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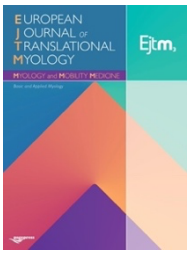

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Deceleration of denervated facial muscle atrophy through functional electrical stimulation: a sonographic quantification in patients with facial nerve paralysis

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Abstract

Functional Electrical Stimulation (FES) is an established intervention for a range of muscular and neurological disorders that has already been studied in numerous publications. However, its application to Peripheral Facial nerve Paralysis (PFP) still needs to be sufficiently investigated. As the first approach known to the authors, this study examines the effect of FES on the facial muscles in complete PFP using ultrasonography as a means of observation. In a prospective single-center observational pilot study, ten patients with complete PFP, confirmed by needle-electromyography (EMG), performed FES of the affected lateral mouth region at home twice daily for 20 minutes. The facial muscles' Cross-Sectional Area (CSA) was regularly assessed using sonographic quantification. While the CSA of most non-stimulated muscles decreased considerably during ongoing paralysis, a significant CSA increase of the Zygomaticus Muscle (ZYG), which was regularly subjected to FES, could be demonstrated. FES can halt the atrophy of denervated ZYG and potentially other facial muscles. Further investigations with a more significant patient collective are recommended. From now on, FES could be established as an additive method in the non-invasive treatment of PFP.

Key words: facial palsy, electrical stimulation, muscle ultrasound, denervated muscle.

Introduction

PFP is caused by lesions of the facial nerve resulting in a complete denervation of facial muscles. Key pathologies associated with facial paralysis are the complete loss of movement

in facial muscles, leading to immediate restrictions of mimic functionality, and the denervation atrophy, leading to a progressive loss of tone and deformation of facial symmetry. Patients suffer from cosmetical and functional losses but also negative perception of their body and social relationships.[1] Being subject to difficulties expressing emotions non-verbally, they often feel to be perceived negatively by others. This leads to distress and possibly even depression, culminating in the withdrawal from social interactions.[2] In addition, a complete loss of eye and mouth muscle tone may result on the functional level in severe constraints such as ulceration of the sclerae, eating or speaking impairments, restricted nasal ventilation, and reduced oral health.[3]

Whereas for many patients, surgical reinnervation procedures are the primary solution for an irreversible paralysis, even in these cases, a supportive treatment to bridge the time until reinnervation after surgery and reduce consequential damages is essential.[4] As patients experience immense suffering in the context of this medical condition, non-invasive approaches need to be investigated in a way that complements surgical treatment options.[2] Several studies show that FES is a non-invasive method to prevent atrophy in denervated muscles and improve facial appearance effectively.[5,6] Some authors suggest electrical stimulation to be a suitable intervention; on one side for treating paralytic disorders of the central and peripheral nervous system and on the other side to prevent denervation atrophy even in facial muscles.[7,8] Even though FES is the subject of scientific controversy in terms of applicability and therapy adherence due to possible side effects such as pain, erythema and discomfort, there are studies indicating FES to be innocuous in patients with facial nerve paralysis.[9,10] Ultrasound (US) of facial muscles allows a reliable and easy-to-repeat quantification of facial muscle sizes.[11] Furthermore, US is inexpensive, fast to perform, and a safe imaging method since it is radiation-free. It also produces high intra- and interrater reliability values.[11] Therefore, this study aims to confirm that FES is indeed a suitable intervention to prevent muscle denervation atrophy associated with complete PFP by using a sonographic monitoring of facial muscles during FES.

Materials and Methods

Study design

This prospective single-centre observational clinical study was registered in the German Clinical Trials Register (DRKS00015015) and approved by the local institutional ethics committee (no. 550503/18). Requirements for study inclusion were patients with a unilateral total PFP confirmed by needle-EMG[12,13], age of ≥18 years, mental and physical aptitude for

home-based surface FES, and high motivation in participating in the clinical study. Exclusion criteria were pregnancy or breastfeeding, signs of reinnervation in EMG, conservative treatment procedures (e.g. botulinum toxin injections) or facial physiotherapy within the last 3 months, medical conditions that influence the results of the clinical investigations (e.g. general muscle diseases; epilepsy; skin diseases), known allergies or intolerances to materials used in the clinical trial, malignant or life-threatening diseases at the time of inclusion, bilateral paralysis, or central facial paralysis. Patients terminated the study after clinical signs of reinnervation, such as voluntary muscle tone of the affected side of the face, visible voluntary movement and visible synkinesis as well as serious adverse events, occurrence of malignant and life-threatening diseases or facial paralysis on the contralateral side as well as on patients' demand. At the latest, the patients' follow-up was terminated after one year of study inclusion. All patients provided written informed consent prior to inclusion.

Study protocol

Ten patients were included. The baseline examination (T0) consisted of an EMG examination to verify complete unilateral PFP. US examination of the facial muscles was performed at every clinical visit. The present investigation is focused on the results of the US examinations. The analyses of score-based paralysis evaluation and of portrait photography examinations will be published separately.[14] Follow-up visits (T2 to T52) took place in the hospital and the same procedure of the baseline examination.

Electrical stimulation protocol

During the baseline examination, the FES parameters were determined in a comfortable sitting position with STMISOLA stimulator (BIOPAC Systems Inc., Germany). The STIWELL® med4 stimulation device (CE 0297; P/N 9001015) developed by MED-EL Elektromedizinische Geräte Gesellschaft m.b.H. (Innsbruck, Austria) was used for home training. Two adhesive electrodes (PALS® Neurostimulation electrodes, oval 4 cm x 6.4 cm, Axelgaard Manufacturing Co., Ltd., Lystrup, Denmark, CE-certified, REF 896230) were placed superficially over the ZYG on the affected side of the face. The cranial electrode was used as the cathode and the caudal electrode as the anode. Both electrodes were positioned as close as necessary to the corner of the mouth to avoid stimulation of the surrounding muscles. Phase duration of 1, 2, 5, 10, 15, 25, 50, 100, 250, 500 and 1000 ms with increasing amplitudes in between 0.1 and 20 mA were then tested. Biphasic triangular and rectangular waveforms with a constant frequency of 1 Hz were used. Amplitudes were noted for each

pulse length at which the ZYG contracted without pain and simultaneous stimulation of surrounding facial muscles. The parameters at maximum triggerable contraction below the pain threshold were then used to program the FES devices. To test tolerance, patients were stimulated for 20 minutes. Only the investigators were able to change stimulation settings by entering a password. The patients could not accidentally stimulate incorrectly or harm themselves.

After the test stimulation, the patients were shown how to apply the electrodes correctly in front of a mirror and how to operate the FES device. Based on the clinically determined FES parameters, patients performed FES at home twice every day (morning and evening with an in-between break of at least 6 hours) for 20 min. For home training, a two-phase stimulation in a triangular waveform with a phase duration of 5 seconds and a pulse pause of 1 second was performed.[14] At each follow-up visit, the parameters and electrode positioning were adjusted again to ensure optimal therapy success and patient safety. On the day of the follow-up visit, only a single run of FES was performed at home. Patients terminated FES when EMG and clinical findings clearly indicated facial reinnervation.

Facial electromyography and ultrasound examinations

Standardised needle-EMG on frontalis (FRO), ZYG, orbicularis oculi (OOC) and oris (OOR) muscles[13] were performed on the paralytic side of the face using VIASYSY Synergy (version 15.0. VIASYS Healthcare UK Ltd. Warwick, United Kingdom). The electrical activity of each muscle was monitored for denervation, synkinesis, and reinnervation.

The US scanner MyLab Seven eHD Crystaline (Esaote, Italy) was employed with exclusive use of the linear transducer SL1543 (18 Hz). An adapted version of an established sonography protocol covering mentalis (MEN), ZYG, depressor anguli oris (DAO), OOC, OOR and FRO muscles was put to use.[15]

US examination was used to quantify absolute and relative changes within the CSA parameter by which the muscle atrophy behaviour can be quantified.[5,11] From this, conclusions could be drawn about the effect of FES on the denervation atrophy of the mimic muscles. The CSA of the muscles provided a valuable indicator for the extent and development of both the atrophy and the muscle status. The assessment of the sonographic data was performed by employing the open-source image editing program ImageJ.[16] Specific muscles were therefore encircled in a region of interest (ROI) for the automatic calculation of its CSA (Figure 1).

Statistics

Linear mixed models were used to detect the significance of CSA value changes over time. Visit numbers were included as fixed effects as well as a random intercept for patients in the model. Continuous values were summarised by mean and standard deviation or median and 25th/75th percentile if the data was not normally distributed. All clinical visit values were analysed in pairwise comparison against Baseline visits. In addition, the data was analysed to detect significant value changes in the CSA for the whole period of investigation (p^a). The significance level was set to $p < 0.05$. The assessed data was documented in Microsoft Excel (Version 2308. Microsoft Corp. Redmond, Wahington, USA) and statistical analysis was performed in IBM SPSS Statistics for Windows (Version 27.0. IBM Corp. Armonk, New York, USA). Statistical graphs were designed using GraphPad Prism (Version 10.2.1, GraphPad Software Inc., Boston, Massachusetts, USA).

Results

Patient characteristics

Ten patients (median 61 years, 25th to 75th percentile 38.3 – 71 years; 4 female, 6 male, median time of denervation 130 d) underwent FES for a mean of 95 days (min. 35, max. 301). Facial paralysis etiologies were vestibular schwannoma (n = 3), parotid cancer (n = 3), benign parotid tumor (n = 1), chronic otitis media (n = 1), zoster oticus (Ramsey-Hunt syndrome; n = 1) and traumatic temporal bone fracture (n = 1) (Table 2). None of the patients experienced any undesired severe side effects of the FES. Minor side effects were skin irritation caused by the adhesive electrodes (n=1) and an unpleasant feeling (n = 1). After T28 (28 ± 2 weeks) all 10 patients had terminated the study either due to reinnervation (n = 6) or other termination criteria: stroke (n = 1), long-term rehabilitation after total hip arthroplasty (n = 1), metastasis of parotid carcinoma (n = 1) and personal reasons (moved away; n = 1).

Muscular Cross Sectional Area (CSA)

For a complete catalogue of all values obtained from the statistical analysis for CSA values, please refer to Table 3. No statistical significance for CSA value changes could be detected for any of the examined muscles when analysing the entire period of investigation ($p^a > 0.05$).

Mentalis muscle

At the baseline visit, MEN showed a mean CSA of 27.9 ± 3.2 mm². No significant CSA value change could be found over the entirety of the examination period ($p^a = 0.976$), and there

were no significant CSA value changes in pairwise comparison. In absolute values, MEN's CSA remained approximately constant. At T28, the CSA was 25.8 ± 5.6 mm².

Orbicularis oris muscle

At baseline visit, OOR produced a mean CSA of 27.5 ± 2.9 mm². A significant CSA decrease in pairwise comparison was observed at T8 study visit (n = 6; p = 0.012). In terms of absolute values, there was a notable CSA decrease over the course of the study (Fig. 2). However, no significant CSA value changes for OOR were found over the whole examination period (p^a = 0.195). At T28, the CSA was approximately 24.4 ± 4.2 mm².

Depressor anguli oris muscle

At baseline visit, DAO produced a mean CSA of 21.7 ± 3.5 mm². Significant CSA decreases in pairwise comparison were observed at study visits T12 (n = 3; p = 0.006) and T16 (n = 2; p = 0.029). In terms of absolute values too, a substantial CSA decrease was observed over the course of the study. However, no significant CSA value changes for DAO were detected over the whole examination period (p^a = 0.098). At T28, the CSA was approximately 16.9 ± 5.1 mm².

Zygomaticus muscle

At baseline visit, ZYG, *i.e.* the muscle that was afterwards stimulated, produced a mean CSA of 45.5 ± 10.2 mm². A significant CSA increase of 43.7 % in pairwise comparison was observed at T8 study visit (n = 6; p = 0.031). In absolute values, an overall increase of the CSA was observed over the course of the study (Figure 2). However, no significant CSA value changes were detected for ZYG when analysing the entirety of the examination period (p^a = 0.217). At T28, the CSA was 70.4 ± 15.2 mm².

Orbicularis oculi muscle

At baseline visit, OOC produced a mean CSA of 7.8 ± 1.4 mm². Significant CSA decreases in pairwise comparison were observed at study visits T8 (n = 6; p = 0.006) and T12 (n = 3; p = 0.013). Absolute values indicate a CSA decrease over the course of the study as well (Figure 2). However, no significant CSA value changes were found for OOC over the whole examination period (p^a = 0.080). At T28, the CSA was approximately 5.3 ± 1.9 mm².

Frontalis muscle

At baseline visit, FRO produced a mean CSA of 50.0 ± 7.9 mm². No statistical significance was found for FRO when analysing the whole examination period ($p^a = 0.957$), neither were there any significant CSA value changes in pairwise comparison. However, FRO produced slightly increasing CSA values over the course of the study in terms of absolute numbers. At T28, the CSA was approximately 54.0 ± 13.5 mm².

Discussion

The applicability of sonography for the visualisation and examination of muscle condition like muscle atrophy is well known.[11,17] This also applies to the mimic muscles being affected by facial nerve paralysis and the procedure has the advantages of being non-invasive, highly reproducible, cost-effective and radiation-free.[17] To the best of our knowledge, this study contains the very first approach to examining the effects of FES on patients with complete PFP by using facial muscle ultrasonography.

FES had no harmful or detrimental effects on the facial muscles or patient well-being over the entire course of this study. None of the enrolled patients reported severe side effects of the FES treatment. This finding is in accordance with many other studies which investigated electrical stimulation.[7,9,10,14,18,19] Furthermore, several studies have described the suitability of FES for therapeutically addressing denervated or atrophied muscles.[9,18,20,21] The academic concerns that have arisen to date regarding its applicability, harmlessness, therapeutic adherence and suitability for home training could be refuted in this three-part pilot study in collaboration with Krauß *et al.* as well as Volk *et al.*[14,22] The advantages of the method outweigh the aforementioned reservations: Doucet et al. describe for skeletal muscles that FES can effectively increase muscle strength and blood flow, minimise muscle atrophy and partial recovery with healing/connective tissue as well as reduce pain.[20] Arnold *et al.* substantiated the investigations regarding the applicability of the method to the facial muscles, particularly to ZYG, which was the target muscle of FES in this study. They outlined essential basic findings and instructions for use, which served as the basis for this pilot study.[9]

The objective of this work was to combine the application of ultrasound's diagnostic suitability for measuring mimic muscles subjected to facial nerve paralysis[17] with the observation of these muscles during the course of the disease and the assessment of the influence of FES on them. In collaboration with Krauß *et al.*, a systematic investigation of FES on the denervated muscles was carried out using multiple methods.[14] In contrast to prior and similar studies such as Tuncay *et al.* and Mäkelä *et al.* who stimulated multiple facial

nerve-innervated mimic muscles, we focused on solely applying FES to the ZYG with regards to the previously studied principles of Arnold et al.

Compared to the default values for healthy ZYG muscles measured by Volk *et al.*, there was identified a noticeably reduced CSA with a mean value of 45.5 ± 10.2 mm² at baseline examination. Standard values for the muscle, on the other hand, are given as an average of 60-75 mm².[11] A significant CSA increase was then found for the ZYG under FES during ongoing facial nerve paralysis in pairwise comparison to the baseline examination. Conversely, significant CSA reductions of the DAO, OOR and OOC could be verified in pairwise comparison to the baseline examination, as would be expected in terms of a progressive atrophy of denervated muscles. This also confirms that a selective FES only targeting the ZYG was achieved without unintended stimulation of the other facial muscles examined.

Based on the study inclusion criterion of complete unilateral PFP and the resulting absence of voluntary residual nerve activity, the presented results impressively demonstrate that FES is capable of halting and even reversing the atrophy of denervated mimic muscles. First results could already be identified within 4-8 weeks after the start of stimulation, shown here for the ZYG (Table 3). That general finding is consistent with those of other studies that used different methods than US to quantify the muscle condition. In their study, Mäkelä *et al.* examined facial palsy patients in the chronic phase of the disease, assuming both completely as well as incompletely denervated facial muscles and additionally applying FES to the FRO, OOC and OOR muscles. According to their results, they describe that FES has the potential for restoring function of the stimulated facial muscles in facial nerve palsies, even if it had been persistent for several years.[7] Tuncay *et al.* in turn examined patients in the acute phase of facial nerve palsy, assuming completely and incompletely denervated muscles as well, and came to the conclusion that the addition of FES to conventional therapeutic approaches such as physiotherapy is superior to omitting this method during the early stages of the disease.[18]

Within the framework of this pilot study, Krauß *et al.* assessed functional-objective changes in the mimics of the same facial nerve paralysis patients using photographic analysis among other methods. Both this work along with Krauß *et al.* obtained compatible results while applying different approaches: For example, the shown substantial CSA decrease of the non-stimulated OOC is consistent with the increase of Krauß *et al.*'s so-called palpebral fissure height parameter,[14] since the functional-structural impairment of the OOC in the lower eyelid caused by atrophy[23] should increase said value. The same applies to the CSA increase of the

ZYG as an important smile muscle and the increase in the smile angle parameter[14] used by Krauß et al. to quantify the ability to smile.

The exclusive recruitment of patients with complete PFP allowed us to examine the isolated effect of FES on the affected muscles without any influence from voluntary residual nerve activity. However, this strict inclusion criterion significantly reduced the number of participants, making it challenging to draw conclusions applicable to a larger patient population. This is generally shown by the results of the statistical data evaluation: numerous significant findings for CSA value changes could be observed accordingly in pairwise analyses to the Baseline examination (OOR, DAO, ZYG, OOC). However, statistical significance over the entire examination period could not be established for any of the muscles' CSA value changes despite the promising trends. Very likely, significance could have been gained with a larger sample size.

This pilot study, along with the complementary work by Krauß *et al.* and Volk *et al.*, primarily focused on assessing the feasibility and tolerability of FES, as well as assessing its impact on denervated facial muscles by performing US measurements.[14,22] Given these aims, the small number of recruitable subjects as well as for practicality purposes, the decision was made during the early planning phase not to include a control group or blinding. To further confirm the causal relationship between FES and improvements in muscle function, facial movement, and the reduction of atrophy in denervated facial muscles, such procedures will be essential to implement in future research. The authors believe the lack of statistical significance in CSA muscle value changes when analysing the entirety of the examination period is mainly due to the limited sample size, the study's design as a pilot or feasibility study lacking a control group and blinding, and possibly also the exclusive use of examiner-dependent imaging. While US has strong intra- and interrater reliability, it is examiner-dependent, unlike stationary imaging techniques such as Magnetic Resonance Imaging (MRI), and thus more prone to variability. It is possible that minor value scattering may have occurred and that, combined with the aforementioned factors, could have biased the statistical outcomes. As a result, future studies could additionally incorporate MRI to assess the affected facial muscles. This approach, with the additional use of MRI measurements of the mimic muscles, was investigated and recommended, for instance, by Mastryukova *et al.* in 2020.[24] Despite the outlined limitations, the findings from this study provide a promising outlook for the non-invasive treatment of PFP by applying FES and offer valuable insights for designing future

studies, which should ideally include a multi-centre, double-blind approach with a control group.

Conclusions

Within the limits of study design, it was demonstrated that FES has great potential to halt and even reverse the atrophy of denervated mimic muscles in facial nerve paralysis patients. It is recommended that the procedure be subjected to further investigations based on this pilot study with a larger patient collective and adapted study design. Looking ahead, FES offers a promising prospect of being established as an additional therapeutic pillar in the conservative treatment of facial nerve paresis and paralysis. Furthermore, the method of US quantification of the effect of FES on the denervated muscles by measuring the muscle CSA parameter is considered to be well suited and applicable in this context.

List of Abbreviations

CSA, cross sectional area

DAO, depressor anguli oris muscle

EMG, electromyography

FES, functional electrical stimulation

FRO, frontalis muscle (venter frontalis

MO, musculi occipitofrontalis)

MEN, mentalis muscle

MRI, magnetic resonance imaging

OOC, orbicularis oculi muscle

OOR, orbicularis oris muscle

PFP, peripheral facial nerve paralysis

US, ultrasound

ZYG, zygomaticus major muscle

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Contributions

GFV, DA, and WM oversaw planning and designing the study. OGL contributed to the study's funding and helped with interpreting the data. The tasks of recruiting patients, collecting ratings and scores, conducting EMG, and adjusting the stimulation parameters during clinical visits were primarily handled by MG, AMK, JB, DA, GFV, OGL, JK, and GM. MG, AMK, JK, and GM were responsible for data collection and digitization. JK and GM conducted the ultrasound examinations. JK, GM, and TL compiled the statistical analysis. JK and GM also created the digital graphics. GM wrote the article in close collaboration with all co-authors.

Conflict of interest

Authors GM, JK, MG, AMK, DA, OGL and GFV have received financial support from MED-El, Innsbruck, Austria. OGL was supported by a grant from the DFG (GU-463/12-1) and MG received a grant from the Interdisciplinary Center for Clinical Research. The remaining authors have no conflicts of interest.

Ethics approval

This prospective single-centre observational clinical study was registered in the German Clinical Trials Register (DRKS00015015) and approved by the local institutional ethics committee (no. 550503/18).

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Table 1. Schedule of visits and remote calls according to study protocol.

Visit	Remote call	T.	Time after baseline in weeks*	EMG	SFGS	PROMs	Photos	US
Baseline		T 0		yes	yes	yes	yes	yes
	1	T 2R	2			yes		
FU 1		T 4	4	yes	yes	yes	yes	yes
	2	T 6R	6			yes		
FU 2		T 8	8	yes	yes	yes	yes	yes
	3	T 10R	10			yes		
FU 3		T 12	12	yes	yes	yes	yes	yes
FU 4		T 16	16	yes	yes	yes	yes	yes
FU 5		T 20	20	yes	yes	yes	yes	yes
FU 6		T 28	28	yes	yes	yes	yes	yes
FU 7		T 40	40	yes	yes	yes	yes	yes
FU 8		T 52	52	yes	yes	yes	yes	yes

*At T2R and T4 a deviation ±1 week and from T6 to T52 of ±2 weeks were allowed. FU, follow up; EMG, needle electromyography, SFGS, Sunnybrook Facial Grading Score, PROMs, Patient Reported Outcome Measures, US, ultrasound

Table 2. Patients' characteristics.

ID	Age (years)	Sex	Etiology	Palsy duration (days) [a]	Reinnervation after 1 year	Termination after	Nerval anastomosis surgery	Reason of termination
1	51	M	vestibular schwannoma	118	yes	T8	none	reinnervation
2	24	F	parotid cancer	188	no	T28	HFJA	personal reasons
3	64	M	chronic otitis media	48	no	T12	none	stroke
4	77	F	zoster oticus	141	yes	T12		reinnervation
5	61	F	vestibular schwannoma	383	yes	T28	HFJA	reinnervation
6	61	M	vestibular schwannoma	34	no	T12	none	rehabilitation after THA
7	71	M	temporal bone fracture	58	yes	T4	none	reinnervation
8	71	M	parotid cancer	674	no	T8	none	metastasis
9	30	M	benign parotid tumor	3	yes	T12	great auricular nerve interposition	reinnervation
10	41	F	parotid cancer	1004	yes	T12	HFJA	reinnervation

[a] clinical onset upon baseline visit; HFJA, hypoglossal-facial nerve anastomosis, THA, total hip arthroplasty; M, male; F, female

Table 3. Sonography results of CSA measurement during electrical stimulation.

			T0	T4			T8			T12		
			Baseline	4 ± 1 w			8 ± 2 w			12 ± 2 w		
Muscle	**F**	**p[a]**	**n**	**Mean ± SD**	**n**	**Mean ± SD**	**p**	**n**	**Mean ± SD**	**p**	**n**	**Mean ± SD**	**p**
Mentalis	0.189	0.976	10	27.9 ± 3.2	9	26.7 ± 3.3	0.686	6	26.6 ± 3.7	0.710	3	24.2 ± 4.8	0.427
Orbicularis oris	1.625	0.195	10	27.5 ± 2.9	9	26.3 ± 3,.0	0.533	6	21.2 ± 3.2	**0.012**	3	26.9 ± 3.7	0.834
Depressor anguli oris	2.139	0.098	10	21.7 ± 3.5	9	18.5 ± 3.6	0.197	6	17.2 ± 3.9	0.121	3	10.1 ± 4.6	**0.006**
Zygomaticus	1.544	0.217	10	45.5 ± 10.2	9	54.9 ± 10.4	0.217	6	65.4 ± 11.3	**0.031**	3	52.1 ± 13.4	0.568
Orbicularis oculi	2.290	0.080	10	7.8 ± 1.4	9	6.4 ± 1.4	0.094	6	5.0 ± 1.5	**0.006**	3	4.5 ± 1.7	**0.013**
Frontalis	0.242	0.957	10	50.0 ± 7.9	9	54.1 ± 8.2	0.577	6	48.7 ± 9.2	0.873	3	52.0 ± 11.7	0.859

			T16			T20			T28		
			16 ± 2 w			20 ± 2 w			28 ± 2 w		
Muscle	**F**	**p[a]**	**n**	**Mean ± SD**	**p**	**n**	**Mean ± SD**	**p**	**n**	**Mean ± SD**	**p**
Mentalis	0.189	0.976	2	24.9 ± 5.6	0.582	2	28.9 ± 5.6	0.853	2	25.8 ± 5.6	0.694
Orbicularis oris	1.625	0.195	2	28.9 ± 4.2	0.702	2	26.5 ± 4.2	0.777	2	24.4 ± 4.2	0.392
Depressor anguli oris	2.139	0.098	2	11.4 ± 5.1	**0.029**	2	13.7 ± 5.1	0.080	2	16.9 ± 5.1	0.285
Zygomaticus	1.544	0.217	2	71.7 ± 15.2	0.062	2	68.8 ± 15.2	0.095	2	70.4 ± 15.2	0.075
Orbicularis oculi	2.290	0.080	2	5.9 ± 1.9	0.191	2	5.5 ± 1.9	0.126	2	5.3 ± 1.9	0.099
Frontalis	0.242	0.957	2	58.7 ± 13.5	0.510	2	61.2 ± 13.5	0.397	2	54.0 ± 13.5	0.762

p^a, overall significance for the whole period of investigation; Mean, mean values for cross-sectional area [mm^2]; SD, standard deviation.

Figure 1. Example of graphic evaluation of ultrasound images using ImageJ: Determination of region of interest for a healthy depressor anguli oris muscle with automatic calculation of its cross-sectional area.

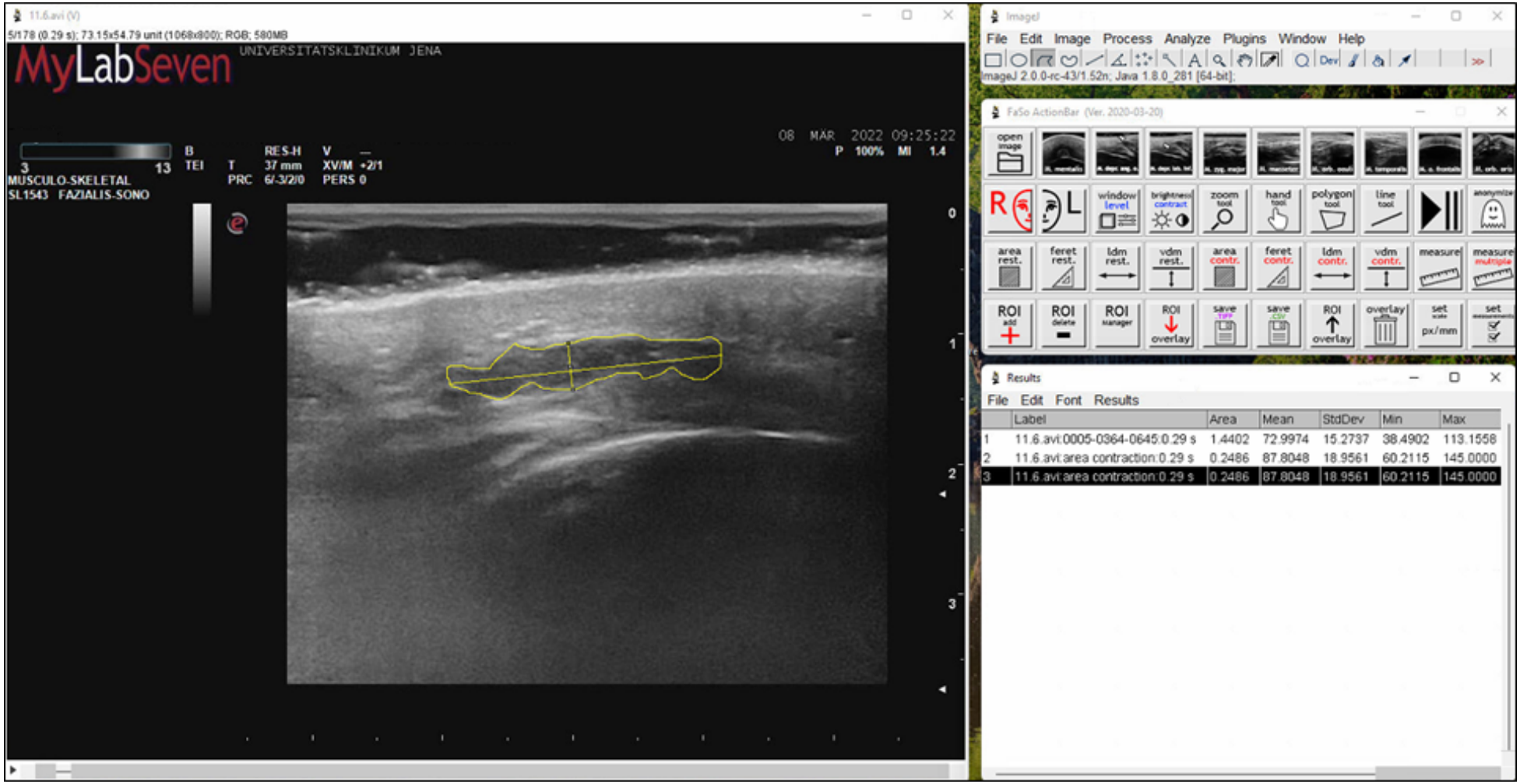

Figure 2. Scatter dot plot visualising significant cross-sectional area changes for paralysed zygomaticus, orbicularis oculi and orbicularis oris muscles during FES at baseline and at T8 examinations. The asterisks indicate a statistically significant value change ($p < 0.05$). For the zygomaticus, the plot visualises the increase in cross-sectional area found. For the other two muscles, their respective reductions in cross-sectional area are shown.