Neurofilament light chain as disease biomarker in a rodent model of chemotherapy induced peripheral neuropathy

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Abstract

The objective of this study is to test the feasibility of using serum neurofilament light chain (NfL) as a disease biomarker in Chemotherapy Induced Peripheral Neuropathy (CIPN) since this easy accessible biological test may have a large impact on clinical management and safety of cancer patients. We performed this preclinical study using a well-characterized rat model based on repeated administration of the cytostatic drug vincristine (VCR, 0.2 mg/kg intravenously via the tail vein once/week for 4 times). Serial NfL serum concentration was measured using the in-house Simoa NfL assay and peripheral neuropathy onset was measured by sensory and motor nerve conduction studies. Serum NfL measure in untreated and VCR-treated rats demonstrated a steady, and significant increase during the course of VCR administration, with a final 4-fold increase with respect to controls ($p < .001$) when sign of axonopathy and loss of intraepidermal nerve fibers were clearly evident and verified by behavioral, neurophysiological and pathological examination. This simple monitoring approach based on serum NfL concentration measures may be easily translated to clinical practice and should be considered as a putative marker of CIPN severity in a typical oncology outpatient setting. Further studies are needed to validate its utility in cancer patients treated with different neurotoxic drugs.

Introduction

Neurofilament light chain (NfL) is a neuron-specific cytoskeletal protein important for cell structural stability. Damage of axons releases NfL into the interstitial fluid and increased concentrations of NfL can be detected in blood samples. Recently, increased NfL concentration has been demonstrated in a cross-sectional study performed in inherited peripheral neuropathies, and their levels correlated with the severity of nerve impairment [1].

Chemotherapy Induced Peripheral Neurotoxicity (CIPN) is a potentially dose-limiting side effect of chemotherapy treatments of several common forms of cancer. Although the neuronal target might be different, the main clinical features of CIPN are those of a length dependent axonopathy [2]. CIPN onset and course must be monitored very closely, since delayed recognition or improper management through chemotherapy schedule modification can lead to permanent nerve damage. However, the scales commonly used by oncologists in daily clinical practice (e.g. National Cancer Institute Common Toxicity Criteria) are not reliable [3], and frequently a formal neurological assessment is not available.

For these reasons, the availability of a simple, fast, effective and reliable biomarker obtained from an easy accessible biological matrix might have a large impact on the clinical management and safety of cancer patients treated with neurotoxic drugs.

To test the possibility to use NfL as a CIPN biomarker we performed this preclinical study using a well-characterized rat model based on repeated administration of the cytostatic drug vincristine, where axonopathy is clearly evident and measurable (VIPN).

Materials and Methods

The study was approved by Animal Care and Use Committee of the University of Milano-Bicocca and adhered to all guidelines for humane treatment of laboratory animals set forth in the Guide for the Care and Use of Laboratory Animals (Office of Laboratory Animal Welfare) as well as to the Italian D.L.vo n. 26/2014.

Adult female Wistar rats (n. 8/group, 175-200g at the beginning of the study, Envigo, Udine, Italy) were left untreated or received vincristine 0.2 mg/kg (VCR) (EVA Pharma B.V., Mijdrecht, Olanda) intravenously via the tail vein once/week for 4 times. At the end of treatment the animals were tested with behavioral methods to assess their mechanical and thermal sensory thresholds (Dynamic and Plantar test) [4]

Sensory and motor nerve conduction studies were performed under deep isoflurane anesthesia and body temperature was kept constant.

At the end of the treatment period, sciatic and caudal nerves were obtained for pathological examination and morphometry [4]. On days 7, 14, 21, and 28 blood samples were collected in XX tubes and serum was obtained after X min coagulation at room temperature through centrifugation at 20°, 3500 g for 15 minutes. Serum was then aliquoted and stored at -80° until NfL measurements.

Serum NfL concentration were determined using the in-house Simoa Nfl assay which has been previously described [5]. Samples were analyzed using one batch of reagents and animal treatment information was blinded to the examiner performing the analysis. The average repeatability of the assay was assessed by measurements of quality control samples and the coefficient of variation was 6.2% for a sample with a mean NfL concentration of 50.7 pg/ml, and 12.3% for a sample with a mean NfL concentration of 22.6 pg/ml.

Statistical analyses were carried out on raw data using GraphPad Prism4 (GraphPad, La Jolla, CA). To compare the behavioral assessments Student's t-test was used, while NfL and neurophysiological data were analyzed using the 2-sided Mann-Whitney U-*test.*

Results

The administration of VCR was well tolerated by the animals, with a normal weight gain (Fig 1A), no behavioral signs of distress and no mortality. The onset of VIPN was confirmed at the end of treatment by the results of the Dynamic test indicating significant mechanical allodynia (Fig. 1B),

while the drop in the Plantar test for thermal threshold with treatment was not statistically significant (Fig. 1C).

No significant changes were observed at the neurophysiological examination of digital nerves in VCR-treated rats. The examination of the caudal nerves evidenced at the end of treatment significant ($p<0.01$) reduction in both conduction velocity and sensory and motor potential amplitude in VCR-treated rats with respect to control animals (Figs. 2A-F).

At the pathologic examination performed on sample obtained at the end of VCR administration, axonopathy was evident particularly in the caudal nerve, while changes in the sciatic nerve were milder (Figs. 2A-C).

Serial NfL serum concentration measured in untreated and VCR-treated rats demonstrated a steady, significant increase during the course of vincristine administration (Fig. 3), with a final 4-fold increase vs. controls ($p<0.001$) when evidence of VIPN was verified by nerve conductivity and pathological examination.

Discussion

Elevated concentration of blood NfL has already been demonstrated in several central nervous system diseases (e.g. dementia, multiple sclerosis, motor neuron disease) [1, 5-7], where NfL is considered a biomarker of axonal damage, and a possible severity biomarker of neuronal injury. In multiple sclerosis, NfL levels correlate with treatment response and disease activity [8], thus adding additional relevance to their measurement.

One of the major limitations in NfL measurement was represented by insufficient standardization and methodological differences. However, recently a highly sensitive and reliable method to measure NfL concentrations was reported and tested in Charcot-Marie-Tooth (CMT) disease showing elevated levels and significant correlation with disease severity [1].

These results are particularly important since no effective serological disease biomarkers are available for peripheral neuropathies. Moreover, the hypothesis that NfL levels might be used to not only assess the *presence* of peripheral nerve damage, but also to *monitor* the course of peripheral neuropathies deserves to be explored.

CIPN represents an ideal model to test this hypothesis since a) each subject can be examined before, during and after chemotherapy, so that individual baseline concentrations could be used as a very precise reference for subsequent samples; b) blood testing are routinely performed in cancer patients undergoing chemotherapy, and therefore no additional invasive procedure would be requested to obtain suitable samples, c) CIPN monitoring is a major challenge for oncologists, particularly when they are not supported by neurologists as is common in daily clinical practice, d) early detection of CIPN might prevent irreversible nerve damage, e) CIPN severity is extremely

variable among patients, and the time course of NfL changes might allow for early detection of high-risk patients.

This latter point is particularly relevant since no effective treatment is currently available for CIPN once it has ensued [9], and debilitating CIPN-related side effects have been reported several years after the end of chemotherapy [10].

In the present study, serial results were obtained in a well-established animal model of repeated VCR administration inducing distal axonopathy with relative sparing of more proximal nerves (i.e. mimicking mild-to-moderate nerve damage). Using the same highly sensitive method used in the cross-sectional human CMT study we showed a progressive increase in serum NfL levels, that is closely correlated to pathologically-confirmed axonopathy and reflect progressive damage.

Therefore, this simple monitoring approach that might be easily translated in clinical practice should be considered as a putative marker of CIPN severity in a typical oncology outpatient setting, and deserve to be tested in cancer patients treated with different neurotoxic drugs.

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Figure 1

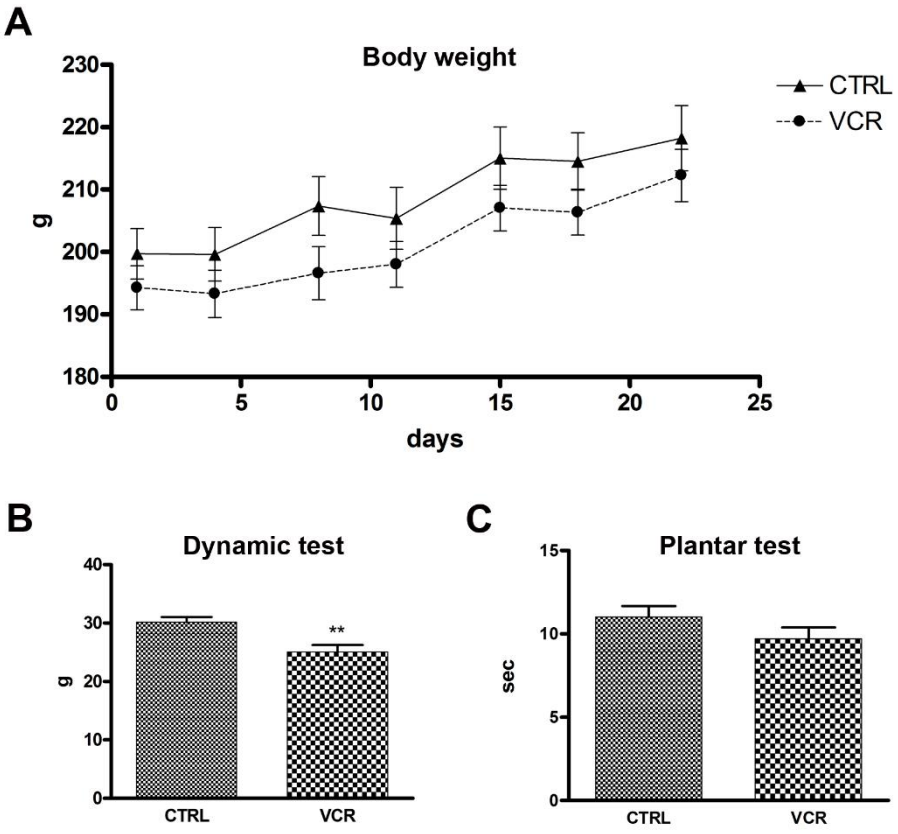

Figure 1. Vincristine induced allodynia in rats. Body weight change during the experiment period (A); assessment of the mechanical hypersensitivity by measuring the force required to induce allodynia after 4 weeks of VCR administration (B); thermal algesimetry tests at the end of experiment (C); **p<0.001.

Figure 2

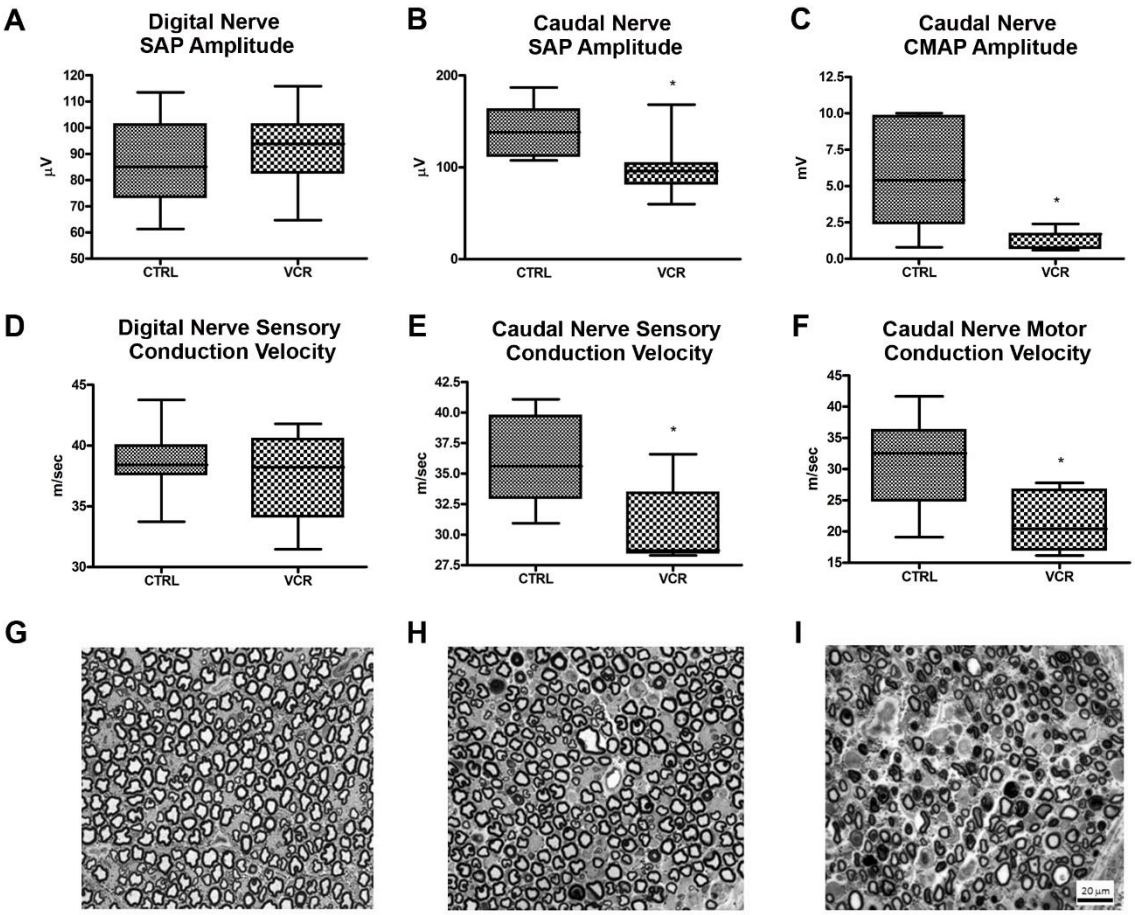

Figure 2. **Vincristine causes distal to proximal axonal degeneration in rats.** Neurophysiological results obtained in the digital nerves (sensory, A, D) and in the caudal nerves (sensory, B, E; motor, C, F), suggesting a distal-to-proximal gradient of nerve damage. These neurophysiological results are in agreement with the pathological examination performed on semithin sections of caudal nerves collected from a control animal (G), and sciatic or caudal nerves from VCR-treated rats (respectively, H and I). *p< 0.01.

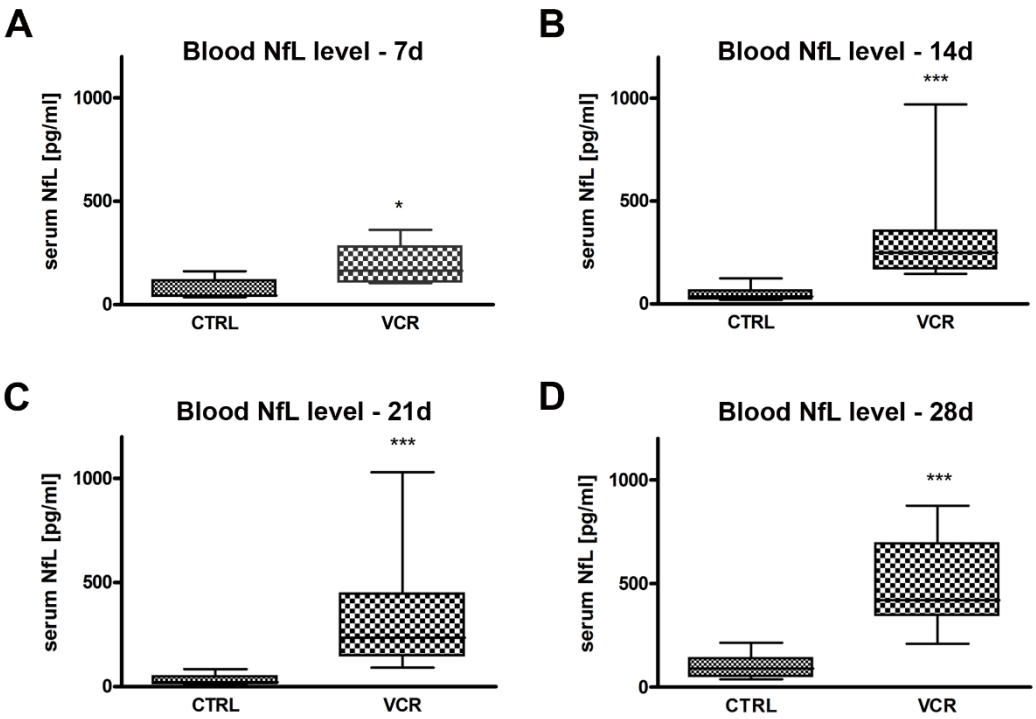

Figure 3: Increased serum neurofilament light (NfL) concentration in VIPN model. Serum NfL analyses performed after 1 weeks of VCR-treated rats (A), 2 weeks of VCR-treated rats (B), 3 weeks of VCR-treated rats (C), 4 weeks of VCR-treated rats (D). Boxplots report median concentrations and interquartile range ?. *p<0.05; ***p<0.001