

Neurofilament Light and Its Association With CNS Involvement in Patients With Classic Infantile Pompe Disease

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Abstract

Background and Objectives

Enzyme replacement therapy (ERT) has substantially improved the outcome of classic infantile Pompe disease, an inheritable muscle disease previously fatal at infancy. However, under treatment, patients develop white matter abnormalities and neurocognitive problems. Therefore, upcoming therapies also target the brain. Currently, biomarkers reflecting CNS involvement are lacking. We aimed to study the association of neurofilament light (NfL) and CNS involvement.

Methods

To investigate the potential of NfL, we analyzed serum samples of patients with classic infantile Pompe disease who were treated with ERT. The samples were collected at ages of <1, 5, and 10 years, as well as around MRI scans. We compared the outcomes with levels in age- and sex-matched peers. Control samples were originally collected as part of routine blood work in children who underwent small surgeries and stored in the biobank of the Erasmus MC/Sophia Children's Hospital.

Results

We analyzed 74 serum samples of 17 patients collected at ages ranging from 22 days to 21.2 years (1–8 samples per patient) and compared these with outcomes of 71 matched peers. In the first year of age, NfL levels in patients and controls were similar (10.3 vs 11.0 pg/mL), but mixed linear model analysis showed a yearly increase of NfL of 6.0% in patients, compared with a decrease of 8.8% in controls ($p < 0.001$). Higher NfL was associated with lower IQ scores ($p = 0.009$) and lower processing speed scores ($p = 0.001$).

Discussion

We found significant differences in NfL levels between patients and controls and a good association between NfL and cognition. NfL deserves further exploration as a biomarker for CNS involvement in patients with classic infantile Pompe disease.

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Glossary

BBB = blood-brain barrier; **CLN2** = ceroid lipofuscinosis type 2; **CRIM** = cross-reactive immunologic material; **DQ** = developmental quotient; **ERT** = enzyme replacement therapy; **GAA** = acid α -glucosidase; **GSDII** = glycogen storage disease type II; **IgG** = immunoglobulin G; **MLD** = metachromatic leukodystrophy; **NfL** = neurofilament light; **PS** = processing speed; **Simoa** = single molecule array; **WAIS-IV** = Wechsler Adult Intelligence Scale–Fourth Edition; **WISC-III/WISC-V** = Wechsler Intelligence Scales for Children, Third/Fifth Edition; **WMA** = white matter abnormalities; **WPPSI** = Wechsler Preschool and Primary Scale of Intelligence.

Introduction

Pompe disease, also known as glycogen storage disease type 2 (GSDII), is a metabolic myopathy caused by the lysosomal deficiency of acid α -glucosidase (GAA). This deficiency leads to the accumulation of glycogen most prominently in muscle cells^{1,2} but, to a lesser extent, also in glial cells, astrocytes, and neurons in the CNS.^{3,4}

Patients with the classic infantile phenotype of Pompe disease have no residual enzyme activity and, consequently, manifest the most severe phenotype of the disease. Untreated, these patients die before the age of 1 year because of cardiorespiratory failure.^{1,5,6} The approval of enzyme replacement therapy (ERT) in 2006 has significantly improved the survival of these patients and enabled them to achieve formerly unmet motor milestones such as the ability to stand and walk.⁷⁻⁹ Globally, the first treated patients with classic infantile disease are now in their 20s.^{10,11}

Neurocognitive development of ERT-treated patients is normal during the first years of life. However, in childhood and adolescence, neuroimaging unveils slowly progressive white matter abnormalities (WMA), whereas cognitive tests show a decrease of processing speed and in a subset of patients a more generalized cognitive decline.^{10,12-14} These symptoms are thought to be the result of the inability of ERT to cross the blood-brain barrier (BBB) and subsequent accumulation of glycogen. This is also illustrated by the fact that autopsy studies in ERT-treated patients have shown glycogen accumulation in the brain.¹⁵ New treatments for classic infantile Pompe disease should therefore include the brain as an additional target.

To appreciate the effect of innovative therapies in upcoming phase 1–2 studies, it is important to fully understand the variability of CNS involvement in patients with classic infantile Pompe disease. Up till now, the extent and rate of CNS involvement has been reported in a limited number of small cohorts of patients with classic infantile Pompe disease and ranges from normal and stable to extensive and fast progressive.^{10,12-14} The methods currently used to monitor CNS involvement have disadvantages. MRI scans are expensive and burdensome because these often require anesthesia. The applied neuropsychological tests vary and are advised not to be performed more than once every 1–2 years because of a potential learning effect. In addition, both methods do not show evident deviations until later in life.^{10,12} Although other

authors describe a correlation between CK, urinary tetrasaccharides, and motor response to ERT,¹⁶ there are no early markers yet that reflect CNS damage. Body fluid biomarkers could be a cheap and easy-to-obtain alternative.

One such potential biomarker is neurofilament light (NfL). Neurofilament is a main protein of the axonal cytoskeleton that consists of different subunits, of which NfL is the most abundant. NfL can be measured in CSF but can also be reliably measured in blood nowadays through single molecule array (Simoa). Levels of NfL in serum, which is more readily available than CSF, are highly correlated with levels in CSF.^{17,18} Increased levels of NfL are associated with neuroaxonal and astroglial damage and correlate with disease activity in many pediatric neurologic diseases, including spinal muscular atrophy, ceroid lipofuscinosis type 2 (CLN2), metachromatic leukodystrophy (MLD), and X-linked adrenoleukodystrophy.¹⁸⁻²³ Unfortunately, reference values of NfL in both serum and CSF for the pediatric populations are lacking.

We hypothesize that NfL is increased in patients with classic infantile Pompe disease and may be correlated with clinical outcomes. In this study, we aimed to determine the potential of NfL as a biomarker for CNS involvement in patients with classic infantile Pompe disease by comparing NfL levels in patients with age- and sex-matched controls and by investigating the correlation of NfL levels with WMA on MRI and cognitive outcomes.

Methods

Patients With Classic Infantile Pompe Disease

All patients with classic infantile Pompe disease in the Netherlands are enrolled in a prospective standardized follow-up study. From this cohort, we included all patients who had a brain MRI scan between 1999 and September 19, 2020 (database lock). Classic infantile Pompe disease was defined as symptom onset before the age of 6 months, presence of a hypertrophic cardiomyopathy, profound enzyme deficiency (<1% of normal values in fibroblasts or below reference values in leukocytes), and 2 severe pathogenic variants in the GAA gene.²⁴⁻²⁶

Patients were treated with alglucosidase alfa at doses ranging from 20 mg/kg per 2 weeks to 40 mg/kg per week.⁸ As per

Table 1 Characteristics of the Patients With Classic Infantile Pompe Disease

No. of patients (% female)	17 (47)
CRIM status, positive/negative/unknown	12/4/1
	Median (range)
Age at diagnosis	2.3 mo (3 d–5.7 mo)
Age at start ERT	2.6 mo (4 d–5.8 mo)
Age at sample <1 y	4.6 mo (22 d–11.1 mo) ^a
Age at the last NfL sample, y	9.1 (3.6–21.2)
No. of blood samples used for NfL measurement per patient	4 (1–8)
No. of cognitive tests per patient	2 (0–6)
Age at the first IQ/DQ measurement	7.2 mo (1.9 mo–10.6 y) ^b
Age at the last IQ/DQ measurement	8.0 y (5.8 mo–21.2 y) ^c
IQ/DQ at the first NfL assessment	89 (64–113) ^c
IQ/DQ at the last NfL assessment	66 (45–102) ^c
Age at the first PS measurement, y	6.8 (5.3–10.6) ^c
Age at the last PS measurement, y	8.5 (5.3–21.2) ^c
PS at the first cognitive assessment	89 (55–111) ^c
PS at the last cognitive assessment	70 (45–111) ^c
Age at the first MRI measurement, y	6.3 (2.7–13.7) ^d
Age at the last MRI measurement, y	8.7 (4.6–21.2) ^d
No. of patients who died (at age, y)	3 (4.4, 14.5, and 15.5)

Abbreviations: DQ = developmental quotient; NfL = neurofilament light; PS = processing speed.

Outcomes are presented as medians with ranges between brackets.

^a Sample not available for 2 patients.

^b No cognitive test data available for 1 patient.

^c PS scores not available for 5 patients.

^d Brain MRI scan data not available for 1 patient.

protocol, blood samples were taken twice to 4 times a year, cognitive evaluations were performed once every 1–2 years, and MRI scans were performed at regular intervals every 1–3 years after 2016 and before 2016 occasionally. We analyzed NfL levels in blood at set time points (at <1 year of age, if available before the start of ERT and otherwise as close to start as possible, as well as at age 5 and 10 years) and around the time an MRI scan was available. We allowed for a window of 1-year difference between the time points at which the different measurements (NfL, cognitive tests, and MRI scans) were taken.

Control Samples

Serum samples from age- and sex-matched controls were obtained from the biobank of the Sophia Children's Hospital. These were taken as part of routine blood work in children who underwent small surgeries (eTable 1, <http://links.lww.com/WNL/C909>).

NfL Analysis Using Simoa Technique

Blood samples were stored in serum tubes at -80°C at the Erasmus Medical Center's Biobank until use. Two hundred fifty microliters of serum was analyzed in the Neurochemistry Lab of the Department of Clinical Chemistry at the Amsterdam University Medical Centers, using the Simoa technique (Simoa HD-1) and the NF-light Kit according to the instructions.²⁷ Before measurement, samples were centrifuged for 10 minutes at room temperature at 1,800g. Measurements were performed by blinded certified technicians in singlicate. Values are expressed in picogram per milliliter.

MRI Scans

We made MRI scans of the brain using a 1.5 or 3 T system (EchoSpeed; GE Healthcare, Milwaukee, WI) and a dedicated 8-channel head coil. MRIs were conducted according to a standardized protocol consisting of T1-weighted, T2-weighted, and fluid-attenuated inversion recovery images.¹⁰ MRIs were graded using the method described before,¹⁰ recognizing 4 stages of involvement: stage 0: no abnormalities; stage 1: periventricular white matter involvement around the centrum semiovale; stage 2: additional abnormalities in the subcortical white matter, internal capsule, external capsule, and corpus callosum; and stage 3: extension to the u fibers, basal ganglia, corticospinal tract, and/or infratentorial white matter. All MRIs were blinded and independently graded by a pediatric neuro-radiologist (M.D.), a pediatric neurologist (J.M.P.v.d.H.), and a PhD student (J.J.A.v.d.D.). Discrepancies were solved by a consensus meeting.

Cognitive Tests

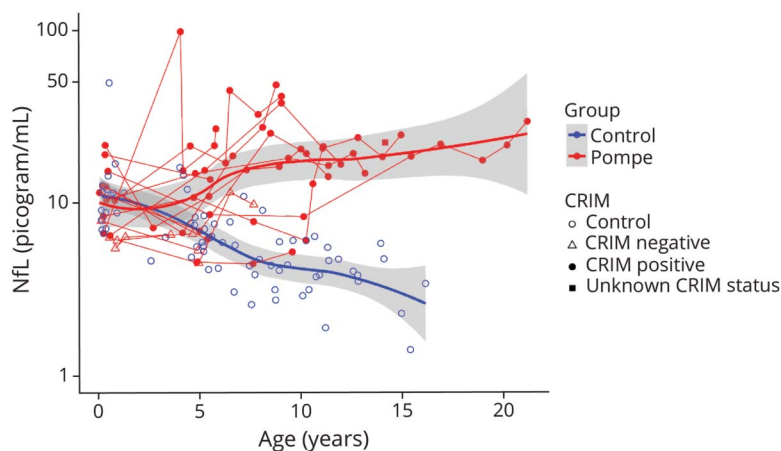
For patients aged 6 years or younger, we used the Griffiths Mental Developmental Scales until the end of 2017²⁸ and the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III-NL) after 2017.²⁹

For children aged 6–16 years and 11 months, we used the Wechsler Intelligence Scales for Children, Third or Fifth Edition (WISC-III-NL or WISC-V-NL),^{30,31} and for patients older than 16 years and 11 months, the Wechsler Adult Intelligence Scale IV-NL (WAIS-IV-NL) was used.³² All neuropsychological tests measured either full scale IQs or developmental quotients (DQs), whereas processing speed (PS) could be tested with the WPPSI, WISC, and WAIS. The scores of all tests were compared with the normative data of the Dutch population. The mean score for the tests is 100, with a SD of 15 points. A score above 85 is indicative of a normal development, a score between 84 and 70 of mild developmental delay, and a score below 70 of intellectual disability.

Statistical Analyses

Statistical analyses were performed using R, version 4.1.3 (2022-03-10, "One Push-Up"). To account for the correlations between repeated measurements in individual patients, we used linear mixed models. Interaction terms between models were tested using the likelihood ratio test. Models' assumptions were checked using residual plots. To correct for skewness of data, NfL levels were log transformed. We used a

Figure 1 NfL Levels in Patients With Classic Infantile Pompe Disease and Controls



NfL levels in patients with classic infantile Pompe disease (red) and controls (blue) visualized with locally estimated scatterplot smoothing curves and the 95% CIs (shadows). The y-axis represents NfL in picogram per milliliter on a logarithmic scale, and the x-axis represents the age in years. Controls are represented by open circles, CRIM-negative Pompe patients by triangle symbols, CRIM-positive Pompe patients by closed circles, and the patient whose CRIM status is unknown signified by a square symbol. CRIM = cross-reactive immunologic material; NfL = neurofilament light.

mixed-effects continuation ratio model for the ordinal data of the brain MRI scans.

The “nlme” and “GLMM adaptive” packages were used for the linear mixed-effects and continuation ratio models; the “lmerTest” package was used for the testing of interaction terms. Descriptive statistics, including median and range, were used to summarize demographic and clinical data. A *p* value of <0.05 was assumed to be significant.

Standard Protocol Approvals, Registrations, and Patient Consents

Study protocols of studies in patients with Pompe disease and controls were approved by the Erasmus MC Medical Ethical Review Committee. Written informed consent was obtained from the patients and of controls and/or their parents.

Data Availability

Unpublished anonymized data within this article are available on reasonable request from a qualified investigator.

Results

General Characteristics

Seventeen patients with classic infantile Pompe disease were included (Table 1). Four (24%) patients were cross-reactive immunologic material (CRIM) negative, 12 (71%) were CRIM positive, and 1 patient’s CRIM status was unknown. The median age at diagnosis was 2.3 months (range 3 days–5.7 months).

All patients were treated with recombinant human GAA. Their median age at the start of ERT was 2.6 months (range 4 days–5.8 months). The median age at the last NfL sample was 9.1 years (3.6–21.2 years), 4.8 years (range 3.6–7.7) for CRIM-negative patients, and 9.9 years (range 5.4–21.2) for CRIM-positive patients. Additional clinical data can be found in Table 1.

NfL Levels in Patients and Controls

NfL blood levels were determined as close as possible to the start of ERT (age <1 year) and at 5 and 10 years of age as well as at the time of MRI scans (Table 1). Five patients had baseline blood samples taken before the initiation of ERT (age range 0.2–0.4 years), 10 had baseline samples taken thereafter (age range 0.1–0.9 years), whereas 2 patients had no baseline sample available before the age of 1 year. The median NfL was 12.4 pg/mL (range 6.7–19.0) for ERT-naïve patients and 9.4 pg/mL (5.5–21.5) in patients with a baseline sample after the start of ERT.

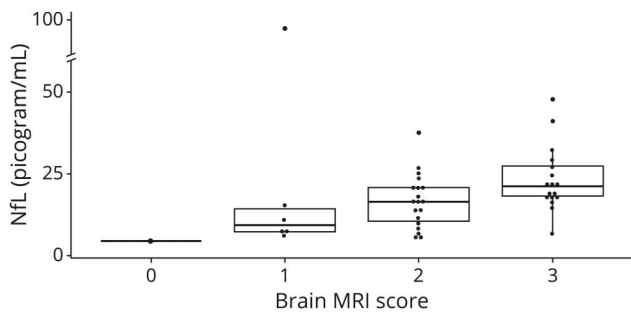
A linear mixed-effects model analysis including all samples (*n* = 74) of the 17 patients (age range 22 days–21.2 years, 1–8 samples per patient) and samples of 71 unaffected age- and sex-matched controls (age range 1.8 months–16.1 years) showed that NfL levels in control patients decreased with 8.8% yearly (95% CI 6.4–11.2), whereas these increased in patients with Pompe with 6.0% yearly (95% CI 3.4–8.0). The difference between these 2 groups was significant (*p* < 0.001).

In our population, CRIM-negative patients (open triangles) had 45% (95% CI 8.4–67.2) lower NfL levels compared with CRIM-positive patients (Figure 1; closed circles). This difference was maintained after correction for age (*p* = 0.028).

Figure 1 shows all individual NfL samples and the locally estimated scatterplot smoothing regression curve as well as the 95% CI (shadows). Figure 1 shows that there is interindividual and intraindividual variation (see also eFigures 1–3, <http://links.lww.com/WNL/C906>, <http://links.lww.com/WNL/C907>, <http://links.lww.com/WNL/C908>).

Selection of 3 time points (age <1, 5, and 10 years) showed the following: At the first NfL assessment (age <1 year), the median NfL value in patients was 10.3 (range 5.4–21.5, *n* = 15) pg/mL and 11.0 (6.7–49.4, *n* = 15) pg/mL in controls; at age 5 years, the median was 7.7 (4.5–21.3, *n* = 16) pg/mL in patients and

Figure 2 NfL Levels Expressed Against Brain MRI Scores



NfL levels in patients with classic infantile Pompe disease expressed against brain MRI scores. The y-axis represents NfL levels in picogram per milliliter and the x-axis the brain MRI score. NfL = neurofilament light.

6.4 (4.1–14.4, $n = 16$) pg/mL in controls; and at 10 years, the median NfL value was 18.6 (5.2–41.1, $n = 8$) pg/mL in patients and 9.8 (8.9–10.7, $n = 8$) pg/mL in controls.

Association Between NfL and Brain Involvement on MRI

Forty-three MRI scans of 16 patients (age range 2.7–21.2 years, 1–6 MRIs per patient) could be paired with 43 NfL blood samples that were taken around the same time (Figure 2). One patient with 1 MRI scan was excluded because of the unavailability of 3-dimensional sequences. One MRI scan was normal (stage 0; age 7.6 years). Six MRI scans were graded as stage 1 (median age 5.4 years [2.7–7.5 years]). Nineteen scans were graded as stage 2 (median age 9.1 years [4.6–12.9 years]), and 17 MRI scans were graded as stage 3 (median age 13.2 years [4.4–21.2 years]).

The NfL level of the patient with a normal MRI was 4.4 pg/mL. The median NfL of patients with stage 1 was 9.3 pg/mL (range 6.1–97.4), 16.6 pg/mL (range 5.3–37.6) of those with stage 2, and 21.3 pg/mL (range 6.7–47.8) of patients with stage 3. Because of the instability of the mixed-effects continuation ratio model, the differences between the different groups could not be tested statistically.

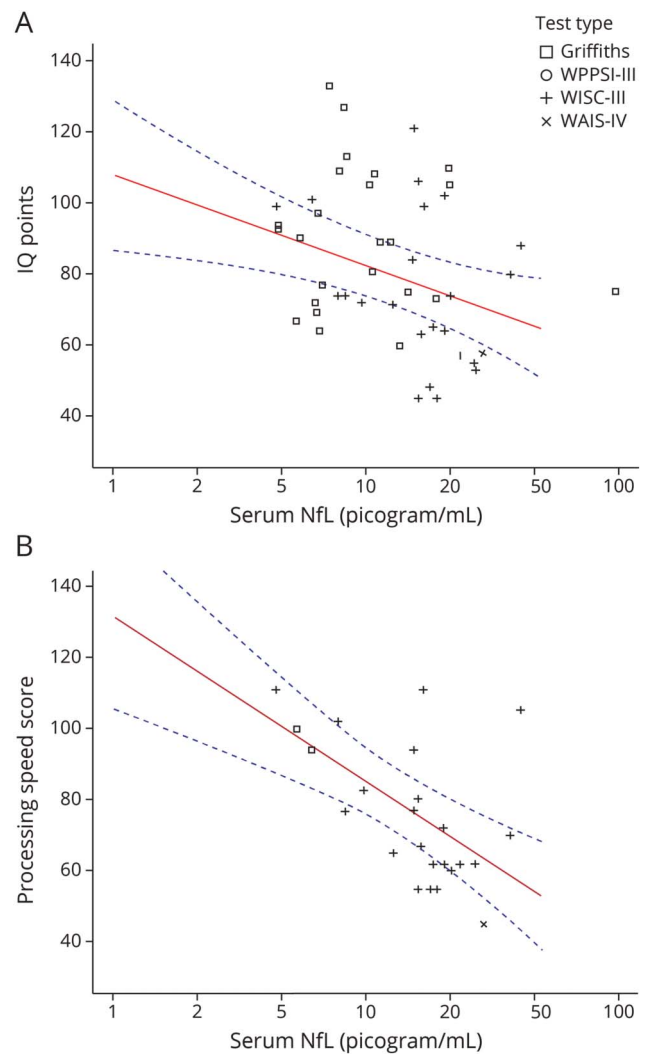
Association Between NfL and Cognitive Outcomes

We compared NfL levels and cognitive scores (Figure 3). Forty-seven serum samples of 16 patients could be paired to 47 total IQ/DQ scores (age range 0.16–21.2 years, 1–5 tests per patient) and 25 samples to 25 results of PS scores (age range 5.3–21.2 years, 1–4 tests per patient). A doubling of NfL corresponded to a decrease of 7.7 IQ points (95% CI –13.2 to –2.1, $p = 0.009$) and a decrease of 13.9 PS score points (95% CI –20.8 to –7.0, $p = 0.001$).

Discussion

We found that NfL levels increased in patients with classic infantile Pompe disease from age 0 to 21.2 years, whereas these decreased over time in healthy age- and sex-matched

Figure 3 Association Between Repeated Measures of (A) Total IQ and (B) PS Scores and NfL Levels in Blood of Patients With Classic Infantile Pompe Disease



The y-axis represents IQ/PS scores, and the x-axis represents NfL levels in picogram per milliliter on a logarithmic scale. Circular symbols represent measurements of the Griffiths Mental Developmental Scales, triangles represent WAIS-IV, square symbols represent scores of the WISC-III, and plus symbols represent measurements of the WPPSI-III-NL. The continuous red line represents the change in cognitive test scores, with the dotted blue lines representing upper and lower limits. NfL = neurofilament light; PS = processing speed; WAIS-IV = Wechsler Adult Intelligence Scale-Fourth Edition; WISC-III = Wechsler Intelligence Scale for Children, Third Edition; WPPSI = Wechsler Preschool and Primary Scale of Intelligence.

controls at the group level. In the first year of life, NfL levels were similar in both groups. After the age of 5 years, NfL levels of controls and patients deviated, NfL levels increased 6.0% per year in patients with Pompe disease, while they decreased by 8.8% per year in controls. With increasing NfL levels, a significant decrease of IQ and PS scores was observed. Although there seemed to be a trend, no statistical relationship could be measured between NfL levels and brain MRI scores.

CRIM-negative patients, who produce no native GAA protein, are thought to have a poorer prognosis than CRIM-

positive patients, who produce some inactive GAA protein. In our study, CRIM-negative patients did not have higher NfL levels than CRIM-positive patients, even after correction for age. As the number of CRIM-negative patients ($n = 4$) in our study was low and their age young, this finding should be interpreted with caution.

Increased levels of NfL are predominantly found in diseases with neuroaxonal or astroglial injury. Therefore, we hypothesize that the gradual increase of NfL in patients with classic infantile Pompe disease is the biochemical result of progressive glycogen accumulation and/or damage to the CNS, leading to the leakage of NfL into CSF and serum.¹² The elevation of NfL reflects the fact that ERT cannot pass the BBB and thus cannot reach glial cells, astrocytes, and neurons, where glycogen deposition has been found in autopsy studies.^{3,4}

Innovative therapies could provide a solution for the still unmet medical needs in classic infantile Pompe disease. Hematopoietic stem cell-mediated lentiviral gene therapy resulted in normalization of glycogen levels and neuroinflammation in the brain of Pompe knock-out mice.^{33,34} In other lysosomal storage disorders such as MPS I and II and CLN2, alternative approaches are effective such as intracerebral ventricular ERT or immunoglobulin G (IgG)-ERT fusion proteins, where the IgG domain is a receptor-specific monoclonal antibody that targets an endogenous BBB receptor transporter.^{35,36} The findings from our study indicate that NfL might be suitable as a biomarker for both follow-up and evaluation of emerging therapies that include the brain as a target.

Although NfL levels increased with age in our cohort of patients with classic infantile Pompe disease on the group level, we also observed interindividual and intraindividual NfL variations, for which no clear explanation could be found. Similar unexplained intraindividual variations are also reported in other diseases such as CLN2 and MLD. More data, preferably in larger cohorts, are needed to provide context on the variability observed.

NfL levels are also elevated in case of a peripheral neuropathy. A recent study in patients with classic infantile Pompe disease who learned to walk reported distal muscle weakness.³⁷ The pathophysiologic process underlying the distal muscle weakness is not yet elucidated. This might be myopathic, neuropathic, or a combination of the both.^{38,39} NfL increases in this cohort therefore might potentially partly be explained by peripheral neuropathy.

The highest NfL level measured in our cohort of patients with classic infantile Pompe disease was 97.4 pg/mL. This was measured in a patient aged 4.1 years. In the oldest patient, aged 21.2 years, the NfL level was measured at 29.2 pg/mL. These NfL levels were both considerably lower than those found in the more rapidly progressive MLD and CLN2.^{18,20} In these diseases, NfL levels were already high (measured around 100–300 pg/mL) around the time of diagnosis. The

median age at diagnosis was 11.1 years in the study of patients with MLD and 4.3 years in the study on patients with CLN2. In the study of MLD, NfL decreased in both treated and untreated patients. This was explained by the rapid progression of CNS damage and a concomitant rapid reduction of healthy brain tissue as a source of NfL release into serum. The decrease in treated and untreated patients with MLD complicates the interpretation of the effect of therapy using NfL as a biomarker.²⁰ Our data show that the CNS progression in Pompe disease is different. Because the CNS involvement in Pompe disease is much slower, and NfL increased in our study over time from childhood through young adulthood in the absence of CNS-targeting therapy, we conclude that NfL holds promise as a biomarker to evaluate the effects of innovative future treatments targeting the brain.

In our study, the NfL levels in controls were relatively high during the first year and showed a decline thereafter during childhood and adolescence. It has been shown by others that during late adulthood, NfL starts to increase again. The decline of NfL during childhood with increasing age was also observed by others and has been hypothesized to be related to factors such as ongoing myelination during the first years of life.^{20,40}

The strength of our study is the longitudinal follow-up combining NfL levels with cognitive tests and neuroimaging as well as the inclusion of a pediatric reference cohort.

A limitation is the small size of our cohort because of the rarity of Pompe disease. Studies in larger groups of patients could provide additional insight in the role of factors such as CRIM status and enable statistical confirmation of the trends we observed between increased NfL levels and brain MRI scan scores. In addition these could provide further insight in the levels of NfL in patients with classic infantile Pompe disease in the first year of life, clarify when NfL levels in patients deviate significantly from normal between the age of 5 and 10 years and elucidate the extent and cause of inter- and intra-individual variations.

In conclusion, we found a significant difference between NfL levels of ERT-treated patients with classic infantile Pompe disease and controls at the group level during follow-up from infancy to young adulthood. In addition, we found that NfL levels were increased in patients with lower IQ and processing speed scores. This study indicates that NfL is a promising biomarker for CNS involvement in classic infantile Pompe disease and deserves additional research as a biomarker to monitor the effect of emerging treatments targeting the brain.

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Disclosure

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Eline A.J. Willemse, PhD	Neurochemistry laboratory, Department of Clinical Chemistry, Amsterdam Neuroscience, Amsterdam University Medical Centres, VU University, the Netherlands; Departments of Biomedizin and Neurology, MS Center and Research Center for Clinical Neuroimmunology and Neuroscience (RC2NB), University Hospital Basel and University of Basel, Switzerland	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
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Charlotte Teunissen	Neurochemistry laboratory, Department of Clinical Chemistry, Amsterdam Neuroscience, Amsterdam University Medical Centres, VU University, the Netherlands	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Ans T. van der Ploeg, MD, PhD	Center for Lysosomal and Metabolic Diseases, Department of Paediatrics, Erasmus University Medical Center, Rotterdam, the Netherlands	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

Appendix (continued)

Name	Location	Contribution
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