Accuracy of chitotriosidase activity and CCL18 concentration in assessing type I Gaucher disease severity. A systematic review with meta-analysis of individual participant data

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METHODS

Study design

This systematic review with IPD meta-analysis was performed according to the current guidelines[1,2] and complied with the *Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA)-IPD* statement.[3] The rationale and methods were pre-specified and reported in a protocol[4] registered at PROSPERO (CRD42015027243).

This meta-analysis was carried out on data from primary studies for which ethical approval had been obtained by the investigators. The Comité de Protection des Personnes Sud Est 6, Clermont-Ferrand, France (IRB 00008526) reviewed the protocol and considered that it did not qualify for biomedical research requiring patient informed consent, provided that no supplementary data would be collected from the participants enrolled in primary studies.[4]

Eligibility criteria

Eligible studies included cross-sectional and cohort studies that measured both chitotriosidase activity and CCL18 concentration at baseline and/or at follow-up. Randomized controlled trials evaluating ERT or substrate reduction therapies were also considered because they are special cases of prospective cohort studies.

To be eligible, primary studies had to enroll consecutive patients with type I GD treated or not with specific therapy. Studies with fewer than 10 participants were excluded from this systematic review.

The relevant methods for the quantification of serum CCL18 concentration included ELISA[5] and dissociation enhanced lanthanide fluoroimmunoassay (DELFIA). The comparator was the quantification of plasma chitotriosidase activity using fluorogenic substrate molecules, such as 4-methyllumbelliferyl-chitobiose, 4-methyllumbelliferyl-chitotriose, and 4-methyllumbelliferyl-deoxy-chitotrioside.[6-8] Pre-specified clinical surrogates

that reflected GD severity included anemia, thrombocytopenia, splenomegaly, hepatomegaly, and symptomatic bone events confirmed by imaging.[9]

Information sources

Studies were identified by searching Medline via PubMed, EMBASE via Ovid, and Cochrane Central Register of Controlled Trials (CENTRAL) via the Wiley interface from January 1995 to June 2017. Our electronic search was supplemented by scanning the reference lists of the retrieved original articles and of previously published review articles to identify additional studies. We also contacted research groups, authors of relevant articles, and prominent clinicians in the field to identify completed relevant studies awaiting publication.

Search strategy

Electronic search strategies were developed by one of the authors (JL) and critically reviewed by a health sciences librarian. The search concepts included plasma chitotriosidase activity, CCL18, biological markers, ERT, and Gaucher disease (*Online supplementary appendices 1-3*). No restriction of document type and language was applied, and no methodology filter was used.

Study selection

Citation titles and abstracts obtained with the literature search were screened against pre-specified eligibility criteria.[4] Two authors (TR and JL) independently assessed potentially relevant full-text articles, using a standardized eligibility form. Duplicate publications reporting data from the same study were identified by comparing the authors' names, study sites, and sample sizes. Disagreements were resolved by discussion between TR and JL, and the reasons for excluding a study were recorded.[4]

Data collection

Two review authors (TR and JL) independently extracted qualitative information using a standardized data extraction form. Where possible, IPD were extracted from published articles. Otherwise, the corresponding authors or principal investigators of the eligible primary studies were invited to collaborate in this systematic review project by supplying de-identified IPD.[4] Pharmaceutical companies that funded clinical trials of ERT or substrate reduction therapies were contacted. Investigators who declined to provide IPD were questioned to identify potential reasons for their refusal.[4] As aggregate data on the comparative accuracy of chitotriosidase activity and CCL18 concentration for the pre-specified outcomes were not reported in the published articles and were not available from the contacted investigators, IPD could not be combined with aggregate data.

Data items

The IPD meta-analysis collaborative group pre-specified in the protocol the data to be collected.[4] Qualitative information on primary studies included country, number of study sites, enrollment period, study design, investigated treatment, sponsorship, fluorogenic substrate used for the chitotriosidase activity assay, technologies for CCL18 quantification, and spleen/liver volume measurement. The requested IPD included baseline characteristics (age, sex, chitotriosidase genotype, previous ERT, splenectomy) and variables collected at baseline and/or at follow-up visits (time to follow-up, current treatment [i.e., untreated, placebo, imiglucerase, velaglucerase alpha, taliglucerase, miglustat, eliglustat, other], plasma chitotriosidase activity, serum CCL18 concentration, hemoglobin concentration, platelet count, liver volume, spleen volume, and symptomatic bone events with imaging confirmation). Bone events included skeletal fracture, osteonecrosis or avascular necrosis that

could be dated and occurred within the previous 12 months of biomarker analysis.[4] Organ volumes were expressed as multiples of normal (MN) adjusted for body weight. When applicable (i.e., patients without splenectomy), the normal spleen volume was calculated as 2 mL/kg body weight. The normal liver volume was computed as 25 mL/kg body weight.

IPD integrity

IPD range, missing values, and consistency were cross-checked with the published reports. For most variables, no or only minor inconsistencies were found compared with the published data. The only exception was the mean platelet count at baseline (i.e., 11.427 versus 23.400 $x10^9$/L) for the group treated with taliglucerase alfa (30 U/kg/2 weeks) in a randomized controlled trial.[10] The Yale's National Gaucher Disease Treatment Center supplied a participant database that was different from the one used in the original publications,[11,12] and therefore IPD integrity could not be assessed. As the relationship between biomarkers (i.e., chitotriosidase activity and CCL18 concentration) and the pre-specified outcomes was observational in nature, randomization integrity and selective outcome reporting were not assessed in randomized controlled trials of ERT.[4]

Risk of bias assessment

Two review authors (TR and JL) independently appraised the methodological quality of the included studies for each outcome of interest, using a checklist adapted from the *Quality Assessment of Diagnostic Accuracy Studies* (QUADAS)-2 tool.[28] The QUADAS-2 tool comprises four domains: patient selection, index test, reference standard, and flow and timing. The risk of bias was evaluated for all four domains, and the applicability to clinical practice was assessed for the first three domains.[28]

Outcomes

Our primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients aged 12 to 59 months), platelet count <100x10^9/L, spleen volume >5 MN, and liver volume >1.25 MN. The secondary outcomes included symptomatic bone manifestations with imaging confirmation, a composite of hemoglobin concentration <8 g/dL (<7 g/dL for patients 12 to 59 months of age), platelet count <50x10^9/L, spleen volume >15 MN, and liver volume >2.5 MN, and individual components of the primary and secondary composite outcomes. All outcomes and cut-off values for continuous parameters were set according to published guidelines or previous studies,[29,30] and were pre-specified.[21]

Statistical analysis

As the chitotriosidase activity and CCL18 concentration distributions were skewed, a logarithm transformation was used and the geometric means and geometric mean ratios were derived with the 95% confidence intervals (CI) for each biomarker.[31] The effect size estimates for the comparative accuracy of serum CCL18 level relative to chitotriosidase activity in discriminating patients with the outcomes of interest were reported as differences in the area under the receiver operating characteristic (AUC-ROC) curves along with the 95% CI.

Data synthesis was performed with one- and two-stage approaches.[32,33] In the one-stage approach, IPD were analyzed in a single step, using a multilevel mixed-effects regression model that accounted for patient clustering within primary studies. For this purpose, three-level models were fit for continuous dependent variables (i.e., chitotriosidase activity or CCL18 concentration), and the three levels were defined by observation, patient, and study. Each pre-specified outcome was entered as a binary independent variable. Estimates and paired-comparisons of AUC-ROC curves were derived using a non-parametric ROC analysis

with bootstrap resampling that accounted for observation clustering within patients and primary studies.[13]

In the two-stage approach, the first stage consisted in analyzing IPD within primary studies to generate study-level effect-size point estimates and variances. In the second stage, point estimates from each primary study were combined using conventional meta-analytical methods. For this purpose, the DerSimonian and Laird's random-effects meta-analysis model was used to combine weighted mean differences in chitotriosidase activity and CCL18 concentration (after logarithm transformation) for patients with and without each pre-specified outcome. Differences in the AUC-ROC curve estimates for chitotriosidase activity and CCL18 concentration were pooled using random-effects meta-analysis models.[14]

Between-study heterogeneity was evaluated graphically by examining forest plots, and statistically by using the I^2 inconsistency index.[4] The I^2 index provides an estimate of the percentage of total variance across studies due to heterogeneity rather than chance. An I^2 index of 0% indicates no evidence of heterogeneity, whereas larger values reflect increasing heterogeneity.

A multilevel mixed-effects regression model that included interaction terms was used to investigate whether summary estimates varied according to the patient (i.e., age <16 versus ≥16 years, and receipt of ERT within the previous year) and study (i.e., fulfilment of five or more QUADAS-2 criteria) characteristics.[4] An unplanned exploratory analysis was performed to assess the summary estimate heterogeneity according to the fluorogenic substrates and assay type (DELFIA versus ELISA) used for measuring chitotriosidase activity and CCL18 concentration, respectively.

The robustness of our findings was assessed by carrying out a sensitivity analysis leaving out one primary study at a time. An additional sensitivity analysis was performed by substituting splenomegaly for splenectomy in the primary and secondary composite outcomes.

Finally, an analysis was performed to test whether the accuracy of CCL18 concentration in discriminating patients with the primary and secondary outcomes varied as a function of the deficiency in chitotriosidase activity. All analyses were performed with Stata Special Edition 14.0 (Stata corp, College Station, Texas, USA).

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Appendix 1. Literature search strategy for MEDLINE via PubMed.

Date range: from January, 1995 to June, 2017, limited to Humans

Search date: 2017.06.28

#1	Chitotriosidase[Supplementary Concept] OR chitotriosidase[Text Word]	414
#2	CCL18 protein, human[Supplementary Concept] OR CCL18[Text Word]	371
#3	Biomarkers[MeSH] OR biomarker[Text Word] OR marker[Text Word]	650,710
#4	#1 OR #2 OR #3	651,098
#5	Enzyme replacement therapy[MeSH] OR enzyme replac*[Text Word]	3,141
#6	(Substrate[Text Word] AND reduc*[Text Word]) OR substrate depriv*[Text Word]	16,999
#7	Miglustat[Supplementary Concept] OR miglustat[Text Word] OR Zavesca[Text Word]	274
#8	Eliglustat[Supplementary Concept] OR eliglustat[Text Word]	30
#9	Imiglucerase[Supplementary Concept] OR imiglucerase[Text Word] OR Cerezyme[Text Word]	314
#10	Velaglucerase alfa, human[Supplementary Concept] OR velaglucerase [Text Word] OR vpriv[Text Word]	50
#11	Taliglucerase alfa[Supplementary Concept] OR taliglucerase[Text Word] OR elelyso[Text Word]	24
#12	#5 OR #6 OR #7 OR #8 OR#9 OR #10 OR #11	20,222
#13	#4 OR #12	669,333
#14	Gaucher disease[MeSH] OR Gaucher[Text Word]	2,616
#15	#13 AND #14	1,091

Appendix 2. Literature search strategy for Embase.

Date range: from January, 1995 to June, 2017, limited to Humans

Search date: 2017.06.28

#1	Chitotriosidase[Emtree] OR chitotriosidase[Text Word]	922
#2	'CCL18 chemokine'/exp [Emtree] OR 'CCL18 protein human'/exp [Emtree] OR CCL18[Text Word]	724
#3	'Biological marker'/exp [Emtree] OR biomarker[Text Word]	211,517
#4	#1 OR #2 OR #3	212,730
#5	'Enzyme replacement'/exp [Emtree] OR 'enzyme replac'[Text Word]	6,451
#6	'Substrate reduction therapy'/exp [Emtree] OR 'substrate reduc' [Text Word]	70
#7	'Miglustat'/exp [Emtree] OR miglustat[Text Word] OR Zavesca[Text Word]	945
#8	'Eliglustat'/exp [Emtree] OR eliglustat[Text Word]	190
#9	'Imiglucerase'/exp [Emtree] OR imiglucerase[Text Word] OR Cerezyme[Text Word]	1,081
#10	'Velaglucerase alfa'/exp [Emtree] OR velaglucerase [Text Word] OR vpriv[Text Word]	288
#11	'Taliglucerase alfa'/exp [Emtree] OR taliglucerase[Text Word] OR elelyso[Text Word]	199
#12	#5 OR #6 OR #7 OR #8 OR#9 OR #10 OR #11	7,614
#13	#4 OR #12	219,781
#14	'Gaucher disease'/exp [Emtree] OR Gaucher[Text Word]	5,578
#15	#13 AND #14	2,326

Appendix 3. Literature search strategy for Central.

Date range: from January, 1995 to June, 2017

Search date: 2017.06.28

#1	Chitotriosidase[Text Word]	37
#2	CCL18[Text Word]	24
#3	Biomarkers[MeSH] OR biomarker[Text Word] OR marker[Text Word]	29,061
#4	#1 OR #2 OR #3	29,099
#5	Enzyme replacement therapy[MeSH] OR enzyme replac*[Text Word]	1,317
#6	(Substrate[Text Word] AND reduc*[Text Word]) OR substrate depriv*[Text Word]	1,227
#7	Miglustat[Text Word] OR Zavesca[Text Word]	27
#8	Eliglustat[Text Word]	38
#9	Imiglucerase[Text Word] OR Cerezyme[Text Word]	46
#10	Velaglucerase [Text Word] OR vpriv[Text Word]	28
#11	Taliglucerase[Text Word] OR elelyso[Text Word]	23
#12	#5 OR #6 OR #7 OR #8 OR#9 OR #10 OR #11	2,563
#13	#4 OR #12	31,387
#14	Gaucher disease[MeSH] OR Gaucher[Text Word]	197
#15	#13 AND #14	136

Appendix 4. Overview of the Primary Studies Included in the Meta-Analysis.

Author, year	Zimran, 2010[31]	Deegan, 2011[17]	Zimran, 2011[34]	Ben Turkia, 2013[28]	Gonzalez, 2013[30]
Study ID registration	NCT00391625	…	NCT00376168	NCT00553631	NCT00430625
Country	International	UK	International	International	International
No. study sites	3	3	11	11	5
Enrolment period	2005	2003-2006	2007–2008	2008-2009	2007-2009
Study design	Single arm trial	Prospective cohort	Parallel group RCT	Parallel group RCT	Parallel group RCT
Investigated treatment	Velaglucerase alpha	…	Taliglucerase alfa	Imiglucerase Velaglucerase alpha	Velaglucerase alpha
Sponsor	Industry	Academic	Industry	Industry	Industry
No. participants	10	103	31	34	25
No. patients with deficient chitotriosidase activity	1	5	1	2	1
No. participants included in MA*	9	98	30	32	24
Female sex, *n* (%)	6 (67)	62 (63)	16 (53)	17 (53)	9 (38)
Age, *y*, median (25^{th}-75^{th} percentiles)	35 (24 to 42)	41 (33 to 50)	35 (29 to 40)	31 (16 to 42)	26 (18 to 31)
Age <16 *y*, *n* (%)	0 (…)	2 (2)	0 (…)	8 (25)	5 (21)
Splenectomy, *n* (%)	0 (…)†	39 (39)	0 (…)†	18 (56)	0 (…)†
ERT within the previous year, *n* (%)	9 (100)	7 (7)	0 (…)	0 (…)	0 (…)
SRT within the previous year, *n* (%)	0 (…)	2 (2)	0 (…)	0 (…)	0 (…)
Length of follow-up, *months*	24	up to 132	68	24	24
No. observations included in MA*	54	220	101	183	136

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Appendix 4. (Continued)

Author, year	Elstein, 2015[29]	Zimran, 2015[35]	Murugesan, 2016[33]	Berger, 2018[32]
Study ID registration	NCT00635427	NCT001132690	…	NCT01951989
Country	International	International	United States	France
No. study sites	15	3	1	8
Enrolment period	2008-2009	2010–2012	2004-2009	2010-2015
Study design	Single arm trial	Parallel group RCT	Cross-sectional	Prospective cohort
Investigated treatment	Velaglucerase alpha	Taliglucerase alfa	-	Imiglucerase
Sponsor	Industry	Industry	Academic	Academic
No. participants	40	11	167	42
No. patients with deficient chitotriosidase activity	1	1	4	2
No. participants included in MA*	39	10	54	38
Female sex, *n* (%)	22 (56)	3 (30)	33 (61)	24 (63)
Age, *y*, median (25^{th}-75^{th} percentiles)	38 (19 to 51)	8 (6 to 12)	46 (28 to 58)	48 (39 to 67)
Age <16 *y*, *n* (%)	8 (21)	10 (100)	9 (17)	1 (3)
Splenectomy, *n* (%)	4 (10)	0 (…)	12 (22)	9 (24)
ERT within the previous year, *n* (%)	39 (100)	0 (…)	12 (22)	25 (66)
SRT within the previous year, *n* (%)	5 (13)	0 (…)	na	na
Length of follow-up, *months*	24	12	0	up to 62
No. observations included in MA*	224	20	54	117

Abbreviations: CT, clinical trial; ERT, enzyme replacement therapy; MA, meta-analysis; na, not available from the authors; RCT, randomized controlled trial; SRT, substrate reduction therapy;

* Patients and observations were included in the individual participant data meta-analysis if they had documented values for chitotriosidase activity, serum CCL18 concentration, and one or more pre-specified outcomes (hemoglobin concentration, platelet count, liver volume, spleen volume, and symptomatic bone event confirmed by X-ray).

† Splenectomy was an exclusion criterion.

Appendix 5. Overview of the primary studies for which individual participant data were not available.

Author, year	Study ID registration	Study design	Sponsor	No. participants	Reason
Boot, 2004[8]	…	Cross-sectional	Academic	55	The PI had changed position. The co-PI declined to provide IPD
Boot, 2006[37]	…	Cross-sectional	Academic	36	The PI had changed position. The co-PI declined to provide IPD
Di Rocco, 2008[38]	…	Retrospective convenience sample	Academic	53	The PI declined to provide IPD
Groener, 2008[42]	…	Prospective cohort	Academic	27	The PI had changed position. The co-PI declined to provide IPD
Giraldo, 2009[39]	…	Prospective cohort	Academic	28	The PI lacked time to assemble IPD
Dekker, 2011[9]	…	Retrospective convenience sample	Academic	64	The PI had changed position. The co-PI declined to provide IPD
Giraldo, 2011[40]	…	Prospective cohort	Academic	50	The PI lacked time to assemble IPD
Lukina, 2014[44]	NCT00358150	Single arm trial	Industry	26	The sponsor declined to share IPD
Pastores, 2014[46]	NCT00712348 NCT00705939	Single arm trial	Industry	11	The sponsor declined to share IPD
Mistry, 2015[45]	NCT00891202	Parallel group RCT	Industry	40	The sponsor declined to share IPD

(Continued)

Appendix 5. (Continued)

Author, year	Study ID registration	Study design	Sponsor	No. participants	Reason
Limgala, 2016[43]	NCT01358188	Cross-sectional	Academic	31	No answer from the PI and corresponding author
Smid, 2016[14]	…	Retrospective convenience sample	Academic	19	The PI declined to provide IPD
Giraldo, 2016[41]	…	Cross-sectional	Academic	108	The PI lacked time to assemble IPD
Andrade-Campos, 2017[36]	…	Prospective cohort	Academic	17	The PI lacked time to assemble IPD

Abbreviations: IPD, individual participant data; PI, principal investigator; RCT, randomized controlled trial.

Appendix 6. Index test and reference methods used in the primary studies.

Author, year	Zimran, 2010[31]	Deegan, 2011[17]	Zimran, 2011[34]	Ben Turkia, 2013[28]	Gonzalez, 2013[30]
Chitotriosidase activity					
Fluorogenic substrate	4MU-deoxy-chitobiose*	4MU-chitotriose	na	4MU-deoxy-chitobiose*	4MU-deoxy-chitobiose*
Median value, *nmol/mL/h*	7,523	2,226	9,128	10,442	9,957
(Range)	(673 to 68,552)	(23 to 30,609)	(68 to 66,628)	(253 to 112,777)	(9 to 82,225)
Serum CCL18 concentration					
Technology	DELFIA*	ELISA	na	DELFIA*	DELFIA*
Median value, *ng/mL*	1,113	496	434	806	1,014
(Range)	(157 to 5,247)	(24 to 2,975)	(38 to 2,229)	(73 to 5,902)	(47 to 4,077)
Liver volume					
Technology	MRI	MRI	MRI	MRI	MRI
Median value, *MN*	1.5	1.0	1.3	1.2	1.3
(Range)	(0.8 to 2.3)	(0.6 to 2.7)	(0.8 to 2.9)	(0.6 to 2.8)	(0.8 to 3.2)
Spleen volume					
Technology	MRI	MRI	MRI	MRI	MRI
Median value, *MN*	10.0	5.8	7.8	5.3	7.4
(Range)	(3.5 to 32.5)	(1.9 to 28.3)	(2.3 to 54.2)	(2.2 to 44.4)	(1.8 to 65.1)
Hemoglobin concentration					
Median value, *g/dL*	12.6	13.5	13.4	12.3	12.3
(Range)	(9.8 to 16.5)	(8.2 to 16.3)	(5.5 to 18.4)	(7.8 to 16.4)	(7.1 to 17.9)
Platelet count					
Median value, x10^9/L	91	179	94	260	82
(Range)	(32 to 178)	(21 to 572)	(27 to 246)	(34 to 603)	(7 to 438)

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Appendix 6. (Continued)

Author, year	Elstein, 2015[29]	Zimran, 2015[35]	Murugesan, 2016[33]	Berger, 2018[32]†
Chitotriosidase activity				
Fluorogenic substrate	4MU-deoxy-chitobiose*	4MU-deoxy-chitobiose*	4MU-deoxy-chitobiose	4MU-chitotriose
Median value, *nmol/mL/h*	2,426	14,809	1,361	1,340
(Range)	(44 to 32,541)	(1,056 to 63,179)	(28 to 22,070)	(20 to 15,822)
Serum CCL18 concentration				
Technology	DELFIA*	DELFIA*	ELISA	ELISA
Median value, *ng/mL*	237	840	269	280
(Range)	(23 to 1,609)	(120 to 2,336)	(45 to 1,961)	(40 to 2,487)
Liver volume				
Technology	MRI	MRI	MRI	-
Median value, *MN*	0.8	1.7	1.0	-
(Range)	(0.5 to 1.5)	(1.0 to 3.0)	(0.6 to 1.9)	-
Spleen volume				
Technology	MRI	MRI	MRI	-
Median value, *MN*	2.7	14.1	5.8	-
(Range)	(1.1 to 15.8)	(6.2 to 69.3)	(1.8 to 27.2)	-
Hemoglobin concentration				
Median value, *g/dL*	13.5	11.7	13.0	14.0
(Range)	(10.4 to 17.5)	(8.2 to 14.2)	(8.1 to 17.2)	(7.0 to 16.1)
Platelet count				
Median value, x10^9/L	166	132	213	152
(Range)	(23 to 434)	(66 to 324)	(26 to 652)	(9 to 919)

Abbreviations: DELFIA = dissociation enhanced lanthanide fluoroimmunoassay; ELISA = enzyme-linked immunosorbent assay; MN = multiple of normal; MRI = magnetic resonance imaging; MU = methylumbelliferyl; na = not available from the authors.

* Chitiotriosidase activity and CCL18 concentration were measured at the Academic Medical Center in Amsterdam, The Netherlands, using validated methods.

† This study did not assess liver and spleen volume.

Appendix 7. Study Quality Assessment According to the QUADAS-2 criteria.

Author, year	Zimran, 2010[31]	Deegan, 2011[17]	Zimran, 2011[34]	Ben Turkia, 2013[28]	Gonzalez, 2013[30]	Elstein, 2015[29]
Risk of bias						
Patient selection*	Low	Low	Low	Low	Low	Low
Index tests†	Low	High	Unclear	Low	Low	Low
Primary composite outcome‡	Low	High	Unclear	Low	Low	Low
Flow and timing§	Low	Low	Low	Low	Low	Low
Applicability concerns						
Patient selection*	High	Low	Low	Low	High	High
Index test†	Low	Low	Unclear	Low	Low	Low
Primary composite outcome‡	Low	Low	Low	Low	Low	Low
No. QUADAS-2 criteria	6	5	4	7	6	6

(Continued on next page)

Appendix 7. (Continued)

Author, year	Zimran, 2015[35]	Murugesan, 2016[33]	Berger, 2018[32]
Risk of bias			
Patient selection*	Low	Low	Low
Index tests†	Low	High	Unclear
Primary composite outcome‡	Unclear	High	…#
Flow and timing§	Low	High	Low
Applicability concerns			
Patient selection*	High	Low	High
Index test†	Low	Low	Unclear
Primary composite outcome‡	Low	Low	…#
No. QUADAS-2 criteria	5	4	…#

* The risk of bias in patient selection was considered low if consecutive or randomly selected patients with Gaucher disease were enrolled. Convenience samples or inappropriate exclusion criteria were potential reasons for rating the risk of bias as high. Applicability concerns were considered high if there were concerns that the setting would not match the study question (e.g., enrolment of pediatrics population only, patients naive to [or untreated for several years with] enzyme replacement therapy only, patients receiving long-term treatment with enzyme replacement therapy only, patients with non-progressive Gaucher disease only).

† The risk of bias for index tests was considered low if chitotriosidase activity and CCL18 concentration were measured at a central core laboratory and interpreted independently from the pre-specified outcomes. Conversely, the risk of bias was considered high if data on chitotriosidase activity and/or CCL18 concentration were collected by a retrospective chart review. The risk of bias was rated unclear if it was not possible to formally determine whether chitotriosidase activity and/or CCL18 concentration assessment were blinded to pre-specified outcomes. Applicability concerns were considered high for

studies that used other flurogenic substrates than 4MU-deoxy-chitobiose for assaying chitotriosidase activity or other assays than ELISA or DELFIA for assessing CCL18 concentration.

‡ The risk of bias for the assessment of pre-specified outcomes was considered low if liver and spleen volumes were quantified using objective tests (i.e., computed tomography, magnetic resonance imaging, or ultrasound technologies) and assessed by independent reviewers blinded to chitotriosidase activity and CCL18 concentration values. Conversely, the risk of bias was considered high if liver or spleen volume was assessed by physical examination, collected by retrospective chart review, or assessed by (local) staff not blinded to chitotriosidase activity and\or CCL18 concentration values. The risk of bias for the assessment of pre-specified outcomes was considered low if hemoglobin concentration and platelet count were assayed by an independent central core laboratory. Conversely, the risk of bias was considered high if hemoglobin concentration or platelet count was collected by retrospective chart review. The risk of bias was rated unclear if it was not possible to formally determine whether hemoglobin concentration or platelet count assessment was blinded to the index test results.

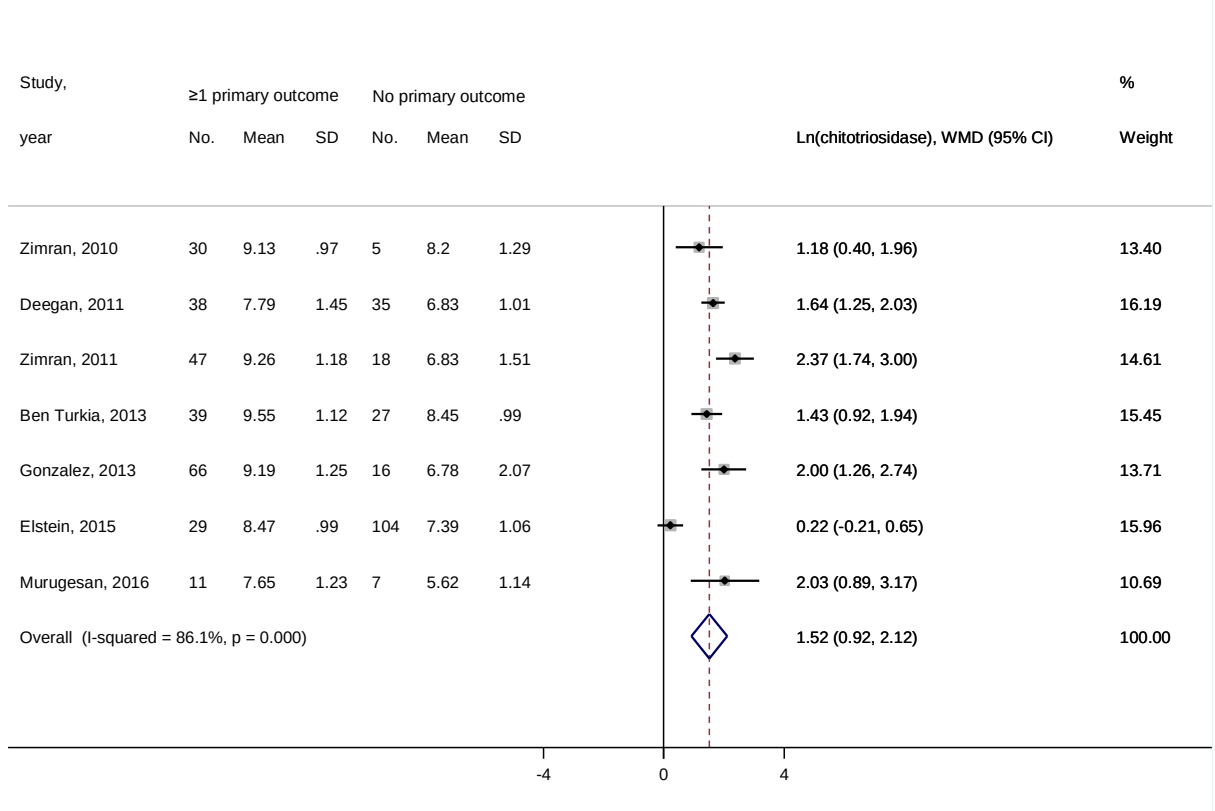
This study did not record liver and spleen volume.

§ The risk of bias for flow and timing was considered high for studies where more than 20% of eligible patients did not undergo chitotriosidase activity or CCL18 concentration measurements, the interval between the index and reference tests were inappropriate (e.g., retrospective measurement of CCL18 concentration), or the same methods for assessing pre-specified outcomes were not used for all patients.

Appendix 8. Random-effect summary estimates (two-stage approach) for differences in chitotriosidase activity (after logarithmic transformation) according to the primary composite outcome.*

Abbreviations: CI = confidence interval; SD = standard deviation; WMD = weighted mean difference.

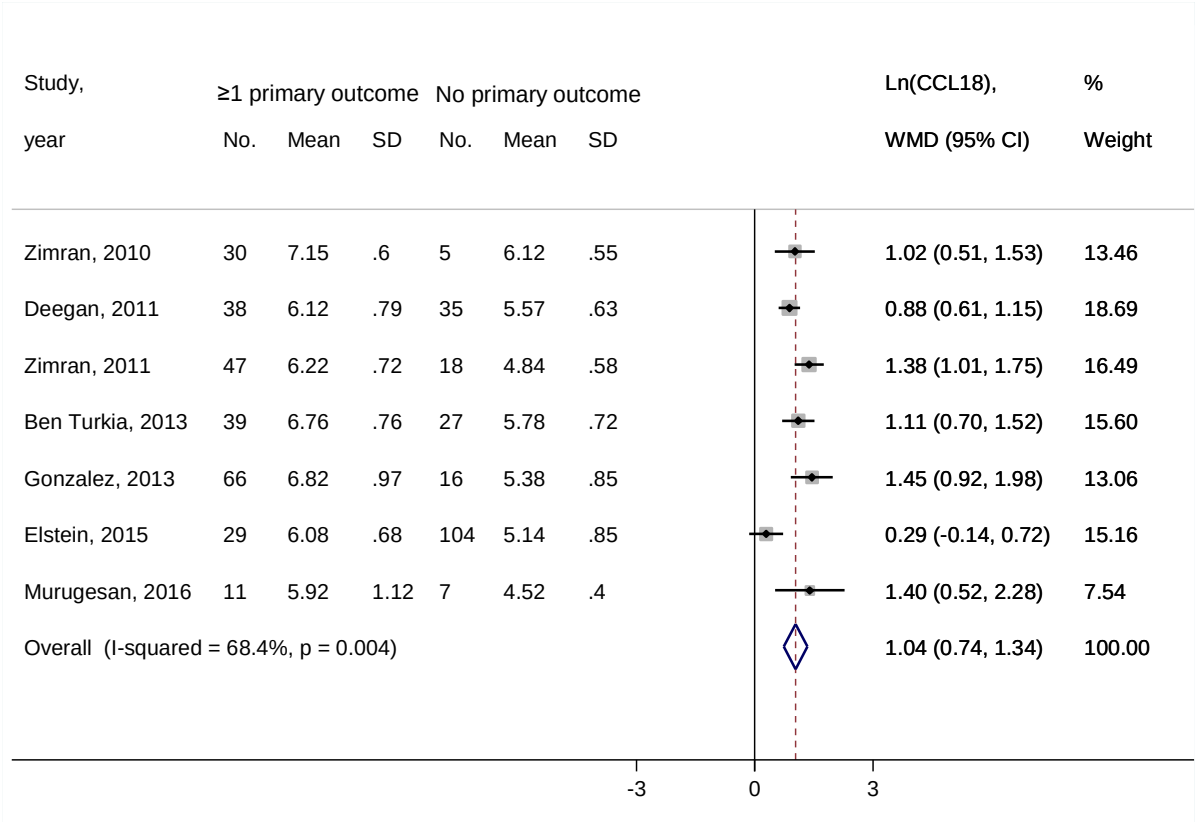
* The geometric mean ratio of chitotriosidase activity associated with the primary outcome was 4.57 (95% CI, 2.51 to 8.33) (*P* <.001). The primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients 12 to 59 months of age), platelet count <100x10^9/L, spleen volume >5 MN, and liver volume >1.25 MN. Patients with splenectomy were excluded from this analysis. As all patients experienced the primary outcome in the study by Zimran et al., 2015, this study was excluded from the two-stage individual participant data meta-analysis.



Appendix 9. Random-effect summary estimates (two-stage approach) for difference in serum CCL18 concentration (after logarithmic transformation) according to the primary composite outcome.*

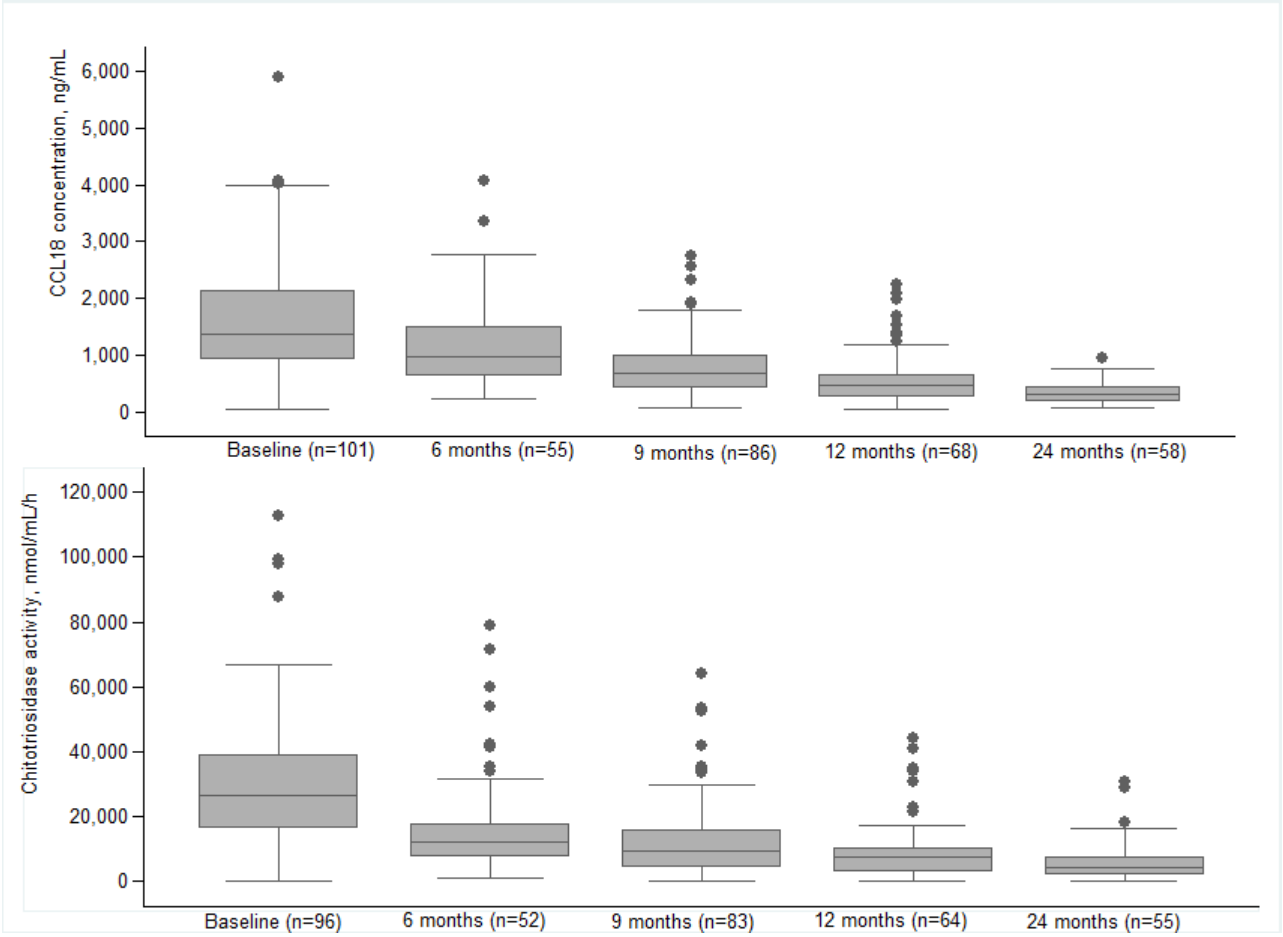
Abbreviations: CI = confidence interval; SD = standard deviation; WMD = weighted mean difference.

* The geometric mean ratio for the serum CCL18 concentration associated with the primary outcome was 2.83 (95% CI, 2.10 to 3.82) (*P* <.001). The primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients 12 to 59 months of age), platelet count <100x10^9/L, spleen volume >5 MN, and liver volume >1.25 MN. Patients with splenectomy were excluded from this analysis. As all patients experienced the primary outcome in the study by Zimran et al., 2015, this study was excluded from the two-stage individual participant data meta-analysis.



Appendix 10. Trends in CCL18 concentration and chitotriosidase activity over 24 months of follow-up, among participants enrolled in four industry-sponsored clinical trials evaluating enzyme replacement therapy.

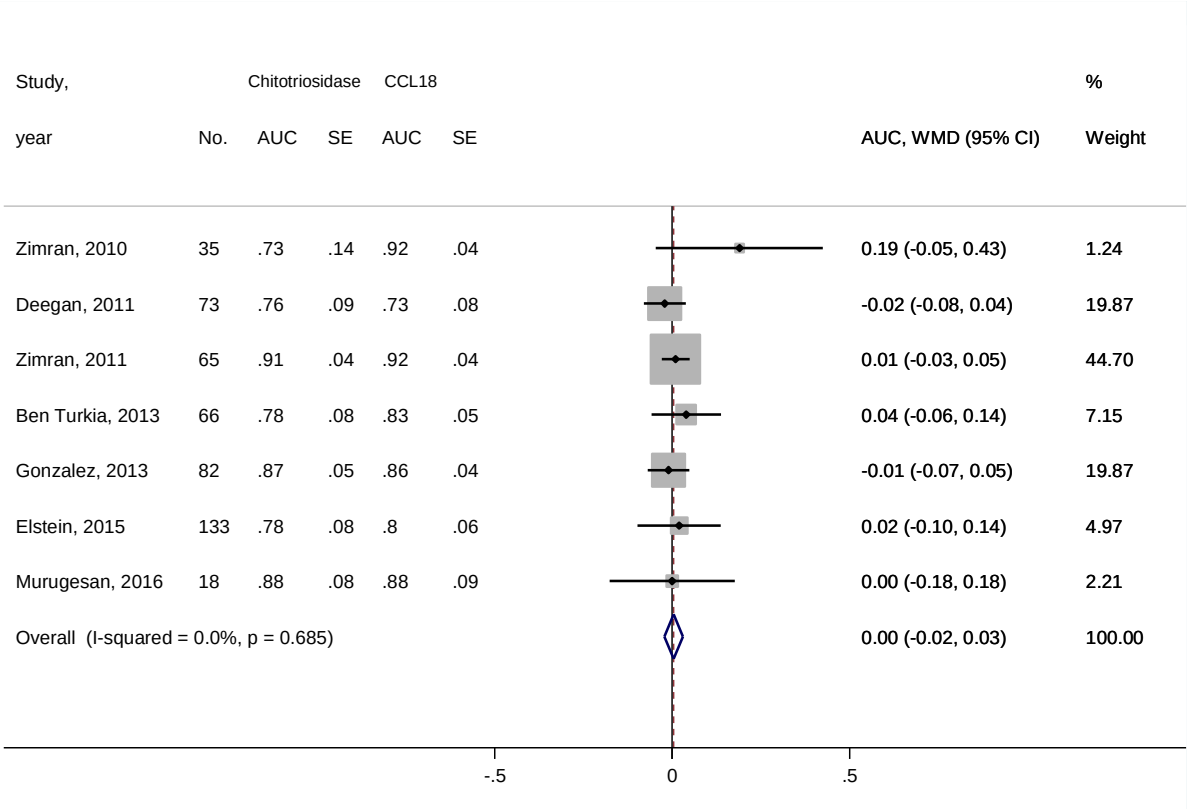
Only clinical trials with participants who were untreated at baseline were included in this analysis (Zimran, 2011[34]; Ben Turkia, 2013[28]; Gonzalez, 2013[30]; and Zimran, 2015[35]).



Appendix 11. Random-effect summary estimates (two-stage approach) for differences in the area under the ROC curves between serum CCL18 concentration and chitotriosidase activity in function of the primary composite outcome.*

Abbreviations: AUC = area under the curve; CI = confidence interval; SE = standard error; WMD = weighted mean difference.

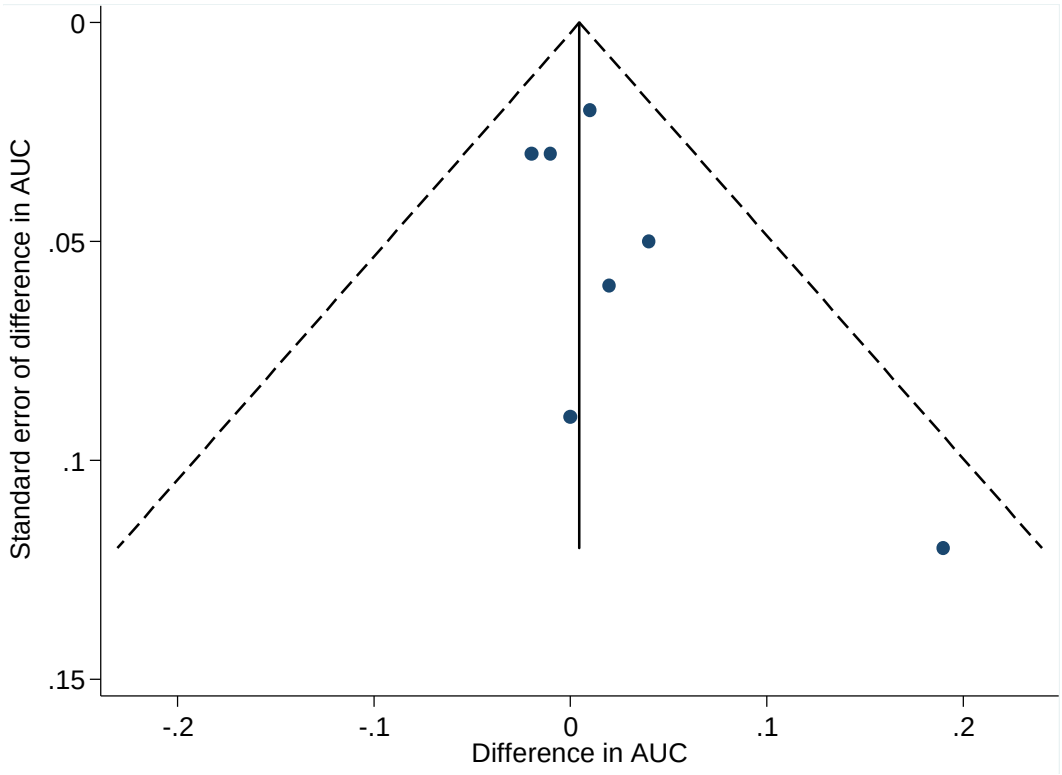
* The primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients 12 to 59 months of age), platelet count $<100x10^9$/L, spleen volume >5 MN, and liver volume >1.25 MN. Patients with splenectomy were excluded from this analysis. As all patients experienced the primary outcome in the study by Zimran et al., 2015, this study was excluded from two-stage individual participant data meta-analysis.



Appendix 12. Funnel plot showing the differences in the areas under the receiver operating characteristic curves for the primary outcome (*P* for weighted regression test of funnel plot asymmetry =0.20).*

Abbreviations: AUC = area under the curve.

* The primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients 12 to 59 months of age), platelet count <100x10^9/L, spleen volume >5 MN, and liver volume >1.25 MN. Patients with splenectomy were excluded from this analysis. As all patients experienced the primary outcome in the study by Zimran et al., 2015, this study was excluded from the two-stage individual participant data meta-analysis.



Appendix 13. Geometric mean ratios of chitotriosidase activity and serum CCL18 concentration associated with the primary outcome according to age, fulfillment of QUADAS-2 criteria, enzyme replacement therapy within the previous year, fluorogenic substrate, and CCL18 assay type among patients with type I Gaucher disease.*†

	No.	Geometric mean ratio (95%CI) for chitotriosidase activity	$P_{for\ interaction}$	Geometric mean ratio (95%CI) for CCL18	$P_{for\ interaction}$
Age			.42		.90
<16 *y*	100	5.09 (2.50 to 10.36)		3.94 (2.54 to 6.11)	
≥16 *y*	392	5.17 (4.10 to 6.51)		2.92 (2.46 to 3.46)	
QUADAS-2 criteria			.001		.10
<5	83	10.06 (5.85 to 17.32)		4.12 (2.93 to 5.81)	
≥5	389	4.33 (3.40 to 5.52)		2.85 (2.36 to 3.44)	
ERT within one year			.32		.48
No	342	5.62 (4.27 to 7.39)		2.95 (2.43 to 3.59)	
Yes	150	3.15 (1.87 to 5.29)		2.59 (1.78 to 3.76)	
Fluorogenic substrate‡			.66		.10
4MU-chitotriose	73	5.21 (3.55 to 7.65)		2.46 (1.88 to 3.22)	
4MU-deoxy-chitobiose	354	4.26 (3.21 to 5.65)		3.16 (2.52 to 3.95)	
CCL18 assay‡			.51		.21
ELISA	91	5.32 (3.70 to 7.64)		2.68 (2.07 to 3.47)	
DELFIA	336	4.21 (3.17 to 5.61)		3.13 (2.49 to 3.94)	

Abbreviations: CI, confidence interval; DELFIA = dissociation enhanced lanthanide fluoroimmunoassay; ELISA = enzyme-linked immunosorbent assay ERT, enzyme replacement therapy; QUADAS-2, quality assessment of diagnostic accuracy studies.

* The primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients 12 to 59 months of age), platelet count $<100x10^9$/L, spleen volume >5 MN, and liver volume >1.25 MN. Patients with splenectomy were excluded from this analysis.

† Geometric mean ratios and *P* values for interaction were derived from 3-level random intercept regression models for continuous dependent variables, with observations nested within patients and studies.

‡ One study (Zimran et al., 2011) was excluded from this analysis because of undocumented fluorogenic substrate for measuring chitotriosidase activity and CCL18 assay.

Appendix 14. Areas under the receiver operating characteristic curves of chitotriosidase activity and serum CCL18 concentration for discriminating patients with type I Gaucher disease with the primary outcome according to the age group, fulfillment of QUADAS-2 criteria, and enzyme replacement therapy within the previous year.*†

Outcome	n/N	AUC (95%CI)				Difference in AUC (95%CI)		*P*
		Chitotriosidase activity		CCL18				
Age								
<16 *y*	55/100	.79	(.65 to .92)	.85	(.75 to .96)	.07	(-.03 to .17)	.17
≥16 *y*	225/392	.83	(.78 to .89)	.83	(.79 to .89)	.00	(-.03 to .04)	.89
QUADAS-2 criteria								
<5	58/83	.89	(.80 to .95)	.92	(.85 to .97)	.03	(-.01 to.08)	.18
≥5	222/409	.81	(.75 to .87)	.84	(.78 to .89)	.03	(-.01 to.07)	.10
ERT within one year								
No	218/342	.82	(.76 to .88)	.82	(.77 to .87)	.00	(-.04 to .04)	.95
Yes	62/150	.82	(.72 to .91)	.89	(.81 to .96)	.07	(.01 to .12)	.02
Fluorogenic substrate‡								
4MU-chitotriose	38/73	.76	(.54 to .89)	.73	(.53 to .86)	-.02	(-.09 to .03)	.44
4MU-deoxy-chitobiose	195/354	.82	(.76 to .89)	.86	(.80 to .91)	.04	(-.00 to .08)	.06
CCL18 assay‡								
ELISA	49/91	.77	(.60 to .89)	.74	(.59 to .85)	-.03	(-.08 to .03)	.28
DELFIA	184/336	.83	(.76 to .89)	.87	(.81 to .91)	.03	(-.01 to .07)	.09

Abbreviations: CI, confidence interval; DELFIA = dissociation enhanced lanthanide fluoroimmunoassay; ELISA = enzyme-linked immunosorbent assay ERT, enzyme replacement therapy; QUADAS-2, quality assessment of diagnostic accuracy studies.

* The primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients 12 to 59 months of age), platelet count $<100x10^9$/L, spleen volume >5 MN, and liver volume >1.25 MN. Patients with splenectomy were excluded from this analysis.

† Summary estimates for the area under the ROC curves and *P*-values for paired comparisons were derived from the non-parametric ROC analysis with bootstrap resampling that accounted for observation clustering within patients and primary studies.

‡ One study (Zimran et al., 2011) was excluded from this analysis because of undocumented fluorogenic substrate for measuring chitotriosidase activity and CCL18 assay.

Appendix 15. Unpaired comparisons (one-stage approach) of serum CCL18 concentration stratified according to chitotriosidase activity deficiency and pre-specified outcomes in patients with type I Gaucher disease.

Outcomes	Wild type or heterozygous				Deficient for chitotriosidase activity				$P_{for\ interaction}$
	No.	Geometric mean (95%CI)	Mean ratio (95%CI)*		No.	Geometric mean (95%CI)	Mean ratio (95%CI)*		
Primary composite outcome†									.12
No outcome	212	198 (177 to 221)	1.00	(…)	13	238 (169 to 334)	1.00	(…)	
≥ 1 outcome	280	679 (612 to 755)	3.04	(2.57 to 3.58)	11	952 (587 to 1545)	4.76	(2.93 to 7.73)	
Secondary composite outcome‡									.38
No outcome	391	311 (283 to 342)	1.00	(…)	19	373 (237 to 588)	1.00	(…)	
≥ 1 outcome	101	1,050 (879 to 1,254)	3.05	(2.53 to 3.68)	5	906 (445 to 1,847)	2.43	(1.05 to 5.61)	

Abbreviations: CI, confidence interval.

* Summary geometric mean ratios and *P*-values for unpaired comparisons were derived from 3-level random intercept regression models for continuous dependent variables, with observations nested within patients and studies.

† The primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients 12 to 59 months of age), platelet count $<100x10^9$/L, spleen volume >5 MN, and liver volume >1.25 MN. Patients with splenectomy were excluded from this analysis.

‡ The secondary outcome was a composite of hemoglobin concentration <8 g/dL (<7 g/dL for patients 12 to 59 months of age), platelet count $<50x10^9$/L, spleen volume >15 MN, and liver volume >2.5 MN. Patients with splenectomy were excluded from this analysis.

Appendix 16. Estimates (one-stage approach) of the area under the receiver operating characteristic curve of serum CCL18 concentration for pre-specified outcomes stratified according to chitotriosidase activity deficiency in patients with type I Gaucher disease.

	Wild type or heterozygous		Deficient for chitotriosidase activity	
Outcomes	No.	AUC (95%CI)*	No.	AUC (95%CI)*
Primary composite outcome†	280/492	.84 (.79 to .88)	11/24	.98 (.85 to 1.00)
Secondary composite outcome‡	101/492	.83 (.74 to .89)	5/24	.83 (.51 to 1.00)

Abbreviations: AUC, area under the (receiver operating characteristics) curve; CI, confidence interval.

* Summary estimates for the area under the ROC curves were derived from a non-parametric ROC analysis with bootstrap resampling that accounted for observation clustering within patients and primary studies.

† The primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients 12 to 59 months of age), platelet count $<100x10^9$/L, spleen volume >5 MN, and liver volume >1.25 MN. Patients with splenectomy were excluded from this analysis.

‡ The secondary outcome was a composite of hemoglobin concentration <8 g/dL (<7 g/dL for patients 12 to 59 months of age), platelet count $<50x10^9$/L, spleen volume >15 MN, and liver volume >2.5 MN. Patients with splenectomy were excluded from this analysis.

Appendix 17. Unpaired comparisons (one-stage approach) of chitotriosidase activity and serum CCL18 concentration according to the primary composite outcome among patients with type I Gaucher disease in the leave-one-out sensitivity analysis.*

Excluded primary study	No.	Chitotriosidase activity, nmol/mL/h				*P*	CCL18, ng/mL				*P*
		Geometric mean (95%CI)		Mean ratio (95%CI)†			Geometric mean (95%CI)		Mean ratio (95%CI)†		
Zimran, 2010[31]						<.001					<.001
No outcome	207	1,446	(1,206 to 1,733)	1.00	(…)		194	(173 to 217)	1.00	(…)	
≥ 1 outcome	250	7,447	(6,283 to 8,828)	5.51	(4.36 to 6.96)		630	(564 to 704)	3.05	(2.56 to 3.63)	
Deegan, 2011[17]						<.001					<.001
No outcome	177	1,621	(1,325 to 1,985)	1.00	(…)		187	(165 to 212)	1.00	(…)	
≥ 1 outcome	242	9,126	(7,823 to 10,646)	5.48	(4.24 to 7.08)		724	(646 to 810)	3.32	(2.74 to 4.03)	
Zimran, 2011[34]						<.001					<.001
No outcome	194	1,543	(1,283 to 1,856)	1.00	(…)		206	(183 to 232)	1.00	(…)	
≥ 1 outcome	233	7,152	(6,008 to 8,513)	4.41	(3.48 to 5.59)		721	(641 to 812)	2.88	(2.39 to 3.46)	
Ben Turkia, 2013[28]						<.001					<.001
No outcome	185	1,248	(1,036 to 1,504)	1.00	(…)		184	(164 to 207)	1.00	(…)	
≥ 1 outcome	241	6,904	(5,827 to 8,180)	5.57	(4.35 to 7.13)		653	(582 to 733)	3.01	(2.51 to 3.60)	
Gonzalez, 2013[30]						<.001					<.001
No outcome	196	1,542	(1,294 to 1,837)	1.00	(…)		196	(175 to 221)	1.00	(…)	
≥ 1 outcome	214	7,057	(5,886 to 8,461)	5.09	(4.07 to 6.36)		619	(552 to 694)	2.91	(2.46 to 3.43)	
Elstein, 2015[29]						<.001					<.001
No outcome	108	1,358	(1,013 to 1,820)	1.00	(…)		228	(197 to 264)	1.00	(…)	
≥ 1 outcome	251	8,044	(6,798 to 9,520)	5.88	(4.52 to 7.66)		714	(639 to 799)	3.12	(2.58 to 3.77)	

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Appendix 17. (Continued)

Excluded primary study	No.	Chitotriosidase activity, nmol/mL/h				*P*†	CCL18, ng/mL				*P*†
		Geometric mean (95%CI)		Mean ratio (95%CI)†			Geometric mean (95%CI)		Mean ratio (95%CI)†		
Zimran, 2015[35]						<.001					<.001
No outcome	212	1,478	(1,235 to 1,768)	1.00	(…)		198	(177 to 221)	1.00	(…)	
≥ 1 outcome	360	7,336	(6,235 to 8,633)	5.26	(4.22 to 6.58)		679	(609 to 756)	3.02	(2.56 to 3.57)	
Murugesan, 2016[33]						<.001					<.001
No outcome	205	1,565	(1,309 to 1,871)	1.00	(…)		203	(181 to 228)	1.00	(…)	
≥ 1 outcome	269	8,034	(6,867 to 9,401)	5.26	(4.19 to 6.59)		696	(627 to 773)	3.00	(2.54 to 3.55)	

Abbreviations: CI, confidence interval.

* The primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients 12 to 59 months of age), platelet count <100x10^9/L, spleen volume >5 MN, and liver volume >1.25 MN. Patients with splenectomy were excluded from this analysis.

† Summary geometric mean ratios and *P*-values for unpaired comparisons were derived from 3-level random intercept regression models for continuous dependent variables, with observations nested within patients and studies.

Appendix 18. Paired comparisons (one-stage approach) of the areas under the receiver operating characteristics curves for chitotriosidase activity and serum CCL18 concentration performance in discriminating patients with type I Gaucher disease according to the primary composite outcome in the leave-one-out sensitivity analysis.*

Excluded primary study	n/N†	AUC (95%CI)† Chitotriosidase activity		CCL18		Difference in AUC (95%CI)†		*P*†
Zimran, 2010[31]	250/457	.82	(.76 to .87)	.83	(.78 to .88)	.01	(-.03 to .04)	.57
Deegan, 2011[17]	242/419	.84	(.78 to .88)	.86	(.80 to .90)	.02	(-.01 to .06)	.24
Zimran, 2011[34]	233/427	.81	(.74 to .86)	.84	(.79 to .89)	.03	(.00 to .07)	.08
Ben Turkia, 2013[28]	241/426	.84	(.78 to .89)	.84	(.79 to .89)	.01	(-.03 to.04)	.70
Gonzalez, 2013[30]	214/410	.81	(.74 to .87)	.83	(.77 to .88)	.02	(-.02 to .06)	.32
Elstein, 2015[29]	251/359	.82	(.74 to .88)	.83	(.77 to .87)	.01	(-.03 to .04)	.73
Zimran, 2015[35]	260/472	.82	(.76 to .87)	.84	(.79 to .88)	.02	(-.01 to .06)	.21
Murugesan, 2016[33]	269/474	.83	(.76 to .88)	.84	(.79 to .89)	.01	(-.02 to .05)	.42

Abbreviations: AUC, area under the (receiver operating characteristics) curve; CI, confidence interval.

* The primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients 12 to 59 months of age), platelet count <100x10^9/L, spleen volume >5 MN, and liver volume >1.25 MN. Patients with splenectomy were excluded from this analysis.

† Summary estimates for the area under the ROC curves and *P*-values for paired comparisons were derived from the non-parametric ROC analysis with bootstrap resampling that accounted for observation clustering within patients and primary studies.

Appendix 19. Unpaired comparisons (one-stage approach) of chitotriosidase activity and serum CCL18 concentration after replacing splenectomy by splenomegaly in patients with type I Gaucher disease.

Outcomes	No.	Chitotriosidase activity, nmol/mL/h				*P**	CCL18, ng/mL				*P**
		Geometric mean (95%CI)		Mean ratio (95%CI)*			Geometric mean (95%CI)		Mean ratio (95%CI)*		
Primary composite outcome†						<.001					<.001
No outcome	212	1,478	(1,235 to 1,768)	1.00	(…)		198	(177 to 221)	1.00	(…)	
≥ 1 outcome	457	5,935	(5,173 to 6809)	4.73	(3.78 to 5.91)		653	(605 to 706)	2.89	(2.48 to 3.37)	
Secondary composite outcome‡						<.001					<.001
No outcome	391	2,701	(2,349 to 3,106)	1.00	(…)		311	(283 to 342)	1.00	(…)	
≥ 1 outcome	278	6,220	(5,097 to 7,590)	3.20	(2.53 to 4.04)		746	(675 to 824)	2.44	(2.09 to 2.84)	

Abbreviations: CI, confidence interval.

* Summary geometric mean ratios and *P*-values for unpaired comparisons were derived from 3-level random intercept regression models for continuous dependent variables, with observations nested within patients and studies.

† The primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients 12 to 59 months of age), platelet count $<100x10^9$/L, spleen volume >5 MN, and liver volume >1.25 MN. Splenectomy was coded as splenomegaly (i.e., spleen volume >15 MN) in this analysis.

‡ The secondary outcome was a composite of hemoglobin concentration <8 g/dL (<7 g/dL for patients 12 to 59 months of age), platelet count $<50x10^9$/L, spleen volume >15 MN, and liver volume >2.5 MN. Splenectomy was coded as splenomegaly (i.e., spleen volume >15 MN) in this analysis.

Appendix 20. Paired comparisons (one-stage approach) of the areas under the receiver operating characteristics curves for chitotriosidase activity and serum CCL18 concentration after replacing splenectomy by splenomegaly in patients with type I Gaucher disease.

Outcome	n/N	AUC (95%CI)*		Difference in AUC (95%CI)*	*P**
		Chitotriosidase activity	CCL18		
Primary composite outcome†	457/669	.78 (.72 to .83)	.84 (.79 to .88)	.06 (.02 to .10)	.005
Secondary composite outcome‡	278/669	.68 (.60 to .74)	.75 (.70 to .80)	.07 (.02 to .13)	. 005

Abbreviations: AUC, area under the (receiver operating characteristics) curve; CI, confidence interval.

* Summary estimates for the area under the ROC curves and *P*-values for paired comparisons were derived from the non-parametric ROC analysis with bootstrap resampling that accounted for observation clustering within patients and primary studies.

† The primary outcome was a composite of hemoglobin concentration <11 g/dL (<10 g/dL for patients 12 to 59 months of age), platelet count $<100x10^9$/L, spleen volume >5 MN, and liver volume >1.25 MN. Splenectomy was coded as splenomegaly (i.e., spleen volume >15 MN) in this analysis.

‡ The secondary outcome was a composite of hemoglobin concentration <8 g/dL (<7 g/dL for patients 12 to 59 months of age), platelet count $<50x10^9$/L, spleen volume >15 MN, and liver volume >2.5 MN. Splenectomy was coded as splenomegaly (i.e., spleen volume >15 MN) in this analysis.