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Original article

Evolution of tongue somatosensory evoked potentials in people with multiple sclerosis

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ABSTRACT

Introduction: The aim of the present study was to investigate the long-term evolution of tongue somatosensory evoked potentials (tSSEP) in people with multiple sclerosis (pwMS).
Methods: Out of initial 121 participants, after two-year follow-up, the data were available for 74 and after four-year follow-up for 58 pwMS. In all pwMS complete neurological examination, brain MRI, cervical spinal cord MRI (if available) and tSSEP were performed at baseline visit (M0). Complete neurological examination and tSSEP were performed 2 and 4 years later (M24 and M48). tSSEP results were interpreted in the form of ordinal tSSEP score and quantitative tSSEP zscore calculated from the sum of z-transformed tSSEP latencies.
Results: Differences in tSSEP scores and tSSEP zscores in three different timepoints showed significant worsening of both scores over time. For the tSSEP score the difference was significant for M0-M24 and M0-M48 visits, but not for M24-M48 visits. For the tSSEP zscore the difference was significant for M0-M48 and M24-M48 visits, but not for M0-M24 visits. The only significant negative predictor found for the tSSEP score improvement was presence of cervical spinal cord lesions on the MRI. A moderate to high correlation was observed between both forms of tSSEP score at all three timepoints.
Conclusion: This study demonstrates a significant deterioration of trigeminal sensory pathway in MS over time, giving further insight into trigeminal system damage in pwMS.

1. Introduction

Trigeminal somatosensory evoked potentials (TSSEPs) are used to evaluate the function of the trigeminal pathway from the periphery to the somatosensory cortex. Although first described in 1970's, this modality of the evoked potentials is not in widespread clinical use (Larsson and Prevec, 1970). Neuroanatomical structure examined by this method is trigeminal pathway, starting with trigeminal nerve (V1, V2, or V3 branches depending on the site of stimulation), which conducts the signal to the trigeminal nuclei in the brainstem, and then crosses the side within the trigeminal lemniscus, accessing the thalamic nuclei and finally projects to the ipsilateral cerebral cortex. Damage to any of these aforementioned structures of the trigeminal pathway may lead to changes in the TSSEPs in the form of delayed latencies, changes in the morphology of the response or a conduction block (Altenmüller et al., 1990). One of the reasons why TSSEPs are not in widespread clinical use, is that there are numerous protocols with different modalities, locations and frequencies of stimulation, and different electrode positions to record the response (Habek, 2013).

Only a small number of studies have been published examining the role of TSSEPs in people with multiple sclerosis (pwMS) (Bergamaschi et al., 1994, Cosi et al., 1989, Soustiel et al., 1996, Gabelić et al., 2013, Koutsis et al., 2016, KrbotSkorić et al., 2016). One of the variants of TSSEPs are tongue somatosensory evoked potentials (tSSEP), which have shown excellent sensitivity in examining the afferent trigeminal pathway, without the myogenic contamination of recorded responses, which is more pronounced when using other TSSEPs (Altenmüller et al., 1990). In our previous work we have shown that tSSEP latencies were significantly prolonged in pwMS compared to healthy controls, and that tSSEP could detect afferent trigeminal pathway damage in a greater percentage of subjects than clinical examination or brain MRI (Gabelić et al., 2013).

The aim of the present study was to investigate the long-term evolution of tSSEP in a cohort of pwMS.

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2. Methods

2.1. Patients

This longitudinal cohort study was performed at the Referral Center for the Autonomic Nervous System Disorders of the Department of Neurology, University Hospital Center Zagreb. Consecutive persons who presented with an acute or subacute episode of neurological dysfunction due to inflammatory demyelination that lasted more than 24 h and occurred in the absence of fever, infection, or encephalopathy, and with at least one demyelinating lesion larger than 3 mm on brain and/or spinal cord MRI were enrolled into the study from August 1st 2014 until the 1st of February 2016. Baseline data on initial 121 patients have previously been reported (KrbotSkorić et al., 2016). Exclusion criteria included corticosteroids within 30 days prior to testing, dental braces and any other piercing in the tongue or lips which could not be removed during testing.

In all patients complete neurological examination, brain MRI, cervical spinal cord MRI (if available) and tSSEP were performed at baseline visit (M0). Complete neurological examination and tSSEP were performed 2 years later (M24 visit) from August 1st 2016 until the 1st of February 2018 and 4 years later (M48 visit) from March 1st 2019 until February 1st 2020.

Ethical committees of the University Hospital Center Zagreb and University of Zagreb, School of Medicine approved the study. All participants signed informed consent.

2.2. Tongue somatosensory evoked potentials

Electrical stimulation with frequency of 3 Hz and duration of 0.2 ms was produced with the constant current stimulator Twister [Germany] and delivered through the modified EEG electrodes, which were applied on the lateral side of the 2/3 of the tongue. In order to avoid stimulation artifacts, the polarity of the stimulation was alternating. Stimulation was performed in the sets of 300 trials and repeated twice on each side of the tongue in order to confirm the reproducibility of the evoked response.

For each participant, the perceptive threshold was determined at the beginning of the recordings, and during the recordings, the stimulation intensity was set at three times the perceptive threshold.

Evoked cortical response was registered from four surface Ag/AgCl disk electrodes situated according to International 10/20 system. Active electrodes were situated on the contralateral side of the scalp (C5 – stimulation right side of the tongue and C6 – stimulation of the left side of the tongue) and referred to the frontal Fz electrode, while vertex electrode, Cz, was used as a ground. Recordings were performed with Brain Products Vision Recorder [Germany] and analyzed with Brain Products Vision Analyzer [Germany]. Sampling rate was 5000 Hz and during the recording signals were filtered with bandpass filter from 0.1 to 1000 Hz. Recorded signals were divided in segments of 70 ms duration (20 ms before stimulus and 50 ms after stimulus) and averaged for each set of stimulation. From two averaged segments for the same side, the grand average was computed and used for further analysis.

Major components of the tSSEP score are wave N1, P1 and N2 (Fig. 1). The information about the latency of the P1 wave, the conduction block and the morphology of the N1, P1 and N2 were analyzed. For the analysis of the P1 latency, for each measurement (M0, M24, M48) the max value of the P1 latency in this sample of patients was determined and set as P1 latency for all patients who had conduction block, because for those patients the latency of P1 wave was missing.

For the purpose of analysis, two forms of the tSSEP score were calculated:

a) Ordinal tSSEP score was calculated as previously published (Crnošija et al., 2019), where tSSEP score was calculated from the raw data compared to the normative values of the laboratory (normal response = 0, prolonged latency = 1, irregular morphology = 2 and conduction block = 3). tSSEP score was calculated for each side and summed in overall tSSEP score (values from 0 to 6).
   Latencies were considered prolonged if there was an increase in more than 2.5 standard deviations compared to the mean value of the laboratory. Irregular morphology was defined if waves N1 or N2 were missing. The missing of the major component P1 was defined as the conduction block. tSSEP score was calculated for each time point (M0, M24 and M48)
   Worsening of the tSSEP score at M24/M48 was defined if the value of tSSEP score at M24/M48 was higher than the value at the baseline.
   Improvement of the tSSEP score at M24/M48 was defined if the value of tSSEP score at the M24/M48 was lower than the value at the baseline.
b) tSSEP zscore was calculated based on the mean and standard deviation (SD) of the P1 latency of the baseline cohort of the patients, separate for the left and the right side. For every value of P1 latency, the zscore was calculated
   $((\text{value-mean}_{\text{baseline}})/\text{SD}_{\text{baseline}})$.
   For each patient the zscore for the right and the left side were averaged in overall score in specific time point (baseline, M24, M48).
   Worsening of the tSSEP zscore at M24/M48 was defined if the value of the tSSEP score at M24/M48 was higher than the value at the baseline for 0.5 or more.
   Improvement of the tSSEP zscore at M24/M48 was defined if the value of the tSSEP score at M24/M48 was lower than the value at the baseline for 0.5 or more.

2.3. Outcomes

The primary aim was to determine the evolution of tSSEP abnormalities in pwMS over four-year follow-up period in three time points (baseline – M24 – M48). tSSEP abnormalities were analyzed in two ways: raw data (latencies, abnormal morphology and conduction block) and in the form of tSSEP scores calculated in two different ways as described above.

Secondary aims were:

1 To determine factors predicting worsening and improvement of tSSEP abnormalities over time.
2 To compare two different ways of calculation of the tSSEP score and their ability to monitor involvement of trigeminal system in pwMS.

2.4. Statistical analysis

Statistical analysis was performed using the IBM SPSS software, version 25. The Kolmogorov–Smirnov test was applied to test whether the data have a normal distribution. Differences in the distribution of qualitative variables were determined with the $\chi 2$ test and McNemar test, while the differences in quantitative variables were determined with the use of parametric independent sample *t*-test or paired *t*-test, and nonparametric Mann–Whitney test or Wilcoxon test. Spearman's coefficient was used to determine if there is a significant correlation between the different forms of the tSSEP score calculation. Univariable and multivariable logistic regression analysis was performed in order to asses which variables are statistically significant predictors for worsening or improvement of tSSEP score and zscore. P values less than 0.05 were considered as significant.

3. Results

Baseline demographic, clinical, MRI and neurophysiological characteristics of the M0 cohort, and cohorts of patients who completed

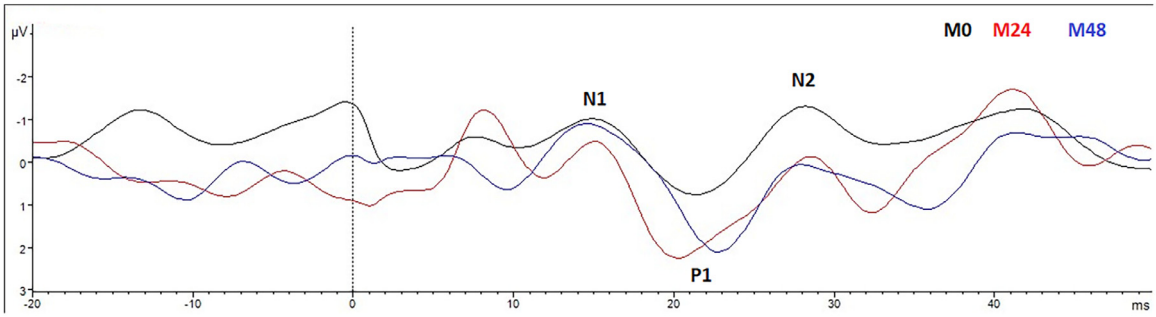

Fig. 1. An example of tracings of tSSEP recordings for the stimulation of the right side from one participant at all three time points.
tSSEP values for specific time points, C5 electrode:
M0 – P1 latency = 21.40 ms, P1_N1 amplitude = 2.20, tSSEP_*R* = 0, tSSEP zscore *R* = −0.305
M24 – P1 latency = 21.20 ms, P1_N1 amplitude = 3.60, tSSEP_*R* = 0, tSSEP_zscore_*R* = −0.395
M48 – P1 latency = 22.00 ms, P1_N1 amplitude = 3.80, tSSEP_*R* = 0, tSSEP_zscore_*R* = −0.036.

Table 1
Baseline demographic, clinical, MRI and neurophysiological characteristics.

	N	Baseline cohort	N	M24 cohort	N	M48 cohort
Clinical data						
Age, years (mean ± SD)	121	32.2 ± 8.7	74	32.3 ± 9.1	58	32.7 ± 9.7
Sex, females (N,%)	121	85 (70.2)	74	51 (68.9)	58	43 (74.1)
Type of CIS (N,%)						
Optic neuritis		35 (28.9)		19 (25.7)		15 (25.9)
Transverse myelitis		40 (33.1)		21 (28.4)		21 (36.2)
Brainstem/cerebellar		28 (23.1)		19 (25.7)		13 (22.4)
Hemispheral		14 (11.6)		12 (16.2)		8 (13.8)
Multifocal		4 (3.3)		3 (4.1)		1 (1.7)
EDSS (median, range)	121	1 (0–3.5)	74	1.5 (0–3.5)	58	1.5 (0–3.5)
BSFS (N,%)	121	19 (15.7)	74	15 (20.3)	58	9 (15.5)
MRI						
Number of T2 lesions on baseline MRI (median, range)	121	11 (0–76)	74	11.5 (0–63)	58	12 (1–76)
Brainstem lesions (N,%)	121	54 (44.6)	74	33 (44.6)	58	26 (44.8)
Cervical spinal cord lesions (N,%)	91	52 (57.1)	59	37 (62.7)	45	29 (64.4)
tSSEP						
P1 latency R (mean ± SD)	115	22.08 ± 2.23	74	22.08 ± 2.31	57	22.37 ± 2.14
P1 latency L (mean ± SD)	115	22.04 ± 2.31	74	22.15 ± 2.48	57	22.61 ± 2.31
P1_N1 amplitude R*	83	2.05 ± 1.20	54	2.09 ± 1.33	40	2.08 ± 1.28
P1_N1 amplitude L*	81	1.60 (0.40–5.60)	50	1.70 ± 0.94	38	2.02 ± 1.21
Conduction block R (N,%)	115	13 (11.3)	74	7 (9.5)	57	7 (12.3)
Conduction block L (N,%)	115	14 (12.2)	74	11 (14.9)	57	10(17.5)
tSSEP score (median, range)	115	1 (0–6)	74	2 (0–6)	57	2 (0–6)
tSSEP zscore (mean ± SD)	115	0.001 ± 0.797	74	0.024 ± 0.840	57	0.189 ± 0.745

CIS Clinically isolated syndrome, EDSS Expanded Disability Status Scale, MRI Magnetic resonance imaging, tSSEP tongue somatosensory evoked potentials.
* median (range) for non-parametric distribution, mean ± standard deviation for parametric distribution.

Table 2
Neurophysiological characteristics at baseline, month 24 and month 48 visits.

	N	Baseline cohort	N	M24 cohort	N	M48 cohort
P1 latency R*	115	22.08 ± 2.23	74	21.6 (18.0–26.2)	58	23.2 (18.6–30.4)
P1 latency L*	115	22.04 ± 2.31	74	22.3 (18.2–26.2)	58	23.63 ± 2.70
P1_N1 amplitude R*	83	2.05 ± 1.20	38	2.73 ± 1.33	35	3.00 ± 1.53
P1_N1 amplitude L*	81	1.60 (0.40–5.60)	29	2.20 ± 1.32	39	2.32 ± 1.14
Conduction block R (N,%)	115	13 (11.3)	74	20 (27%)	58	14 (24.1%)
Conduction block L (N,%)	115	14 (12.2)	74	25 (33.8%)	58	14 (24.1%)
tSSEP score*	115	1 (0–6)	74	3.5 (0–6)	58	3 (0–6)
tSSEP z score*	115	0.001 ± 0.797	74	0.237 ± 1.053	58	0.861 ± 1.261

tSSEP tongue somatosensory evoked potentials.
* median (range) for non-parametric distribution, mean ± standard deviation for parametric distribution.

M24 and M48 visits are presented in Table 1. Due to technical difficulties, six participants out of 121 could not perform tSSEP at the baseline (one participant refused to perform examination, five had dental braces).

During the 4-year follow-up period, 46 (79.3%) pwMS who completed the M48 visit were started on disease modifying therapy (9 on glatiramer acetate, 13 on teriflunomide, 12 on dimethyl fumarate, 11 on interferons, 1 on cladribine). Furthermore, in 11 (23.9%) participants, DMT has been changed due to disease activity (1 to fingolimod, 2 to alemtuzumab, 2 to cladribine and 6 to ocrelizumab).

After two-year of follow-up data were available for 74 participants and after four-year of follow-up for 58 participants (baseline

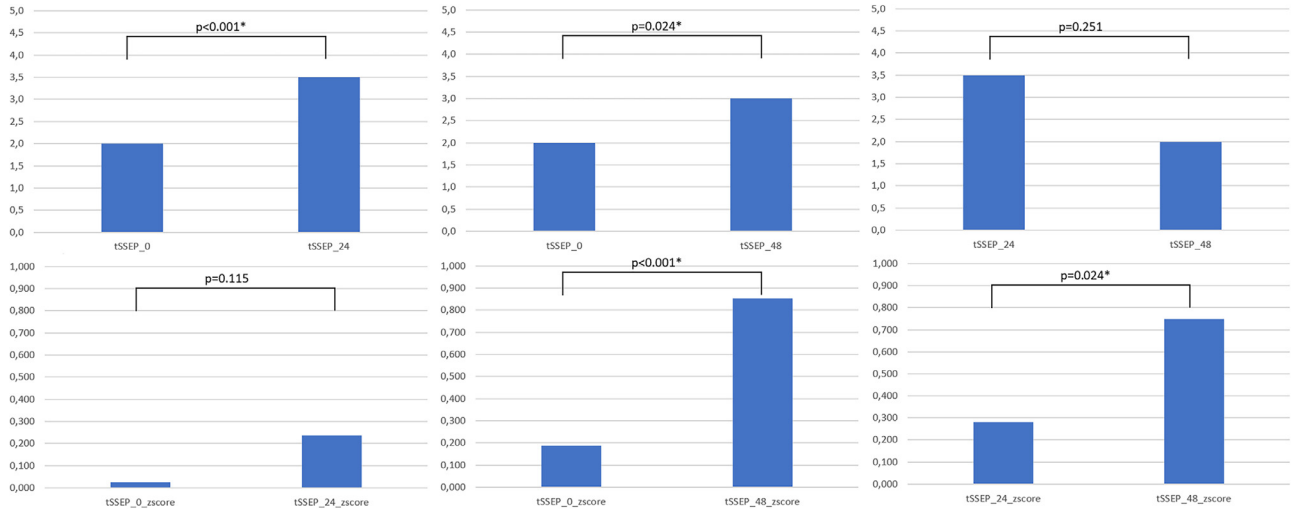

Fig. 2. Differences in tSSEP scores and tSSEP zscores in three different timepoints.

characteristics are presented in Table 1). The main reason for the drop-out rate on M24 visit was long distance travel for some patients required to come to the center and M48 visits were stopped in February 2020 due to COVID-19 pandemic (we plan to continue with M72 visits in September 2020).

3.1. Primary outcomes

Values of the tSSEP variables at all three timepoints are presented in Table 2. While there was no difference in absolute values of latencies (combined for the left and right side) between baseline and M24 visits (22.11 ± 1.91 vs 22.60 ± 2.39, $p = 0.113$), latencies were significantly prolonged at M48 compared to the baseline visit (24.00 ± 2.87 vs 22.49 ± 1.69, $p < 0.001$). An example of tracings of tSSEP from one participant at all three time points is presented in Fig. 1.

Conduction blocks (combined for the left and the right side) were more prevalent on M24 visit compared to baseline visit ($p = 0.001$), while there was no significant difference between M48 and baseline visits ($p = 0.118$).

Differences in tSSEP scores and tSSEP zscores in three different timepoints showed significant worsening of both scores over time (Fig. 2). For the tSSEP score the difference was significant for M0-M24 and M0-M48 visits, but not for M24-M48 visits. For the tSSEP zscore the difference was significant for M0-M48 and M24-M48 visits, but not for M0-M24 visits.

3.2. Secondary outcomes

An univariable logistic regression analysis was used to investigate which variables are possible predictors for worsening or improvement of both tSSEP score and tSSEP zscore (Table 3.). Variables with the highest significance were included in further analysis in the form of multivariable regression model (Table 3). The only significant negative predictor found for the tSSEP score improvement was presence of cervical spinal cord lesions on the baseline visit MRI. There were no variables predicting tSSEP worsening over time.

Finally, a correlation analysis was performed to compare the two different ways of calculation of the tSSEP score. We observed a moderate to high correlation between two tSSEP scores at all three timepoints (Fig. 3).

4. Discussion

The main results of this study indicate that during a 4-year follow-up period a significant deterioration of the trigeminal sensory pathway function occurs in pwMS. This is an important finding since trigeminal damage is one of the most frequent brainstem symptoms and brainstem impairment is one of the important predictors of unfavorable disease course in pwMS (Zadro et al., 2008, Tintore et al., 2010).

There are several neurophysiological methods which can evaluate brainstem function like auditory EP (AEP), vestibular evoked myogenic potentials (VEMP) and TSSEP. Among the first authors to investigate the role of TSSEPs in pwMS were Bergamaschi et al. who published a study in 1994 showing that TSSEP has a high sensitivity to demonstrate trigeminal pathway damage in patients with appropriate clinical signs (Bergamaschi et al., 1994). In addition, TSSEP pointed to impaired trigeminal pathways in patients without appropriate clinical signs, and the authors had already advocated the inclusion of this method in multimodal EPs to monitor pwMS (Bergamaschi et al., 1994). In an earlier study, this group of authors showed that AEP and TSSEP were equally sensitive in the detection of brainstem lesions in pwMS, but the results also showed that combined administration of AEP and TSSEP could reveal brainstem damage in a larger percentage of patients than using only one of the above methods (Cosi et al., 1989). Furthermore, the authors also analyzed part of the SSEP response of the median nerve (mSSEP) related to the brainstem and found that the combination of mSSEP and TSSEP was pathologically altered in 44 of 53 (83%) participants, whereas the combination of mSSEP, TSSEP, and AEP found brainstem damage in just one additional patient, suggesting the relative insensitivity of AEP (Cosi et al., 1989). On the other hand, Soustiel et al. showed that the combination of AEP and TSSEP was more sensitive than MR in detecting brainstem damage (Soustiel et al., 1996). However, this study also points to the insensitivity of AEP in patients with MS and the clinical signs of brainstem damage, since AEP was pathologically altered in only 53% of these patients, unlike TSSEP which was pathologically altered in 14 of 15 of these patients.

Although several lines of evidence suggest that TSSEP are superior to AEP in pwMS, the problem with this EP modality are numerous existing protocols with different modalities, locations and frequencies of stimulation, and different electrode positions to record the response. We have previously shown in a relatively large group of subjects with CIS that one of the TSSEP variants, tSSEP was pathologically altered in 32% of participants (KrbotSkorić et al., 2016). In addition, the absence of response to tSSEP was statistically significantly correlated with MRI-proven lesions in the brainstem, more specifically in the pons and the midbrain. In the same study, the tSSEP score was developed, and the results showed that subjects with MRI-proven lesions in the area of the midbrain and pons had a significantly higher tSSEP score, while binary logistic regression showed that the tSSEP score was significant predictor of lesions in the midbrain (KrbotSkorić et al., 2016). Furthermore, there

Table 3
Results of the univariable and multivariable logistic regression model.

	Univariable Exp(B)	95% C.I. for EXP(B)	p value	Multivariable Exp(B)	95% C.I. for EXP(B)	p value
M24 tSSEP score worsening						
Age	0.990	0.931–1.054	0.757			
Sex	1.255	0.373–4.211	0.713			
EDSS	1.481	0.824–2.663	0.189	1.602	0.789–3.252	0.192
BSFS	0.880	0.469–1.649	0.689			
Total number of baseline T2 lesions	0.966	0.927–1.007	0.102	0.987	0.934–1.042	0.629
Presence of brainstem lesions on the baseline MRI	1.166	0.381–3.563	0.788			
Presence of spinal cord lesion on the baseline MRI	0.299	0.058–1.550	0.150	0.301	0.049–1.835	0.193
M24 tSSEPzscore worsening						
Age	0.981	0.926–1.038	0.505	0.982	0.926–1.040	0.533
Sex	0.632	0.192–2.078	0.450	0.671	0.198–2.279	0.522
EDSS	1.522	0.869–2.668	0.142	1.497	0.850–2.634	0.162
BSFS	1.427	0.763–2.669	0.265			
Total number of baseline T2 lesions	0.991	0.952–1.032	0.673			
Presence of brainstem lesions on the baseline MRI	1.594	0.541–4.696	0.397			
Presence of spinal cord lesion on the baseline MRI	1.125	0.337–3.760	0.848			
M48 tSSEP score worsening						
Age	0.960	0.894–1.030	0.253	0.961	0.890–1.036	0.298
Sex	7.333	0.834–64.454	0.072	5.641	0.617–51.579	0.125
EDSS	0.554	0.272–1.131	0.105	0.595	0.284–1.247	0.169
BSFS	0.829	0.262–2.619	0.749			
Total number of baseline T2 lesions	0.991	0.961–1.022	0.566			
Presence of brainstem lesions on the baseline MRI	0.824	0.221–3.074	0.773			
Presence of spinal cord lesion on the baseline MRI	0.444	0.075–2.637	0.372			
M48 tSSEPzscore worsening						
Age	1.021	0.961–1.085	0.492			
Sex	0.390	0.097–1.569	0.185	0.450	0.068–2.981	0.408
EDSS	1.359	0.780–2.368	0.279			
BSFS	1.330	0.645–2.742	0.440			
Total number of baseline T2 lesions	0.976	0.946–1.006	0.116	0.989	0.950–1.029	0.574
Presence of brainstem lesions on the baseline MRI	0.611	0.195–1.919	0.399			
Presence of spinal cord lesion on the baseline MRI	0.333	0.072–1.543	0.160	0.449	0.080–2.504	0.361
M24 tSSEP score improving						
Age	1.011	0.942–1.084	0.763			
Sex	1.750	0.385–7.951	0.469			
EDSS	1.243	0.629–2.455	0.532			
BSFS	0.755	0.293–1.943	0.560			
Total number of baseline T2 lesions	1.001	0.953–1.050	0.980			
Presence of brainstem lesions on the baseline MRI	3.857	0.859–17.322	0.078	3.595	0.452–28.566	0.226
Presence of spinal cord lesion on the baseline MRI	0.091	0.012–0.704	0.022	0.105	0.012–0.880	0.038
M24 tSSEPzscore improving						
Age	1.002	0.939–1.069	0.949			
Sex	1.167	0.351–3.882	0.802			
EDSS	1.306	0.740–2.304	0.356			
BSFS	0.748	0.298–1.875	0.536			
Total number of baseline T2 lesions	1.004	0.967–1.041	0.846			
Presence of brainstem lesions on the baseline MRI	1.919	0.597–6.166	0.274			
Presence of spinal cord lesion on the baseline MRI	1.157	0.292–4.586	0.835			
M48 tSSEP score improving						
Age	0.956	0.886–1.032	0.249	0.943	0.851–1.044	0.258
Sex	3.000	0.272–33.085	0.370	2.964	0.175–50.313	0.452
EDSS	0.808	0.420–1.555	0.524	0.615	0.248–1.529	0.296
BSFS	1.741	0.681–4.455	0.247			
Total number of baseline T2 lesions	0.984	0.942–1.028	0.470			
Presence of brainstem lesions on the baseline MRI	1.333	0.301–5.912	0.705			
Presence of spinal cord lesion on the baseline MRI	0.190	0.029–1.249	0.084	0.062	0.005–0.814	0.034
M48 tSSEPzscore improving						
Age	0.946	0.853–1.050	0.296	0.936	0.839–1.044	0.237
Sex	1.714	0.349–8.421	0.507	0.913	0.157–5.317	0.919
EDSS	0.993	0.464–2.125	0.985			
BSFS	0.000	0.000	0.999			
Total number of baseline T2 lesions	0.965	0.918–1.014	0.159	0.961	0.911–1.013	0.140
Presence of brainstem lesions on the baseline MRI	0.417	0.082–2.106	0.290			
Presence of spinal cord lesion on the baseline MRI	0.313	0.050–1.938	0.212			

are no studies investigating the longitudinal behavior of TSSEP and only few studies investigated longitudinal behavior of AEP. Leocani et al. investigated AEP in a cohort of 64 pwMS and did not found changes in percentage of abnormalities of AEP over time, however they observed a significant worsening of AEP score on follow-up evaluation (Leocani et al., Sep).

The current study confirmed that cervical spinal cord lesions influence tSSEP results, as the only significant negative predictor for the tSSEP score improvement was presence of cervical spinal cord lesions on the MRI. This finding is not surprising knowing the anatomical location of the spinal nuclei of trigeminal nerve. It has been shown that in trigeminal neuralgia, pain can also originate from damage to the central

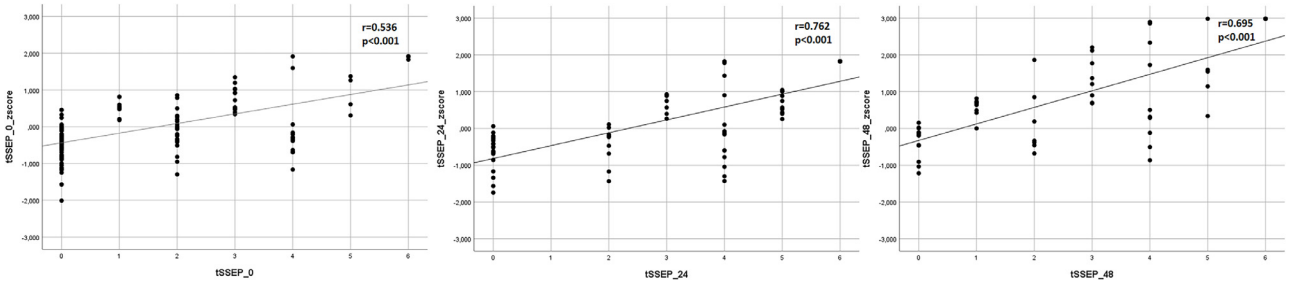

Fig. 3. Correlations between two tSSEP scores at all three timepoints.

trigeminal system in the upper spinal cord (Knibestöl et al., 1990). Moreover, it has been shown that brainstem pathology can serve as a biomarker of spinal cord damage and disability in MS, indicating the close relationship between brainstem and upper cervical spinal cord damage in MS. [Liptak et al., 2008] On the other hand it is surprising that we haven't found that cervical spinal cord lesions predict tSSEP worsening over time. One possible explanation is the combined influence of brainstem lesions on the tSSEP results, however do to relatively small sample size and the fact that not all participant had baseline cervical spinal cord MRI, we couldn't analyze this possible association.

The final question remains which method of EP score calculation will yield the best results.

Three different approaches have been investigated on a heterogeneous population of people with MS; apart from ordinal and quantitative approach exampled by tSSEP score and tSSEP zscore used in this study, a semi-quantitative approach combining features of both ordinal and quantitative scores can also be used (Schlaeger et al., 2016). Results have shown that all three methods allow disease course monitoring in MS, however, the quantitative EP score detects clinically relevant short-term changes with a smaller sample size than semi-quantitative or ordinal EP score. In the current study we didn't observe significant differences between two forms of tSSEP score calculations. Although both ordinal and quantitative approaches were able to detect significant changes in tSSEP results in 24-month periods, tSSEP score did not detect a significant change in M24-M48 period as tSSEP zscore did. Therefore, it may be useful to implement both approaches when following-up an MS patient.

The limitations of this study are relatively small sample size and high drop-out rate. Furthermore, not all participants completed all three visits.

Nevertheless, this study demonstrates a significant deterioration of trigeminal sensory system in MS over time, giving further insight into trigeminal system damage in pwMS.

CRediT authorship contribution statement

Magdalena Krbot Skorić: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Luka Crnošija:** Data curation, Formal analysis, Writing - review & editing. **Berislav Ruška:** Data curation, Formal analysis, Writing - review & editing. **Tereza Gabelić:** Data curation, Formal analysis. **Barbara Barun:** Data curation, Formal analysis, Writing - review & editing. **Ivan Adamec:** Data curation, Formal analysis, Writing - review & editing. **Mario Habek:** Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

None of the authors have relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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