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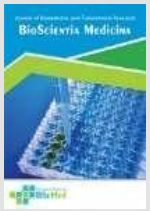

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Chemotherapy-Induced Cognitive Impairment and Neuroaxonal Damage: Investigating the Role of Serum Neurofilament Light Chain

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A B S T R A C T

Background: Chemotherapy-induced cognitive impairment (CICI), colloquially termed "chemobrain," represents a significant challenge for cancer survivors, potentially affecting up to 85% of patients undergoing treatment. Diagnosis often relies on neuropsychological testing and imaging, which may lack sensitivity for early detection or reflect chronic changes. Neurofilament light chain (NfL), a neuronal structural protein released into biofluids upon neuroaxonal damage, emerges as a promising biomarker. This study investigated the relationship between serum NfL levels and the degree of cognitive impairment in patients receiving chemotherapy. **Methods:** An observational, cross-sectional study was conducted involving 50 cancer patients undergoing chemotherapy at Dr. M. Djamil General Hospital Padang between October and December 2024. Cognitive function was assessed using the Montreal Cognitive Assessment Indonesian version (MoCA-Ina), and depression was screened using the Patient Health Questionnaire-9 (PHQ-9). Serum NfL levels were quantified using an Enzyme-Linked Immunosorbent Assay (ELISA) method. The Kruskal-Wallis test was employed to analyze the relationship between serum NfL levels and cognitive function status (normal, mild impairment, moderate-severe impairment). **Results:** Cognitive impairment (MoCA-Ina assessed) was identified in 41 (82%) of the 50 participants, with 30 (60%) exhibiting mild and 11 (22%) exhibiting moderate to severe impairment. The median serum NfL level across all subjects was 23.44 pg/ml (range: 13.81-68.71 pg/ml). A statistically significant relationship was observed between serum NfL levels and the presence and severity of cognitive impairment ($p = 0.02$). Median NfL levels progressively increased from the cognitively normal group (18.49 pg/ml) to the mild impairment group (23.5 pg/ml) and the moderate-severe impairment group (24.5 pg/ml). Post-hoc analysis revealed significant differences in NfL levels between the normal group and both the mild ($p=0.03$) and moderate-severe ($p=0.01$) impairment groups. **Conclusion:** This study demonstrated a significant positive association between serum NfL levels and the presence and severity of cognitive impairment in cancer patients undergoing chemotherapy. These findings support the potential utility of serum NfL as an accessible biomarker for detecting chemotherapy-associated neuroaxonal damage and concomitant cognitive decline.

1. Introduction

Cancer poses a persistent and significant threat to global health, accounting for nearly 10 million deaths worldwide in 2020 alone. However, advancements in early detection and treatment strategies have led to improved survival rates, resulting in an increasing population of cancer survivors. This positive shift in survival, however, is frequently accompanied by long-term consequences stemming from cancer treatments. Chemotherapy, a fundamental component in the treatment of various malignancies, is often associated with a range of adverse effects, notably a cluster of

cognitive deficits known as chemotherapy-induced cognitive impairment (CICI), commonly referred to as "chemobrain". CICI presents with a range of cognitive difficulties, primarily affecting attention, memory recall, information processing speed, and executive functions. These deficits can have a profound impact on patients' quality of life, their ability to perform daily activities, and their capacity to return to work. The prevalence of CICI is a significant concern, with estimates suggesting that up to 85% of patients undergoing chemotherapy may experience some degree of cognitive impairment. This wide range underscores the variability in CICI presentation and the challenges in its accurate assessment. The precise mechanisms underlying CICI are complex and not fully understood. However, it is believed to involve a combination of factors, including the direct neurotoxic effects of chemotherapeutic agents, neuroinflammation, dysregulation of cytokines, disruption of the blood-brain barrier (BBB), oxidative stress leading to DNA damage, impaired neurogenesis, and glial cell dysfunction. The heterogeneity of these contributing factors likely contributes to the variability in CICI severity and presentation across patients. A variety of chemotherapeutic agents have been implicated in the development of CICI. These include platinum compounds, antimetabolites (such as 5-FU), anthracyclines (such as doxorubicin), and taxanes. These agents may exert their neurotoxic effects by crossing the BBB, thereby directly affecting the central nervous system, or by inducing systemic changes that indirectly impact brain function and structure. The specific mechanisms of action of these drugs vary, contributing to the complexity of CICI pathophysiology.[1-4]

The diagnosis of CICI remains a challenge, as there are currently no standardized guidelines. Clinical assessment typically relies on patient self-reports, often gathered through questionnaires, and formal neuropsychological testing. Patient self-reports can provide valuable insights into the subjective experience of cognitive difficulties, but they may be influenced by factors such as recall bias and individual variations in symptom perception. Neuropsychological tests offer more objective measures of cognitive function. These tests assess various cognitive domains, including memory, attention, and executive function. However, neuropsychological testing can be time-consuming and costly, limiting its widespread use in clinical settings. Additionally, the results of these tests can be influenced by factors such as the patient's education level, fatigue, and emotional state, including depression. Furthermore, neuropsychological tests may lack sensitivity for detecting early or subtle cognitive changes and may primarily reflect more chronic or established cognitive deficits. Neuroimaging techniques, such as magnetic resonance imaging (MRI), can be used to investigate structural and functional changes in the brain. MRI may reveal alterations in brain volume, white matter integrity, or functional connectivity. However, these changes often reflect more chronic or advanced processes and may not be sensitive enough to detect early or subtle neuroaxonal damage associated with CICI. Therefore, there is a pressing need for objective, accessible, and sensitive biomarkers that can aid in the early detection, monitoring, and prediction of CICI. Blood-based biomarkers offer a minimally invasive approach to assess the underlying physiological and pathological processes involved in CICI. These biomarkers can provide valuable information about the biological changes occurring in the brain in response to chemotherapy. Neurofilament light chain (NfL) has emerged as a promising biomarker in this context.[5-7]

NfL is a 68 kDa cytoskeletal protein predominantly expressed in large myelinated axons of the central and peripheral nervous system. It plays a crucial role in maintaining axonal structure and providing support to nerve fibers. Upon axonal damage or degeneration, NfL is released into the cerebrospinal fluid (CSF) and subsequently enters the bloodstream. In the bloodstream, its concentration can be measured using highly sensitive immunoassays. Elevated levels of NfL in CSF and blood have been consistently reported in a

variety of neurological disorders characterized by neuroaxonal damage. These disorders include multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, traumatic brain injury, and stroke. In these conditions, NfL levels correlate with the extent of axonal injury and disease severity, highlighting its potential as a marker of neurodegeneration. Emerging evidence suggests that NfL levels may also increase in response to neurotoxicity induced by cancer therapy. Studies have indicated that certain chemotherapeutic agents can cause neuroaxonal damage and that serum NfL levels may be elevated in cancer patients undergoing chemotherapy compared to healthy controls or cancer patients not receiving chemotherapy. This suggests that NfL may serve as a marker of chemotherapy-induced neuroaxonal damage. However, the relationship between serum NfL levels and the degree of cognitive impairment in chemotherapy recipients has yielded inconsistent results. Some studies have demonstrated a correlation between elevated NfL levels and cognitive decline, while others have not found a significant association. These inconsistencies may be due to variations in study design, patient populations, chemotherapy regimens, and cognitive assessment methods. Furthermore, there is a lack of data from diverse populations, including Indonesia, regarding the utility of NfL as a biomarker for CICI.[8-10] To address these gaps in knowledge, this study aimed to investigate the relationship between serum NfL concentrations and the severity of cognitive impairment, assessed using the MoCA-Ina, in a cohort of Indonesian cancer patients undergoing chemotherapy.

2. Methods

This investigation was structured as an observational study, employing a cross-sectional design. This approach was chosen to examine the relationship between serum neurofilament light chain (NfL) levels and the severity of cognitive impairment in cancer patients undergoing chemotherapy at a single point in time. The cross-sectional design allowed for the assessment of the prevalence of cognitive impairment and the measurement of NfL levels concurrently, providing a snapshot of this association within the specified study population.

The research was carried out over a three-month period, specifically from October 2024 to December 2024. The study was conducted at the Dr. M. Djamil General Hospital, a tertiary referral center located in Padang, Indonesia. The study population consisted of all cancer patients who were registered and actively receiving chemotherapy regimens at this hospital during the defined study period.

Participants were recruited for the study using a consecutive sampling method. This approach ensured that all patients meeting the study's inclusion and exclusion criteria and who presented at the hospital during the study period had an equal opportunity to be included in the research. To be eligible for participation in the study, patients were required to meet several inclusion criteria. These criteria typically included having a confirmed diagnosis of cancer, receiving systemic chemotherapy as part of their treatment regimen, being 18 years of age or older, and demonstrating the ability to provide informed consent. Additionally, participants were required to be able to complete the necessary assessment procedures. These criteria ensured that the study focused on adult cancer patients undergoing active chemotherapy treatment and that participants were capable of understanding and participating in the research. Patients were excluded from the study if they met any of the specified exclusion criteria. These criteria typically involved the presence of pre-existing neurological or psychiatric conditions that were known to independently affect cognitive function. Additionally, patients with concurrent central nervous system (CNS) metastases were excluded, as CNS involvement by cancer could directly impact cognitive function, potentially confounding the results. Patients who were unable to cooperate with the testing procedures were also excluded from the study. These exclusion criteria were implemented to minimize the influence of factors other than chemotherapy on

cognitive function, thereby increasing the internal validity of the study. A total of 50 patients met all the eligibility criteria and provided informed consent to participate in the study. This final sample of 50 participants formed the basis for all subsequent data collection and analysis.

The study protocol adhered strictly to the ethical principles outlined in the Declaration of Helsinki. This ensured that the research was conducted in an ethical and responsible manner, protecting the rights and welfare of all participants. Prior to the commencement of the study, ethical approval was obtained from the relevant institutional ethics committee. This approval (Ethics Certification No: DP.04.03/D.XVI.XI/408/2024) confirmed that the study design and procedures were in compliance with ethical guidelines. All participants were provided with comprehensive information about the study's objectives, the procedures involved, and any potential risks and benefits associated with their participation. Written informed consent was obtained from each participant only after they had received and understood this detailed explanation. Participants were explicitly informed that their participation was voluntary and that they had the right to withdraw from the study at any time without any negative consequences. The confidentiality of all participant data was maintained throughout the duration of the study. All data was anonymized, and access to the data was restricted to authorized research personnel only.

Upon enrollment in the study, several types of data were collected from each participant. This included basic demographic and clinical information, assessment of cognitive function, screening for depressive symptoms, and collection of blood samples for NfL analysis. Basic demographic and clinical information was recorded for each participant. This information included the participant's name, medical record number, age, gender, and education level. Further clinical details were also collected, such as the date of hospital admission, the specific type of cancer diagnosis, and detailed information about the participant's current chemotherapy regimen. The chemotherapy regimen details included the specific drugs being used (whether it was a single-agent or combination therapy) and the current cycle number of the treatment.

Cognitive function was formally assessed in each participant using the Indonesian version of the Montreal Cognitive Assessment (MoCA-Ina). The MoCA-Ina is a validated screening tool specifically designed to detect mild cognitive impairment. This tool evaluates various cognitive domains, providing a comprehensive assessment of cognitive function. The cognitive domains assessed by the MoCA-Ina include attention, concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. The MoCA-Ina produces a total score ranging from 0 to 30. Lower scores on the MoCA-Ina indicate a greater degree of cognitive impairment. Standardized cut-off scores for the MoCA-Ina were used to categorize participants into different cognitive function groups. These cut-off scores were potentially adjusted for the participant's education level, in accordance with recommendations from validation studies of the MoCA-Ina. Participants were classified into one of three groups based on their MoCA-Ina scores: normal cognitive function, mild cognitive impairment, or moderate-to-severe cognitive impairment.

To account for the potential confounding effects of depression on cognitive performance, all participants were screened for depressive symptoms. This screening was conducted using the Patient Health Questionnaire-9 (PHQ-9). The PHQ-9 is a widely used and validated instrument designed to assess the severity of depression symptoms. For the analysis of NfL, a venous blood sample was collected from each participant. The volume of blood collected was approximately 3 cubic centimeters (cc). These blood samples were collected using standard phlebotomy procedures and processed according to established laboratory protocols to obtain serum. Following processing, the serum samples were promptly transported to the Biomedical Laboratory of the

Faculty of Medicine, Andalas University, located in Padang, for the analysis of NfL concentrations. Serum NfL concentrations were quantified using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit. The ELISA procedure was performed strictly in accordance with the manufacturer's instructions provided with the kit. All measurements were carried out by trained laboratory personnel who were blinded to the participants' clinical and cognitive status. This blinding procedure was implemented to minimize potential bias in the laboratory analysis. The NfL concentrations were expressed in picograms per milliliter (pg/ml).

IBM SPSS Statistics software, version 25.0 for Windows, was used for all data management and statistical analyses. Descriptive statistics were calculated to provide a comprehensive summary of the characteristics of the study cohort. For continuous variables, such as age and NfL levels, the data were assessed for normality. Given the expected non-normal distribution of these variables, they were reported as median and range (minimum-maximum). Categorical variables, including gender, education level, cancer type, and cognitive status, were presented as frequencies and percentages (n, %). Univariate analysis was initially conducted to describe the sample's characteristics. Following this, bivariate analyses were performed to explore the relationships between different variables. The Kruskal-Wallis test was used as the primary statistical test to analyze the relationship between serum NfL levels and the categorized cognitive impairment groups. The Kruskal-Wallis test is a non-parametric method suitable for comparing median values across three or more independent groups. In this study, serum NfL levels represented the dependent variable, while the categorized cognitive impairment groups (normal, mild, and moderate-severe) represented the independent variable. To assess associations between categorical baseline characteristics and cognitive impairment status, Chi-square tests, Fisher's exact tests, or Kolmogorov-Smirnov tests were used. Post-hoc tests were conducted to determine which specific cognitive impairment groups differed significantly in their NfL levels, but only if the overall Kruskal-Wallis test indicated a significant result. These post-hoc tests included Mann-Whitney U tests with Bonferroni correction or other specific post-hoc comparisons. For all statistical analyses, a p-value of less than 0.05 was considered to indicate statistical significance.

3. Results

Table 1 provides an overview of the key demographic and clinical characteristics of the 50 participants included in the study. The median age of the participants was 41.5 years, with the ages ranging from 24 to 49 years. This indicates that the study population included a range of adult ages, with the midpoint being around 41 years old. The study cohort was predominantly female, with 42 participants being female (84%) and 8 participants being male (16%). This reflects a notable gender imbalance in the study population. Regarding education level, the majority of participants (37, or 74%) had completed 12 or more years of schooling, while 13 participants (26%) had less than 12 years of education. This shows that most participants had at least a high school level of education. Depression status, as assessed by the PHQ-9, showed that 36 participants (72%) had no depression, 14 participants (28%) had mild depression, and no participants (0%) had moderate or severe depression. This indicates that a significant portion of the participants exhibited mild depressive symptoms, while none showed moderate to severe depression. The type of cancer was categorized as either solid tumor or hematopoietic tumor. The majority of participants (42, or 84%) had solid tumors, with breast cancer being the primary type (52% of the total participants). Only 8 participants (16%) had hematopoietic tumors, which included Hodgkin and Non-Hodgkin lymphoma. Chemotherapy regimens were categorized as either single-agent or combination. Most participants (38, or 76%) received combination chemotherapy regimens, while 12 participants (24%) received single-agent regimens.

Table 1. Baseline demographic and clinical characteristics of study participants (N=50).

Characteristic	Category / Statistic	Value
Age	Median (Range), years	41.5 (24 - 49)
Gender	Female	42 (84.0%)
	Male	8 (16.0%)
Education level	< 12 years	13 (26.0%)
	≥ 12 years	37 (74.0%)
Depression status (PHQ-9)	No Depression	36 (72.0%)
	Mild Depression	14 (28.0%)
	Moderate/Severe Depression	0 (0.0%)
Type of cancer	Solid Tumor*	42 (84.0%)
	Hematopoietic Tumor#	8 (16.0%)
Chemotherapy regimen	Single Agent	12 (24.0%)
	Combination Regimen	38 (76.0%)

Notes: N (%) represents count and percentage of participants; Median (Range) presented for age;* Primarily breast cancer (n=26, 52% of total participants);# Included Hodgkin and Non-Hodgkin lymphoma; PHQ-9: Patient Health Questionnaire-9.

Table 2 summarizes the distribution of cognitive impairment among the 50 study participants, as categorized by the Montreal Cognitive Assessment Indonesian version (MoCA-Ina). The table reveals that the majority of participants exhibited some level of cognitive impairment. Specifically, 41 out of the 50 participants (82%) were classified as having cognitive impairment. Within the cognitively impaired group, 30 participants (60% of the total sample) were classified as having mild impairment. A smaller subset of 11 participants (22% of the total sample) were classified as having moderate-to-severe cognitive impairment. Notably, this moderate-to-severe group included one participant with severe impairment. Conversely, 9 participants (18% of the total sample) were classified as having normal cognitive function based on the MoCA-Ina assessment.

Table 2. Distribution of cognitive impairment based on MoCA-Ina assessment (N=50).

Cognitive status category	Number of participants (n)	Percentage of total cohort (%)
Normal cognitive function	9	18.0%
Cognitive impairment	**41**	**82.0%**
- Mild impairment	30	60.0%
- Moderate-severe impairment	11	22.0%
Total participants	**50**	**100.0%**

Notes: The Moderate-Severe category includes one participant classified with severe cognitive impairment.

Table 3 explores the relationship between various baseline characteristics of the study participants and their cognitive impairment status, as categorized into "Normal Cognition," "Mild Cognitive Impairment," and "Moderate-Severe Cognitive Impairment"; Age: The median age across all cognitive impairment groups was relatively similar, ranging from 38 to 46 years. The statistical test (Kruskal-Wallis) indicated no significant difference in age distribution across the cognitive impairment groups ($p = 0.94$). This suggests that age was not a significant factor in determining the degree of cognitive impairment in this study; Gender:

The majority of participants in each cognitive impairment group were female. The distribution of gender across the cognitive impairment groups was not significantly different (p = 0.74). This suggests that gender was not associated with the level of cognitive impairment in this study; Education Level: A notable trend was observed with education level. In the "Moderate-Severe Cognitive Impairment" group, a higher proportion of individuals had less than 12 years of education (61.5%) compared to those with 12 or more years of education (8.1%). Conversely, in the "Normal Cognition" group, all participants had 12 or more years of education. The p-value for education level was 0.08. While not reaching the conventional significance level of 0.05, it indicates a trend towards a statistically significant relationship. This suggests that lower education level might be associated with more severe cognitive impairment; Depression (PHQ-9): Most participants in each cognitive impairment group had no depression. The distribution of depression status (no depression vs. mild depression) was not significantly different across the cognitive impairment groups (p = 0.57). This suggests that the presence of mild depression, as measured by PHQ-9, was not strongly associated with the degree of cognitive impairment in this study; Cancer Type: The majority of participants in each cognitive impairment group had solid tumors. The distribution of cancer type (solid vs. hematopoietic) was not significantly different across the cognitive impairment groups (p = 0.74). This suggests that the type of cancer was not a significant factor in determining the level of cognitive impairment; Chemotherapy Regimen: Most participants in each cognitive impairment group received combination chemotherapy regimens. The distribution of chemotherapy regimen (single vs. combination) was not significantly different across the cognitive impairment groups (p = 0.85). This suggests that the type of chemotherapy regimen was not associated with the degree of cognitive impairment in this study.

Table 3. Relationship between baseline characteristics and cognitive impairment status in chemotherapy patients (n=50).

Characteristic	Category	Normal cognition (n=9)	Mild cognitive impairment (n=30)	Moderate-Severe cognitive impairment (n=11)	p-value
Age (years)	Median (Min-Max)	38 (24-49)	40 (24-49)	46 (32-49)	0.94
Gender	Female (n=42)	8 (19.0%)	23 (54.8%)	11 (26.2%)	0.74
	Male (n=8)	1 (12.5%)	7 (87.5%)	0 (0%)	
Education level	< 12 years (n=13)	0 (0%)	5 (38.5%)	8 (61.5%)	**0.08**
	≥ 12 years (n=37)	9 (24.3%)	25 (67.6%)	3 (8.1%)	
Depression (PHQ-9)	No Depression (n=36)	4 (11.1%)	23 (63.9%)	9 (25.0%)	0.57
	Mild Depression (n=14)	5 (35.7%)	7 (50.0%)	2 (14.3%)	
Cancer type	Solid Tumor (n=42)	7 (16.7%)	24 (57.1%)	11 (26.2%)	0.74
	Hematopoietic Tumor (n=8)	2 (25.0%)	6 (75.0%)	0 (0%)	
Chemotherapy regimen	Single Regimen (n=12)	4 (33.3%)	5 (41.7%)	3 (25.0%)	0.85
	Combination Regimen (n=38)	5 (13.2%)	25 (65.8%)	(21.1%)	

Notes: Values are presented as n (%) unless otherwise specified. Percentages within gender, education, depression, cancer type, and regimen categories represent the proportion of patients in that specific category who fall into each cognitive impairment group; Cognitive impairment assessed using MoCA-Ina; Kruskal-Wallis test used for Age comparison; Kolmogorov-Smirnov test used for categorical variable comparisons; A p-value < 0.05 was considered statistically significant.

Table 4 displays the serum Neurofilament Light Chain (NfL) levels measured in the chemotherapy patients participating in the study. The table shows that the overall median serum NfL level for the entire cohort of 50 participants was 23.44 pg/ml. The range of NfL levels observed in the cohort spanned from 13.81 pg/ml (the minimum value) to 68.71 pg/ml (the maximum value). This indicates a considerable variability in NfL levels among the participants. The table further breaks down the NfL levels based on the cognitive function groups; Normal Cognitive Function: The median NfL level for participants with normal cognitive function (n=9) was 18.49 pg/ml, with a range of 13.81 to 27.93 pg/ml; Mild Cognitive Impairment: The median NfL level for participants with mild cognitive impairment (n=30) was 23.50 pg/ml, with a range of 14.66 to 49.51 pg/ml; Moderate-Severe Cognitive Impairment: The median NfL level for participants with moderate-severe cognitive impairment (n=11) was 24.50 pg/ml, with a range of 18.06 to 68.71 pg/ml.

Table 4. Serum neurofilament light chain (NfL) levels in chemotherapy patients (n=50).

Group description	**Number of subjects (n)**	**Median NfL level (pg/ml)**	**Range (Min-Max) of NfL levels (pg/ml)**
Overall cohort	50	23.44	13.81 – 68.71
Cognitive function groups:			
- Normal cognitive function	9	18.49	13.81 – 27.93
- Mild cognitive impairment	30	23.50	14.66 – 49.51
- Moderate-Severe cognitive impairment	11	24.50	18.06 – 68.71

Notes: Serum NfL levels are presented as median (minimum-maximum).

Table 5 examines the relationship between serum Neurofilament Light Chain (NfL) levels and the degree of cognitive impairment in the study participants, categorized using the MoCA-Ina. It also includes the results of post-hoc analyses conducted to compare NfL levels between specific cognitive impairment groups. The table shows the following median serum NfL levels and ranges for each cognitive impairment group; Normal Cognitive Function: Participants with normal cognitive function (n=9) had a median serum NfL level of 18.49 pg/ml, with a range of 13.81 to 27.93 pg/ml; Mild Cognitive Impairment: Participants with mild cognitive impairment (n=30) had a median serum NfL level of 23.50 pg/ml, with a range of 14.66 to 49.51 pg/ml; Moderate-Severe Cognitive Impairment: Participants with moderate-severe cognitive impairment (n=11) had a median serum NfL level of 24.50 pg/ml, with a range of 18.06 to 68.71 pg/ml. The overall Kruskal-Wallis test, used to compare NfL levels across the three cognitive impairment groups, revealed a statistically significant difference ($p = 0.02$). This indicates that there is a significant association between NfL levels and cognitive impairment status. Post-hoc analyses, performed to determine which specific groups differed significantly from each other, yielded these results; Serum NfL levels were significantly higher in the mild cognitive impairment group compared to the normal cognitive function group ($p = 0.03$); Serum NfL levels were significantly higher in the moderate-severe cognitive impairment group compared to the normal cognitive function group ($p = 0.01$); However, there was no statistically significant difference in serum NfL levels between the mild cognitive impairment group and the moderate-severe cognitive impairment group ($p = 0.27$).

Table 5. Relationship between serum neurofilament light chain (NfL) levels and cognitive impairment, including post-hoc analysis.

Cognitive Impairment Group (MoCA-Ina Classification)	Number of subjects (n)	Median serum NfL level (pg/ml)	Serum NfL range (Min-Max, pg/ml)	Pairwise comparison (Post-Hoc p-value)
Normal cognitive function	9	18.49	13.81 - 27.93	vs. Mild: p = 0.03 vs. Mod-Sev: p = 0.01
Mild cognitive impairment	30	23.50	14.66 - 49.51	vs. Normal: p = 0.03 vs. Mod-Sev: p = 0.27
Moderate-Severe cognitive impairment	11	24.50	18.06 - 68.71	vs. Normal: p = 0.01 vs. Mild: p = 0.27
Overall comparison (Kruskal-Wallis test)				**p = 0.02**

4. Discussion

Our findings revealed a high prevalence of cognitive impairment (82%) in this cohort of chemotherapy patients, with 60% experiencing mild and 22% moderate-to-severe impairment. This rate is substantial and aligns with the upper range reported in the literature, where prevalence estimates for CICI vary widely (from roughly 20% to over 85%) depending on the assessment methods (self-report vs. objective testing), timing of assessment relative to treatment, cancer type, chemotherapy regimens used, and study populations. The high rate observed here underscores the significance of CICI as a clinical issue in our patient population. The variability in reported CICI prevalence highlights the complexities in defining and assessing this condition. Self-report measures, while valuable for capturing patients' subjective experiences, can be influenced by individual differences in symptom perception and reporting biases. Objective neuropsychological testing aims to provide a more standardized assessment of cognitive function, but the sensitivity and specificity of these tests can vary. Furthermore, the timing of cognitive assessments in relation to chemotherapy cycles plays a crucial role. Cognitive deficits may fluctuate during treatment, with some patients experiencing acute changes while others develop more chronic impairments. The type of cancer and the specific chemotherapy regimens employed also contribute to the heterogeneity of CICI. Different chemotherapeutic agents have varying degrees of neurotoxicity and may affect specific cognitive domains differently. Finally, differences in study populations, including demographic and clinical characteristics, can influence the observed prevalence of CICI. In our study, the use of the MoCA-Ina, a validated screening tool for mild cognitive impairment, allowed for a relatively objective assessment of cognitive function. The high prevalence of cognitive impairment observed suggests that a significant proportion of cancer patients undergoing chemotherapy experience cognitive difficulties. This finding emphasizes the need for increased awareness and monitoring of CICI in clinical practice. The finding that education level potentially influenced cognitive scores (p=0.08) is consistent with known effects of education on neuropsychological test performance and highlights the importance of using appropriately normed or adjusted tools like the MoCA-Ina, particularly in diverse educational backgrounds. While not statistically significant in this sample size, lower education has been previously linked to greater cognitive decline post-treatment. Education is a complex variable that can influence cognitive reserve, which is the brain's ability to withstand damage and maintain cognitive function. Individuals with higher levels of education may have greater cognitive reserve, enabling them to better compensate for the neurotoxic

effects of chemotherapy. Conversely, individuals with lower levels of education may be more vulnerable to cognitive decline following chemotherapy treatment. This highlights the importance of considering individual patient characteristics, such as education level, when assessing and managing CICI. The use of appropriately normed or adjusted cognitive assessment tools is crucial to minimize the impact of education and other sociodemographic factors on test results.[11-13]

The median serum NfL level in our cohort was 23.44 pg/ml. Notably, the median NfL levels observed in all cognitive groups (normal: 18.49, mild: 23.50, moderate-severe: 24.50 pg/ml) appear elevated compared to reference intervals reported for similarly aged healthy individuals in some studies (e.g., 4.6-21.4 pg/ml for ages 41-65). This suggests that even patients classified as cognitively normal via MoCA-Ina might be experiencing subclinical neuroaxonal effects related to their cancer or treatment. Neurofilament light chain (NfL) is a structural protein found predominantly in large myelinated axons. It is released into the cerebrospinal fluid (CSF) and subsequently enters the bloodstream following axonal damage or degeneration. Elevated levels of NfL in the blood are considered a marker of neuroaxonal injury across a range of neurological conditions. The observation of elevated NfL levels in our cohort, even in those classified as cognitively normal by the MoCA-Ina, suggests that chemotherapy may induce neuroaxonal damage even in the absence of overt cognitive impairment. This highlights the potential of NfL as a more sensitive biomarker for detecting early or subclinical neurotoxicity associated with chemotherapy. It also raises the possibility that some patients may be experiencing subtle neurological changes that are not captured by traditional cognitive assessments. Our findings are broadly consistent with prior research demonstrating increased NfL levels in cancer patients undergoing chemotherapy. For instance, Study reported significantly increased NfL levels post-chemotherapy compared to baseline and healthy controls, linking this to neuroinflammation and white matter changes. A Study also found elevated median NfL in a chemotherapy group. These elevations are biologically plausible, as various chemotherapeutic agents can induce neurotoxicity through mechanisms like mitochondrial damage, oxidative stress, impaired neurogenesis, DNA damage accumulation, and BBB disruption, ultimately leading to neuronal stress or death and NfL release. NfL release is considered a specific indicator of such neuroaxonal damage. Chemotherapeutic agents can exert their neurotoxic effects through a variety of mechanisms. Mitochondrial damage can disrupt cellular energy production, leading to neuronal dysfunction and death. Oxidative stress, an imbalance between the production of reactive oxygen species and the body's ability to neutralize them, can damage cellular components, including DNA and proteins. Impaired neurogenesis, the process of generating new neurons, can compromise the brain's ability to repair and adapt. DNA damage accumulation can trigger cellular senescence and apoptosis, contributing to neuronal loss. Disruption of the blood-brain barrier (BBB) can allow neurotoxic substances to enter the brain, further exacerbating neurotoxicity. The observed elevations in NfL levels in chemotherapy patients, as seen in our study and previous research, likely reflect the cumulative effect of these various neurotoxic mechanisms. The release of NfL into the bloodstream serves as a marker of the resulting neuroaxonal damage.[14-16]

The core finding of this study is the significant positive association between serum NfL levels and cognitive impairment severity. Participants with mild or moderate-severe cognitive impairment had significantly higher median NfL levels compared to those with normal cognitive function. Furthermore, there was a trend of increasing median NfL levels with greater impairment severity. This suggests that the extent of chemotherapy-associated neuroaxonal damage, as reflected by serum NfL concentration, correlates with the degree of measurable cognitive dysfunction. This aligns with the hypothesis that NfL serves as a biomarker linking the neuropathological

process (neuroaxonal injury) to the clinical outcome (cognitive impairment) in the context of CICI. The significant positive association between NfL levels and cognitive impairment severity supports the potential utility of NfL as a biomarker for CICI. The progressive increase in median NfL levels from the normal cognition group to the mild and moderate-severe impairment groups suggests that higher NfL levels are associated with greater cognitive dysfunction. This finding is consistent with the understanding that NfL release reflects neuroaxonal damage, a key pathological process implicated in CICI. Our results corroborate findings from studies, which associated NfL increases with cognitive worsening and neuroinflammation. However, it is important to note that not all studies have found this association. A Study did not find a significant correlation between serum NfL and MoCA scores in breast cancer patients receiving paclitaxel, although they did observe higher NfL in patients with CICI compared to those without in some analyses. The discrepancies in findings across studies highlight the complexity of CICI and the challenges in identifying consistent biomarkers. Differences in study design, including cross-sectional versus longitudinal approaches, sample size, patient populations, and specific chemotherapy regimens, can contribute to variations in results. Furthermore, the timing of NfL and cognitive assessments relative to chemotherapy cycles, the cognitive tests used, and the sensitivity of NfL assays can also influence the observed associations. In our study, the use of a cross-sectional design limits our ability to establish causality or track longitudinal changes in NfL levels and cognitive function. However, the significant association observed between NfL levels and cognitive impairment severity provides further evidence for the potential role of NfL as a biomarker for CICI. The lack of a significant difference in NfL levels between the mild and moderate-severe groups in our post-hoc analysis might suggest that while NfL elevation distinguishes impaired from unimpaired individuals, it may be less sensitive in differentiating finer grades of impairment severity, or it could be due to limited statistical power in the smaller moderate-severe group. The post-hoc analyses in our study revealed significant differences in NfL levels between the normal cognition group and both the mild and moderate-severe impairment groups. However, no significant difference was observed between the mild and moderate-severe impairment groups. This suggests that NfL elevation may be useful in distinguishing between individuals with and without cognitive impairment, but it may not be as sensitive in differentiating between different levels of impairment severity. It is also possible that the lack of a significant difference between the mild and moderate-severe groups is due to limited statistical power. The moderate-severe impairment group had a smaller sample size (n=11) compared to the mild impairment group (n=30). This difference in sample size may have reduced the power to detect a statistically significant difference between these two groups.[17-20]

5. Conclusion

In conclusion, this study provides compelling evidence for a significant positive association between serum NfL levels and the presence and severity of cognitive impairment in cancer patients undergoing chemotherapy. The findings suggest that elevated serum NfL levels correlate with the degree of cognitive dysfunction, as assessed by the MoCA-Ina. Specifically, patients with mild and moderate-severe cognitive impairment exhibited significantly higher NfL levels compared to those with normal cognition. These results support the potential utility of serum NfL as a biomarker for detecting chemotherapy-associated neuroaxonal damage and concomitant cognitive decline. The measurement of serum NfL offers a minimally invasive approach to assess neurotoxicity in these patients, potentially aiding in earlier detection and monitoring of CICI. While this study contributes valuable insights, it is important to acknowledge the limitations of its cross-sectional design, which precludes the establishment of causality or the examination of longitudinal changes in NfL levels and cognitive function. Further research,

employing longitudinal study designs and larger sample sizes, is warranted to validate these findings, explore the predictive value of NfL for CICI, and investigate the potential clinical utility of NfL monitoring in guiding interventions aimed at mitigating chemotherapy-induced neurotoxicity and preserving cognitive function in cancer survivors.

6. References

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