



Prediction of Chemotherapy-Induced Peripheral Neuropathy in Patients with Lymphoma and Myeloma: the Roles of Brain-Derived Neurotrophic Factor Protein Levels and A Gene Polymorphism

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Background and Purpose Brain-derived neurotrophic factor (BDNF) is a neuronal growth factor that plays an essential role in the maintenance of the nervous system. We have evaluated the peripheral blood protein levels of BDNF and the valine-to-methionine substitution at codon 66 (Val66Met) single-nucleotide polymorphism (SNP) as potential biomarkers for the early recognition of chemotherapy-induced peripheral neuropathy (CIPN) in non-Hodgkin lymphoma and multiple myeloma patients.

Methods CIPN was assessed in 45 patients at the diagnosis and during vincristine or bortezomib-based therapy using objective [reduced version of the Total Neuropathy Score (TNSr)] and subjective (FACT-GOG-NTx) tools. Depression was assessed using the Patient Health Questionnaire-9 (PHQ-9) questionnaire. BDNF protein levels and the Val66Met SNP were determined using ELISA and Sanger sequencing.

Results The pretreatment BDNF protein level was inversely correlated with the maximum TNSr, FACT-GOG-NTx, and PHQ-9 scores in both genotypes. BDNF patients with the Val/Val genotype demonstrated significantly higher maximum FACT-GOG-NTx and PHQ-9 scores than those with the Val/Met and Met/Met genotypes (Met-BDNF carriers). Correlations between PHQ-9 and TNSr score were found only in Met-BDNF carriers, suggesting that peripheral neuropathy and depression coincide in Met-BDNF carriers.

Conclusions Determining the BDNF protein levels before initiating chemotherapy might be a useful tool for CIPN risk assessment and preemptive dose modification. The present data should be validated in larger studies that include other neurotoxic agents.

Key Words BDNF, chemotherapy-induced peripheral neuropathy, Val66Met single-nucleotide polymorphism, non-Hodgkin lymphoma, multiple myeloma.

Received March 14, 2019

Revised May 2, 2019

Accepted May 2, 2019

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INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor (NGF) family that plays an essential part in the development,¹ maintenance,² and repair³ of the central and peripheral nervous systems. The human BDNF gene frequently carries a no conserved single-nucleotide polymorphism (SNP; dbSNP: rs6265) that results in a valine-to-methionine substitution at codon 66 (Val66Met). This SNP does not affect BDNF signaling but is reported to cause a deficit in the cellular distribution and dysregulated secretion of BDNF in neurons.⁴ BDNF SNP exists solely in humans and is associated with increased susceptibility to memory and cognitive impairment in various neurological and

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neuropsychiatric disorders.^{5,6}

BDNF protein is synthesized as proBDNF (a 32-kDa precursor peptide) that is proteolytically cleaved to mBDNF (its 14-kDa mature form) that acts as a 28-kDa dimer and exerts its cellular effects via high-affinity interactions with tropomyosin-related kinase receptor B (TrkB) and the nonselective low-affinity TNF- α -related p75 (p75NTR) neurotrophin receptor). The peripheral sources of BDNF are complex and have not been fully elucidated. However, it has recently become evident that BDNF is abundant in the peripheral blood. Lymphocytes and monocytes have been previously shown to produce and secrete BDNF and participate in neuronal tissue healing processes by increasing the availability and levels of BDNF in injured areas.^{7,8} Consistent with these observations, dysregulated BDNF production and diminished peripheral levels of BDNF in the circulating blood have been identified in chronic neurological impairments.⁹ Platelets are the main BDNF-containing compartment in the blood.^{10,11} Recent data suggest that in the peripheral blood, BDNF is mostly produced by megakaryocytes and delivered to platelets.¹²

Chemotherapy-induced peripheral neuropathy (CIPN) is a known adverse effect associated with significant morbidity during and even after completing anticancer therapy.¹³ Moreover, the development of CIPN is a dose-limiting factor and therefore may diminish the rate of response.¹⁴ Bortezomib and vincristine are anticancer agents with neurotoxic potential that are extensively used for the treatment of multiple myeloma (MM) and non-Hodgkin lymphoma (NHL). The impact of neurotrophic factors on the mechanism of CIPN development has been identified in several studies. For example, reduced levels of the BDNF family member NGF were found to be associated with CIPN development in patients with cervical carcinoma.¹⁵ Another study demonstrated a direct correlation between the level of BDNF and the combined score for diabetes-related peripheral neuropathy, which is a known risk factor for CIPN.¹⁶ Preliminary studies by our group showing a link between CIPN and BDNF protein levels in blood¹⁷⁻¹⁹ emphasize the need for further investigations of BDNF as a potential biomarker for CIPN. The current study aimed to determine the associations of serum BDNF protein levels and the Val66Met SNP with the development of CIPN in NHL and MM patients.

METHODS

Study population

Newly diagnosed NHL and MM patients between 18 and 70 years of age who were assigned to receive treatment with vincristine- or bortezomib-based protocols signed an informed consent form (IRB No. 0195-15-RMB) and were recruited to

the study at the Hematology Ambulatory Unit of the Rambam Health Care Campus, Haifa, Israel. The patients were recruited during 2017 and 2018. The following exclusion criteria were applied: NHL with central nervous system involvement, pregnancy, or inadequate judgment capabilities.

Neurological assessment

The NHL patients were assessed neurologically prior to treatment initiation (baseline), after three treatment cycles, and at the end of the treatment protocol, while the MM patients were assessed before treatment initiation and following three treatment cycles. Additional neurological assessments were performed upon the completion of five treatment cycles in eight MM patients. Objective symptoms of polyneuropathy were graded using the reduced version of the Total Neuropathy Score (TNSr).²⁰ In addition, each patient completed the Functional Assessment of Cancer Therapy/Gynecologic Oncology Group-Neurotoxicity (FACT-GOG-NTX) questionnaire in order to evaluate their subjective polyneuropathy symptoms. The patients also completed the Patient Health Questionnaire-9 (PHQ-9) depression screening questionnaire.²¹

BDNF Val66Met-SNP genotyping

DNA was purified from peripheral blood using the QIAamp DNA Mini kit (cat# 51106, Qiagen; Hilden, Germany) according to the manufacturer's instructions. The BDNF-gene DNA region containing the rs6265 polymorphism (Val66Met) was amplified by PCR using the following primers: P1 (forward) CCTACAGTTCCACCAGGTGAGAAGAGTG and P2 (reverse) TCATGGACATGTTTGCAGCATCTAGGTA. The 401-bp PCR product containing the SNP of interest was sequenced by Sanger sequencing, and the allele-specific G→A substitution was determined with a sequence analyzer (Abi).

Quantification of BDNF protein levels in patient serum

At each of the neurological assessment time points, 4 mL of peripheral blood were drawn and serum was isolated immediately by centrifugation of the tube containing the clotted blood at 1,200 g. The serum was transferred into a new 1.5-mL tube and stored at -80°C until further analysis. The total BDNF level in the serum was quantified using the DuoSet ELISA Development System kit (DY278, R&D Systems; Minneapolis, MN, USA).

Clinical data

The demographic and clinical parameters obtained from the study participants included sex, age, comorbidities, and complete blood cell count.

Data analysis

Two-way ANOVA with multiple comparisons was used to compare BDNF levels at each time point as well as the patient clinical score at baseline in different genotype groups. To address inter-patients variations in CIPN development, we analyzed the maximum clinical score that each patient achieved during treatment. A logistic regression model was used to test the correlation between BDNF levels and clinical scores. All statistical analyses were performed using JMP statistical software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Patient demographics and BDNF genotype frequency

This study included 33 NHL and 12 MM patients, whose characteristics are detailed in Table 1. All NHL patients were treated with the R-CHOP protocol (comprising rituximab, cyclophosphamide, vincristine, doxorubicin, and prednisone), with 30 patients receiving 6 courses of therapy, 2 receiving 4 courses, and 1 receiving 3 courses. Eight MM patients were treated with VCD (bortezomib, cyclophosphamide, and dexamethasone), three received VD (bortezomib and dexamethasone), and one received VDT (bortezomib, thalidomide, and dexamethasone), with eight patients receiving five courses of therapy and four receiving three courses before they underwent autologous stem cell transplantation or continued with bortezomib-based treatment. The BDNF Val/Val genotype (Val-BDNF) was identified in 30 patients (66.7%), while the Val/Met and Met/Met genotypes (Met-BDNF) were detected in 14 (31.1%) and 1 (2.2%) patients, respectively. Nine of our patients (three with MM and six with NHL) were diagnosed with non-insulin-dependent diabetes mellitus, all of whom carried the Val-BDNF genotype ($p=0.0038$). There were no other significant differences in demographic parameters between the Val-BDNF and Met-BDNF carriers.

Low pretreatment serum BDNF protein levels are correlated with CIPN development

To establish a possible link between the serum BDNF level and CIPN severity, a linear regression analysis of the BDNF baseline level and the maximum TNSr, FACT-GOG-NTx,

and PHQ-9 scores was performed. Pretreatment serum BDNF levels were normally distributed and did not differ between NHL and MM [BDNF=10.24±6.07 ng/mL (mean±SD), range=0–22.8 ng/mL]. Moreover, the serum BDNF level only showed a nonsignificant trend of being lower in Val-BDNF patients (Supplementary Fig. 1 in the online-only Data Supplement). It was particularly notable that we found significant inverse correlations between the baseline BDNF level and the maximum values of the clinical scores for TNSr ($r=0.60$, $p=0.0001$), FACT-GOG-NTx ($r=0.61$, $p=0.0002$), and PHQ-9 ($r=0.45$, $p=0.01$) in both genotypes (Fig. 1). These data suggest that high pretreatment BDNF levels are protective against CIPN development and that BDNF serves as a biomarker for predicting the severity of CIPN. It was especially notable that no correlation was found between any of these scores and baseline platelet counts (Supplementary Fig. 2 in the online-only Data Supplement). Similar results were obtained when the same analysis was performed separately for MM and NHL patients (data not shown).

FACT-GOG-NTx and PHQ-9 scores are higher in Val-BDNF than Met-BDNF patients

The baseline and maximum TNSr scores did not differ significantly between Val-BDNF and Met-BDNF patients (Supplementary Fig. 3 in the online-only Data Supplement). The baseline FACT-GOG-NTx score was significantly higher in Val-BDNF patients ($2.87±2.55$) than in Met-BDNF patients ($1.00±1.29$, $p=0.017$), as was the maximum FACT-GOG-NTx scores ($8.03±4.55$ and $4.77±2.58$, respectively; $p=0.02$) (Fig. 2A). There was a trend for the baseline PHQ-9 score being higher in Val-BDNF patients ($4.60±4.97$) than in Met-BDNF patients ($1.92±2.13$, $p=0.07$), while the maximum PHQ-9 score was significantly higher in Val-BDNF patients ($7.93±5.32$) than in Met-BDNF patients ($3.31±2.32$, $p=0.004$) (Fig. 2B).

PHQ-9 and TNSr scores are correlated in Met-BDNF but not Val-BDNF patients

Given that BDNF plays a role in depression and that depression can influence CIPN, we analyzed the correlation between symptoms of depression and peripheral neuropathy among the different BDNF genotypes. A significant correlation was found between the maximum PHQ-9 and

Table 1. Demographic and clinical characteristics of the study participants ($n=45$)

	All patients	Val-BDNF	Met-BDNF	<i>p</i>
Age (years)±SD	58.86±12.41	60.50±13.51	55.60±9.42	0.21
Sex (male:female)	22:23	15:15	7:8	0.8
Diagnosis (NHL vs. MM)	33:12	22:8	11:4	1.0
Diabetes type II	9	9	0	0.003

BDNF: brain-derived neurotrophic factor, MM: multiple myeloma, NHL: non-Hodgkin lymphoma.

FACT-GOG-NTx scores in both the Val-BDNF patients ($r=0.57, p=0.001$) and Met-BDNF patients ($r=0.77, p=0.0008$) (Table 2). It was particularly notable that we found a significant correlation between the maximum PHQ-9 and TNSr

scores in Met-BDNF patients ($r=0.7, p<0.0034$) but not in Val-BDNF patients ($r=0.28, p=0.12$). These data suggest that the objective symptoms of peripheral neuropathy and depression are associated with Met-BDNF carriers.

DISCUSSION

The current study explored the roles of serum BDNF protein levels and the BDNF Val66Met SNP in NHL and MM patients who develop CIPN. We identified inverse correlations between the pretreatment serum BDNF level and the maximum TNSr, FACT-GOG-NTx, and PHQ-9 scores, which point to a high serum BDNF level being associated with lower

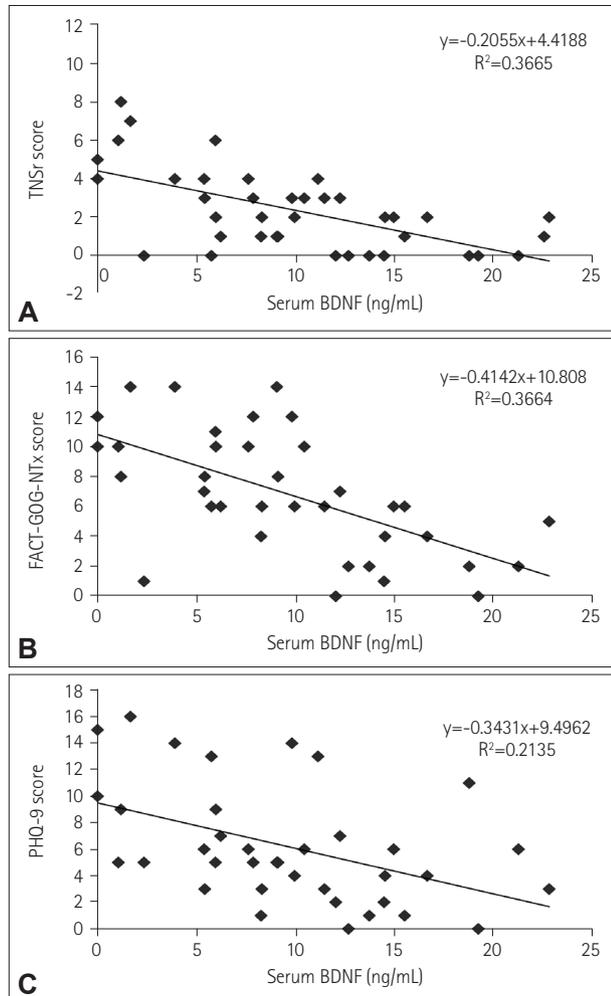


Fig. 1. Correlations between the pretreatment BDNF level and the objective symptoms (A), subjective symptoms (B), and depression (C) in CIPN patients. The graphs include linear regression lines for the relationships between pretreatment BDNF levels and the maximum TNSr, FACT-GOG-NTx, and PHQ-9 scores. BDNF: brain-derived neurotrophic factor, CIPN: chemotherapy-induced peripheral neuropathy, FACT-GOG-NTx: Functional Assessment of Cancer Therapy/Gynecologic Oncology Group-Neurotoxicity, PHQ-9: Patient Health Questionnaire-9, TNSr: reduced version of the Total Neuropathy Score.

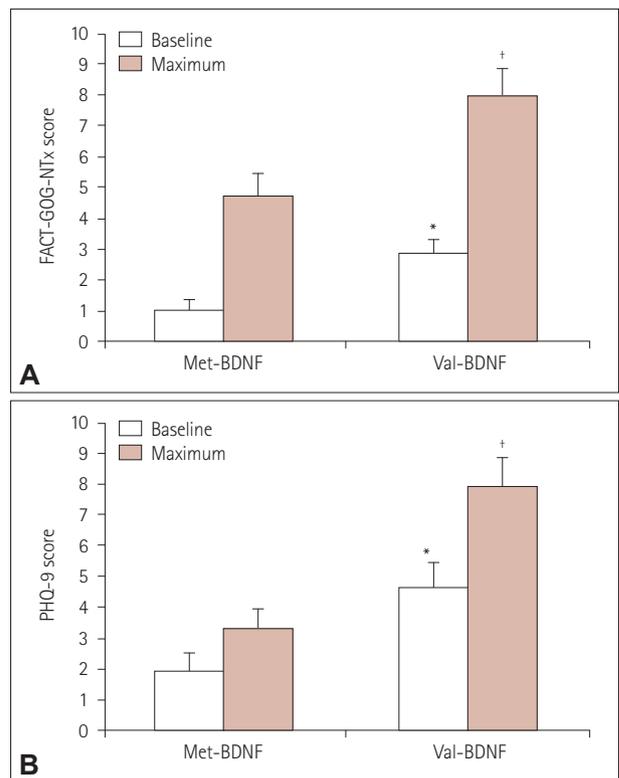


Fig. 2. Baseline and maximum FACT-GOG-NTx scores (A) and PHQ-9 scores in Val-BDNF and Met-BDNF patients (B). Data are mean and SEM values. * $p<0.05$, † $p<0.01$. BDNF: brain-derived neurotrophic factor, FACT-GOG-NTx: Functional Assessment of Cancer Therapy/Gynecologic Oncology Group-Neurotoxicity, PHQ-9: Patient Health Questionnaire-9.

Table 2. Correlation coefficient values (r) between PHQ-9 and FACT-GOG-NTx and TNSr in Val-BDNF and Met-BDNF patients

	All patients	Val-BDNF	Met-BDNF	p
PHQ-9 vs. FACT-GOG-NTx	0.6*	0.57 [†]	0.77 [†]	0.0001*, 0.001 [†] , 0.0008 [†]
PHQ-9 vs. TNSr	0.37*	0.28	0.7 [†]	0.01*, 0.003 [†]

*Correlation coefficient value (r) between PHQ-9 and FACT-GOG-NTx and TNSr in all patients, [†]Correlation coefficient value (r) between PHQ-9 and FACT-GOG-NTx and TNSr in Val-BDNF patients, [‡]Correlation coefficient value (r) between PHQ-9 and FACT-GOG-NTx and TNSr in Met-BDNF patients. BDNF: brain-derived neurotrophic factor, FACT-GOG-NTx: Functional Assessment of Cancer Therapy/Gynecologic Oncology Group-Neurotoxicity, PHQ-9: Patient Health Questionnaire-9, TNSr: reduced version of the Total Neuropathy Score.

risks of developing CIPN symptoms and depression. Remarkably, unlike serum BDNF levels, the baseline platelet count was not correlated with the maximum clinical scores that we investigated, despite it being well known that platelets are the main source of BDNF in the peripheral blood.^{10-12,22,23} This suggests that the intensity of CIPN and depression are related to the content of BDNF in platelets or other peripheral sources of BDNF, such as lymphocytes and monocytes, rather than to the number of platelets *per se*. The serum is a major source of BDNF, and the current findings further support the neuroprotective role of peripheral BDNF in CIPN. One plausible explanation is that patients with a larger baseline peripheral BDNF reservoir are more protected from CIPN than patients with a diminished BDNF pool.

The significantly higher maximum FACT-GOG-NTx and PHQ-9 scores observed in Val-BDNF patients suggest that in our cohort, depression and the perception of neuropathy symptoms were influenced by the BDNF genotype. The Met-BDNF allele has been previously shown to affect the cellular distribution and activity-related secretion of BDNF in neurons.^{4,24} Although this hypothesis needs further exploration, we suggest that Met-BDNF carriers could benefit from the favorable effects that result from reduced neuronal activity, which is potentially involved in depression and the perception of neuropathy symptoms. Ng et al.²⁵ found the Met-BDNF allele to be protective against chemotherapy-induced cognitive impairment which, despite differences in diseases, treatment, and Met-BDNF allele frequency from our study, may support our assumption.

While the role of BDNF SNPs as mediators of depression is well established,^{26,27} the correlation that we found between TNSr and PHQ-9 scores among the Met-BDNF carriers further supports the role of depression as a contributing factor in CIPN. This finding might indicate that the development of objective CIPN symptoms influences the development of depression in Met-BDNF but not Val-BDNF carriers. This interpretation also explains why there was no significant effect on the TNSr score in Val-BDNF patients that paralleled its effects on FACT-GOG-NTx and PHQ-9 scores.

In conclusion, higher baseline serum BDNF levels are related to the development of lower objective and subjective symptoms of CIPN in patients with NHL and MM. Objective CIPN symptoms and depression coincide in Met-BDNF patients; however, these patients seem to be more protected against the development of depression and the perception of CIPN symptoms. While confirmatory studies involving more patients with other neurotoxic agents are needed, we suggest that the BDNF level can serve as a biomarker for predicting the evolution of CIPN in NHL and MM patients.

Supplementary Materials

The online-only Data Supplement is available with this article at <https://doi.org/10.3988/jcn.2019.15.4.511>.

Author Contributions

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Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Acknowledgements

Supported by grant from the ICRF and from the Israeli ministry of galilee development.

We thank Mrs. Kamenetsky for editing the final manuscript and preparing the layout.

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