



Research Article

Comparison of neurofilament light chain protein levels in the blood of patients with multiple sclerosis and amyotrophic lateral sclerosis

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ABSTRACT

Neurodegenerative disorders involve a gradual loss of neurons. This includes multiple sclerosis and amyotrophic lateral sclerosis. Yet there is still a shortage of dependable blood-based indicators. These indicators would aid in diagnosis and track therapeutic effectiveness. The neurofilament light chain, which is an essential component of the neuronal cytoskeleton, enters the cerebrospinal fluid and bloodstream in the event of axonal injury. While most previous studies have reported increased neurofilament light chain concentrations in untreated patients, evidence from cohorts that have undergone long-term treatment is scarce. This study measured plasma neurofilament light chain concentrations in 86 participants (33 with multiple sclerosis, 38 with amyotrophic lateral sclerosis and 15 healthy controls) using the Bioassay Technology Laboratory neurofilament light chain ELISA assay. Median plasma neurofilament light chain levels were significantly lower in patients with multiple sclerosis ($p=0.0035$), whereas the reduction observed in patients with amyotrophic lateral sclerosis did not reach statistical significance ($p=0.0728$) compared with healthy controls. This suggests a treatment-associated reduction in neuronal damage, despite ongoing neurodegeneration. No significant sex-based differences were detected ($p=0.879$). The results suggest that plasma neurofilament light chain levels could function not only as an auxiliary tool for diagnosis but also as a marker reflecting therapeutic effects in chronically treated neurodegenerative conditions. A key contribution of this study is the observation that treated patients exhibited lower plasma concentrations than healthy controls, a finding that contrasts with previous reports of elevated levels in untreated groups. This emphasizes the potential clinical utility of plasma neurofilament light chain monitoring and sets the stage for future longitudinal validation efforts.

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INTRODUCTION

Neurodegenerative diseases, particularly multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS), present significant challenges in diagnosis and treatment due to their biological complexity and progressive nature [1]. MS is a disease affecting the central nervous system characterized by immune-mediated demyelination and neuroinflammation [2]. ALS, on the other hand, is characterized by progressive degeneration of upper and lower motor neurons, leading to motor dysfunction and disability [3]. Given the progressive and heterogeneous nature of these disorders, identifying a reliable biomarker for the diagnosis and monitoring is of considerable clinical importance [4]. According to the findings from the studies, the idea that NFL could be a biomarker with potential for use in its release into biological fluids during neuroaxonal damage [5,6]. In this context, the study evaluates the potential of plasma NFL levels as a biomarker to distinguish ALS or MS patients from healthy individuals.

Neurofilaments represent a component of the cytoskeleton of neurons, exhibiting a high degree of abundance, particularly within the axons of these cells. Through their structural role, neurofilaments contribute to the maintenance of axonal size, shape, and diameter [7]. Neurofilaments belong to the intermediate filament family and consist of triple formations of the three subunits; NFL, neurofilament medium chain (NFM) and neurofilament heavy chain (NFH) [8,9]. Located on chromosome 8p21, the NFL gene encodes a 543-amino acid protein weighing approximately 61.5 kDa. Similarly, the NFM gene, which also lies on chromosome 8p21, encodes a protein of 916 amino acids that is essential for axonal radial growth. The NFH gene, however, is found on chromosome 22q12.2 and produces a 1020-amino acid protein with a molecular mass of around 112.5 kDa [10,11]. The production of these proteins takes place in the nerve cell body, after which they are incorporated into intermediate filaments. These filaments provide stability to the axon [12].

Neurodegenerative diseases are progressive, multifactorial conditions that affect nerve cells in the central and peripheral nervous systems. They arise as a result of nerve cell loss in the nervous system [13]. Typical characteristics of neurodegenerative diseases include sporadic or hereditary transmission, gradual progression, early onset, contributions from both genetic and environmental factors, accumulation of protein deposits in the nervous system, and the deterioration of specific neurons [14]. There is no effective treatment currently for neurodegenerative diseases (NDDs); ongoing therapies are mainly aimed at treating the most unpleasant symptoms [15]. There is currently no effective treatment for NDDs; the treatments applied nowadays are actually aimed at removing the most disturbing symptoms [16]. Early treatment is crucial to mitigate the effects of these diseases in NDDs, unique and sensitive biomarkers are needed to early diagnosis [17].

It is difficult to diagnose diseases that cause the nervous system to degenerate, especially in conditions such as ALS and MS. Currently, there are no clinically validated diagnostic biomarkers in routine clinical practice. Most biomarker research focuses on the analysis of cerebrospinal fluid (CSF) and blood samples to identify diagnostic and prognostic markers. In this context, NFL has gained significant interest due to its ability to reflect axonal damage [18]. In addition GFAP is associated with astroglial activation and gliosis and is more indicative of neuroinflammation, whereas it does not directly reflect axonal damage [19]. Tau protein is a known biomarker, particularly in Alzheimer's disease, and has also been studied in ALS and MS [20]; however, it shows less consistent association with disease progression compared to NFL. UCH-L1 has been proposed as a marker of neuronal damage [21], while CHI3L1 has been proposed as a marker of glial activation [22]; however, the specificity of these biomarkers for ALS and MS is limited.

MS is an autoimmune inflammatory disease affecting the central nervous system [23,24]. The number of MS cases is increasing in both developed and developing countries [25]. The defining pathological feature of MS is the occurrence of demyelination in the brain and spinal cord, usually associated with damage to the axons of neurons [26]. ALS, also known as Lou Gehrig's disease, is characterized by degeneration of both upper (UMN) and lower (LMN) motor neurons in the brainstem and spinal cord [27,28]. Although there have been several studies investigating the use of cytoplasmic components (e.g., tau protein, neurofilaments, and S100) as a measure of disease activity and progression [29], there are currently no proven CSF or serum biomarkers for diagnosing or predicting the prognosis of ALS [30]. The use of diagnostic biomarkers in the early stages of ALS could shorten the diagnostic process. Studies have shown that NFL and pNFH levels in cerebrospinal fluid (CSF) are significantly higher in the early stages of the disease [31–34].

Research on neurofilaments is rapidly expanding. Levels of neurofilaments are being explored as markers. These markers show disease activity and progression. Elevated neurofilament levels have been associated with disease activity and progression across a broad spectrum of neurological disorders, including stroke [35], ALS [34], frontotemporal dementia [36], and MS [37]. However, a comparison of NFL and NFH chain levels shows that NFL is a more specific marker of MS than NFH [38]. As neurofilaments are specific to neuronal tissue, their presence in the bloodstream implies leakage or diffusion through the blood–brain barrier. Furthermore, NFL can reach the circulation via CSF venous drainage pathways [39].

Recent systematic reviews and guidance reports indicate that blood NFL is consistently elevated in untreated multiple sclerosis and correlates with relapse activity, MRI lesion burden and risk of future disability, supporting its utility as a prognostic biomarker in routine care [6,40]. However,

evidence is limited on how long-term immunomodulatory or neuroprotective therapies modify peripheral NFL concentrations in real-world treated cohorts, and whether such therapy-related changes alter the biomarker's diagnostic or prognostic performance [41,42]. Moreover, assay and platform differences (single molecule array [Simoa], electrochemiluminescence [ECL], high-sensitivity ELISA) can produce systematic differences in absolute NFL values and sensitivity for low concentrations, complicating direct comparisons across studies [43,44]. To address these gaps, the present study measured plasma NFL using a standardized ELISA approach in well-characterized, predominantly long-term treated cohorts of multiple sclerosis and amyotrophic lateral sclerosis patients and compared them with healthy controls, with the goal of assessing whether treatment-associated modulation of plasma NFL is observable in routine clinical samples. The novelty of this work is that it directly compares treated multiple sclerosis and amyotrophic lateral sclerosis cohorts using the same assay and provides evidence that long-term therapy may attenuate circulating NFL levels — a topic that has not been extensively explored in prior, predominantly untreated or therapy-heterogeneous cohorts [40,45].

MATERIALS AND METHODS

Test Subjects

A total of 86 participants were recruited from the Istanbul University Faculty of Medicine, Department of Neurology. Blood samples were collected and analyzed at Yildiz Technical University, Department of Molecular Biology and Genetics. The cohort consisted of 43 women (50%, mean age: 42.80 ± 1.93 years) and 43 men (50%, mean

age: 43.19 ± 2.15 years). Participants were further classified into diagnostic groups. The participants who provided the samples used in the experiment gave their written informed consent, and their demographic information has been anonymized. The sample size ($n=86$) was determined by clinical availability during the study period rather than a predefined statistical power calculation. This approach is consistent with other MS and ALS biomarker studies that typically include 50–120 participants, and was sufficient to detect significant intergroup differences. The participant demographics are summarised in Table 1.

As a result of the tests performed, the mean NFL protein concentration in the MS subject group was 1.35 ± 0.14 . The mean NFL protein concentration was 1.24 ± 0.21 in men and 1.45 ± 0.18 in women. Similarly, the mean NFL protein concentration in the ALS subject group was 1.31 ± 0.11 . The mean NFL protein concentration was 1.42 ± 0.18 in men and 1.74 ± 0.10 in women. In the control group, the NFL protein amounts are as follows: the overall mean is 1.91 ± 0.29 . The mean NFL protein concentration was 1.13 ± 0.09 in men and 2.26 ± 0.37 in women. In the whole subject group, the mean NFL protein concentration was 1.51 ± 0.13 in women and 1.32 ± 0.12 in men. The average concentration of NFL protein is presented in Table 2.

Samples

Blood samples were collected into 10 mL EDTA tubes at the Department of Neurology, Faculty of Medicine, Istanbul University, and transported on ice to the Molecular Biology and Genetics Research Laboratories at Yildiz Technical University. The samples were then centrifuged at $1000 \times g$ for 30 minutes at 24°C . The plasma obtained from two tubes per subject was pooled into a 15 mL tube and divided into 1.5 mL cryotubes. To ensure accurate data processing

Table 1. Participants demographic information

Groups	Total Subjects	Number of Women	Mean Age $\pm$ SD (Women)	Number of Men	Mean Age $\pm$ SD (Men)
MS	33	17	38.94 ± 4.12	16	35.38 ± 2.82
ALS	38	16	53.81 ± 2.64	22	49.73 ± 2.56
Healthy Control	15	10	33.40 ± 4.40	5	36.20 ± 2.89
Total Participants	86	43	42.80 ± 1.93	43	43.19 ± 2.15

Table 2. Mean Concentration of NFL protein (ng/ml)

Groups	Overall Mean NFL (ng/ml) $\pm$ SD	Women- Mean NFL (ng/ml) $\pm$ SD	Men- Mean NFL (ng/ml) $\pm$ SD
MS	1.35 ± 0.14	1.45 ± 0.18	1.24 ± 0.21
ALS	1.31 ± 0.11	1.74 ± 0.10	1.41 ± 0.18
Healthy Control	1.91 ± 0.30	2.26 ± 0.37	1.13 ± 0.08
Total Participants	1.51 ± 0.13	1.51 ± 0.13	1.32 ± 0.12

and sample tracking, each participant was assigned a unique identifier, and samples were stored at -80°C until protein analysis was performed.

Analytical Methods

Plasma NFL protein concentrations were measured using a 96-well Human NEFL ELISA Kit (Cat. No: E3350Hu, Bioassay Technology Laboratory). The kit used for ELISA analysis was prepared according to the manufacturer's instructions. The analytical sensitivity of the kit was 0.051 ng/mL. All samples were measured twice, and the mean of the two measurements was used for statistical analysis. Optical density was determined using a Biotek ELISA multi-plate reader (GEN5 software, version 1.08).

Hypotheses

The study tested the following hypotheses:

- i. **Gender:** NFL concentrations are consistent across genders (H0) or differ between genders (H1).
- ii. **Age groups:** NFL concentrations are consistent across age groups (H0) or differ between at least two age groups (H1).
- iii. **Disease type:** NFL concentrations are consistent across all groups (H0) or differ between at least two groups (H1).

Statistical Methods

The analyses in this study were performed using SPSS 22.0 (IBM Corp.) software. In this context, plasma NFL levels were compared in the MS group, the ALS group, and the healthy control group. Due to the non-normal distribution of the data, intergroup comparisons were performed using the Kruskal–Wallis test, and p-values below 0.05 were accepted as statistically significant.

Ethics Approval

Ethical approval for the study was obtained from the Istanbul University Faculty of Medicine Clinical Research Ethics Committee (Decision No. 689, File No. 2016/665, Meeting Number 10, May 24, 2016). All participants whose blood samples were used in the study provided written informed consent for the use of their biological materials in this study.

RESULTS AND DISCUSSION

Statistical tests were selected based on the distribution and nature of the dataset. Since the normality assumption was not met, the Kruskal–Wallis test was used to compare NFL levels between the ALS, MS, and control groups. Pairwise comparisons were performed using Dunn's post-hoc test with a healthy control group as a reference. In women, NFL levels were statistically significantly decreased in both the MS ($p=0.0171$) and ALS ($p=0.0135$) groups compared to controls. No significant difference was observed in men. In the overall cohort, NFL was significantly decreased in the MS group compared to controls ($p=0.0035$), while the decrease was not statistically significant in the ALS group ($p=0.0728$). No significant difference was found between the multiple sclerosis and amyotrophic lateral sclerosis groups (Fig. 1).

The figure illustrates mean plasma NFL concentrations (ng/mL) in female and male participants in the MS, ALS and healthy control groups, as well as in the total study population. Female patients with MS and ALS exhibited significantly lower NFL levels than healthy controls. However, in the entire cohort, a significant reduction was only observed in the MS group. No significant difference was found between male participants or between the MS and ALS groups. Data are presented as mean $\pm$ standard deviation. Statistical significance is defined as * $p<0.05$ and ** $p<0.01$ in the graph.

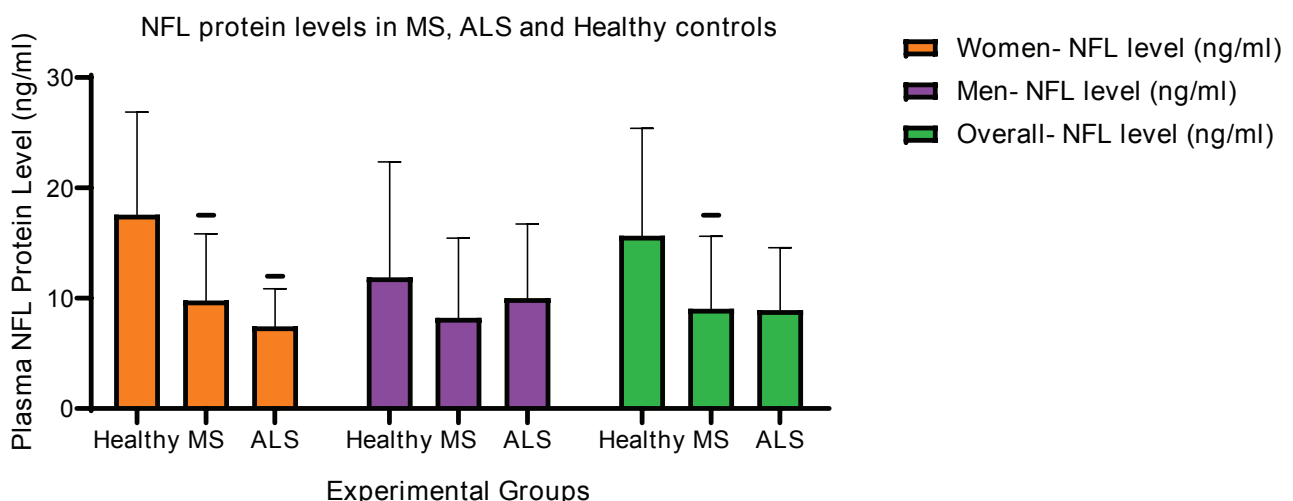


Figure 1. Plasma NFL protein levels in MS, ALS, and healthy controls.

NFLs are specific to neuronal cells and pass into the CSF. They can be detected in the bloodstream, even at low concentrations. The concentration of NFLs in the CSF increases in various diseases that cause neuronal damage [37,46]. NFL is one of the proteins that make up the structure of neurons and is released into the space around them when there is damage to the nerves. It then enters the fluid around the brain and spinal cord and the blood [47-49]. High levels of NFL in the fluid around the brain and spine are seen in diseases that cause the nervous system to weaken or break down, like multiple sclerosis, a type of muscular wasting, and problems like memory loss and paralysis [50-55]. Due to its specificity to neurons, NFL is considered a potential biomarker for assessing damage to the central and peripheral nervous systems.

Although diagnosing neurodegenerative diseases such as ALS and MS remains a complex process, neurofilament light chain (NFL) has gained increasing importance as a biomarker particularly for neurodegeneration. Most studies investigating biomarkers focus on the analysis of CSF and blood samples. In this work, plasma NFL levels in both ALS and MS patients were evaluated for their biomarker potential. Blood samples were collected from 38 ALS patients, 33 MS patients, and 15 healthy controls. Plasma fractions were isolated from these samples, and NFL protein levels were quantified using the sandwich ELISA method. The resulting data were statistically analyzed using the SPSS 22.0 software. Given that the data did not follow a normal distribution, the Kruskal-Wallis test was employed as a non-parametric alternative to the one-way ANOVA, which does not assume normality [56]. This test was chosen because it allows for the comparison of NFL protein levels across three independent groups assessing whether there are statistically significant differences among them. Results were considered significant at $p < 0.05$.

Statistical analysis revealed a significant difference in NFL levels between the healthy control group and the MS group ($p = 0.0035$). In a study by Bjornevik et al. [57], NFL levels were investigated among US military personnel whose serum samples were stored in the US Department of Defense Serum Depot. Serum samples collected between 2000 and 2011 were later analyzed for NFL concentrations between 2018 and 2019 using an ultra-sensitive Simoa. Notably, NFL levels were elevated up to six years before the onset of clinical MS symptoms, indicating that multiple sclerosis may have a prodromal phase characterized by neuroaxonal damage that begins several years before clinical manifestation.

In our study, NFL levels were lower in MS patients compared to healthy controls; this decrease is consistent with findings that it is associated with long-term immunomodulatory treatment. A study by Bar-Or et al. [40] showed that ocrelizumab significantly reduced NFL levels and decreased physical symptoms in MS. High levels of the protein have been shown to cause progression of clinical signs. Furthermore, the findings in our study resulted in

increased disease activity and neuroaxonal damage after discontinuation of natalizumab treatment, and NFL protein levels increased [58]. In MS patients treated with glatiramer acetate as an immunomodulator, NFL protein levels were statistically reduced in the first 9 months ($p < 0.05$) and this reduction became even more pronounced at 12 months ($p = 0.013$) [59]. Our study was conducted with patients diagnosed with MS who have been on medication treatment for a duration ranging from at least 1 year to a maximum of 20 years [60].

The next statistical comparison was conducted between the healthy control group and the ALS group. This revealed a reduction in NFL levels which did not reach statistical significance ($p = 0.0728$). In a study by Verde et al. [61], serum samples were taken from 124 patients with ALS, 50 individuals without neurodegenerative diseases and 44 patients undergoing diagnosis for differential diagnosis of ALS (disease controls). Additionally, serum samples from 65 patients with other neurodegenerative disorders were included, consisting of 20 patients with frontotemporal dementia, 20 with Alzheimer's disease, 19 with Parkinson's disease and 6 with Creutzfeldt-Jakob disease (CJD). In patients other than those diagnosed with ALS and CJD, NFL protein levels, as measured by ultra-sensitive Simoa technology, were significantly higher. These outcomes imply that serum NFL could be used as a diagnostic and prognostic biomarker in ALS [57].

In our study, the NFL levels were found to be lower in the ALS patient group compared to the healthy controls. Clinically, the lower NFL levels observed in ALS patients compared with healthy controls may reflect a treatment effect, where long-term therapy reduces ongoing axonal damage and decreases neurofilament release. In this respect, NFL is considered not only a disease marker but also a potential indicator in disease monitoring. In practice these findings suggest that the decrease in NFL protein levels may be related to disease-modifying therapies (DMTs) [45,60,61]. It has been determined that Riluzole, used in ALS patients, reduces axonal damage by slowing down neurofilament transport. In the study by Esselin et al. [62], it was observed that serum NFL protein levels were higher in ALS patients compared to healthy controls and that this level increased when patients were not treated. No statistically significant difference was found in serum NFL protein levels with Riluzole treatment. This suggests that the use of serum NFL for treatment monitoring is limited. According to the study by Stevenson et al. [63], Riluzole's anti-glutamatergic properties have been shown to protect against glutamate-mediated changes in neurofilament phosphorylation and transport, both of which are crucial pathological features of ALS. Given that the ALS patients in our study were treated with Riluzole (Rilutek), this may explain the lower NFL levels observed in this group compared to healthy controls. Our study included ALS patients who had been receiving medication for a period ranging from 1 to 10 years.

Kuhle et al. [64] measured serum NFL levels in 589 MS and 35 healthy controls. There was no clear link found between the levels of NFL in the blood and age or gender. Similarly, a meta-analysis by Cai et al. [65] included 2,651 participants from thirteen studies, comprising 795 cerebrospinal fluid samples and 1,856 blood samples. In the experimental groups, MS patients were aged 31–44, and healthy volunteers were aged 28–44.3. NFL protein levels in CSF and serum were measured using ELISA and electrochemical luminescence (ECL), and higher NFL concentrations were detected in ECL, which is more sensitive than ELISA. This observation in MS patients may be due to the high sensitivity of ECL [37,64]. Prior reports also confirmed that elevated NFL concentrations in both CSF and blood of the patients, independent of age, gender and age.[65]

In our study, a statistically significant difference was observed in NFL concentration levels based on gender at the $\alpha=0.10$ significance level ($p=0.076$). However, no difference was found in this protein among the diseases. The absence of a difference may indicate a response related to the medications used.

When considered together, our results support the reported findings related to changes in NFL protein levels in neurodegenerative diseases. The lower NFL levels detected in ALS and MS patient groups compared to healthy controls suggest that this decrease may be related to treatment approaches for reducing axonal damage over time. The results, consistent with previous studies, showed that immunomodulatory therapies used in MS and neuroprotective agents such as Riluzole used in ALS reduced NFL levels. While NFL levels are considered promising biomarkers for early diagnosis and monitoring disease progression in neurodegenerative diseases, therapeutic background was observed to be a significant factor in NFL measurements. Therefore, further studies involving larger patient cohorts and analyses classified according to treatment duration and treatment type are needed to establish the diagnostic and prognostic value of NFL more clearly in neurodegeneration. Furthermore, increasing the sample size of the healthy control group will contribute to stronger validation of the results and make the findings transferable to larger populations. When the aforementioned points are taken into consideration plasma NFL measurements can be used as a useful tool for clinicians in monitoring disease progression and treatment response. Beyond this, plasma NFL levels could be a valuable instrument for researchers to use when selecting patients for long-term follow-up studies.

CONCLUSION

The study shows that measuring neurofilament light chain protein levels could be a way to spot neurodegenerative diseases including multiple sclerosis and amyotrophic lateral sclerosis. Although previous studies have consistently reported elevated levels of this protein in these conditions, our findings suggest that long-term therapeutic

interventions may significantly impact these levels by reducing axonal damage. The observed reduction in neurofilament light chain concentrations among treated patients emphasizes the importance of considering treatment history when interpreting neurofilament light chain measurements. Although our findings provide valuable insights, larger and more diverse sample groups are needed to improve the clinical relevance of the results. These findings may contribute to a broader understanding of neurodegenerative diseases and raise questions about the clinical role of biomarkers, particularly in patients receiving treatment. Larger prospective studies with bigger cohorts are needed to confirm these findings. Future research should also evaluate treatment duration and treatment type to clarify the diagnostic and prognostic role of neurofilament light chain in distinct neurodegenerative diseases.

AUTHORSHIP CONTRIBUTIONS

Bertan Emrah: Data curation, Writing – original draft. **Günel Aslıhan:** ELISA experiments, Editing. **Çelik Reşit:** Statistical Analysis. **İdrisoğlu Halil Atila:** Diagnosis and follow-up of patients; collection of blood samples. **Vural Korkut Senay:** Writing – review & editing.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ETHICS

Ethical approval for the study was obtained from the Istanbul University Faculty of Medicine Clinical Research Ethics Committee (Decision No. 689, File No. 2016/665, Meeting Number 10, May 24, 2016).

STATEMENT ON THE USE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence was not used in the preparation of the article.

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