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ORIGINAL ARTICLE

Exploring biomarkers in trigeminal neuralgia patients operated with microvascular decompression: A comparison with multiple sclerosis patients and non-neurological controls

Muataz S. Lafta[1] | **Aleksandr V. Sokolov**[1] | **Anne-Marie Landtblom**[2,3] | **Hans Ericson**[4] | **Helgi B. Schiöth**[1] | **Sami Abu Hamdeh**[4]

[1]Department of Surgical Sciences, Functional Pharmacology and Neuroscience, Uppsala University, Uppsala, Sweden

[2]Department of Medical Sciences, Neurology, Uppsala University, Uppsala, Sweden

[3]Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden

[4]Department of Medical Sciences, Section of Neurosurgery, Uppsala University, Uppsala, Sweden

Correspondence
Sami Abu Hamdeh, Department of Medical Sciences, Section of Neurosurgery, Uppsala University Hospital, SE-751 85 Uppsala, Sweden.
Email: sami.abu.hamdeh@neuro.uu.se

Abstract

Background: Trigeminal neuralgia (TN) is a severe facial pain condition often associated with a neurovascular conflict. However, neuroinflammation has also been implicated in TN, as it frequently co-occurs with multiple sclerosis (MS).

Methods: We analysed protein expression levels of TN patients compared to MS patients and controls. Proximity Extension Assay technology was used to analyse the levels of 92 proteins with the Multiplex Neuro-Exploratory panel provided by SciLifeLab, Uppsala, Sweden. Serum and CSF samples were collected from TN patients before ($n = 33$ and $n = 27$, respectively) and after ($n = 28$ and $n = 8$, respectively) microvascular decompression surgery. Additionally, we included samples from MS patients ($n = 20$) and controls ($n = 20$) for comparison.

Results: In both serum and CSF, several proteins were found increased in TN patients compared to either MS patients, controls, or both, including EIF4B, PTPN1, EREG, TBCB, PMVK, FKBP5, CD63, CRADD, BST2, CD302, CRIP2, CCL27, PPP3R1, WWP2, KLB, PLA2G10, TDGF1, SMOC1, RBKS, LTBP3, CLSTN1, NXPH1, SFRP1, HMOX2, and GGT5. The overall expression of the 92 proteins in postoperative TN samples seems to shift towards the levels of MS patients and controls in both serum and CSF, as compared to preoperative samples. Interestingly, there was no difference in protein levels between MS patients and controls.

Conclusions: We conclude that TN patients showed increased serum and CSF levels of specific proteins and that successful surgery normalizes these protein levels, highlighting its potential as an effective treatment. However, the similarity between MS and controls challenges the idea of shared pathophysiology with TN, suggesting distinct underlying mechanisms in these conditions.

Significance: This study advances our understanding of trigeminal neuralgia (TN) and its association with multiple sclerosis (MS). By analysing 92 protein

biomarkers, we identified distinctive molecular profiles in TN patients, shedding light on potential pathophysiological mechanisms. The observation that successful surgery normalizes many protein levels suggests a promising avenue for TN treatment. Furthermore, the contrasting protein patterns between TN and MS challenge prevailing assumptions of similarity between the two conditions and point to distinct pathophysiological mechanisms.

1 | INTRODUCTION

Trigeminal neuralgia (TN) is an extremely painful neurological disorder characterized by short-lasting episodes of intense stabbing pain within the distribution of one or more branches of the trigeminal nerve (CN V) (Bendtsen et al., 2020). The condition leads to severe suffering reducing the quality of life of patients (Tölle et al., 2006) and affects about twice as many women as men (Maarbjerg et al., 2014; Zakrzewska et al., 2017). The lifetime prevalence in the general population is estimated to be between 0.1% and 0.3% (Mueller et al., 2011; Sjaastad & Bakketeig, 2007), which is similar to the prevalence of other chronic neurological conditions such as multiple sclerosis (MS) and Parkinson's disease (GBD 2015 Neurological Disorders Collaborator Group, 2017). TN is classified into classical, which is the most common and is caused by neurovascular conflict (NVC) as described below; secondary, accounting for approximately 15% of cases and caused by an underlying disease, usually a tumour or MS; and idiopathic, without evidence of morphological changes to the nerve or other underlying disease (Cruccu et al., 2020; Gobel, 2021).

Mechanisms underlying TN are still poorly understood, though the leading hypothesis of classical TN pathophysiology is NVC with compression of CN V by a cerebral vessel, most frequently the superior cerebellar artery (Maarbjerg et al., 2014). This compression may cause morphological changes at the root entry zone (REZ), including demyelination, distortion, dislocation, and atrophy of CN V. The injured, demyelinated nerve fibres become hyperexcitable and start firing more easily according to the 'ignition hypothesis', which leads to severe facial pain in the corresponding nerve territory (Gambeta et al., 2020). Although an NVC is thought to be an important etiological factor, it does not seem to be the only aetiology. The NVC hypothesis is supported by evidence that surgery with microvascular decompression (MVD) is effective for the relief of symptoms (Ferguson et al., 1981; Jannetta, 1967; Zakrzewska & Akram, 2011), while it is contradicted by the fact that not all patients with NVC develop TN (Antonini et al., 2014; DeSouza et al., 2016; Maarbjerg et al., 2017). Moreover, the morphological alterations can be observed in TN patients without an NVC (Lutz et al., 2016) and be absent in patients with an NVC without TN (Lin et al., 2014). Other mechanisms are therefore likely to contribute to the development of the disorder. For example, it has been suggested that ischaemia and neuroinflammation may play a role in TN (DeSouza et al., 2014; Goadsby et al., 1988; Love & Coakham, 2001; Strittmatter et al., 1997). Arachnoiditis is often observed around the trigeminal root during surgery and is prognostically negative (Dumot et al., 2017; Sindou et al., 2007). Furthermore, abnormal functioning of ion channels, especially sodium and calcium channels, may have an impact on nerve transmission and the development of TN (Smith et al., 2021). Studies of familial TN have linked a mutation in the calcium channel TRPM7 to increased hyperexcitability that may contribute to pain (Gualdani et al., 2022).

The role of neuroinflammation in the development of TN is of particular interest. The prevalence of TN among patients with MS, a chronic autoimmune neuroinflammatory disorder is approximately 20-fold higher than the general population (Burkholder et al., 2017). TN occurs frequently and precedes the MS diagnosis in 15% of individuals (Fallata et al., 2017; Foley et al., 2013). In classical TN, there is a neurovascular compression to the nerve, whereas in TN secondary to MS, an inflammatory lesion in the trigeminal nucleus or on the proximal part of the trigeminal root is causal. This suggests that there may not necessarily be a shared pathophysiology (Noory et al., 2021). Nevertheless, the association of TN with MS remains widely unaddressed, and there are significant variations in the results of studies regarding demyelinating lesions and NVC as aetiological factors of TN in MS patients (Laakso et al., 2020; Lummel et al., 2015; Truini et al., 2016).

We have previously conducted proteomic studies to investigate the molecular mechanisms involved in the pathogenesis of TN. Recent studies by our team have identified candidate biomarkers related to neuroinflammation, demyelination, and cell death in the cerebrospinal fluid (CSF) of TN patients compared with controls (Abu Hamdeh et al., 2020; Ericson et al., 2019; Svedung Wettervik et al., 2022).The aim of this study was to analyse the expression levels of 92 protein biomarkers with the Proseek Multiplex Neuro-Exploratory panel using Proximity Extension Assay (PEA) technology provided

by SciLifeLab, Uppsala, Sweden. We explored protein expression in both CSF and serum of TN patients in relation with MS patients and controls, respectively. We also studied protein expression in both CSF and serum before and after MVD surgery.

2 | MATERIALS AND METHODS

2.1 | TN patients and the study design

Patients who were referred to the Department of Neurosurgery at Uppsala University Hospital as candidates for surgical treatment with MVD between 2015 and 2017 were included (Figure S1). All the patients were assessed for classical TN according to the beta version of the International Classification of Headache disorders, third edition (Headache classification Committee of the International Headache Society (IHS), 2013). Patients without surgical contraindications, who fulfilled the criteria for TN with NVC and morphological changes (e.g. atrophy or displacement) in the nerve root (classical TN), or without morphological changes in the nerve (idiopathic TN) but still considered to benefit from surgery by the attending surgeon were scheduled for the surgical procedure and invited to participate in the study. All study participants provided written consent after both oral and written information. The study was conducted in accordance with the Helsinki Declaration and was approved by the Regional Ethical Review Committee of Uppsala, Sweden (DNR 2014-178).

2.2 | MS patients and controls

Two groups of participants composed of volunteers from Sweden were used in the current study (Figure S1). One group consisted of participants seeking medical care for neurological symptoms but with normal investigation, referred to as controls. The second group consisted of patients with MS but no other neurological disorders. No MS patients had co-existing TN, and vice versa, among TN patients. Both lumbar CSF and serum samples were collected and analysed in the study. The collection of samples for both groups was approved by the Regional Ethical Review Committee of Uppsala, Sweden (DNR 2023-01368-01).

2.3 | Sample collection

Spinal CSF was collected from 27 TN patients the day before surgery by a standardized lumbar puncture procedure without anaesthesia using a 25- or a 27-gauge needle. The CSF was collected by free flow in sterile test tubes and stored in −80°C freezer until analysis. For 8 of the 27 TN patients, spinal CSF was also collected 10–30 months postoperatively. The postoperative sample collection was not initially planned during patient recruitment, causing a delay in obtaining them. The collection of 20 spinal CSF samples from each of the MS and control groups was handled according to the same protocol.

Serum samples were also collected from both TN patients and each of the MS and control groups. For TN patients, serum samples were collected from 33 patients preoperatively the day before surgery and from 28 patients at 10–30 months postoperatively. For the MS and control groups, 20 serum samples were collected from each group.

2.4 | Proximity extension assay

We conducted a multiplexed PEA using high-throughput Olink Proseek Multiplex Neuro-Exploratory 96×96 kits which measures 92 protein biomarkers simultaneously. The proteins included in the Neuro-Exploratory panel are functionally associated with different neurological disorders, and they are linked to a broad range of biological processes such as cellular metabolic processes, immune response, inflammatory response, and response to stress (Table S19). Both CSF and serum were analysed on the same occasion, and the samples were randomly distributed over plates in a balanced manner with respect to group affiliation.

The Olink methodology has been described in detail on the company's website and in a previous study by our team (Ericson et al., 2019). Briefly, a mixture of 1 μL of CSF or serum and a 3-μL incubation mix was incubated at 8°C overnight. Then, a 96-μL extension mix with PEA enzyme and PCR reagents was added and incubated for 5 min at room temperature. Afterwards, an extension reaction in a thermal cycler was performed followed by 17 cycles of DNA amplification. In the next step, 92 oligonucleotide-labelled pairs of antibodies were allowed to bind to their respective target protein in the sample. Once the antibody probes bound to the targeted protein and the attached DNA oligonucleotides were in close proximity, the oligonucleotides hybridized and were extended by enzymatic polymerization. The oligonucleotide templates were then amplified and quantified using real-time polymerase chain reaction (PCR). The generated data obtained by real-time PCR are expressed as Normalized Protein eXpression (NPX) on a log2 scale. The NPX values represent relative protein

values where a larger number represents a higher protein level in the sample.

2.5 | Statistical/data analyses

To investigate global differences between TN patients and each of the MS and control groups, principal component analysis (PCA) was used to overview the pattern of biomarkers related to each group. Four different groups of samples were compared with each other, including TN patients before surgery (referred to as TN-pre-surgery), TN patients after surgery (referred to as TN-post-surgery), MS group and controls. Only protein biomarkers with more than 80% detectable values were included in the analysis which was performed in GraphPad Prism.

To explore the differences in protein biomarker expression levels between TN patients and controls, linear regression models were fit independently for each biomarker with NPX values as the dependent variable, adjusted for age and sex. Separate models were created for CSF and serum samples to investigate the differences in expression levels between both TN-pre-surgery and TN-post-surgery in relation to each of the MS and control groups. We also investigated the differences in expression levels between MS patients and controls. To account for multiple testing, the Bonferroni method was used implying a significance threshold of $0.05/25 = 0.002$ for CSF samples and $0.05/48 = 0.001$ for serum samples.

To investigate the differences in concentration levels before and after surgery, TN-post-surgery was compared with TN-pre-surgery using linear mixed models, with patient ID as random effect, adjusted for age and sex. Separate models were created for both CSF and serum samples with a p-value of $0.05/25 = 0.002$ for CSF samples and $0.05/48 = 0.001$ for serum samples.

In both linear regression analyses and linear mixed model analysis, only biomarkers with more than 80% detectable values were included. All the analyses were conducted using IBM SPSS Statistics version 26.0 (IBM SPSS, Armonk, NY, USA) for Windows.

2.6 | Network and enrichment analysis

To perform the network analysis, we used a publicly available database STRING to download protein–protein interactions. We used only physical interactions from experimental data with a default threshold confidence score (0.400). We downloaded the top 50 1st shell interactions for proteins, while secondary interactions were not analysed. Visualization of the graph networks for all figures has been performed, utilizing the R package '*visNetwork*' and custom R scripts. Enrichment analyses have been performed with the R package 'clusterProfiler' and R scripts for data preparation (Yu et al., 2012). Entrez gene IDs for enrichment analysis were obtained from the NIH gene portal (Brown et al., 2015).

3 | RESULTS

3.1 | Demographic and clinical data

Demographic details of the TN, MS, and controls are presented in Table 1. The study encompassed 27 TN patients, among whom 19 were identified as classic and 8 as idiopathic according to the beta version of the International Classification of Headache Disorders, third edition, published in 2018 (Headache classification Committee of the International Headache Society (IHS), 2013). After the initial follow-up at 6 weeks after surgery, 93% (25 out of 27) of the patients reported being pain-free without medication according to the Barrow Neurological Institute (BNI) pain scale. Six months later, 78% (21 patients) maintained the BNI grade I classification. When postoperative CSF samples were collected from 8 patients, 10–30 months after their surgery, 6 of them (75%) remained pain-free without medication (BNI grade I). The remaining 2 patients showed significant improvement compared to their pre-surgery condition, with one not requiring medication (BNI grade II) and the other effectively managing her pain with medication (BNI grade III).

The two other groups of participants encompassed 20 MS patients and 20 controls. Within the MS group, there was a higher proportion of women than men (13/7), while the control group had fewer women than men (6/14), similar to the distribution observed among TN patients (11/16). Furthermore, the controls shared the same average age as the TN patients (63 years), while the MS group had a lower average age (51 years). The average disease duration for the MS patients was 4.6 years, and seven out of 20 had active MS lesions as indicated by Gadolinium enhancing lesions at the time of sampling. Only five MS patients were receiving medication, including Rituximab (2 patients), Copaxone, Dimethyl fumarate (DMF), and Tysabri, while the remaining 15 patients were enrolled in the study at the diagnostic stage before starting any medication. These five MS patients who were medicated had a longer duration of progressive MS, and they underwent the lumbar puncture later to measure CSF neurofilament due to their active disease.

TABLE 1 Demographic characteristics of the final sample populations of controls, MS patients and TN patients.

Variable	Controls ($N = 20$)	MS patients ($N = 20$)	TN patients ($N = 27$)
Age (years)	63	51	63
Sex (female/male)	6/14	13/7	11/16
BMI	*	*	27.7
Tobacco use	*	*	11
Alcohol use	*	*	18
TN diagnosis (classical/idiopathic)	*	*	19/8
ASA			
10	*	*	6
20	*	*	18
30	*	*	3
High blood pressure	*	*	14
Diabetes	*	*	0
Cardiovascular disease			
IHD	*	*	3
Stroke	*	*	2
Peripheral	*	*	1
Cancer	*	*	3
Pain (other than TN)			
Fibromyalgia	*	*	2
Osteoarthritis	*	*	1
Low back pain	*	*	1
Rheumatoid arthritis	*	*	1
MS parameters			
Gadolinium enhancing lesions at sample time	*	7	*
Disease duration (years)	*	4.6	*
EDSS score (mean/median)	*	2.7/2	*
Medication	*	5	*

Abbreviations: ASA, American Society of Anesthesiologists Score; BMI, body mass index; EDSS, Expanded Disability Status Scale; IHD, ischaemic heart disease; MS, multiple sclerosis; *N*, number of patients; TN, trigeminal neuralgia.

*Data not available.

3.2 | Principal component analysis

Out of the 92 protein biomarkers measured using the Proseek Multiplex Neuro-Exploratory panel, 48 proteins were detected in more than 80% of serum samples and 25 proteins were detected in more than 80% of CSF samples. Therefore, 44 and 67 proteins were excluded from the analyses in serum and CSF, respectively.

We sought to overview the protein expression levels of all TN patients and control samples in both serum and CSF using PCA with PC scores and corresponding loadings (Figure 1a–d). A high PC1 score can be interpreted as overall high levels of protein expression. As illustrated by the score plot in Figure 1b, the PCA analysis of serum biomarker concentrations showed that TN patients before surgery in general had lower levels of protein biomarkers than both MS patients and controls that seemed to have similar levels. Samples from TN patients after surgery moved longitudinally from preoperative levels towards levels in the control samples.

The results were similar for CSF samples as illustrated by the score plot in Figure 1d. TN patients had overall lower levels of protein expression compared to both MS patients and controls which had similar levels. After surgery, there was again a shift in expression levels in the same direction as serum samples towards levels in the groups of MS patients and controls. However, unlike the serum samples, the variation between the CSF samples was smaller.

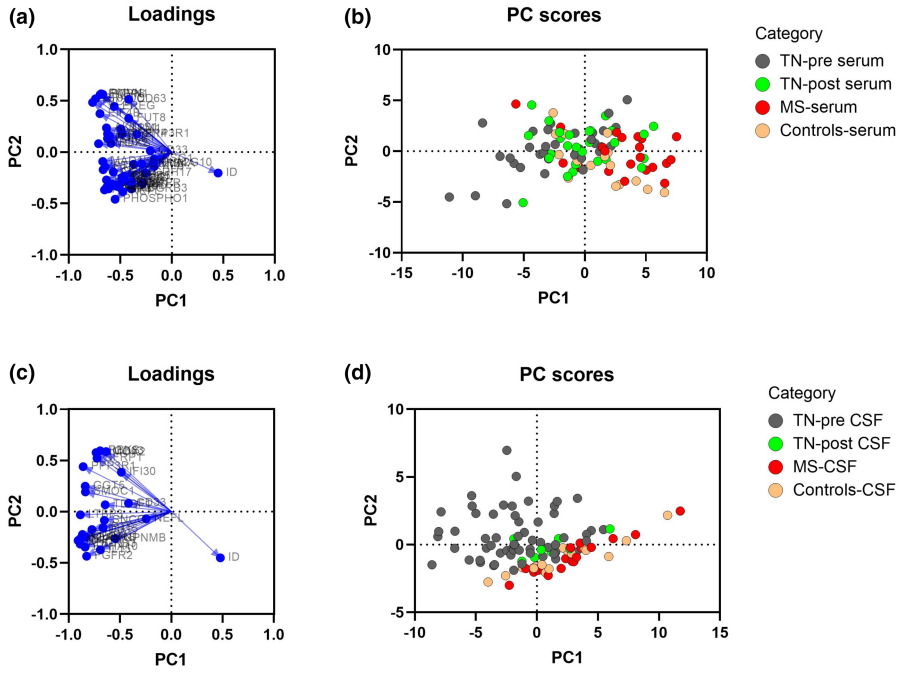

FIGURE 1 Principal component analysis (PCA) loadings and score plots. The PCA is based on preoperative (TN-pre) and postoperative (TN-post) trigeminal neuralgia (TN) samples, as well as multiple sclerosis (MS) and control samples in serum (a, b) and CSF (c, d).

3.3 | Linear regression analysis of the levels of protein expression

We conducted linear regression models, adjusted for age and sex, to investigate the differences in biomarker concentrations between TN patients and each of the MS and control groups, between the MS patients and controls and before and after MVD surgery in TN patients.

At a significance level of 0.05/48 = 0.001 for serum samples, seven proteins (EIF4B, PTPN1, EREG, TBCB, PMVK, FKBP5, and CD63) were significantly altered between TN-pre-surgery and controls, with all seven proteins significantly higher in TN patients (Table S1). Compared to the MS patients, 11 proteins (EIF4B, CRADD, BST2, PTPN1, EREG, CD302, CRIP2, CCL27, PMVK, FKBP5, and CD63) were significantly altered, with all proteins significantly higher in TN-pre-surgery and six (EIF4B, PTPN1, EREG, PMVK, FKBP5, and CD63) of the 11 proteins overlapping with the controls (Table S2). Except for one protein (EREG), the protein levels observed after surgery showed no significant difference between the TN-post-surgery and either the MS patients or controls (Table S3 and S4). EREG was higher in TN patients before surgery compared to both MS patients and controls but the level had decreased after surgery. No proteins showed significant differences in concentration level between the MS patients and controls (Table S5).

At a significance level of 0.05/25 = 0.002 for CSF samples, nine proteins (TDGF1, SMOC1, RBKS, LTBP3, CLSTN1, NXPH1, SFRP1, HMOX2, and GGT5) were significantly altered in TN-pre-surgery compared to controls, with all proteins significantly higher in TN-pre-surgery and without an overlap with the corresponding serum groups (Table S6). Compared to MS patients, ten proteins (TDGF1, SMOC1, RBKS, LTBP3, NXPH1, SFRP1, HMOX2, PPP3R1, GGT5, and CD63) were significantly higher in TN-pre-surgery, and one of these biomarkers (CD63) was found to overlap with both MS patients and controls in serum (Table S7). There were no significant differences in protein levels between either TN-post-surgery and any of the MS or control group (Tables S8 and S9), or between the MS and control groups themselves (Table S10).

3.4 | Linear mixed models with the patients as their own controls

To investigate the differences in protein levels in TN-post-surgery compared to TN-pre-surgery, we performed separate linear mixed models in both serum and CSF, adjusted for age and sex, and with patient ID as random effect. In serum, five proteins (KLB, CD302, PLA2G10, PMVK, and WWP2) were significantly lower after surgery than before surgery while one protein (PPP3R1) was significantly higher (Table S11). In CSF, no protein showed significantly altered level after surgery (Table S12).

3.5 | Sensitivity analyses

To investigate potential differences in protein levels among the two subgroups of TN patients, that is, classical and idiopathic, separate linear regression models were conducted for both serum and CSF samples before and after surgery. None of the analyses revealed significant differences in protein levels based on subgroup (Tables S13–S16).

Additionally, linear regression models were employed to examine differences in protein levels in both serum and CSF between patients with BNI grade I and those reporting more pain after surgery, classified as BNI grade >I. The results indicated no significant differences in protein levels after surgery in relation to the pain level (Tables S17 and S18).

3.6 | Network and enrichment analysis

Protein biomarkers that were statistically significant in both serum and CSF from both linear regression and linear mixed model analyses and overlapping proteins between different groups are shown in Figure 2. Network analysis was conducted using STRING (Szklarczyk et al., 2015) to explore any physical or functional interactions between the significant proteins and other proteins. As illustrated in Figures 3

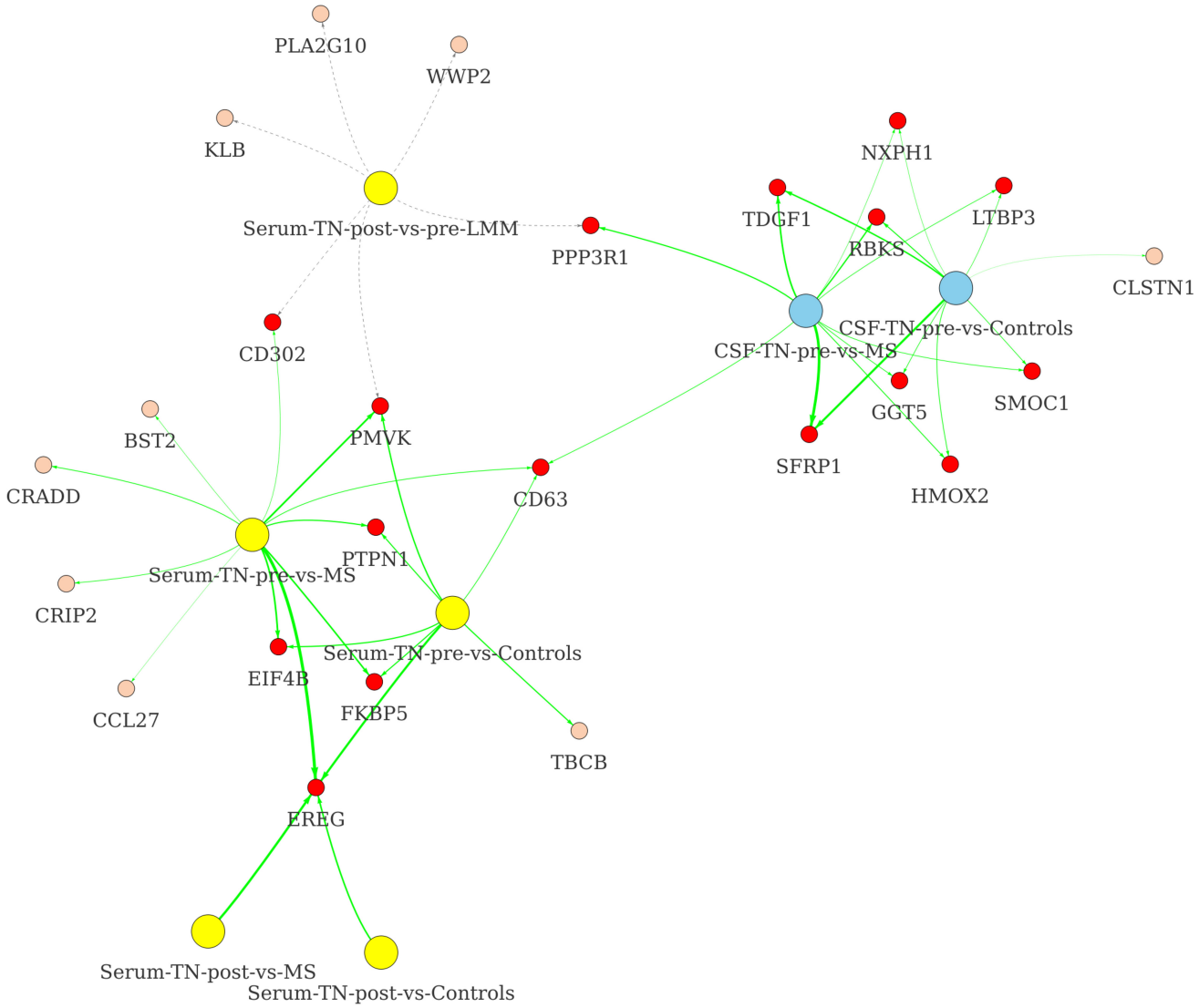

FIGURE 2 Network graph of differentially expressed proteins. This figure summarizes the results for differential expression analysis based on the sample source. Yellow and blue nodes indicate the sample source (serum or CSF), whether a sample was obtained before or after surgical intervention, and whether it comes from MS patients or controls. Beige and red nodes indicate investigated proteins. Red nodes indicate proteins that were significant in more than one sample source. The size of the arrows is proportional to the effect size (beta coefficient). Dashed arrows indicate a linear mixed model where effect size data is not available. CSF, cerebrospinal fluid.

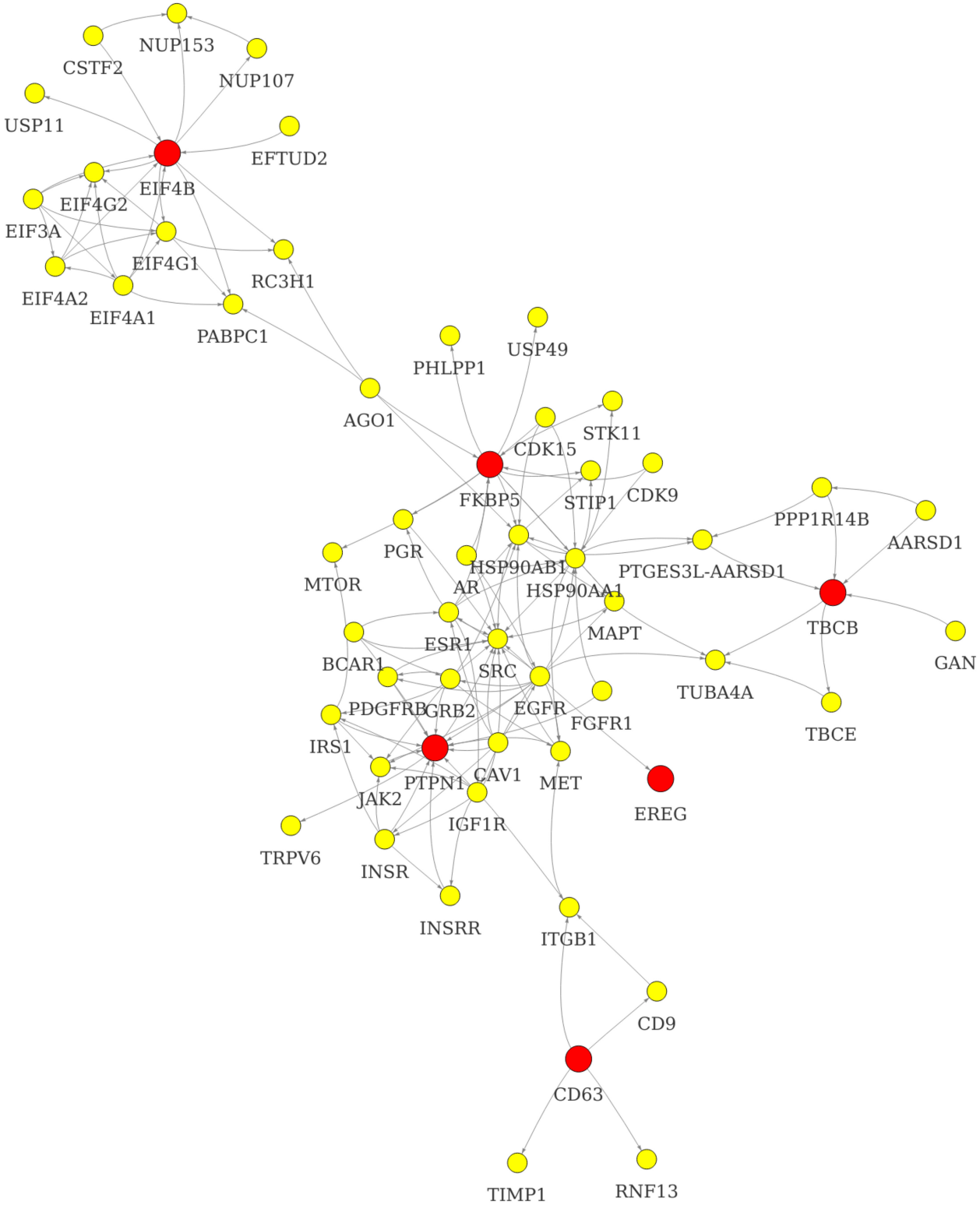

FIGURE 3 Interaction partners for serum proteins. This network graph shows protein interactions for significant proteins identified in serum samples. Red colour nodes show investigated proteins. Yellow colour nodes show other proteins.

and 4 for serum and CSF, respectively, there were no direct interactions between the proteins. However, some indirect interactions can be seen through other mediating proteins.

To investigate whether any specific biological pathways were over-represented, separate enrichment analyses were performed both in serum and CSF for the significant proteins, with proteins from the PEA analysis that

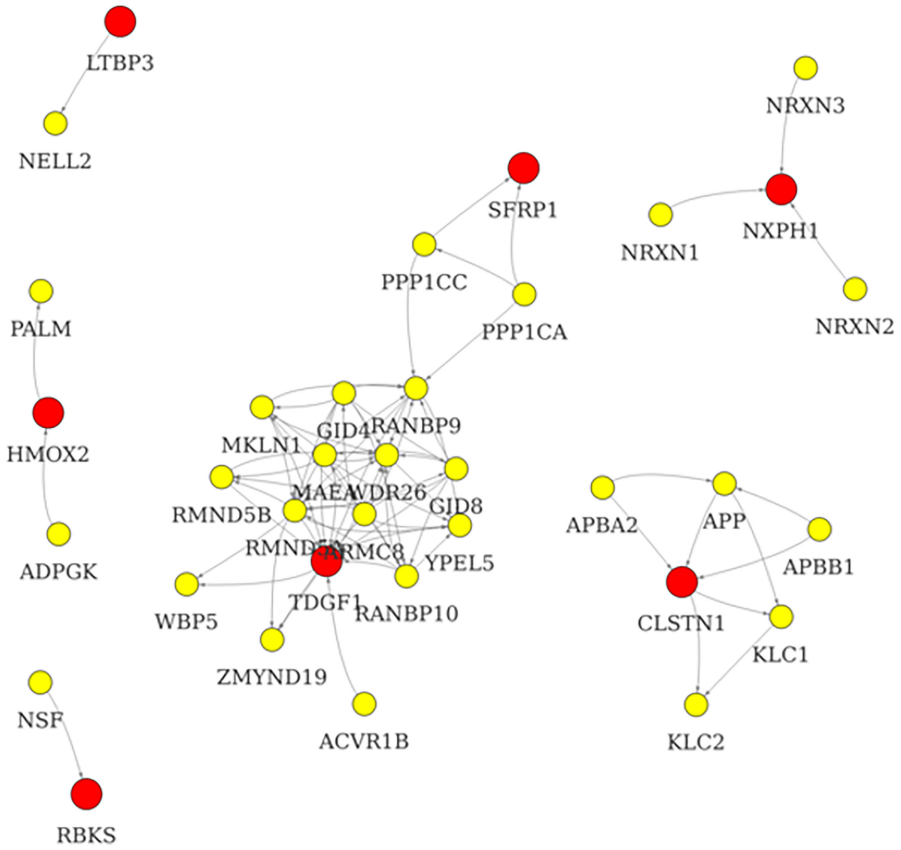

FIGURE 4 Interaction partners for CSF proteins. This network graph shows protein interactions for significant proteins identified in CSF samples. Red colour nodes show investigated proteins. Yellow colour nodes show other proteins. CSF, cerebrospinal fluid.

were detected in more than 80% of the samples, as reference. The enrichment analyses showed no significant results (Tables S14 and S15). Thus, there were no over-represented pathways with reference to the different biological pathways represented by the proteins included in the Proseek Multiplex Neuro-Exploratory panel.

4 | DISCUSSION

In this exploratory study, we analysed the expression levels of 92 protein biomarkers in both CSF and serum of TN patients compared to both MS patients and controls. In both serum and CSF, several proteins were found increased in TN patients compared to either MS patients, controls, or both, including EIF4B, PTPN1, EREG, TBCB, PMVK, FKBP5, CD63, CRADD, BST2, CD302, CRIP2, CCL27, PPP3R1, WWP2, KLB, PLA2G10, TDGF1, SMOC1, RBKS, LTBP3, CLSTN1, NXPH1, SFRP1, HMOX2, and GGT5. While the relation of most proteins to neuropathology remains poorly understood, some of them may provide novel insight into the underlying mechanisms. Among the proteins identified, SFRP1, FKBP5, and TBCB seem to be particularly interesting in the context of TN and MS. While previous studies have reported their involvement in distinct mechanistic pathways, these findings shed new light on their potential implications for the neuropathological basis of TN and MS.

SFRP1 levels were 2.43-fold higher in CSF from TN patients compared to MS patients and 1.90-fold higher compared to controls. SFRP1 is a multifunctional regulator of cell-to-cell communication, mainly modulating Wnt signalling pathways (Esteve & Bovolenta, 2010), which is one of the most common cell–cell communication pathways in development and tissue homoeostasis (Logan & Nusse, 2004). It also inhibits ADAM10 activity (Esteve et al., 2011), which is relevant for neurodegeneration (Lichtenthaler et al., 2018; Saftig & Lichtenthaler, 2015). SFRP1 is down-regulated in the adult brain (Augustine et al., 2001; Esteve et al., 2019; Zhang et al., 2016) but upregulated in many inflammatory conditions including periodontitis, pulmonary emphysema, and rheumatoid arthritis (Claudel et al., 2019; Esteve & Bovolenta, 2010). The upregulation of SFRP1 has also been linked to the low-grade chronic inflammation in the aged brain (Folke et al., 2019; Youm et al., 2013). In mouse models of acute and chronic neuroinflammation, SFRP1 mediates astrocyte-microglia cross-talk and promotes chronic neuroinflammation by maintaining microglial activation (Rueda-Carrasco et al., 2021). Our findings about SFRP1

are consistent with previous evidence of neuroinflammation in neuropathic pain conditions such as TN (Bäckryd, Lind, et al., 2017; Bäckryd, Tanum, et al., 2017; Ericson et al., 2019; Svedung Wettervik et al., 2022) and align with evidence of neuroinflammation in TN. Further studies are needed to establish causality and explore the therapeutic potential by targeting SFRP1 pathways to mitigate chronic inflammation in TN and other diseases.

Another interesting biomarker is FKBP5, whose levels were 1.38-fold higher in the serum of TN patients compared to MS patients and 0.97-fold lower compared to controls. Its most well-known mechanistic functions have been described in the context of immunoregulation and regulation of basic cellular processes and protein activity (Hähle et al., 2019). FKBP5 is known to modulate AKT and glucocorticoid signalling pathways in response to acute or chronic stress (Daneri-Becerra et al., 2019; Fries et al., 2017; Häusl et al., 2019; Yu et al., 2017). Dysregulation of glucocorticoid receptor (GR) signalling, influenced by FKBP5 competition with glucocorticoids, is associated with various medical conditions, including anxiety (Minelli et al., 2013), depression (Binder et al., 2004; Han et al., 2017), and the effects of early trauma and stress in general (Ising et al., 2008; Lessard & Holman, 2014; Yeo et al., 2017). FKBP5 also acts as a co-chaperone in the heat shock protein 90 (HSP90) machinery critical for stress regulation (Schiene-Fischer & Yu, 2001). Its interaction with HSP90 has implications in the pathogenesis of Alzheimer's disease by affecting tau degradation and accumulation (Blair et al., 2013). Given the evidence linking FKBP5 dysregulation to stress-related phenotypes, it may reflect the effects of chronic stress in TN pathology. There is both a direct mechanical stress resulting from the NVC, but also the significant amount of stress related to chronic pain.

TBCB levels were 1.02-fold higher in the serum of TN patients compared to controls. TBCB, which is involved in microtubule dynamics, is one of five conserved tubulin-folding cofactors (TBCA to TBCE) that regulates microtubule homeostasis by controlling the composition of tubulin heterodimers (Lewis et al., 1997; Serna et al., 2015). The physiological dimerization of TBCB and TBCE has been associated with dissociation of the microtubule cytoskeleton, forming the core of a neurodegenerative disease pathway that leads to the neurodegenerative disease amyotrophic lateral sclerosis (Kortazar et al., 2007). Interestingly, both downregulation and reduction of TBCE levels or overexpression and increased TBCB function have been linked to neurodegeneration. Reduced gene function caused by a hypomorphic TBCE mutation has been linked to the neurodevelopmental disorder spinal muscular atrophy (Sferra et al., 2016). In mice, the loss of TBCE protein expression caused by a genetic mutation resulted in progressive motor neuropathy (Martin et al., 2002; Schäfer et al., 2017). On the other hand, excess of TBCB has been associated with axonal growth cone abnormalities with axonal retraction followed by neuronal degeneration (Lopez-Fanarraga et al., 2007). Our findings about the increased serum levels of TBCB in TN patients may indicate a neurodegenerative process in TN pathophysiology.

A noteworthy finding in our results is the resemblance between the MS and control groups, as MS patients showed no distinct expression patterns in CSF or serum compared to controls. While it could be speculated that medication could be responsible for shifting the proteome pattern of MS patients towards that of controls, our data indicate that only a few MS patients were on medication, and they were all in active disease. Most of the patients included in the study were at the diagnostic stage with an active disease before medication was initiated. Patients who were already on medication at the time of CSF sampling were also in active MS as they were selected for CSF neurofilament analysis, a common practice at the neurology department of our hospital for relapsing MS patients. Hence, we can reasonably conclude that the disparity in protein levels between the MS group and TN patients, as well as their resemblance to the controls, was unrelated to medication. Alternatively, the observed results may be influenced by the matching process based on age and sex. Furthermore, both the MS and control groups included individuals with other non-neurological diseases, which may also have potential implications. Both the similarities in protein levels between MS and controls, and the differences between TN and MS, challenge the hypothesis of shared pathophysiology between TN and MS, suggesting that they may be distinct entities with separate pathophysiological mechanisms. The inclusion of MS patients without TN helped minimize potential overlaps between these two conditions, allowing us to better isolate differences in protein levels between TN, primarily caused by NVC, and MS as a distinct condition.

Finally, our study results indicated that, preoperatively, TN patients had a higher overall expression of the 92 proteins analysed in the panel in both CSF and serum compared to both MS patients and controls. These protein levels were normalized postoperatively as we could not detect any significant difference in their expression between operated TN patients and neither MS patients nor controls after surgery. Successful surgery may directly impact the proteome pattern, leading to postoperative normalization of protein levels. This is consistent with earlier findings from our team, indicating the potential efficacy of surgery in

treating medically refractory TN (Ericson et al., 2019). Additionally, our sensitivity analyses revealed no difference in protein levels between the subgroups of TN patients, supporting a recent finding by our team that suggests patients with idiopathic TN, though to a lesser extent, also experience an effect from surgery (Loayza et al., 2023). Furthermore, we also showed that the patterns of expression were similar in both serum and CSF as significant differences were existing in both CSF and serum before but not after surgery, suggesting that serum levels could be valuable for disease monitoring. If further evidence confirms the correlation between serum and CSF levels, clinicians could potentially utilize a blood sample instead of invasive CSF collection procedures.

4.1 | Methodological consideration

There are several limitations that should be considered in future studies. First, the study is limited by a low number of study participants, which in combination with the high quantity of proteins examined, increases the risk of false positive findings. Despite efforts to match participants using all available data at our department, finding perfectly matched control groups based on age and sex proved challenging. In addition, TN and MS patients commonly take medications that can possibly affect the proteome pattern and may have confounded our results. Patients with TN were receiving anti-epileptic medications before surgery, and their post-surgery regimen involved either reduced or no use of such drugs. The discontinuation of anti-epileptics may have influenced the observed changes in protein levels after surgery. Additionally, other factors, including reduced suffering from pain, improved mood, sleep, and overall quality of life, may impact the post-surgery protein levels. It was not possible to adjust for these variables in the current study and the results should be interpreted with awareness of these potential confounding factors.

It is also important to consider that all TN patients that were included in the study were candidates for surgical treatment, which causes a selection bias and limits the possibility to draw general conclusions about the broad group of TN patients with possibly different proteome patterns. Moreover, there are some limitations associated with the PEA technology which can potentially be subject to measurement bias. For our study, we opted for the neuro-exploratory panel, as it contained numerous proteins that are functionally linked to various neurological disorders. However, the panel is limited to 92 proteins, and thus, there may be other relevant markers that were not included in the analysis. Despite those limitations, we found interesting biomarkers, with similar expression patterns in both serum and CSF, that may be implicated in the pathophysiology of TN or MS.

5 | CONCLUSIONS

In conclusion, our study identified specific protein biomarkers with similar expression patterns in serum and CSF, potentially relevant to the pathophysiology of TN and MS. We found increased levels of SFRP1, FKBP5, and TBCB in TN patients, suggesting involvement in neuroinflammation, chronic stress, and neurodegeneration, respectively. In addition, successful surgery normalized protein levels in TN patients, indicating treatment potential. The resemblance between MS and controls challenges the hypothesis of shared pathophysiology between TN and MS, suggesting distinct mechanisms. These findings contribute to our understanding of TN and MS and may guide future research and therapeutic strategies.

AUTHOR CONTRIBUTIONS

Muataz S. Lafta: conceptualization, data curation, data analysis, and writing—original draft preparation. **Aleksandr V. Sokolov**: data analysis (network and enrichment analysis), writing—methods related to network and enrichment analysis, and reviewing. **Anne-Marie Landtblom**: conceptualization, data interpretation, and writing—reviewing and editing. **Gull Rukh**: data analysis (linear mixed models) and writing—reviewing. **Hans Ericson**: conceptualization and writing—reviewing and editing. **Helgi B. Schiöth**: conceptualization, resources, supervision, and writing—reviewing and editing. **Sami Abu Hamdeh**: conceptualization, data curation, data analysis, supervision, and writing—reviewing and editing.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest. The preliminary results of this study were presented at the Walter E. Dandy 9th World Congress, January 2023, Jaipur, India.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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