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BRIEF REPORT

Cerebrospinal fluid neurofilament light levels in CLN2 disease patients treated with enzyme replacement therapy normalise after two years on treatment [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Classic late infantile neuronal ceroid lipofuscinosis (CLN2 disease) is caused by a deficiency of tripeptidyl-peptidase-1. In 2017, the first CLN2 enzyme replacement therapy (ERT) cerliponase alfa (Brineura) was approved by the FDA and EMA. The CLN2 disease clinical rating scale (CLN2 CRS) was developed to monitor loss of motor function, language and vision as well as frequency of generalised tonic clonic seizures. Using CLN2 CRS in an open label clinical trial it was shown that Brineura slowed down the progression of CLN2 symptoms. Neurofilament light chain (NfL) is a protein highly expressed in myelinated axons. An increase of cerebrospinal fluid (CSF) and blood NfL is found in a variety of neuroinflammatory, neurodegenerative, traumatic, and cerebrovascular diseases. We analysed CSF NfL in CLN2 patients treated with Brineura to establish whether it can be used as a possible biomarker of response to therapy. Newly diagnosed patients had CSF samples collected and analysed at first treatment dose and up to 12 weeks post-treatment to look at acute changes. Patients on a compassionate use programme who were already receiving ERT for approximately 1yr had CSF samples collected and NfL analysed over the following 1.3 years (2.3 years post-initiation of ERT) to look at long-term changes.

All newly diagnosed patients we investigated with classical late infantile phenotype had high NfL levels >2000 pg/ml at start of treatment. No significant change was observed in NfL up to 12 weeks

Open Peer Review

Approval Status

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1. **Michel Boutin** , Université de Sherbrooke, Sherbrooke, Canada

2. **Daniel Erskine**, Newcastle University, Newcastle upon Tyne, UK

Any reports and responses or comments on the article can be found at the end of the article.

post-treatment. After one year of ERT, two out of six patients still had high NfL levels, but all patients showed a continued decrease, and all had low NfL levels after two years on ERT. NfL levels appear to correspond and predict improved clinical status of patients on ERT and could be useful as a biomarker to monitor neurodegeneration and verify disease modification in CLN2 patients on ERT.

Keywords

Neuronal Ceroid lipofuscinosis, Enzyme replacement therapy, Neurofilament light

This article is included in the **UCL Child Health** gateway.

Corresponding author: Wendy E. Heywood (wendy.heywood@ucl.ac.uk)

Author roles: **Iwan K:** Data Curation, Formal Analysis, Writing – Review & Editing; **Patel N:** Data Curation; **Heslegrave A:** Formal Analysis, Writing – Review & Editing; **Borisova M:** Formal Analysis; **Lee L:** Data Curation, Resources; **Bower R:** Data Curation, Resources; **Mole SE:** Conceptualization, Writing – Review & Editing; **Mills PB:** Resources, Writing – Review & Editing; **Zetterberg H:** Resources, Visualization, Writing – Review & Editing; **Mills K:** Resources; **Gissen P:** Data Curation, Investigation, Methodology, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; **Heywood WE:** Conceptualization, Formal Analysis, Funding Acquisition, Project Administration, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: Henrik Zetterberg has served on scientific advisory boards and/or as a consultant for Alector, Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, CogRx and Red Abbey Labs, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). Sara E. Mole receives funding from Biomarin to support the NCL mutation database.

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Introduction

Classic late infantile neuronal ceroid lipofuscinosis (CLN2 disease) is the second most common type of neuronal ceroid lipofuscinosis (NCL), a group of inherited progressive neurodegenerative diseases in children.¹ The classical form of CLN2 disease presents in most patients with early language delay followed by onset of seizures at around three years of age, ataxia, motor and cognitive decline, loss of vision, with death by early adolescence. Atypical forms of CLN2 exist, where patients may present at an older age and with much slower pace of neurodegeneration.² It is estimated that atypical patients constitute 10-20% of the total CLN2 patient cohort, although this may vary in different populations.

In the UK, 5-6 children are diagnosed with CLN2 disease each year, and it is estimated that 30-50 children are currently living with the disease.³ CLN2 disease is caused by mutations in the tripeptidyl peptidase 1 (*TPP1*) gene, which result in either loss or deficiency of the TPP1 lysosomal hydrolase. TPP1 deficiency leads to the characteristic autofluorescent neuronal ceroid lipofuscin accumulation of NCL.⁴

Brineura (cerliponase alfa) is a human recombinant form of TPP1 enzyme replacement therapy (ERT) that was approved for treatment in the US and EU in 2017. It is administered as an intracerebroventricular infusion every two weeks.⁵ The effect of ERT on functional decline can be measured using the CLN2 Disease Clinical Rating Scale (CLN2 CRS).⁶ Patients on ERT are less likely to have an unreversed two-point decline in a combined motor and language function score when compared to untreated patients.⁵ Currently the only way to monitor treatment is through the CLN2 CRS as there are no biomarkers used to monitor neurodegeneration. In this study, we measured neurofilament light (NfL) a known biomarker of neuroaxonal damage and degeneration⁷ in patients undergoing ERT either in the initial 18 weeks of treatment or, in another cohort of patients, who were receiving ERT for at least one year, in whom we monitored NfL over the following 12-18 month period. This allowed us to determine the timing of the response of NfL levels in patients on ERT.

Methods

Ethical statement

The collection of samples for this study has ethical approval (13/LO/0168; IRAS ID 95005; London-Bloomsbury Research Ethics Committee) and Health Research Authority (HRA) approval and all participants provided informed written consent to participate. The study was conducted between May 2019 to October 2020 at Great Ormond Street hospital, UCL Institute of Child Health and UCL Dementia Research Institute.

Samples

All patients were receiving intraventricular infusion of Brineura (cerliponase alfa) from BioMarin pharmaceutical.⁸ Samples were collected for two groups of patients. A short-term/acute change ($n = 5$) group consisted of samples from patients at first dose of ERT and followed from 20-80 days after the start of treatment. A long-term group ($n = 6$) consisted of samples collected from patients who had been receiving ERT for approximately one year. Samples were collected over an additional 12-18 month period from one year of ERT. CLN2 score was performed as in Schulz et al, 2018.⁵ The lowest initial combined CLN2 score was 2 in the short-term group and 1 in the long-term group. All patients receiving Brineura infusions at Great Ormond Street Hospital were included into the study provided the families signed an informed consent form.

Ventricular CSF samples were acquired from surplus material taken for routine infection monitoring. Collected CSF was frozen at -80°C within 24 hours of collection. CSF NfL protein concentration was measured on a Simoa HD-X analyser using the Simoa NF-light Advantage assay kit (Quanterix, Billerica, MA) after being diluted $100\times$, as per manufacturer's instructions. The measurements were performed in one round of experiments using one batch of reagents with the analyst blinded to clinical data. Intra-assay coefficients of variation, monitored using internal quality control samples, were 0.3-8.9%.

Analysis

Results were exported to Microsoft Excel¹⁵ and data analysed using GraphPad Prism v 6 for statistical analysis. Non-parametric paired t-test was applied.

Results and discussion

NfL had first been suggested as a biomarker for future treatment-monitoring of CLN3 disease as elevated CSF (2096 ± 1202 pg/ml) has been observed in patients compared to controls (345 ± 610 pg/ml).⁹ Serum NfL concentration in CLN2 disease has been described to decrease with treatment in canine models and paediatric patients where levels were 48-fold higher than controls pre-treatment but decreased by 50% each year over more than three years of treatment. CSF NfL was monitored for the canine model and observed to correlate with serum NfL; however, the degree of change of NfL with

Table 1. Patient clinical information.

Patient	Sex	Age at start of ERT (years)	CLN2 language and motor score	Days from first ERT to first NfL measurement	CLN2 L+M score after most recent NFL measurement	Genetics allele 1	Genetics allele 2	Disease subtype: classical vs atypical	Severe adverse reactions
Short term group									
1480	Female	4	2+2	0	2+2	c.622C>T p.(Arg208*)	c.1094G>A p.(Cys365Tyr)	Classical phenotype	no
1479	Female	3	2+2	0	2+1	c.509-1G>C	c.622C>T, p.(Arg208*)	Classical phenotype	Anaphylactic reaction to enzyme
1488	Male	4	1+1	0	1+1	c.622C>T p.(Arg208*)	c.1678_1679del	Classical phenotype	no
1498	Male	8	2+2	77	2+2	c.622C>T p.(Arg208*)	c.511G>C	Atypical phenotype	Infection with Initial infusion. Device removed and replaced.
1502	Female	4	2+2	0	2+2	c.509-1G>C	c.509-1G>C	Classical phenotype	no
1504	Male	4	2+2	0	2+2	c.379C>T p.(Arg127*)	c.509-1G>C	Classical phenotype	no
Long term group									
				Months from first ERT to first NfL measurement					
1305	Male	4	2+0	9	1+0	c.1052G>T, p.Gly351Val	c.1052G>T, p.Gly351Val	Classical phenotype, Movement disorder and seizures settled over last 2 years	no
1306	Female	4	1+2	15	1+2	c.509-1G>C	c.509-1G>C	Classical phenotype, Severe movement disorder. Slight improvement observed with ERT	no
330	Male	5	1+1	15	0+0	c.509-1 G>C	c.509-1 G>C	Classical phenotype	no
331	Female	4	3+2	12	3+2	c.509-1 G>C	c.509-1 G>C		vomiting and myoclonic jerks after infusion
1128	Female	4	1+0	14	0+0	c.89+5G>A	c.509-1 G>C	Classical phenotype, Poorly controlled seizures	no
667	Female	15	2+2	12	2+2	c.89+5G>C	c.1340G>A p.(Arg447His)	Atypical phenotype	no

disease progression was observed to be greater in CSF (by 100%) than in serum (56%).¹⁰ A healthy paediatric NfL range has so far not been defined. NfL is observed to increase with age with the lowest range observed for 20-25 year olds as <300 pg/ml.¹¹ Therefore, values above 300 pg/ml are likely pathological.

We describe here the analysis of two separate patient cohorts collected to assess short-term change (n = 5) and long-term change (n = 6) in clinical parameters and CSF NfL over 1-2 years after the first infusion of cerliponase alfa. We observed levels of NfL at the first infusion/baseline over 2000 pg/ml for all patients. Patient information is given in [Table 1](#).

In the first cohort of “treatment-naïve” patients, the post-treatment samples collected 2-3 weeks after the first cerliponase alfa infusion showed that NfL had decreased in two patients ([Figure 1](#)) but these values were still far higher than levels observed for patients on more than two years of ERT ([Figure 2A](#)). The other three patients showed either no change or an increase in NfL.

In this cohort of six patients, the patient (1488) with the lowest combined language and motor CLN2 score had the highest NfL level. Patient 1498 has an atypical form of the disease with later onset of symptoms and much slower progression and had the lowest initial NfL levels in this cohort, but this increased after start of treatment. This increase is likely due to NfL levels being affected by an intracerebroventricular (ICV) device infection and repeat surgery in this patient, as NfL is a non-specific marker of neuroinflammation.

The second (long-term ERT) cohort started their treatment prior to approval of Brineura and had been receiving infusions as part of a compassionate use programme for a year before collection of CSF. For this group CSF began to be collected from 299 days (~10 months) to 600 days (~1.64 years) after the start of treatment and then continued to be collected up to

Figure 1. CSF NfL levels (pg/ml) in CSF, showing response of patients in the short term group at start of treatment and followed up from 11-84 days after.

Figure 2. CSF NfL levels in the long term group (A) Longer term changes in a separate cohort of patients from 299 (9-10 months) to 911 days (~2.5 yrs) post treatment. (B) Paired analysis of the median values of NfL at 1-1.15 of treatment vs 2.5-3 yrs treatment. * $p < 0.05$ Wilcoxon paired t-test.

990 days (2.7 years) post treatment. Most patients had lower levels of NfL that were in the normal adult range (<300pg/ml), apart from two patients. These two patients (1305 and 1306) had more severe movement disorders compared with the other patients. Improvement in involuntary movements temporarily correlated with a decrease of NfL over the next 18 months. Patient 1128 has a particularly severe seizure disorder with poor response to pharmacotherapy. Their NfL level after one year of treatment was low suggesting that NfL level is not likely to be a useful biomarker of seizure control. This observation is supported by a previous study which reported that serum NfL was not altered in children with febrile seizures.¹² However, it had also previously been reported that NfL may reflect the contribution of seizure status to CLN3 disease severity.¹³

The lowest NfL levels in this cohort was in the atypical patient 667. For the long-term ERT cohort, the one-year NfL levels fluctuated moderately before dropping to their lowest levels at two years after start of ERT. When taking the median value of 1-1.5 yrs post-ERT and comparing with levels >2.5 years after ERT there was a significant decrease in NfL levels ($p < 0.03$ by paired t-test (Figure 2B)). This confirms previous observations where serum NfL levels were seen to continually decrease over three years on treatment.¹⁰ That study also revealed that some patients' early serum NfL levels (approximately two months post-ERT) increased and did not begin to decline until nearly one year on treatment. This previous study and our observations here for CSF NfL indicate that in some patients it may take up to a year to start seeing the positive effect of ERT on preventing axonal damage. Moreover, the complete normalisation of NfL levels is likely to take longer. Once the levels normalise, they are likely to stay in the normal range which corresponds to the stabilisation of the patients' clinical parameters.

Conclusions

CSF NfL levels are increased in CLN2 disease patients with lower levels observed in patients with an atypical phenotype therefore NfL levels potentially indicate disease progression and show the effect of treatment with Brineura, correlating with the decline of neuroaxonal damage to very low levels after 2.8 years of ERT. However, in some patients we observed a delayed decline to low levels in NfL compared to other CLN2 patients. CSF NfL may therefore be a good marker to identify these patients who could then receive an adjusted treatment regimen to achieve a faster improved clinical outcome. The results reported here are also relevant to interpretation of NfL changes with time in a clinical trial of potentially disease-modifying drug candidates in adult neurodegeneration.¹⁴

Data availability

Underlying data

UCL data repository: CSF NfL results CLN2 disease. <https://doi.org/10.5522/04/14822463.v1>.¹⁵

The project contains CSF NfL results for Group 1 and Group 2 (CSF NfL CLN2 disease Iwan et al.xlsx).

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

Acknowledgments

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Open Peer Review

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Version 1

Reviewer Report 09 November 2021

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Daniel Erskine

Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK

This manuscript, by Iwan and colleagues, set out to longitudinally follow patients with the CLN2 form of Neuronal Ceroid Lipofuscinosis, to detect response to enzyme replacement therapy by evaluating CSF levels of NfL. There is a pressing need to better evaluate response to treatment in CLN2 patients receiving enzyme replacement therapy, as current measures are limited to clinical instruments. Whilst clinical scales are useful to show functional improvement, clinical features likely follow from progressive degeneration over time, and thus may lack the sensitivity to detect changes in neurodegeneration. Evaluation of NfL levels in CSF has been gaining traction as a relatively broad measure of axonal degeneration across a range of neurodegenerative conditions, and the authors quite reasonably suggest this may be a more sensitive measure of treatment response compared to clinical tests alone. NfL levels were measured in CSF using the highly sensitive SIMOA platform that is optimised for detecting even low levels of the analyte of interest. They identified a reduction in NfL over time in most cases following enzyme replacement therapy, but this was often 2-3 years following treatment initiation. The authors note the variability in response and discuss the potential of using this information to monitor treatment response and potentially adjust regimens.

Overall, I thought this was an excellent manuscript. Given the rarity of CLN2 and the longitudinal nature of this study, the number of participants was particularly impressive. I have some suggestions for the authors to consider:

- I felt there was a lack of relationship with clinical variables. Given that the stated aim of the study was to augment current monitoring with CLN2 CRS, I was surprised to see no attempt at associating changes in CLN2 CRS with NfL levels over time.
- Was any imaging performed to corroborate the suggestion that changes in NfL are associated with reduced axonal degeneration or neurodegeneration?

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neurodegenerative disease pathology, neurometabolic disease pathology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 03 Dec 2021

Wendy Heywood, University College London, London, UK

Many thanks for reviewing the report.

We tried to analyse whether the speed of loss of points on CLN2 clinical score is correlated with the slope of NFL normalisation, i.e. whether NFL levels can act as a biomarker of response to enzyme replacement therapy (ERT). We did indeed look at this and did not find a statistically significant correlation. This could be due to a few factors such as the small number of patients in the study, the fact that CLN2 score is not sufficiently sensitive to detect small changes in response or there is no real correlation between the NFL levels and the patients response to ERT.

In response to your other question the MRI scans are performed yearly in the patients which is similar to the clinical trial previously reported by Schulz et al. NEJM 2018, where MRI grey matter volume changes was one of the outcome measures. The results in our patients were in line with the clinical trial report where patients lost approximately 10.5% of total grey matter volume in the first year of treatment and 3.3% in the second year which indeed indicates neurodegenerative processes.

Competing Interests: No competing interests were disclosed.

Reviewer Report 11 August 2021

<https://doi.org/10.5256/f1000research.58055.r90536>

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Michel Boutin

Division of Medical Genetics, Department of Pediatrics, Centre de Recherche-CHUS, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada

The authors of this brief report have tested the possibility to use the neurofilament light chain (NfL) levels in cerebrospinal fluid as a biomarker to monitor the response to cerliponase alfa for patients suffering from CLN2, the classic late infantile neuronal ceroid lipofuscinosis. The biomarkers were analysed in specimens of six patients at the initiation of treatment and followed up from 14-84 days after, and for six other patients from 9-10 months to 2.5 yrs post-treatment. Since CLN2 is an extremely rare genetic disease, I think that it was a major accomplishment to longitudinally monitor the biomarkers for a cohort of 12 patients. The results obtained revealed a statistically significant long-term decrease of NfL levels in cerebrospinal fluid of CLN2 patients treated with cerliponase alfa. However, soon after the initiation of ERT (≤ 84 days), the biomarker levels increased for some patients and decreased for others. It suggests that the NfL levels might be used to adjust patient regimen, but further studies will be needed to confirm it. Moreover, we must keep in mind that NfL is a non-specific marker of neuroinflammation, and its level is also affected by repeat surgeries and infections due to the intracerebroventricular device used to dispense the enzyme replacement therapy.

Minor revisions:

1. In the title, I suggest adding "chain" between "light" and "levels".
2. In the second paragraph of the "Samples" section, I suggest adding the limit of detection and the limit of quantification of the Simoa kit, as well as the concentration of the internal quality control sample.
3. In the "Analysis section", I suggest replacing "Results were exported to Microsoft Excel¹⁵" with "Results were downloaded in the UCL data repository¹⁵".
4. I think that it can be less confusing for the reader if the patients are labeled 1 to 12, instead of 1480, 1479, 1488, 330 ... in the figures, the table, and in the core of the manuscript.
5. In the "Results and Discussion" section, second paragraph, first line: Please replace (n = 5) by (n = 6).
6. For Figures 1 and 2, the shape of the data point marks might be changed for the different patients (Ex: square, triangle ...) to facilitate the comprehension of the graphs when the article is printed in black and white.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I have a Ph.D. in chemistry and I performed postdoctoral trainings in proteomics and metabolomics. Since 2011, I am the technical director of the Waters-CHUS Expertise Centre in Clinical Mass Spectrometry (Sherbrooke, QC, Canada). My main area of research concerns the discovery of biomarkers for rare genetic diseases using untargeted metabolomics.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 03 Dec 2021

Wendy Heywood, University College London, London, UK

Many thanks. All the recommended changes have been made to the manuscript.

Competing Interests: No competing interests were disclosed.

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