Plasma neurofilament heavy chain is not a useful biomarker in Charcot-Marie-Tooth Disease

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Abstract

Background: The negative trials of vitamin C in Charcot-Marie-Tooth disease (CMT) type 1A have highlighted the lack of sensitive outcome measures. Neurofilaments are abundant neuronal cytoskeletal proteins and their concentration in blood is likely to reflect axonal breakdown. We therefore examined plasma neurofilament heavy chain (NfH) concentration as a potential biomarker in CMT.

Methods: Blood samples were collected from healthy controls and patients with CMT over a 2-year period. Disease severity was measured using the CMT Examination Score. An in-house ELISA was used to measure plasma NfH levels.

Results: There was no significant difference in plasma NfH concentrations between CMT patients and controls (*P*=0.449). There was also no significant difference in plasma NfH levels in the CMT group over 1 year (mean difference = -0.02, Standard error of the mean = 4.44, *P*=0.98).

Conclusions: Plasma NfH levels are not altered in patients with CMT and are not a suitable biomarker of disease activity.

Keywords:

Charcot-Marie-Tooth disease

CMT

Outcome measure

Neurofilament heavy chain

ELISA

Plasma

Introduction

Charcot-Marie-Tooth disease (CMT) is the commonest genetic neuromuscular disease; it has a population prevalence of 1 in 2500 (1). Although no pharmacological treatments exist, there have been several successful pre-clinical trials in mouse models of the disease (2–5). Vitamin C was shown to be effective in a mouse model of CMT1A (6), but this effect was not borne out in several double-blind randomized placebo-controlled trials (7–10). These trials, however, have highlighted the insensitivity of current outcome measures to detect changes in disease progression over a 2-year period and the need for a sensitive biomarker.

To date, plasma neurofilament heavy chain (NfH) levels have been shown to be raised in several central nervous system diseases including amyotrophic lateral sclerosis (ALS) and optic neuritis (11–15). Neurofilaments are the most abundant neuronal cytoskeletal protein in peripheral nerves, and their concentration in blood likely reflects axonal breakdown and provides a biomarker of peripheral nerve disease (16). In further support of this hypothesis has been the demonstration of raised plasma NfH levels in a mouse model of lower motor neuron degeneration and in patients with diabetic polyneuropathy (17,18).

The aims of this study were to determine whether plasma NfH levels are elevated in patients with CMT. If they are raised, we questioned whether they correlate with disease severity and whether they change over a 12-month follow up period.

Materials and methods

This study was approved by The National Hospital for Neurology and Neurosurgery Research Ethics Committee/ Central London REC 3 09/H0716/61.

Blood samples were collected prospectively, with informed consent, from CMT patients attending the inherited neuropathy clinic at the NHNN. CMT1A is the commonest type of CMT and the only type of CMT in which clinical trials have taken place. Although it is classified as a demyelinating neuropathy, CMT1A patients were included in this study, as their clinical deficit is known to be due to axonal damage. Only patients with a genetically confirmed diagnosis of CMT were included, and patients with other neurological diseases were excluded. The disease severity, as measured by the Charcot-Marie-Tooth Examination Score (CMTES, second version), was recorded at the same time as plasma was collected (19). The CMTES is a validated outcome measure for assessing the severity of CMT. It is a composite score that includes the patient's symptoms and examination findings.

Blood samples were obtained from healthy relatives of patients attending the inherited neuropathy clinic and staff at the UCL Institute of Neurology. Healthy subjects were excluded if they had co-existent neurological disease as determined by a symptom- and past medical history-based questionnaire. Plasma from healthy controls was also acquired with informed consent from the relatives of patients with ALS recruited as part of a separate study [East London and the City Research Ethics Committee 1 (09/H0703/27)].

A modified in-house sandwich ELISA was used to measure plasma hyper-phosphorylated NfH levels in the plasma (SMI-34R, Covance, USA)(20).

All statistical analysis was performed using SPSS version 14.00. Plasma NfH levels were compared using a Mann-Whitney U test, and correlation of plasma NfH and disease severity and age were assessed using Pearson correlation coefficients.

Results

A similar number of patients with CMT (n=90) and healthy controls (n=79) were included in this study. Controls were significantly older than the CMT cohort (*P*=0.039, *t*-test) although the mean difference was only 5 years. There was also a significant difference in the ratio of men to women in the 2 groups [46:44 (CMT), 56:23 (Controls), χ^2=6.876 *P*= 0.009], with significantly more women in the controls. Nevertheless, there were no gender differences in plasma NfH levels within the CMT (unpaired *t*-test, *P*=0.3) and control groups (*P*=0.9). The percentage of patients with demyelinating forms of CMT (71%) was higher than for axonal forms (29%).

There was no difference in the primary outcome measure of plasma NfH concentration between patients with CMT (27.4 ng/ml) and controls (21.5 ng/ml) (*P*=0.449, Mann Whitney U test) and no significant difference in plasma NfH levels between patients with axonal (27.8 ng/ml) and demyelinating (27.3 ng/ml) forms of CMT (*P*=0.833) (see Figure 1).

There was a small but significant difference in the mean age of CMT patients (44) and controls (48.9) (*P*= 0.02, *t*-test), although there was no significant correlation between plasma NfH levels and age in the controls (Pearson correlation coefficient = -0.039, *P*=0.730).

To determine whether the lack of a difference in plasma NfH levels in CMT is due to the inclusion of patients with a lower CMTES score (<10), and hence lower levels of axonal

degeneration, NfH levels were correlated against the CMTES score. Unexpectedly, there was a trend toward a reduction in NfH levels with increasing disease severity as defined by the CMTES (Pearson Rank -0.173, *P*=0.104) (Figure 1).

There was no significant difference in plasma NfH levels in the CMT group over 1 year (mean difference = -0.02, SEM=4.44, paired *t*-test, *P*=0.98). This is despite a mean increase in the CMTES of 1 point for all forms of CMT (SEM=0.41, *P*=0.01).

Discussion

A cross-sectional comparison of plasma NfH levels revealed no difference between CMT patients and controls. There are several reasons why this may be the case. First, CMT is a slowly progressive disease in comparison to more rapidly progressive neurodegenerative disorders in which plasma and serum NfH levels were elevated (15). Second, the timing of clinical progression due to axonal degeneration and timing of sampling is critical, and this cohort might, while being systematic, not have covered random but clinically relevant events. The third possibility is that chronic release of NfH into the peripheral circulation induces an immune response that leads to the production of anti-NfH antibodies and a consequent reduction in detectable plasma NfH levels. This phenomenon has been observed in other neurological conditions, including primary and secondary progressive MS, in which higher antibody levels against neurofilament light chain (NfL) and NfH have recently been described (21,22). It is also worth noting that the mean age of subjects was 44 years. It is possible that plasma NfH levels may be elevated in pediatric patients with CMT in whom the volume of distribution is smaller and in whom antibodies against NfH and NfL (if present) may not yet have been produced.

Much of the work examining NfL and NfH has focused on levels in both plasma and CSF (23,24). It is possible that NfH levels are elevated in the CSF of patients with CMT but are degraded rapidly in the plasma. Against this hypothesis is the fact that CMT is a length-dependent, dying-back neuropathy in which neurodegeneration occurs in the distal limbs. The degree of neurodegeneration at the level of the intradural nerve root is therefore likely to be low.

In order for plasma NfH to be a biomarker of axonal degeneration in CMT there should be a significant difference between patients with CMT and controls, and the levels should correlate with disease severity. Unfortunately, plasma NfH is not higher in CMT patients than controls, and there is a decrease in plasma NfH with increasing disease severity. Plasma NfH levels are therefore not suitable as a biomarker of CMT disease severity or as a potential biomarker for natural history studies or clinical trials.

Abbreviations:

CMT = Charcot-Marie-Tooth disease

NfL = Neurofilament light chain

NfH = Neurofilament heavy chain

CMTES = Charcot-Marie-Tooth examination score

CMTNS = Charcot-Marie-Tooth neuropathy score

ALS = Amyotrophic Lateral Sclerosis

SEM = Standard error of the mean

ELISA = Enzyme Linked Immuno- absorbant Assay

NHNN = National Hospital for Neurology and Neurosurgery

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Figure 1. Plasma neurofilament heavy chain levels in patients with CMT versus controls. **(A)** the genetic breakdown of all CMT patients included in the study. **(B)** plasma NFH levels (black dots) of all CMT patients and controls. The horizontal dotted line shows mean plasma NfH levels of CMT patients (27.4 ng/ml) versus controls (21.5 ng/ml)(p=0.449). **(C)** plasma NfH levels plotted against disease severity as determined by the CMTES. Unexpectedly, there was a trend toward a reduction in NfH levels with increasing disease severity (Pearson Rank -0.173, *P*=0.104). **(D)** mean difference in plasma NfH levels over 1 year in 19 CMT patients and 10 controls. There was no significant difference in plasma NfH levels in CMT patients (mean difference = -0.02, *P*=0.98) or controls (mean difference=-7, *P*=0.37) over 1 year.