World Cancer Research Journal

WCRJ 2026; 13 : e2999
DOI: 10.32113/wcrj_20262_2999

CYTOKINES AS POTENTIAL BIOMARKERS FOR DETECTING CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

U. NATARAJAN[1,2], S. S. JAGANATHAN[1,2], G. WALDRON[1], A. RATHINAVELU[1,2]

• • •

[1]Rumbaugh-Goodwin Institute for Cancer Research, Nova Southeastern University, Ft. Lauderdale, FL, USA
[2]Barry & Judy Silverman College of Pharmacy, Health Professions Division, Nova Southeastern University, Ft. Lauderdale, FL, USA

Corresponding Author
Appu Rathinavelu, Ph.D; email: appu@nova.edu

ABSTRACT – *Objective:* Chemotherapy-induced peripheral neuropathy (CIPN) is a common and debilitating side effect of chemotherapeutic agents used in the treatment of cancer patients. Peripheral Neuropathy (PN) may arise due to the disease itself or as a consequence of treatment with chemotherapeutic agents, such as proteasome inhibitors, platinum-based compounds, and vinca alkaloids, which are widely known as CIPN. However, the underlying mechanisms of CIPN remain poorly understood. The objective of this study was to investigate the presence of CIPN in a rat model and identify potential biomarkers associated with the induction of neuronal damage and consequent neuropathy.

Materials and Methods: CIPN was induced in experimental rats by administering Bortezomib (BTZ), Cisplatin (CIS), and Vincristine (VIN). The onset and progression of CIPN in these rats were assessed by the Hot and Cold plate experiments. The ELISA method was used to measure the levels of neuronal damage-related biomarkers NSE, S100B, and inflammatory biomarkers IL-6, TNFα, and IL-10 using plasma and sciatic nerve, collected from the experimental rats.

Results: Our findings revealed significant alterations in body weight, as well as abnormal responses in the hot plate and cold plate tests, which are indicative of CIPN. Our results showed a significant increase in NSE, S100B, IL-6, and TNFα levels in the plasma of rats that showed symptoms of CIPN. However, the IL-10 levels were elevated in the sciatic nerve and plasma of the CIPN rats.

Conclusion: These findings suggest that NSE, S100B, IL-6, TNFα, and IL-10 could serve as biomarkers for detecting CIPN induced by chemotherapeutic agents. Further research is necessary to fully understand the roles of these biomarkers in the development and progression of CIPN.

KEYWORDS: NSE, S100B, IL-6, TNFα, IL-10, Chemotherapy drugs, Peripheral neuropathy.

INTRODUCTION

Cancer poses a significant threat to human health, with its incidence rising each year. Chemotherapy is one of the most important modalities for treating cancers, offering the potential to cure or prolong the survival of cancer patients. However, it is often accompanied by distressing side effects[1]. Chemotherapy-Induced Peripheral Neuropathy (CIPN) is one of the most common side effects and a challenging obstacle that arises from treatment with several classes of anti-cancer agents such as proteasome inhibitors, platinum-based antineoplastics, vinca alkaloids, and taxanes[2]. Statistically, CIPN occurs acutely in 65% to 96% of patients, with 40% to 93% experiencing chronic symptoms after oxaliplatin treatment. The incidence of CIPN following cisplatin use ranges from 12% to 85%, while paclitaxel is associated with the highest incidence, affecting 61% to 92% of patients. CIPN is highly prevalent, affecting up to 68% of patients within the first month of treatment, and approximately 30% will continue to experience persistent symptoms for more than six

months after chemotherapy. Furthermore, some studies confirmed that 47% of female cancer survivors reported CIPN symptoms six years after chemotherapy cessation[3-8]. Despite their efficacy in combating cancer, the neurotoxic effects of these drugs pose significant challenges in the clinical management of the disease. The incidence of CIPN is related to the duration of chemotherapy, with symptoms primarily observed in the first six months. Common symptoms include pain, numbness, tingling, and weakness in the extremities. The precise mechanisms underlying CIPN remain unclear, and current therapeutic approaches are largely aimed at relieving symptoms rather than alleviating the cause. Furthermore, existing treatments are often insufficient to effectively manage the underlying causes, hinder the treatment progress, and may lead to premature termination of chemotherapy. Given that the severity of CIPN is linked to the dosage of neurotoxic anticancer drugs, identifying sensitive and specific biomarkers could enable clinicians to adjust treatment protocols and reduce both the occurrence and severity of CIPN[4,5,9].

Despite their distinct mechanisms of action, the CIPN-inducing drugs share the common consequence of causing PN, highlighting the need for a comprehensive investigation into understanding the fundamental pathways. In general, chemotherapy-induced immunomodulatory effects trigger cytokine-driven neuroinflammation, which in turn plays a critical role in the pathogenesis of CIPN[10]. In particular, cytokines such as Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNFα), and Interleukin-10 (IL-10) are implicated in various neurological disorders. Neuroinflammation in neuropathic pain involves the infiltration of immune cells, activation of glial cells, and the production of inflammatory mediators in both the peripheral and central nervous systems[11,12]. Moreover, neuron-specific enolase (NSE), an intracytoplasmic enzyme, has been shown to increase in serum following neuronal damage and serves as a marker of neuronal dysfunction. Elevated serum NSE is a marker of neuronal dysfunction, and increased serum levels indicate neuron damage[13]. In parallel, S100B (S100B calcium-binding protein), predominantly expressed in astrocytes, serves as a marker of astroglial dysfunction. It is released following damage to glial cells, further emphasizing the role of glial cells in the inflammatory response[14]. These findings highlight the complexity of the inflammatory pathways involved in the onset and progression of CIPN, marking them as critical targets for further research aimed at developing effective treatments[15,16].

Preliminary studies indicated decreased intracellular levels of NSE and S100B, suggesting their involvement in neurodegenerative processes relevant to our *in vivo* model[17]. In continuation of our preliminary findings, the present study investigated the neuronal damage-specific biomarkers NSE and S100B to examine their roles in CIPN induced by chemotherapeutic agents, including Bortezomib (BTZ), Cisplatin (CIS), and Vincristine (VIN). Since there is limited understanding of how these biomarkers change during CIPN, our study also examined the relationship between CIPN and levels of inflammatory cytokines (IL-6, TNFα, and IL-10), alongside NSE and S100B, in experimental rats. Our findings provide compelling evidence that the levels of these biomarkers are significantly altered in the plasma of rats with CIPN symptoms, suggesting a potential role in CIPN pathophysiology.

MATERIALS AND METHODS

Animal Model

The study used 5-week-old male Sprague Dawley rats (Envigo, Tampa, FL, USA). Rats were housed in a standard rat cage and maintained in a 12 h light/12 h dark cycle, with 50% humidity and 20 ± 2°C temperature. The rats had free access to food and water. All procedures involving animals were in compliance with the Institutional Animal Care and Use Committee (IACUC) of NSU (Nova Southeastern University) and the guidelines of the US National Research Council[18]. The investigation and management of pain in animals followed guidelines outlined by the International Association for the Study of Pain (IASP). All animal experiments included in this study were conducted in accordance with institutional and national guidelines using the workflow shown in Figure 1A. The study was approved by the Institutional Animal Care and Use Committee of Nova Southeastern University, under protocol number 2020.03.08. AR3 (approved on 08/03/2020).

Drugs and Chemicals

The chemotherapeutic drugs bortezomib (BTZ), cisplatin (CIS), and vincristine (VIN) were obtained from Selleckchem (Houston, TX, USA). Enzyme-Linked Immunosorbent Assay (ELISA) kits for neuro biomarkers of NSE (NBP2-76684) were purchased from Novus Biologicals (Centennial, CO, USA). The S100B (LS-

F23898) assay kit was purchased from LSBio (Seattle, WA, USA), and the assay kits for IL-6 (ELR-IL-6), TNFα (ELR-TNFα), and IL-10 (ELR-IL-10) were purchased from Ray Biotech (Norcross, GA, USA).

Establishment of CIPN in Rats

Rats were divided into four groups of five animals each (n = 5). Following an acclimatization period, three groups received chemotherapeutic treatments – bortezomib (BTZ), cisplatin (CIS), and vincristine (VIN) – *via* intraperitoneal (i.p.) injection. BTZ was administered at 0.15 mg/kg (cumulative dose 4.2 mg/kg), CIS at 0.80 mg/kg (cumulative dose 22.4 mg/kg), and VIN at 0.2 mg/kg (cumulative dose 5.6 mg/kg), according to the schedule indicated in Table 1. The fourth group served as untreated controls.

Preliminary studies showed that higher drug doses caused mortality, while animals with obesity were resistant to CIPN induction. Administration of lower doses more frequently, every other day for eight weeks (Figure 1B), successfully induced CIPN, consistent with previous studies[19,20]. Following the treatment period, animals were maintained without further injections until week 18 (day 120). Blood and sciatic nerve samples were collected at this time for biomarker analysis (Figure 1B). Euthanasia was performed *via* CO_2 asphyxiation, followed by cardiac puncture for blood collection and sciatic nerve isolation.

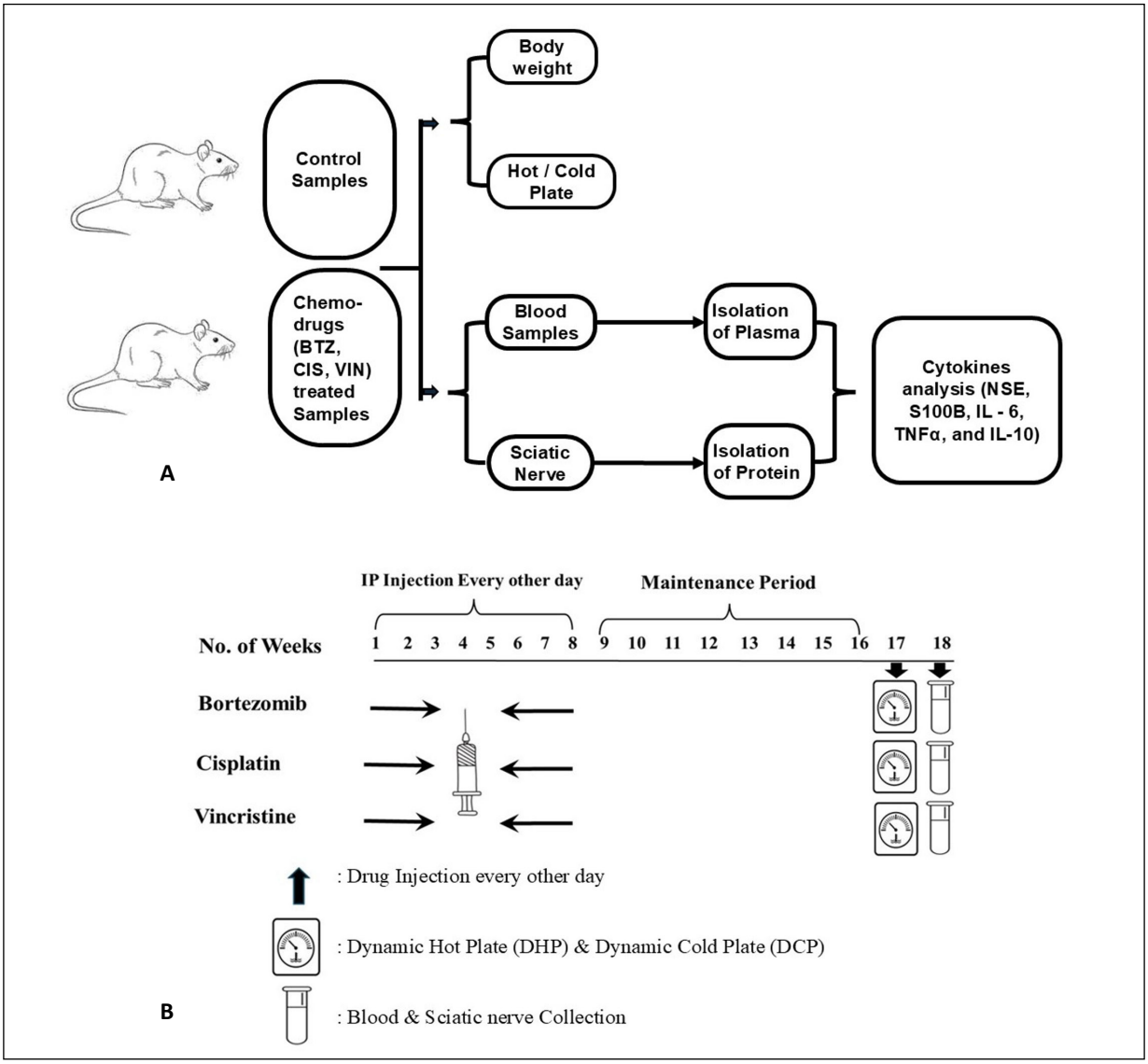

Figure 1. A, Schematic representation of the establishment of chemotherapy drug-induced peripheral neuropathy (CIPN) in rat models and subsequent evaluation of behavioral analysis. **B**, Schematic overview of the integrated experimental workflow.

Table 1. Dosage and timeline for drug administration and sample collection.

S. No.	Drug for CIPN Induction	Dosage for CIPN Induction	Intraperitoneal injection for CIPN induction (up to 8th week)	Blood, and Sciatic Nerve collection (On 18th Week)
1.	Bortezomib (BTZ)	0.15 mg/kg	Every other day	120th Day
2.	Cisplatin (CIS)	0.80 mg/kg	Every other day	120th Day
3.	Vincristine (VIN)	0.20 mg/kg	Every other day	120th Day

Dynamic Hot and Cold Plate Tests

The Dynamic Hot Plate (DHP) experiment was performed using a Hot and Cold Plate Analgesia Meter (Bioseb, Chaville, France), with a slight modification of the DHP procedures that were previously utilized[21]. Each animal was marked on its tail with the group numbers (e.g., 1-4) and the letters A-E. In the conventional dynamic hot plate experiment, unrestrained rats were placed on a metal surface maintained at 35 ± 0.1°C, and the plate temperature was increased to 55°C at 1°C/sec. Once the initial gradient reached 55°C, the temperature was maintained at that level. For each animal, the time to hind paw withdrawal, forepaw withdrawal, licking, jumping, and leaning posture from the start of the temperature gradient was recorded by the investigator. If neuropathic pain behaviors were not evident, the experimental animals were withdrawn from the dynamic hot plate experiment after a predetermined cutoff of 150 seconds. The cutoff was set to avoid paw damage, and care was taken to ensure that the maximum temperature and duration used did not induce tissue damage in the experimental animals. Additionally, the dynamic cold plate assessment is one of the simplest methods for evaluating cold-evoked behavioral responses to both noxious and harmless cold temperatures in experimental rats. Similar to the dynamic hot plate assessment, animals were placed on the pre-cooled plate at a specific temperature (typically 25°C) for a maximum observation period of 10-30 seconds to minimize the risk of tissue damage.

During the dynamic cold plate (DCP) experiment, the starting temperature was set to 25°C ± 0.1°C, and the plate temperature was gradually decreased to 1°C. The behaviors were determined as described with DHP measurements. Three measures of the paw withdrawal latency were taken and averaged for each hind paw. The DHP and DCP measurements were performed during the 17th week of the experiment over five consecutive days. For each day, DCP assessments were conducted after completing the DHP measurements for all animals across the four groups, with a minimum one-hour gap between the two tests to avoid carry-over effects. Behavioral responses were averaged across testing days to reduce variability and improve data reliability, enabling assessment of the chronic neuropathic phenotype.

Plasma Isolation

The blood was collected using EDTA tubes from the control and experimental animals by cardiac puncture under deep anesthesia with isoflurane[22,23]. The tubes were gently inverted to ensure proper mixing of the blood with the anticoagulant. The plasma samples were separated using centrifugation at 2,000 x g for 15 minutes at 4°C. The plasma layer was carefully separated using a pipette, ensuring the buffy coat was not disturbed, and transferred into sterile tubes for storage. Plasma samples were frozen at -20°C for long-term storage to preserve biomarker integrity. Repeated freeze-thaw cycles were avoided to maintain sample quality. Plasma samples from each animal were processed separately and analyzed individually for biomarker detection, and the results obtained will be averaged for statistical significance.

Protein Extraction

Sciatic nerves of the control and experimental rats were collected and immediately stored at -80°C until further processing. For protein extraction, the whole sciatic nerves from the left and right thighs of the rats were homogenized in 1X Lysis buffer (Ray Biotech, Norcross, GA, USA) supplemented with protease and phosphatase inhibitors to stabilize protein. The homogenates were then centrifuged at 14,000 rpm for 20 minutes at 4°C to remove debris, and the supernatants containing the total protein were collected. Protein concentrations were determined using the bicinchoninic acid (BCA) assay according to the manufacturer's protocol (Thermo Fisher Scientific, Pierce, Cat. No: 23227). The extracted proteins were subsequently stored at -80°C for further analysis.

Measurement of Neurospecific Markers and Cytokines

ELISA was used for measuring the levels of NSE, S100B, IL-6, TNFα, and IL-10, from the plasma of control animals and animals treated with BTZ, CIS, and VIN, following the manufacturer's protocol. The levels of IL-6, TNFα, and IL-10 were also assessed in the homogenates prepared from the sciatic nerve of rats treated with the chemotherapeutic drugs. Briefly, 100 µl of plasma or 100 µg of protein collected from the control and experimental rats were diluted in 100 µl of the assay buffer. The samples were added to the wells of ELISA plates coated with capture antibodies, and the plate was incubated for 3 h at 37°C. After removing the unbound samples and gently washing the plate, the microwells were incubated with a detection antibody followed by an HRP-conjugated secondary antibody. The color for the measurement was developed using a TMB (3,3',5,5'-tetramethylbenzidine) substrate. The absorbance was measured at 450 nm immediately after adding the stop solution, and the results were recorded with a microplate reader (Molecular Devices, San Jose, CA, USA).

Statistical Analysis

The data presented in this study represent the mean ± standard deviation (SD) of values from at least 5 experimental animals or independent experiments. The statistical significance between the groups shown in the graphs was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Pearson correlation analysis was performed to evaluate the relationships between hot threshold, cold threshold, and the biomarkers. A *p*-value of less than 0.05 was considered significant.

RESULTS

The Effect of Chemotherapy Drugs on the Body Weight of CIPN Model Rats

After treating the experimental animals for the periods indicated in Table 1, the drug-treated animals showed lower body weight than the control group, which showed normal weight gain during the treatment period. The average initial weight of the rats in all four groups was 96 ± 3 grams. There was a significant reduction in body weight in the chemotherapy-treated groups compared with the control group. Specifically, VIN- and BTZ-treated animals showed significant weight loss, weighing 315 ± 6 grams and 335 ± 3 grams, respectively. The CIS-treated rats weighed 333 ± 5 grams, compared with the control group, which weighed 466 ± 28 grams, as shown in Figures 2A and 2B. This corresponds to decreases of 28.09%, 28.41%, and 32.35% in body weight for the VIN-, BTZ-, and CIS-treated groups, respectively, compared to the control group. No significant difference in weight between the treated animals and the control ones was reported on day zero.

The Effect of Chemotherapy Drugs on the Hot and Cold Pain Threshold in Experimental Rats

The administration of chemotherapy drugs significantly elevated the withdrawal threshold time in the Dynamic Hot Plate (DHP) test for the CIS and BTZ groups by 110 and 104 seconds, respectively, compared to the 53-second threshold time of the control group, at which time the temperature reached 53°C. The CIS and BTZ rats were able to withstand the 55°C for an additional 55 and 51 seconds, respectively, compared to the control groups. However, the VIN-treated group showed only a slight increase in the withdrawal time of 3 seconds after the temperature reached 55°C (Figure 3). In the dynamic cold plate (DCP) test, we observed a significant reduction in cold allodynia thresholds in CIS-, BTZ-, and VIN-treated rats. Paw withdrawal occurred at 14, 15, and 16 seconds, corresponding to temperatures of 11°C, 10°C, and 9°C, respectively. In contrast, control rats withdrew their paws at 23 seconds, which was 3 seconds after the temperature had stabilized at 1°C. Notably, these withdrawal times were significantly shorter than those observed in the control group (Figure 3). Consequently, peripheral neuropathy induced by the chemotherapeutic agents BTZ, CIS, and VIN delayed sensory responses to hot stimuli and early withdrawal responses to cold stimuli. Thus, our DHP and DCP results provided sufficient evidence supporting the development of peripheral neuropathy following BTZ, CIS, and VIN treatment in the experimental rats.

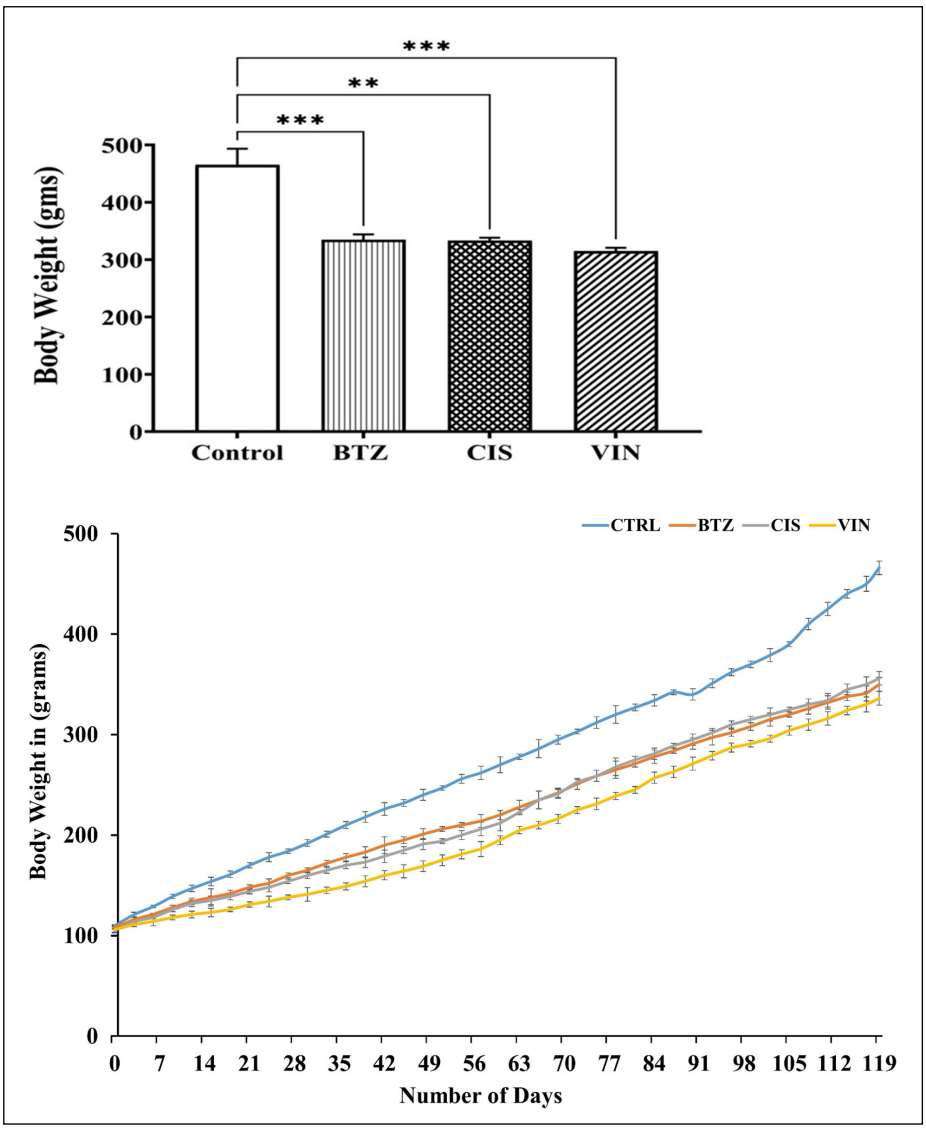

Figure 2. A, The effect of chemotherapy drugs on the body weight of CIPN model rats. Values are mean ± SD, of the groups (n = 5). The level of significance was indicated by *** = $p <$ 0.001, and ** = $p < 0.01$. **B**, Body weight changes over the course of the study. Body weight was measured twice weekly for all experimental groups. Measurements were taken consistently to monitor the effects of treatments on animal growth and health throughout the experimental period.

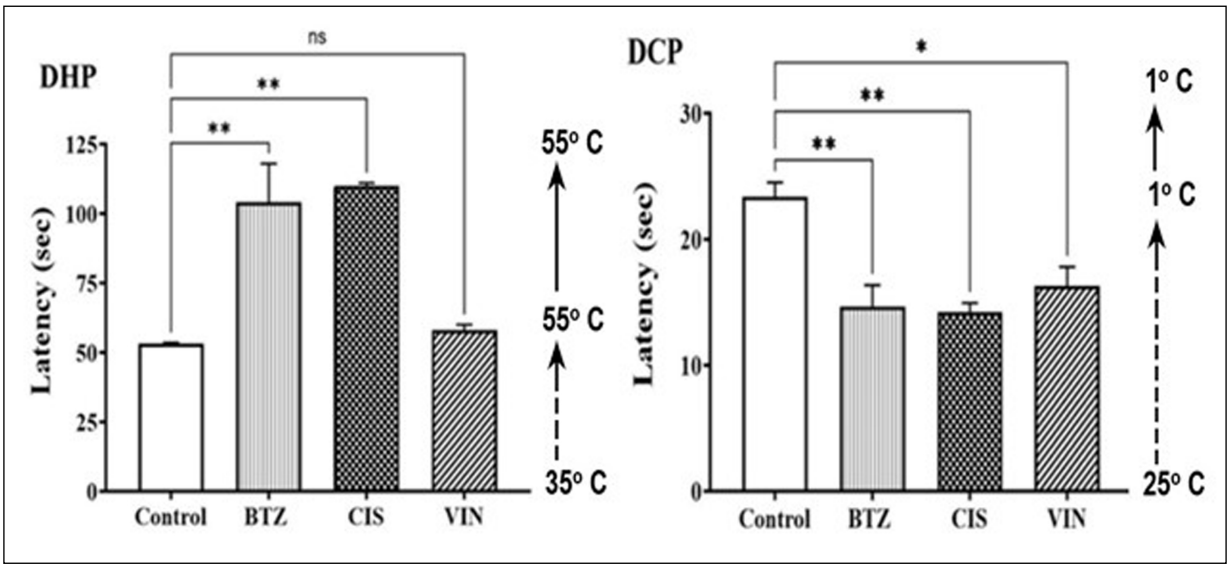

Figure 3. The effect of chemotherapy drugs on the hot withdrawal (left) and cold withdrawal (right) Threshold of the CIPN model rats. Values are mean ± SD of the groups (n = 5). The level of significance was indicated by **= $p < 0.01$, * = $p < 0.05$, and ns = non-significant.

The Effect of Chemotherapy Drugs on the Levels of NSE & S100B in the Plasma of the CIPN Experimental Rats

During our experiments, NSE and S100B were assessed as biomarkers for CIPN. The effects of chemotherapy drugs on plasma NSE & S100B levels in control and CIPN rats were analyzed at the 120th day. As shown in Figure 4, the plasma NSE level in rats treated with BTZ was approximately 101% higher than in the control. Meanwhile, the NSE levels in rats treated with CIS and VIN showed an increase of 58% and 50%, respectively, compared with the control. Similarly, analysis of S100B levels in the plasma of experimental rats revealed that VIN-treated animals showed a significant increase of nearly 105% compared with the control, while BTZ- and CIS-treated rats showed increases of 61% and 50%, respectively, compared with the control.

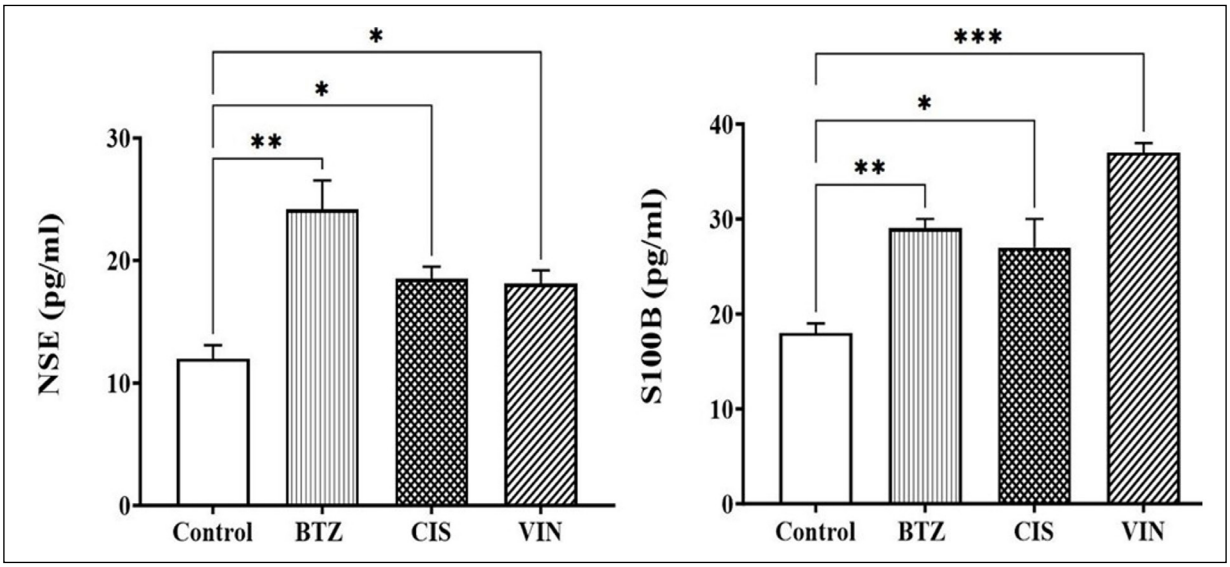

Figure 4. NSE (Neuron Specific Enolase) and S100B levels in the plasma of CIPN rats. Values are mean ± SD, of the groups (n = 5). The level of significance was indicated by *** = $p < 0.001$, ** = $p < 0.01$, and * = $p < 0.05$.

The Effect of Chemotherapy Drugs on the Levels of IL-6, TNFα, and IL-10 in the Plasma of CIPN Experimental Rats

Inflammatory cytokines, such as IL-6, TNFα, and IL-10, have been shown to contribute to neuropathic pain; therefore, receptor antagonists for these cytokines have been found to be effective in alleviating hyperalgesia in animal models of neuropathic pain[24]. In our study, the levels of both pro-inflammatory and anti-inflammatory cytokines were analyzed in the experimental rats that exhibited CIPN. Plasma IL-6 levels in rats treated with VIN were significantly increased by 110% compared with the control group. However, the BTZ-treated group showed only a mild 13% increase, while the CIS-treated group showed a 9% decrease relative to the control. Similarly, TNFα levels in the plasma of VIN-treated animals increased by 344%, while those of CIS- and BTZ-treated animals increased by 257% and 229%, respectively, compared to the control. The anti-inflammatory marker IL-10 was also notably elevated in the plasma of VIN-treated rats by 263% compared to the control. Similarly, CIS- and BTZ-treated rats also exhibited a significant elevation of plasma IL-10 by 154% and 133%, respectively, compared with the control (Figure 5).

The Effect of Chemotherapy Drugs on the Levels of IL-6, TNFα, and IL-10 in the Sciatic Nerve of CIPN Experimental Rats

When we analyzed the levels of cytokines in the whole homogenates of the sciatic nerve isolated from the experimental rats, a significant increase in IL-6 levels was observed in the sciatic nerve isolated from the rats treated with bortezomib (BTZ) and vincristine (VIN), with increases of 78% and 74%, respectively, followed by the cisplatin (CIS)-treated group, which showed a 47% increase compared to the control.

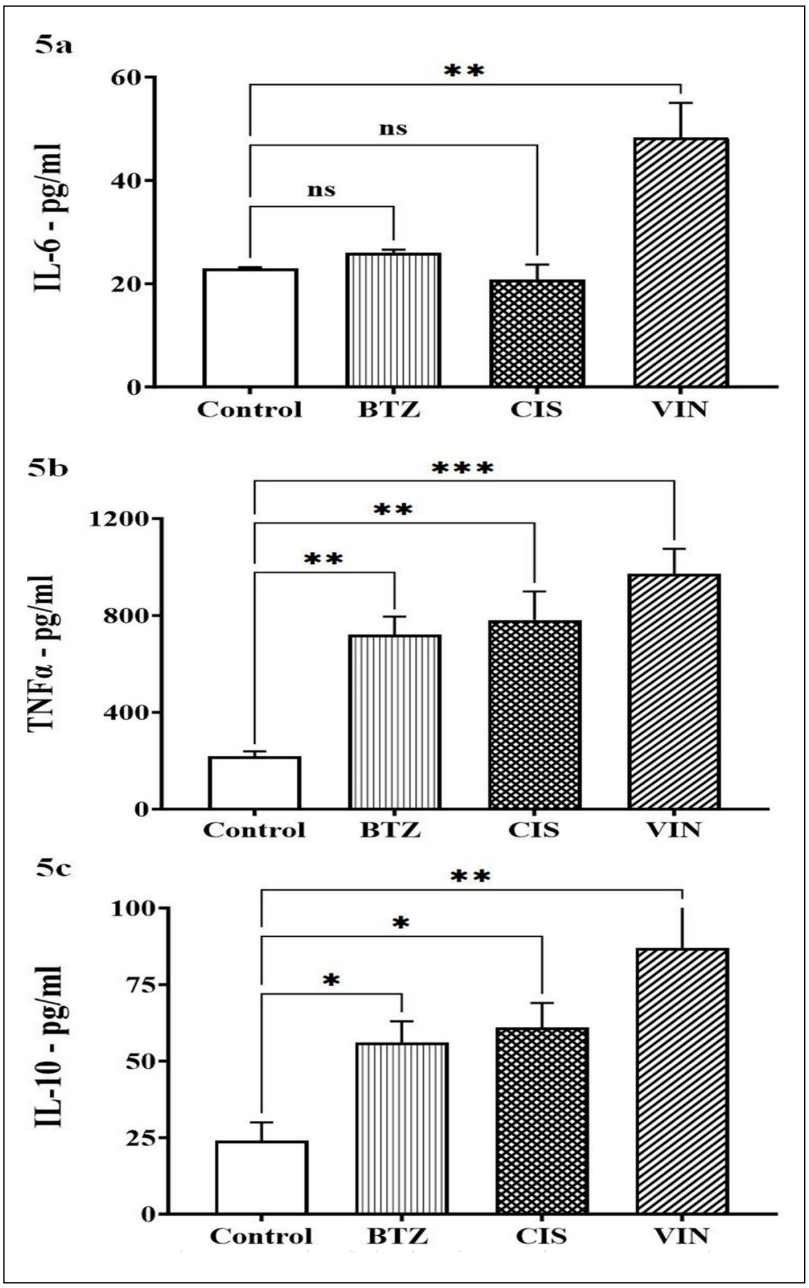

Figure 5. A, IL-6, (**B**) TNFα, and (**C**) IL-10 levels in the plasma of CIPN rats. Values are mean ± SD, of the groups (n = 5). The level of significance was indicated by *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, and ns = nonsignificant.

Additionally, TNFα levels in the sciatic nerve of BTZ-treated rats were elevated by 27%, whereas CIS- and VIN-treated rats showed only 19% and 4% increases, respectively, compared to the control. However, the anti-inflammatory marker IL-10 was significantly elevated in the sciatic nerve isolated from CIS-treated rats, showing a 10-fold increase, while VIN-treated rats showed a 9-fold increase compared to the control. Similarly, BTZ-treated rats demonstrated a 6-fold increase in IL-10 levels in the sciatic nerve compared to controls (Figure 6).

Heat Map of Inflammatory Cytokines and Neuron-Specific Biomarkers.

The heat map of the results shown in Figure 7 confirms that NSE, S100B, and the inflammatory cytokines IL-6, TNFα, and IL-10 were differentially expressed within the lower and higher limits of <3.5-fold (down-regulation indicated in green) and >3.5-fold (up-regulation indicated in red). The most significant elevation of IL-10 was observed in the sciatic nerve of the experimental groups in all three (BTZ, CIS, and VIN) treatment groups, as indicated with the dotted lines in the heatmap. However, in plasma, although the maximum fold change was observed in the VIN-treated groups, TNFα and IL-10 were elevated in all three treatment groups compared to the control.

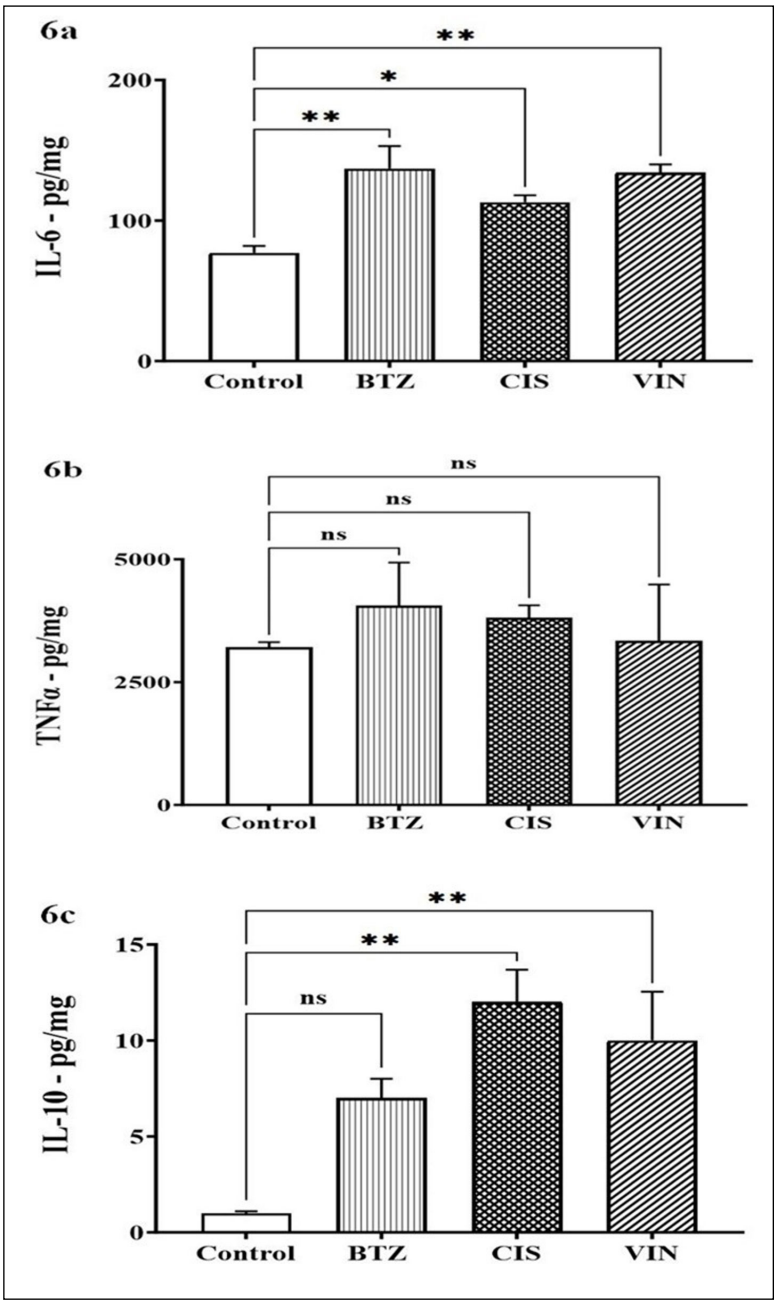

Figure 6. A, IL-6, (**B**) TNFα, and (**C**) IL-10 levels in sciatic nerve of CIPN rats. Values are mean ± SD, of the groups (n = 5). The level of significance was indicated by ** = $p < 0.01$, * = $p < 0.05$, and ns = non significant.

	Plasma					Sciatic Nerve		
	NSE	S100B	IL-6	TNFα	IL-10	IL-6	TNFα	IL-10
BTZ	1	0.7	0.2	1.7	1.2	0.8	0.3	2.6
CIS	0.6	0.6	-0.1	1.8	1.3	0.6	0.2	3.3
VIN	0.5	1	1.1	2.1	1.8	0.8	0.1	3.1

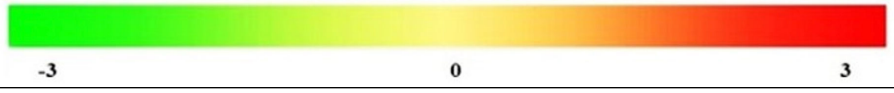

Figure 7. Heat map showing differentially expressed cytokines in plasma, and sciatic nerve, of CIPN rats compared to the controls. Blocks in red and green colors represent a high and low level of expressions, respectively.

Correlations Analysis of Biomarker Levels with the Hot Plate Threshold Changes

The data presented in Table 2 summarize the Pearson correlation coefficients (R values), *p*-values, and levels of statistical significance for biomarkers levels in plasma (NSE, S100B, IL-6, TNFα, and IL-10), and in sciatic nerve (IL-6, TNFα, and IL-10) across three treatment groups (CIS, BTZ, and VIN) compared to the increase in the hot plate threshold values of the experimental rats.

In BTZ-treated animals, biomarker levels showed significant correlations, with highly significant p-values for NSE, S100B, and TNFα ($p < 0.001$), and significant correlations for IL-6 and IL-10 ($p < 0.01$). Similarly, CIS-treated groups showed significant correlations for NSE ($p = 0.010$), S100B ($p < 0.001$), TNFα ($p = 0.002$), and IL-10 ($p < 0.001$), whereas IL-6 did not show a significant correlation ($p = 0.230$). In addition,

Table 2. This table shows the correlation analysis between the plasma and sciatic nerve biomarker changes with the hot plate threshold values. The table compares the following groups: control *vs.* BTZ, control *vs.* CIS, and control *vs.* VIN. The R-values indicate the strength of the correlation, and the *p*-values indicate the statistical significance. The level of significance was also indicated by *** = $p < 0.001$, * = $p < 0.05$, and ns = non-significant.

	Hot Plate Threshold / Biomarkers - Plasma				
	NSE	**S100B**	**IL-6**	**TNFα**	**IL-10**
Control *vs*. BTZ					
R-value	0.96	0.98	0.93	0.98	0.85
p-value	0.003	0.001	0.007	0.000	0.030
Significance	**	***	**	***	*
Control *vs*. CIS					
R-value	0.91	0.99	-0.57	0.97	0.98
p-value	0.010	0.000	0.230	0.002	0.000
Significance	*	***	ns	**	***
Control *vs*. VIN					
R-value	0.73	0.85	0.82	0.84	0.89
p-value	0.088	0.023	0.039	0.028	0.012
Significance	ns	*	*	*	*

	Hot Plate Threshold / Biomarkers - Sciatic Nerve		
	IL-6	**TNFα**	**IL-10**
Control *vs*. BTZ			
R-value	0.98	0.60	0.74
p-value	0.001	0.214	0.091
Significance	***	ns	ns
Control *vs*. CIS			
R-value	1.00	0.92	0.99
p-value	0.000	0.009	0.000
Significance	***	**	***
Control *vs*. VIN			
R-value	0.80	0.42	0.92
p-value	0.045	0.400	0.006
Significance	*	ns	**

the increase in hot plate threshold values in VIN-treated animals showed a moderate correlation with increases in biomarker levels, with significant p-values for S100B, IL-6, TNFα, and IL-10 ($p < 0.05$), whereas NSE was not statistically significant ($p = 0.088$). The results from the plasma in Table 2 show that the biomarker changes in the plasma of BTZ-treated animals showed the strongest overall correlations with the increase in the hot plate threshold due to CIPN.

When the changes in the biomarker levels of the sciatic nerve in BTZ-treated animals were analyzed, IL-6 showed a strong positive correlation ($R = 0.98$, $p = 0.001$), which was highly significant. However, TNFα ($R = 0.60$, $p = 0.214$) and IL-10 ($R = 0.74$, $p = 0.091$) did not demonstrate statistically significant correlations. Similarly, CIS-treated animals showed strong correlations for IL-6 ($R = 1.00$, $p < 0.001$), TNFα ($R = 0.92$, $p = 0.009$), and IL-10 ($R = 0.99$, $p < 0.001$), all highly significant ($p < 0.01$). However, the VIN-treated animals showed a moderate correlation for IL-6 ($R = 0.80$, $p = 0.045$) and IL-10 ($R = 0.92$, $p = 0.006$). Conversely, TNFα levels in VIN-treated animals did not show a significant correlation ($R = 0.42$, $p = 0.400$). The results in Table 2 show that among the biomarker changes observed in the sciatic nerve, CIS treatment showed the strongest overall correlations, and VIN-treated animals showed a moderately significant correlation with the increase in hot plate threshold values due to CIPN.

Inverse Correlations Analysis of Biomarker Changes with the Cold Plate Threshold Changes

The data presented in Table 3 summarize the Pearson correlation coefficients (R values), *p*-values, and levels of statistical significance for biomarker changes in plasma (NSE, S100B, IL-6, TNFα, and IL-10), and sciatic nerve (IL-6, TNFα, and IL-10) from across the three treatment groups (CIS, BTZ, and VIN) compared to the control using cold plate threshold values.

In BTZ-treated groups, changes in plasma biomarker levels showed an inverse correlation with the decrease in threshold temperature, with R-values ranging from −0.97 to −0.91. Significant correlations were observed for NSE ($R = -0.97$, $p = 0.001$), S100B ($R = -0.96$, $p = 0.002$), IL-6 ($R = -0.91$, $p = 0.012$), TNFα ($R = -0.97$, $p = 0.002$), and IL-10 ($R = -0.91$, $p = 0.011$). Similarly, CIS-treated animals showed a strong inverse correlation for changes in NSE, S100B, TNFα, and IL-10 (R-values ranging from -0.96 to -0.92), while IL-6 showed a weaker correlation (R = 0.61). The correlation for IL-6 ($p = 0.196$) was not significant (ns). The biomarker changes in VIN-treated animals also showed an inverse correlation for all biomarkers (R-values ranging from -0.98 to -0.77). Significant correlations were noted for S100B ($R = -0.95$, $p = 0.004$), IL-6 ($R = -0.98$, $p = 0.001$), TNFα ($R = -0.93$, $p = 0.007$), and IL-10 ($R = -0.94$, $p = 0.006$). The correlation for NSE ($p = 0.071$) was not significant (ns). These results confirmed a significant inverse correlation between most changes in plasma biomarker levels and the decrease in threshold temperatures, indicative of an increase in the threshold.

In line with the results observed during the hot plate experiments, the sciatic nerve samples obtained from BTZ-treated groups showed an inverse correlation with increases in all biomarkers (R-values ranging from -0.96 to -0.71). The most significant correlations were identified for IL-6 ($R = -0.96$, $p = 0.002$) and IL-10 ($R = -0.85$, $p = 0.031$). However, the correlation for TNFα ($R = -0.71$, $p = 0.117$) was not significant (ns). The changes in the sciatic nerve of CIS-treated groups also exhibited a strong inverse correlation for all biomarker changes (R-values ranging from -0.98 to -0.94). Significant correlations were identified for IL-6 ($R = -0.98$, $p = 0.001$), TNFα ($R = -0.94$, $p = 0.005$), and IL-10 ($R = -0.98$, $p = 0.000$). Interestingly, the changes in VIN-treated animals also showed an inverse correlation between IL-6 and IL-10, but no significant correlation with TNFα. Finally, results from the cold plate experiments substantiated the inverse correlation between IL-6 and the treatment-induced increase in the cold plate thresholds, particularly in the CIS and BTZ groups.

DISCUSSION

The aim of this study was to assess pre-selected plasma proteins in the CIPN animal model to identify the most relevant biomarkers. Nearly 80% of cancer patients receiving chemotherapy drugs are treated for CIPN[25]. As the number of cancer survivors increases, the necessity for treating this debilitating side effect has become one of the top priorities because of its impact on the treatment and patient outcomes. As discussed earlier, CIPN can compromise the quality of life of patients and sometimes force them to discontinue chemotherapy[26,27]. Despite the high prevalence and impact of CIPN, there is a lack of clear guidelines on its optimal management[28-30]. Although several drugs have been considered in

Table 3. This table represents the inverse correlation analysis between biomarkers from the plasma and sciatic nerve of rats with cold plate threshold values. The figure compares the following groups: control *vs.* BTZ, control *vs.* CIS, and control *vs.* VIN. The R-values indicate the strength of the inverse correlation, and the *p*-values assess statistical significance. The level of significance was indicated by *** = $p < 0.001$, * = $p < 0.05$, and ns = non-significant.

	Cold Plate Threshold / Biomarkers - Plasma				
	NSE	**S100B**	**IL-6**	**TNFα**	**IL-10**
Control *vs*. BTZ					
R-value	-0.97	-0.96	-0.91	-0.97	-0.91
p-value	0.001	0.002	0.012	0.002	0.011
Significance	***	**	*	**	*
Control *vs*. CIS					
R-value	-0.92	-0.96	0.61	-0.96	-0.95
p-value	0.009	0.002	0.196	0.003	0.004
Significance	*	***	ns	**	***
Control *vs*. VIN					
R-value	-0.77	-0.95	-0.98	-0.93	-0.94
p-value	0.071	0.004	0.001	0.007	0.006
Significance	ns	**	***	**	**

	Cold Plate Threshold / Biomarkers - Sciatic Nerve		
	IL-6	**TNFα**	**IL-10**
Control *vs*. BTZ			
R-value	-0.96	-0.71	-0.85
p-value	0.002	0.117	0.031
Significance	**	ns	*
Control *vs*. CIS			
R-value	-0.98	-0.94	-0.98
p-value	0.001	0.005	0.000
Significance	***	**	***
Control *vs*. VIN			
R-value	-0.96	0.04	-0.74
p-value	0.002	0.941	0.093
Significance	**	ns	ns

experimental research for preventing or treating CIPN, only a few have been used effectively in clinical practice[31]. Moreover, it has been widely accepted that detecting the onset of CIPN with specific biomarkers would offer the best chance of managing this side effect more successfully. Hence, our study was aimed to assess a panel of potential biomarker proteins, primarily in plasma. We also analyzed the same proteins obtained from sciatic nerve samples to identify the most relevant correlations with the plasma levels of these biomarkers in animals with CIPN. To confirm the induction and progression of CIPN, body weight and changes in the threshold for the DHP and DCP experiments were assessed. The induction of CIPN with BTZ, CIS, and VIN in Sprague-Dawley rats resulted in a significant reduction in body weight, which could be due to loss of muscle and body fat associated with sarcopenia and cachexia[32]. Another interesting observation during the onset of PN was the occurrence of swollen paws[33].

Interestingly, the swollen paws and the loss of muscle and fat mass were reported to lead to delayed response with temperatures (hot and cold), confirming slow neurotransmission rates due to the onset of damage to the myelin sheath[34]. The results from the present study confirmed that the administration of CIPN-inducing chemotherapy drugs significantly reduced the weight gain ability of the rats (Figure 2) and also changed the hot pain and cold pain threshold in CIPN-exhibiting rats (Figure 3).

In our search for the identification of CIPN related biomarkers, the initial selection of NSE and S100B for analysis was due to the fact that NSE is a critical enzyme required for energy metabolism in neurons. Also, NSE is one of the major soluble proteins in the neuron and is responsible for catalyzing the conversion of 2-phosphoglycerate to phosphoenolpyruvate. Furthermore, NSE was considered a marker for peripheral nervous system damage, particularly in disorders like peripheral neuropathies, which qualified it as a potential biomarker for monitoring CIPN. The NSE level has been reported to be highly useful in assessing the quantitative measures of brain damage, thereby improving the diagnosis and outcome evaluation in several pathophysiological conditions including ischemic stroke, intracerebral hemorrhage, seizures, and coma after cardiopulmonary resuscitation for cardiac arrest and traumatic brain injury[35]. Correlations between the size of cerebral infarctions and levels of NSE in the serum and CSF (cerebrospinal fluid) have been observed in animal models as well as in humans[36]. Interestingly, multiple myeloma (MM) patients treated with either thalidomide and dexamethasone combination or BTZ were previously reported to exhibit significant elevations in serum NSE levels[37]. Similarly, elevation of serum NSE was observed in non-small cell lung cancer patients undergoing treatment with CIS[38]. A similar elevation was observed in our experimental rats exhibiting CIPN after 56 days of treatment with BTZ, CIS, and VIN (Figure 4). The elevation of NSE was the highest in BTZ treatment groups compared to others. Furthermore, S100B was selected as another useful marker, which is also typically used to assess the brain damage caused by circulatory arrest, stroke, and traumatic brain injury. In addition, S100B has been reported to have prognostic use during the progression of chronic neurological diseases[39]. In the nervous system, S100B has been shown to be concentrated in astrocytes, oligodendrocytes, Schwann cells, ependymal cells, retinal Muller cells, and enteric glial cells[40]. Interestingly, patients with migraine were reported to have significantly higher serum levels of S100B and NSE, suggesting neurodegeneration as the possible cause of the elevation of these proteins in the serum[41]. Even though S100B has been reported to be elevated in the CSF after severe traumatic brain injury in infants and children, no reports are available so far regarding its elevation in CIPN[42]. However, our study showed a significant elevation of S100B in experimental rats that developed CIPN symptoms following BTZ, CIS, and VIN treatments (Figure 4). Also, S100B was highly correlated with the hot plate threshold values with strong statistical significance (Table 2). Thus, both NSE and S100B are released by neurons and peripheral neuroendocrine cells in response to various brain disorders and peripheral nerve damage. Accordingly, our observation also reveals a significant elevation of these biomarkers in the plasma of experimental rats. Based on our results seen in the experimental rats, it can be concluded that following the induction of CIPN by BTZ, CIS, and VIN treatments. Although significant elevations in NSE and S100B levels were detected in the plasma of all three treatment groups, the increase in S100B levels was more pronounced.

Though the onset and progression of CIPN are frequent with chemotherapy, the underlying mechanism is complex due to the interaction of multiple intersecting pathways. Several studies have shown that excessive inflammation observed in damaged neurons is one of the major factors responsible for the initiation and maintenance of CIPN[43]. Recent studies have confirmed that neuroinflammation certainly plays a key role in the pathogenesis and maintenance of CIPN[12,44-47]. Accordingly, during the onset and progression of CIPN, increased levels of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNFα, along with enhanced activation of CD4+ and CD8+ T cells, have been reported in the dorsal root ganglia[48]. The release of cytokines during the neuroinflammatory processes can occur in response to various stimuli, such as nerve injury, exposure to toxic compounds, or other pathological conditions that directly affect the nervous system's integrity. Also, cytokines can directly or indirectly act on primary afferent fibers, DRG, and spinal dorsal horn neurons, leading to pain sensitization. Therefore, increased cytokine expression can be expected during the onset of neuronal damage and associated neuropathic pain. The contributions of chemokines to platinum drug-related neurotoxicity have been demonstrated in several studies[49-52]. In addition, scholars strongly suggest that proinflammatory cytokines such as TNFα, IL-1β, and IL-6 play a crucial role in the nociceptive process of CIPN[16].22 In this regard, our study also demonstrates that the levels of TNFα, along with IL-6 and IL-10, are increased in the plasma of animals treated with BTZ, CIS, and VIN (Figure 5). This is similar to recent studies showing that significantly higher levels of TNFα, IL-1β, and IL-6 played an important role in the development of neuropathic pain, which was also shown to decrease the responsiveness of neurons to alleviate mechanical hyperalgesia in rats[53-55]. These observations are further substantiated by other studies in the literature that have

shown that chemotherapeutic agents such as paclitaxel, vincristine, and oxaliplatin can induce painful peripheral neuropathy by elevating pro-inflammatory cytokines such as IL-1β, IL-2, IL-4**,** and IL-6[56,57]. Also, the cytokines IL-6, TNFα, and IL-10 were found to be highly correlated with increased threshold values, with high statistical significance in the treated groups. On the other hand, IL-10 is considered an important anti-inflammatory modulator of glial activation, preventing inflammation-mediated neuronal degeneration under pathological conditions[58]. However, the elevation of IL-10 levels seen in the plasma of experimental rats exhibiting CIPN in our study could be due to a protective mechanism that was activated to limit neurodegenerative processes. In this regard, several recent experimental findings have shown that administering the anti-inflammatory marker IL-10 prevents mechanical allodynia induced by paclitaxel[59]. Following peripheral nerve injury, immune-modulating cells such as lymphocytes and macrophages contribute to the process by releasing pro-inflammatory cytokines that recruit additional immune cells to the site of injury. These cells then infiltrate through the blood-nerve barrier, exposing the injured tissue to a host of inflammatory mediators[60]. Increased levels of infiltrating macrophages were also observed after paclitaxel administration[61]. Our results indicate that VIN treatment increased the IL-6 levels non-significantly in the plasma; however, the elevation was significant in the sciatic nerve of rats exhibiting symptoms of CIPN. Peripheral IL-6 may be an important factor involved in the pathophysiological changes that cause pain and CIPN-related symptoms. This was evident when a neutralizing anti-IL-6 antibody was injected into the area surrounding the sciatic nerve, which suppressed the development of vincristine-induced mechanical allodynia[62]. Thus, though the elevation of IL-6 was significant in the sciatic nerve, the level of elevation was mild in the plasma of animals exhibiting symptoms of CIPN. Therefore, it appears that other cytokines may also be involved in the cascade of events leading to the development and progression of CIPN. Our current study demonstrates elevated levels of IL-6, IL-10, and TNFα, as well as increased S100B in the plasma of CIPN rats. This is similar to the observation in patients with atypical and idiopathic parkinsonism, where they observed a marked elevation of both pro- and anti-inflammatory cytokines in all groups with Parkinson's Disease (PD). The pro-inflammatory cytokines (i.e., IL-1β, TNFα, and INF-γ), which were measured in their study, were suspected to be involved in the induction of nitric oxide (NO), ROS (reactive oxygen species), or prostaglandin production[63]. Alternatively, it was suggested that the elevated levels of anti-inflammatory cytokines (i.e., IL-2, IL-4, or IL-10) in PD patients also provided neuroprotective function through scavenging free radicals and promoting trophic factors[64]. For example, a recent study investigated mRNA levels of proinflammatory cytokines, including IL-2 and TNFα, as well as anti-inflammatory cytokines IL-4 and IL-10, among patients with painful neuropathy, non-painful neuropathy, and control participants. Patients with painful neuropathy had a 2-fold increase in IL-2 and TNFα levels, while patients with non-painful neuropathy had significantly higher mRNA levels of IL-4 and IL-10[65,66]. Previous research has clearly indicated that IL-10 is essential for controlling neuroinflammation in CIPN. Accordingly, several studies have shown that endogenous IL-10 facilitates recovery from neuropathy induced by chemotherapeutic agents like paclitaxel[67-73]. The functional relevance of the observed biomarker changes is interesting and warrants further discussion. Upregulated markers, such as NSE, S100B, IL-6, and TNFα, reflect activation of pro-inflammatory pathways that have been strongly implicated in the initiation and persistence of neuropathic pain. Elevated levels can enhance neuronal excitability, promote peripheral sensitization, and contribute to structural damage within peripheral nerves. In contrast, the limited increase of anti-inflammatory mediator IL-10 suggests an insufficient systemic counterbalance to this pro-inflammatory drive. Interestingly, IL-10 upregulation in the sciatic nerve may represent a compensatory mechanism that mitigates inflammation and nerve injury. Together, these differential patterns highlight the complex interplay between pro- and anti-inflammatory signaling, suggesting that the balance between upregulated and downregulated biomarkers may be a critical determinant of CIPN severity and progression. To further elucidate the inflammatory profile associated with CIPN, we analyzed plasma and sciatic nerve cytokine expression patterns using heat map visualization. Plasma TNFα levels were modestly elevated in BTZ, CIS, and VIN groups (1.7, 1.8, and 2.1, respectively), reflecting a consistent pro-inflammatory response following chemotherapy. As a key mediator of neuroinflammation, TNFα has been implicated in the initiation and maintenance of neuropathic pain, underscoring its relevance to CIPN pathogenesis. In contrast, plasma IL-10 levels showed only minor increases in BTZ, CIS, and VIN groups (1.2, 1.3, and 1.8), suggesting a limited systemic anti-inflammatory response. However, as discussed above, the elevation of IL-10 levels, as indicated by the heatmap scores (BTZ: 2.6; CIS: 3.3; VIN: 3.1), is possibly due to a compensatory response developed in the experimental animals, primarily aimed at attenuating pro-inflammatory signaling. These trends highlight differential cytokine regulation at systemic *vs.* nerve-specific sites and underscore the importance of assessing localized immune modulation during CIPN development. As an anti-inflammatory cytokine, IL-10 is known to prevent neuroinflammation and reduce the

activation of pro-inflammatory pathways that contribute to neuronal damage. Therefore, the elevation of IL-10 seen in our experiments could reflect a protective response aimed at limiting tissue injury and promoting neuronal survival during the late stages of CIPN. These findings not only deepen our understanding of CIPN-related pathogenesis but also pave the way for validating relevant biomarkers. Thus, several recent studies have reported increased plasma levels of IL-6, TNFα, and IL-10, similar to what was observed in our experimental animals, which were confirmed to exhibit symptoms of CIPN[67-76].

Limitations

Despite the strengths of our study, certain limitations should be acknowledged. Though our experiments were well controlled by excluding the animals with no symptoms of CIPN from our study, a relatively small (n = 5) sample size may limit the statistical power of our analyses. However, the strong correlations observed (R > 0.95) in our experiments may be due to the elimination of the outliers. Therefore, further validation of these findings with a large sample size would validate the CIPN occurrence in a larger population of cancer patients.

CONCLUSIONS

In conclusion, our study induced CIPN in Sprague-Dawley rats by treating them with BTZ, CIS, and VIN every other day for 56 days. The experimental animals exhibiting symptoms of CIPN showed alterations in body weight, and changes in the pain thresholds, along with elevation of NSE and S100B levels in the plasma. Our results also suggest that changes in NSE, S100B, IL-6, and TNFα levels correlated well with the onset and progression of CIPN in the experimental rats, as evidenced by the strong Pearson Correlation Coefficient values. The elevation of IL-10 observed in CIPN rats may serve a neuroprotective function. To our knowledge, this study is the first of its kind to quantitatively assess a cluster of biomarkers in plasma that included assessment of NSE, S100B, IL-6, IL-10, and TNFα levels in rats exhibiting CIPN. Our study provides valuable insights into potential pathways and cytokines that are activated during CIPN induced by chemotherapy drugs, which can also pave the way for future clinical assessments and the development of strategies to alleviate CIPN-related complications. However, further studies with human subjects are needed to fully validate the changes in the biomarker levels observed in the CIPN experimental model.

Funding:
This work was supported by the President's Faculty Research and Development Grant (PFRDG) – (NSU Grant Number: 334818), and the authors would like to thank the Royal Dames of Cancer Research, Inc. (Ft. Lauderdale, FL, USA) for their financial support in conducting this research.

Authors' Contributions:
Umamaheswari Natarajan contributed to Conceptualization, Writing Original Draft, Methodology, Sample Collection, Data curation, Data validation, and Software analysis. Shyam Sundar Jaganathan contributed in Writing the Original Draft, Methodology, Data curation, Data validation, and Software analysis. Grace Waldron contributed to the Sample Collection. Appu Rathinavelu contributed in Conceptualization, Review & Editing Original Draft, Data Validation, Software analysis, and over all Supervision.

Conflict of Interest:
The authors declare that they have no conflict of interest to disclose.

Data Availability Statement:
The data used in this study are included within the manuscript and will be made available on request.

REFERENCES

1. Amjad MT, Chidharla A, Kasi A. Cancer chemotherapy. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan.
2. Ni W, Zheng X, Hu L, Kong C, Xu Q. Preventing oxaliplatin-induced neuropathic pain: Using berberine to inhibit the activation of NF-κB and release of pro-inflammatory cytokines in dorsal root ganglions in rats. Exp Ther Med 2021; 21: 135.
3. Sałat K. Chemotherapy-induced peripheral neuropathy-part 2: focus on the prevention of oxaliplatin-induced neurotoxicity. Pharmacol Rep 2020; 72: 508-527.
4. Boyette-Davis JA, Hou S, Abdi S, Dougherty PM. An updated understanding of the mechanisms involved in chemotherapy-induced neuropathy. Pain Manag. 2018; 8(5): 363-375.
5. Ibrahim EY, Ehrlich BE. Prevention of chemotherapy-induced peripheral neuropathy: A review of recent findings. Crit Rev Oncol Hematol 2020; 145: 102831.
6. Hiramoto S, Asano H, Miyamoto T, Takegami M, Kawabata A. Risk factors and pharmacotherapy for chemotherapy-induced peripheral neuropathy in paclitaxel-treated female cancer survivors: A retrospective study in Japan. PLoS One 2021; 16: e0261473.
7. Seretny M, Currie GL, Sena ES, Ramnarine S, Grant R, MacLeod MR, Colvin LA, Fallon M. Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: a systematic review and meta-analysis. Pain 2014; 155: 2461-2470.
8. Winters-Stone KM, Horak F, Jacobs PG, Trubowitz P, Dieckmann NF, Stoyles S, Faith S. Falls, functioning, and disability among women with persistent symptoms of chemotherapy-induced peripheral neuropathy. J Clin Oncol 2017; 35: 2604-2612.
9. Rodwin LR., Siddiq A, Ehrlich N, Lustberg M. Biomarkers of chemotherapy-induced peripheral neuropathy: Current evidence and clinical implications. Frontiers in Pain Research. 2022
10. Livni L, Lees JG, Barkl-Luke ME, Goldstein D, Moalem-Taylor G. Dorsal root ganglion explants derived from chemotherapy-treated mice have reduced neurite outgrowth in culture. Neurosci Lett 2019; 694: 14-19.
11. Miguel CA, Raggio MC, Villar MJ, Gonzalez SL, Coronel MF. Anti-allodynic and anti-inflammatory effects of 17α-hydroxyprogesterone caproate in oxaliplatin-induced peripheral neuropathy. J Peripher Nerv Syst. 2019; 24(1):100-110.
12. Fumagalli G, Monza L, Cavaletti G, Rigolio R, Meregalli C. Neuroinflammatory process involved in different preclinical models of chemotherapy-induced peripheral neuropathy. Frontiers in immunology, 2021. 11, p.626687.
13. Shaik AJ, Reddy K, Mohammed N, Tandra SR, Rukmini Mridula Kandadai, Baba Kss S. Neuron specific enolase as a marker of seizure related neuronal injury. Neurochem Int. 2019 Dec;131:104509. doi: 10.1016/j.neuint.2019.104509. Epub 2019 Aug 9. PMID: 31404559.
14. Di Cesare Mannelli L, Pacini A, Bonaccini L, Zanardelli M, Mello T, Ghelardini C. Morphologic features and glial activation in rat oxaliplatin-dependent neuropathic pain. J Pain (2013) 14:1585–600. 10.1016/j.jpain.2013.08.002 [DOI] [PubMed] [Google Scholar]
15. Haque A, Polcyn R, Matzelle D, Banik NL. New insights into the role of neuron-specific enolase in neuro-inflammation, neurodegeneration, and neuroprotection. Brain Sci 2018; 8: 33.
16. Lawrence JM, Schardien K, Wigdahl B, Nonnemacher MR. Roles of neuropathology-associated reactive astrocytes: a systematic review. Acta Neuropathol Commun 2023; 11: 42.
17. Priya V, Shriya S, Umamaheshwari N, Rathinavelu A. Abstract 4767: Changes in the NSE and S100B levels following treatment with peripheral neuropathy-inducing drugs. Cancer Res 2024; 84: 4767.
18. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the care and use of laboratory animals. 8th ed. Washington (DC): National Academies Press (US); 2011.
19. Duggett NA, Flatters SJL. Characterization of a rat model of bortezomib-induced painful neuropathy. Br J Pharmacol 2017; 174: 4812-4825.
20. Li Y, Adamek P, Zhang H, Tatsui CE, Rhines LD, Mrozkova P, et al. The cancer chemotherapeutic paclitaxel increases human and rodent sensory neuron responses to TRPV1 by activation of TLR4. J Neurosci 2015; 35: 13487-13500.
21. Yalcin I, Charlet A, Freund-Mercier MJ, Barrot M, Poisbeau P. Differentiating thermal allodynia and hyperalgesia using dynamic hot and cold plate in rodents. J Pain 2009; 10: 767-773.
22. Beeton C, Garcia A, Chandy KG. Drawing blood from rats through the saphenous vein and by cardiac puncture. J Vis Exp 2007; 7: 266.
23. Upton RA. Simple and reliable method for serial sampling of blood from rats. J Pharm Sci 1975; 64: 112-114.
24. Hung AL, Lim M, Doshi TL. Targeting cytokines for treatment of neuropathic pain. Scand J Pain 2017; 17: 287-293.
25. Sisignano M, Baron R, Scholich K, Geisslinger G. Mechanism-based treatment for chemotherapy-induced peripheral neuropathic pain. Nat Rev Neurol 2014; 10: 694-707.
26. Mora E, Smith EM, Donohoe C, Hertz DL. Vincristine-induced peripheral neuropathy in pediatric cancer patients. Am J Cancer Res 2016; 6: 2416-2430.
27. Alberti P, Salvalaggio A, Argyriou AA, Bruna J, Visentin A, Cavaletti G, Briani C. Neurological complications of conventional and novel anticancer treatments. Cancers 2022; 14: 6088.
28. Kim EY, Hong SJ. Real-life experiences of chemotherapy-induced peripheral neuropathy in patients with cancer: a qualitative meta-synthesis study. Semin Oncol Nurs 2023; 39: 151499.
29. Gewandter JS, Mohile SG, Heckler CE, Ryan JL, Kirshner JJ, Flynn PJ, Hopkins JO, Morrow GR. A phase III randomized, placebo-controlled study of topical amitriptyline and ketamine for chemotherapy-induced peripheral neuropathy (CIPN): a University of Rochester CCOP study of 462 cancer survivors. Support Care Cancer 2014; 22: 1807-1814.
30. Hammack JE, Michalak JC, Loprinzi CL, et al. Phase III evaluation of nortriptyline for alleviation of symptoms of cis-platinum-induced peripheral neuropathy. Pain 2002; 98: 195-203.
31. Majithia N, Loprinzi CL, Smith TJ. New practical approaches to chemotherapy-induced neuropathic pain: Prevention, assessment, and treatment. Oncology (Williston Park) 2016; 30: 1020-1029.
32. Kang J, Lee SH, Son JH, Lee JW, Choi YH, Choi JH, Paik WH, Ryu JK, Kim YT. Body mass index and weight change during initial period of chemotherapy affect survival outcome in advanced biliary tract cancer patients. PLoS One 2018; 13: e0195118.
33. Izenberg A, Perkins BA, Bril V. Diabetic neuropathies. Semin Neurol 2015; 35: 424-430.

34. Halliday AM, McDonald WI. Pathophysiology of demyelinating disease. Br Med Bull 1977; 33: 21-27.
35. Wunderlich MT, Lins H, Skalej M, Wallesch CW, Goertler M. Neuron-specific enolase and tau protein as neurobiochemical markers of neuronal damage are related to early clinical course and long-term outcome in acute ischemic stroke. Clin Neurol Neurosurg 2006; 108: 558-563.
36. Sankar R, Shin DH, Wasterlain CG. Serum neuron-specific enolase is a marker for neuronal damage following status epilepticus in the rat. Epilepsy Res 1997; 27: 129-136.
37. Yang H, Mi R, Wang Q, Wei X, Yin Q, Chen L, Zhu X, Song Y. Expression of neuron-specific enolase in multiple myeloma and implications for clinical diagnosis and treatment. PLoS One 2014; 9: e94304.
38. Andoh M, Gemma A, Takenaka K, Yamada K, Usuki J, Hasegawa K, Sakonji M, Kudoh S, Tsuboi E, Niitani H. Serum neuron specific enolase level as a prognostic factor in non-small cell lung cancer. Intern Med 1994; 33: 271-276.
39. Hajduková L, Sobek O, Prchalová D, Bílková Z, Koudelková M, Lukášková J, Matuchová I. Biomarkers of brain damage: S100B and NSE concentrations in cerebrospinal fluid—a normative study. Biomed Res Int 2015; 2015: 379071.
40. Michetti F, D'Ambrosi N, Toesca A, Puglisi MA, Serrano A, Marchese E, Corvino V, Geloso MC. The S100B story: from biomarker to active factor in neural injury. J Neurochem 2019; 148: 168-187.
41. Yilmaz N, Karaali K, Ozdem S, Turkay M, Unal A, Dora B. Elevated S100B and neuron specific enolase levels in patients with migraine-without aura: evidence for neurodegeneration? Cell Mol Neurobiol 2011; 31: 579-585.
42. Berger RP, Pierce MC, Wisniewski SR, Adelson PD, Clark RS, Ruppel RA, Kochanek PM. Neuron-specific enolase and S100B in cerebrospinal fluid after severe traumatic brain injury in infants and children. Pediatrics 2002; 109: e31.
43. Makker PG, Duffy SS, Lees JG, Perera CJ, Tonkin RS, Butovsky O, Park SB, Goldstein D, Moalem-Taylor G. Characterisation of immune and neuroinflammatory changes associated with chemotherapy-induced peripheral neuropathy. PLoS One 2017; 12: e0170814.
44. Brandolini L, D'Angelo M, Antonosante A, Allegretti M, Cimini A. Chemokine Signaling in Chemotherapy-Induced Neuropathic Pain. Int J Mol Sci. 2019; 20.
45. Starobova H, Mueller A, Deuis JR, Carter DA, Vetter I. Inflammatory and Neuropathic Gene Expression Signatures of Chemotherapy-Induced Neuropathy Induced by Vincristine, Cisplatin, and Oxaliplatin in C57BL/6J Mice. J Pain. 2020; 21:182–94.
46. Duan Z, Su Z, Wang H, Pang X. Involvement of pro-inflammation signal pathway in inhibitory effects of rapamycin on oxaliplatin-induced neuropathic pain. Mol Pain. 2018;
47. Ian Kleckner, Todd Jusko, Eva Culakova, Amber Kleckner, Matthew Asare, Julia Ellen Inglis, Kah Poh Loh, Luke Joseph Peppone, Jeffrey L. Berenberg, Alison Katherine Conlin, Deborah J. Ossip, and Karen Michelle Mustian. Inflammatory and clinical risk factors for chemotherapy-induced peripheral neuropathy (CIPN): A nationwide longitudinal study in 143 cancer patients during chemotherapy. J Clin Oncol, 2020; 38, 12099-12099.
48. Tay N, Laakso EL, Schweitzer D, Endersby R, Vetter I, Starobova H. Chemotherapy-induced peripheral neuropathy in children and adolescent cancer patients. Front Mol Biosci, 2022; 9: 1015746.
49. Ta LE, Espeset L, Podratz J, Windebank AJ. Neurotoxicity of oxaliplatin and cisplatin for dorsal root ganglion neurons correlates with platinum-DNA binding. Neurotoxicology. 2006; 27(6):992–1002.
50. Gornstein E, Schwarz TL. The paradox of paclitaxel neurotoxicity: Mechanisms and unanswered questions. Neuropharmacology. 2014; 76 ,175–83.
51. Zhirui Tao, Zhiqin Chen, Xiaochen Zeng, Jiujie Cui, Ming Quan, An emerging aspect of cancer neuroscience: A literature review on chemotherapy-induced peripheral neuropathy, Cancer Letters, Volume 611, 2025; 217433.
52. Makker PG, Duffy SS, Lees JG, Perera CJ, Tonkin RS, Butovsky O, Park SB, Goldstein D, Moalem-Taylor G. Characterization of immune and neuroinflammatory changes associated with chemotherapy-induced peripheral neuropathy. PloS one. 2017;12(1).
53. Ramesh G, MacLean AG, Philipp MT. Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. Mediators Inflamm 2013; 2013: 480739.
54. Liang F, Liu M, Fu X, Zhou X, Chen P, Han F. Dexmedetomidine attenuates neuropathic pain in chronic constriction injury by suppressing NR2B, NF-κB, and iNOS activation. Saudi Pharm J 2017; 25: 649-654.
55. Huh Y, Ji RR, Chen G. Neuroinflammation, bone marrow stem cells, and chronic pain. Front Immunol 2017; 8: 1014.
56. Yoon SY, Oh J. Neuropathic cancer pain: prevalence, pathophysiology, and management. Korean J Intern Med 2018; 33: 1058-1069.
57. Carozzi VA, Canta A, Chiorazzi A. Chemotherapy-induced peripheral neuropathy: What do we know about mechanisms? Neurosci Lett 2015; 596: 90-107.
58. Porro C, Cianciulli A, Panaro MA. The regulatory role of IL-10 in neurodegenerative diseases. Biomolecules 2020; 10: 1017.
59. Kanat O, Ertas H, Caner B. Platinum-induced neurotoxicity: a review of possible mechanisms. World J Clin Oncol 2017; 8: 329-335.
60. Ma W, Quirion R. Up-regulation of interleukin-6 induced by prostaglandin E from invading macrophages following nerve injury: an in vivo and in vitro study. J Neurochem 2005; 93: 664-673.
61. Peters CM, Jimenez-Andrade JM, Jonas BM, Sevcik MA, Koewler NJ, Ghilardi JR, Wong GY, Mantyh PW. Intravenous paclitaxel administration in the rat induces a peripheral sensory neuropathy characterized by macrophage infiltration and injury to sensory neurons and their supporting cells. Exp Neurol 2007; 203: 42-54.
62. Starkweather A. Increased interleukin-6 activity associated with painful chemotherapy-induced peripheral neuropathy in women after breast cancer treatment. Nurs Res Pract 2010; 281531.
63. 63.Brodacki B, Staszewski J, Toczyłowska B, Kozłowska E, Drela N, Chalimoniuk M, Stepien A. Serum interleukin (IL-2, IL-10, IL-6, IL-4), TNFalpha, and INFgamma concentrations are elevated in patients with atypical and idiopathic parkinsonism. Neurosci Lett 2008; 441: 158-162.
64. Vila M, Jackson-Lewis V, Guegan C, Wu D, Teismann P, Choi DK, Tieu K, Przedborski S. The role of glial cells in Parkinson's disease. Curr Opin Neurol 2001; 14: 483-489.
65. Uceyler N, Rogausch JP, Toyka KV, Sommer C. Differential expression of cytokines in painful and painless neuropathies. Neurology 2007; 69: 42-49.
66. Al-Mazidi S, Alotaibi M, Nedjadi T, Chaudhary A, Alzoghaibi M, Djouhri L. Blocking of cytokines signalling attenuates evoked and spontaneous neuropathic pain behaviours in the paclitaxel rat model of chemotherapy-induced neuropathy. Eur J Pain 2018; 22: 810-821.

67. Abel M, Sene D, Pol S, Bourliere M, Poynard T, Charlotte F, Cacoub P, Caillat-Zucman S. Intrahepatic virus-specific IL-10-producing CD8 T cells prevent liver damage during chronic hepatitis C virus infection. Hepatology. 2006; 44:1607–1616.
68. Sankaranarayanan I, Tavares-Ferreira D, Mwirigi J.M. Inducible co-stimulatory molecule (ICOS) alleviates paclitaxel-induced neuropathic pain via an IL-10-mediated mechanism in female mice. J Neuroinflammation 2023; 20, 32.
69. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. Nat Rev Immunol. 2010; 10:170–181.
70. Gabrysova L, Howes A, Saraiva M, O'Garra A. The regulation of IL-10 expression. Curr Top Microbiol Immunol. 2014; 380:157–190.
71. Kashi VP, Ortega SB, Karandikar NJ. Neuroantigen-specific autoregulatory CD8+ T cells inhibit autoimmune demyelination through modulation of dendritic cell function. PLoS One. 2014; 9:e105763.
72. 72.Krukowski K, Eijkelkamp N, Laumet G, Hack CE, Li Y, Dougherty PM, Heijnen CJ, Kavelaars A. CD8+ T cells and endogenous IL-10 are required for resolution of chemotherapy-induced neuropathic pain. J Neurosci 2016; 36: 11074-11083.
73. Ledeboer A, Jekich BM, Sloane EM, Mahoney JH, Langer SJ, Milligan ED, Martin D, Maier SF, Johnson KW, Leinwand LA, Chavez RA, Watkins LR. Intrathecal interleukin-10 gene therapy attenuates paclitaxel-induced mechanical allodynia and proinflammatory cytokine expression in dorsal root ganglia in rats. Brain Behav Immun. 2007; 21:686–698.
74. Wang XM, Lehky TJ, Brell JM, Dorsey SG. Discovering cytokines as targets for chemotherapy-induced painful peripheral neuropathy. Cytokine. 2012; 59(1):3-9.
75. Tarasiuk O, Chiorazzi A, Argentini A. Interleukin-6 as a keystone cytokine in experimental rat models of chemotherapy-induced peripheral neurotoxicity. Sci Rep. 2026;
76. García-Domínguez, M. The Role of TNF-α in Neuropathic Pain: An Immunotherapeutic Perspective. Life 2025; 15, 785.