medical Center Lubljana

Serum chitotriosidase is a readily detected and inexpensive marker of sarcoidosis with adequate sensitivity and specificity. It could be used in clinical practice together with other markers to evaluate severity and monitor follow-up.

Human Chitotriosidase: A Potential New Marker of Sarcoidosis Severity (Respiration 2008;76:234–238). Bargagli et al, Siena University.



Boot et al, Clin. Chimica Acta 411 (2010) 31–36.



MS

Correlation Between Chit Ratio (X Variable) and Cerebrospinal Fluid/Clinical Findings (Y Variables) in the Multiple Sclerosis Patients Series

Variables		Statistics	
X	Y	r	p
Chit ratio	Albumin quotient	-0.12	NS
Chit ratio	EDSS at entry	0.24	NS
Chit ratio	EDSS at follow-up	0.91	<0.001

Correlation coefficient (r) and significance (p) values are indicated. EDSS = Extended Disability Status Scale; NS = not significant.
Source: Sotgiu et al., 2006.48

MULTIPLE SCLEROSIS

This study not only confirms the important role of the innate immune response in MS phenomenology, but also allows Chit determination in CSF and plasma to be proposed as a diagnostic and monitoring method in an MS laboratory. As a result of our analysis, plasma and, to a larger extent, CSF Chit levels better correlate with the extent of CNS damage compared with the previously proposed macrophage-derived markers such as TNF- α , IL-6, IL-1, NO and ROS.



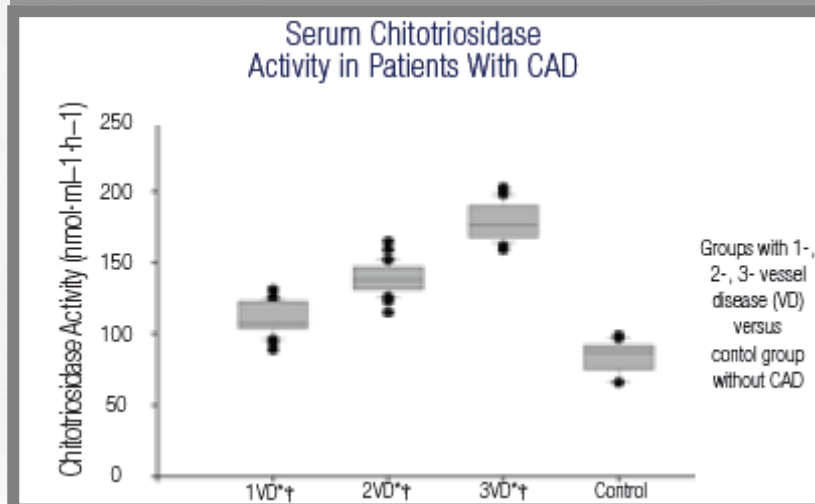
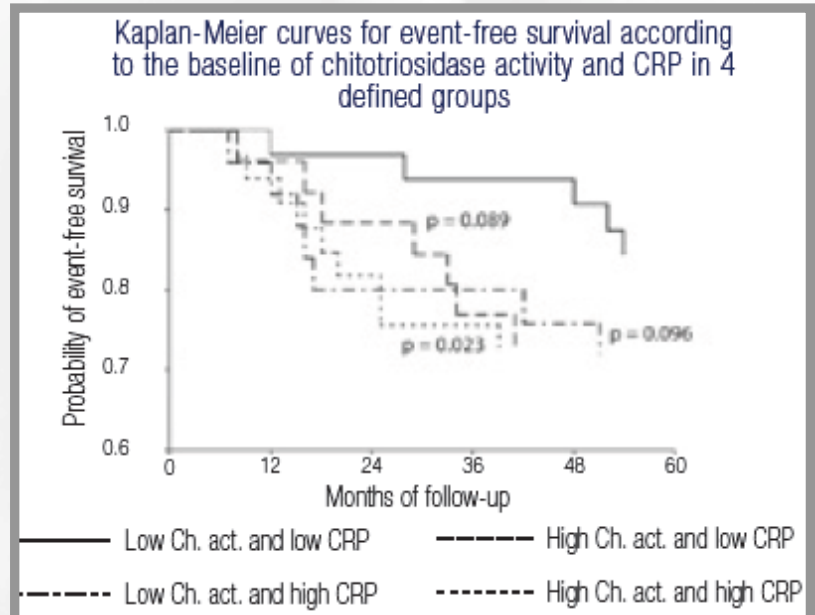
Atherosclerosis, CAD

Serum chitotriosidase activity predicts the risk of new cardiovascular events in the following 4 years and is a better marker of events occurring after 2 years of follow-up than in the first 2 years; furthermore, it has a predictive value similar to CRP. This new cardiovascular risk marker is independent of CRP and, when combined, the risk prediction of new cardiovascular events and the identification of a lower-risk group seem to improve.

Serum Chitotriosidase Activity, a Marker of Activated Macrophages, Predicts New Cardiovascular Events Independently of C-Reactive Protein (Cardiol. 2007;108:297–306). Artieda et al, Hospital Universitario Miguel Servet.

Serum chitotriosidase activity can be considered a strong inflammatory marker of CAD. Moreover, plasma chitotriosidase activity may be also regarded as a quantitative indicator of disease extent, as well as being a marker of disease presence.

Serum Chitotriosidase Activity in Patients With Coronary Artery Disease (Circ J 2008;72:71–75). Karadag et al, Istanbul University.





Tangier disease

- **UltraRare** autosomal recessive disorder with ~50 cases identified worldwide, but many more carriers.
- Alpha-lipoprotein deficiency caused by mutation in gene for [ABCA1](#) transporter;
- develop premature [atherosclerosis](#), characterized by fatty deposits and scar-like tissue lining the arteries.
- Hepatosplenomegaly related to the accumulation of cholesteryl esters in tissues and macrophages;
- No treatment (eventually surgery);



For discussion..

- Improved CT assay will require GD experts to change to new CT level standards;
- When CT is qualified as an official surrogate endpoint, will this only be required for clinical trials or also follow-up monitoring?
- CT surrogate for more diseases than Gaucher?
 - Does this offer opportunity for accelerated review?