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# La ricerca clinica tra sperimentazione e osservazione

## ATTI

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# NONPARAMETRIC METHODS FOR A CLINICAL TRIAL WITH MULTIPLE ENDPOINTS AND SMALL SAMPLE SIZE

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## *Aim of the Work*

This study is a preliminary double-blind, placebo controlled, randomized clinical trial with a six-months follow-up period, aiming to assess the efficacy of type A botulinum toxin (Botox®) to treat myofascial pain symptoms and to reduce muscle hyperactivity in bruxers. Twenty patients (10 males, 10 females; age range 25-45) with clinical diagnosis of bruxism and with myofascial pain of masticatory muscles were enrolled at the Division of Maxillo-Facial Surgery, University Hospital in Padova, for this a double-blind, placebo controlled, randomized clinical trial, with a treatment (10 subjects treated with botulinum toxin injections-BTX-A) and a control group (10 subjects treated with saline placebo injections). The presence of bruxism was diagnosed according to a validated set of screening oriented clinical diagnostic criteria, so that in the present work bruxism is only approached in terms of its clinical impact on the masticatory apparatus and not as a more complex pathophysiological disorder affecting central nervous system. Diagnosis of bruxism was made when the patient exhibited, at least five nights a week, grinding bruxism sounds during sleep during the last six months, as reported by his/her bed partner, and at least one of the following adjunctive criteria: observation of tooth wear or shiny spots on restorations; report of morning masticatory muscle fatigue or pain; masseteric hypertrophy upon digital palpation.

The design of the study provided a double-blind, placebo controlled, randomized clinical trial, with a treatment (10 subjects treated with botulinum toxin injections) and a control group (10 subjects treated with saline placebo injections).

Criteria for the exclusion from the study were the following: a history of any treatment for bruxism and/or temporomandibular disorders during the six months before the study; the presence of neuromuscular pathologies preventing the use of botulinum toxin (i.e.: miastenia gravis); reported hypersensitivity to Clostridium Botulinum type A neurotoxin.

The treatment protocol provided 4 Type A botulinum toxin (BTX-A) (Botox®, Allergan, Inc, Irvine, CA, USA) intramuscular injections for each side (30 U) within the masseter muscles and 3 injections (20 U) within the anterior temporalis muscles, for a treatment total of 100 U. The injections were made during a single appointment under anatomotopographic and/or ultrasonographic control. All injections were performed by the same expertise maxillo-facial surgeon.

The following clinical parameters were assessed at baseline time, and at three follow-up appointments at one week, one month and six months respectively:

- pain at rest and at chewing, assessed by means of a Visual Analogue Scale (VAS) from 0 to 10, with the extremes being “no pain” and “pain as bad as the patient ever experienced” respectively;
- mastication efficiency, assessed by a VAS from 0 to 10, the extremes of which were “eating only semi-liquid” and “eating solid hard food”;
- maximum non-assisted and assisted mouth opening, protrusive and laterotrusive movements (in mm);
- functional limitation during usual jaw movements (0, absent; 1, slight; 2, moderate; 3, intense, 4, severe);
- subjective efficacy of the treatment (0, poor; 1, slight, 2, moderate; 3, good; 4, excellent);
- tolerability of the treatment (0, poor; 1, slight, 2, moderate; 3, good; 4, excellent).

At the same time of the clinical evaluations, all patients underwent electromyography (EMG) recordings of masseter and temporalis muscles activity under different experimental conditions:

- at rest;
- during maximal voluntary clenching;
- during maximal clenching on cotton rolls.

Surface electrodes were positioned using anatomic-topographic masks to achieve repeatability of electrodes localization.

Patients were informed of the possible side effects of botulinum toxin injections (tenderness after the injection and fatigue at chewing) and gave informed consent prior to the start of the study.

## **Methods**

Three groups of outcome variables were identified for statistical analysis:

- symptoms: pain at rest and at chewing (VAS values from 0=“no pain” to 10= “pain as bad as the patient ever experienced”); mastication efficiency (VAS values from 0=“eating only semi-liquid” to 10=“eating solid hard food”); functional limitation during usual jaw movements (rating from 0=absent to 4= severe); subjective efficacy of the treatment (rating from 0=poor to 4=excellent); tolerability of the treatment (rating from 0=poor to 4=excellent);
- signs: maximum non-assisted and assisted mouth opening, protrusive and laterotrusive movements (in mm);
- Electromyography (EMG) values: left and right anterior and posterior temporalis muscles; left and right masseter muscles; left and right anterior temporalis muscles during maximum voluntary clenching and during clenching on cotton rolls; left and right posterior temporalis muscles during maximum voluntary clenching and during clenching on cotton rolls; masseter muscles during maximum voluntary clenching and during clenching on cotton rolls.

To control for the differences between groups in baseline values, differences between the baseline and the three follow-up values for the outcome variables were considered for statistical analysis. The new variables defined as differences were not created for the variables “subjective efficacy of the treatment” and “tolerability of the treatment”, which were not assessed at baseline.

Since the sample size is quite low we preferred to perform a robust nonparametric approach, i.e. a two-sample permutation tests in order to compare the two groups of patients (botulinum toxin group and control group) in the outcome variables with respect to time. For the ordinal variables (functional limitation during usual jaw movements; subjective

efficacy of the treatment; tolerability of the treatment) an Anderson-Darling permutation test was performed (Pesarin, 2001).

For the variables defined as differences included in the statistical analysis, the alternative hypothesis was that patients treated with botulinum toxin had higher values than those treated with placebo, except that for the differences in pain at mastication, pain at rest and functional limitation, for which placebo group was expected to have higher values than botox group.

The Bonferroni-Holm (method for multiple tests) was also applied in order to control for multiplicity since several tests are applied to the same variables (Finos and Salmaso, 2006). The cut-off significance level was set at  $p < 0.05$ .

## **Results**

Descriptive analysis showed that values of maximum non-assisted and assisted mouth opening, protrusive and laterotrusive movements (in mm) showed a slight increase in the botox group (differences between baseline and follow-up values tend to increase) and seem to be unaltered in the placebo group (Figures 1-4). As for symptoms, pain at rest and at chewing decreased in the botox group while remaining constant in the placebo group, even though mastication efficiency did not improve neither in the botox nor in the placebo group (Figures 5-8). Similarly, changes in functional limitation with time did not differ between the two groups of patients. With regard to subjective parameters of efficacy and tolerability, botox patients referred a higher improvement with time in their perception of treatment efficacy than placebo patients (Figure 9). Tolerability of the treatment was good for both group of patients (Figure 10).

Similarly, EMG activity values tend to decrease with time in patients treated with botox (i.e: differences between baseline values and follow-up values tend to increase), while EMG values did not change with time in patients treated with placebo. Such trends were mostly evident in the case of EMG activity values during clenching on cotton rolls (Figures 11-14).

Permutation tests performed on the outcome variables defined as differences showed significant differences between the two groups at one week and at one month in EMG activity values during maximum clenching on cotton rolls for the anterior right temporalis and both the right and left masseter muscles (Table 1). As for symptoms, improvement in pain at chewing and patients' perception of treatment efficacy were significantly higher in botox than in placebo patients at six-months (Table 2). No significant differences between the two groups were showed in the other outcome variables.

## **Conclusions**

Results from the present pilot study give an indication towards a possible efficacy of BTX-A to reduce myofascial pain symptoms and to decrease EMG activity in bruxers, even though some differences with placebo were not significant.

Descriptive analysis showed that improvements in both objective (EMG masseter and temporalis muscles activity; range of mandibular movements) and subjective (pain at rest; pain at chewing) outcome variables were higher in botox than in placebo patients. Besides, patients treated with BTX-A referred a higher subjective improvement with time in their perception of treatment efficacy than placebo patients. The small sample size obviously limits generalization of results. This study was intended to provide exploratory results on this particular issue, since sample size was small due to the difficulties to recruit patients for ethic reasons. Such observations suggest the need for a RCT conducted on an appropriate sample, whose size must be determined by a power analysis taking into account the present preliminary findings.

Figure 1-4. BOTULINUM TOXIN, Descriptive analysis - EXPLORATORY RESULTS (signs)  
(median of the differences between follow-up and baseline values: variations respect to time 0)

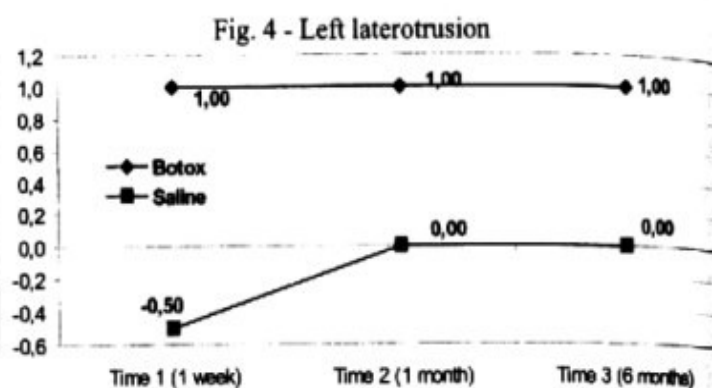
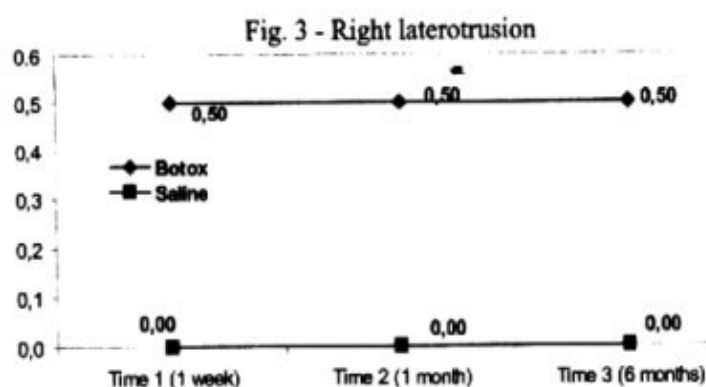
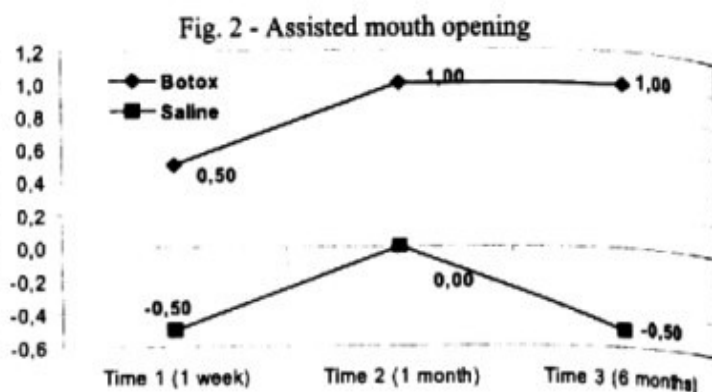
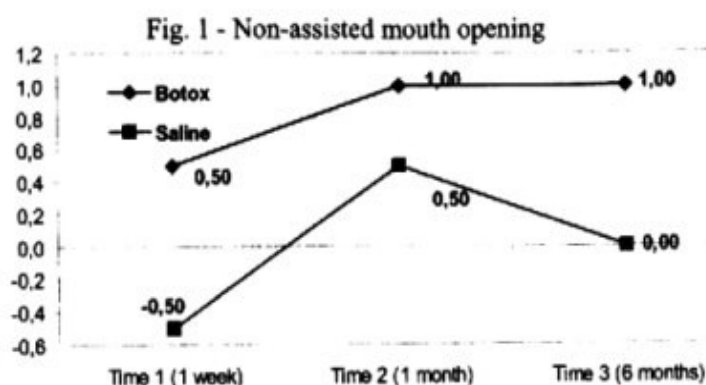


Figure 5-8. BOTULINUM TOXIN, Descriptive analysis - EXPLORATORY RESULTS (symptoms)  
(median of the differences between follow-up and baseline values: variations respect to time 0)

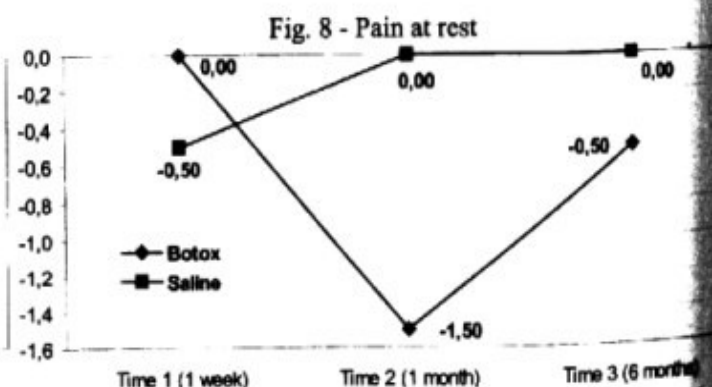
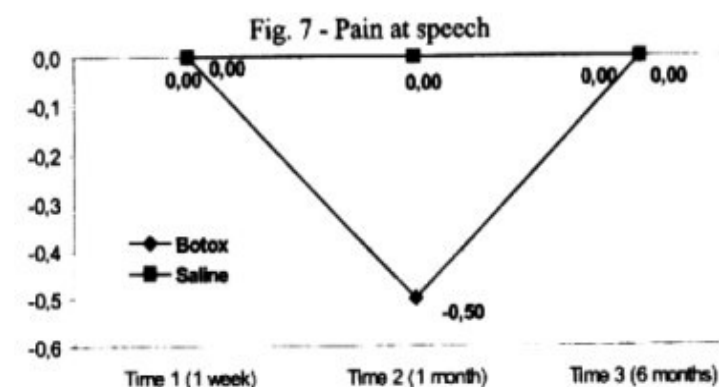
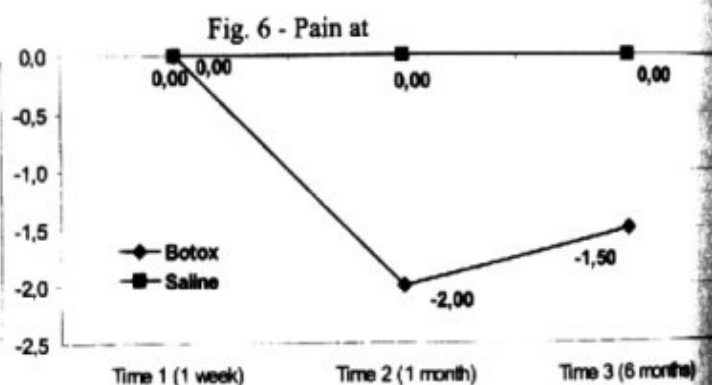
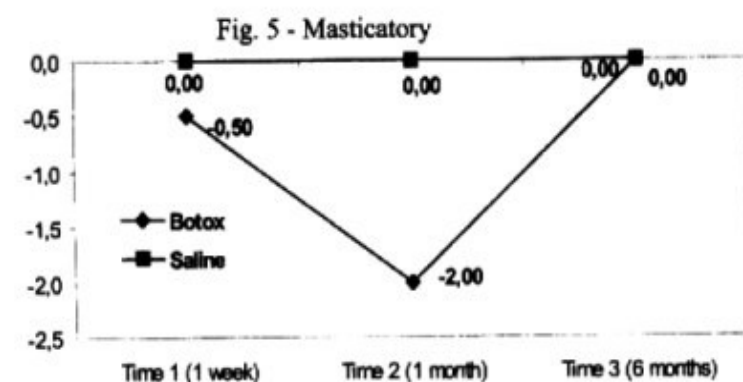


Figure 9 - 10: BOTULINUM TOXIN, Descriptive analysis (symptoms)  
(percentage distribution of patients)

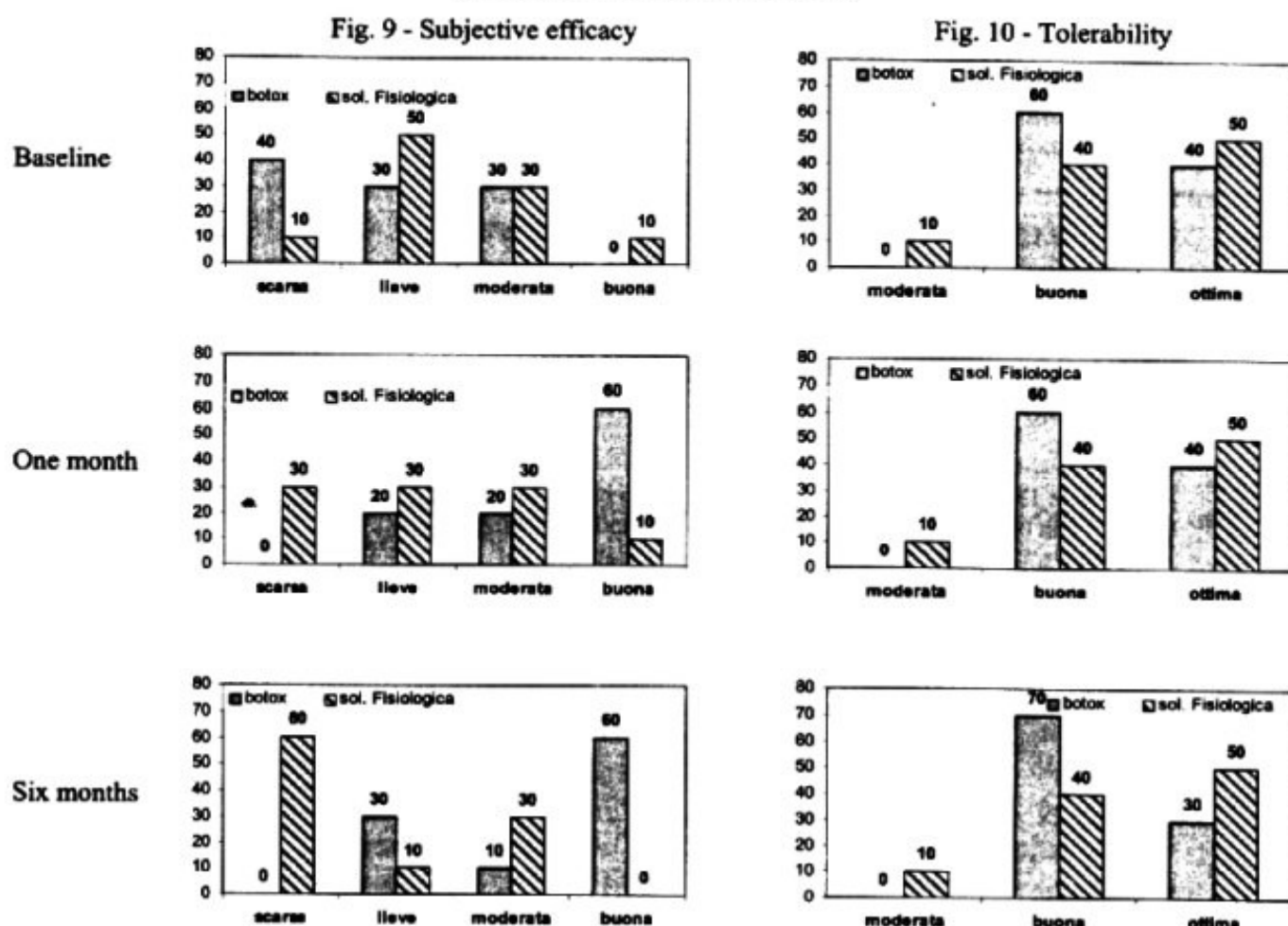


Figure 11. Boxplot. Left anterior temporalis EMG activity during clenching on cotton rolls (difference between baseline and one-week, one-month and six-months values). BTX-A (left) vs. placebo (right).

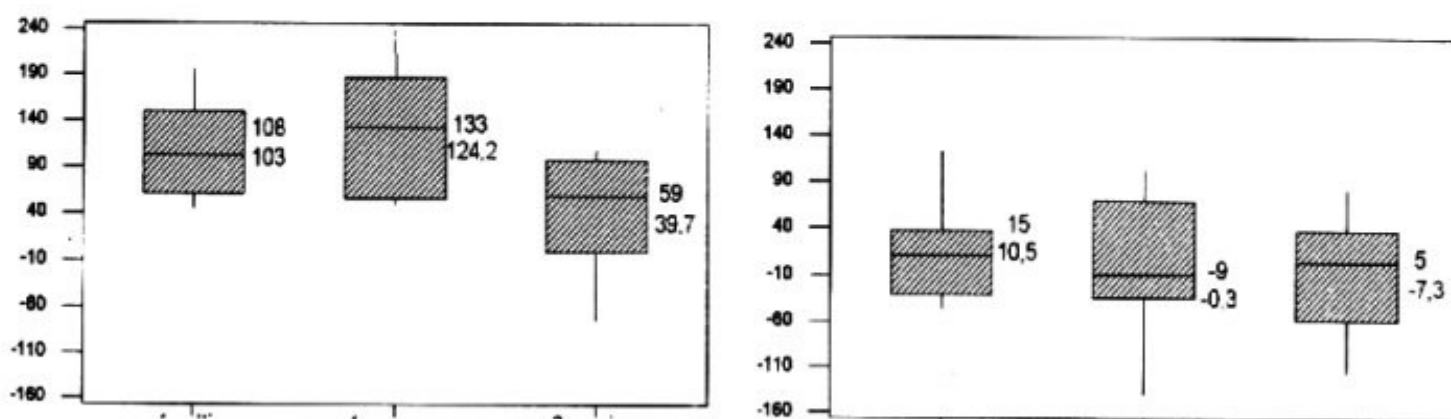


Figure 12. Boxplot. Right anterior temporalis EMG activity during clenching on cotton rolls (difference between baseline and one-week, one-month and six-months values). BTX-A (left) vs. placebo (right).

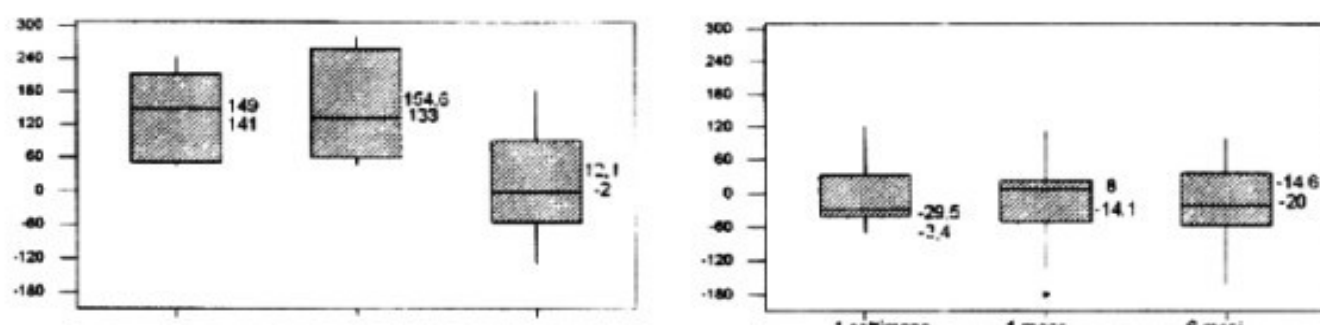


Figure 13. Boxplot. Left masseter EMG activity during clenching on cotton rolls (difference between baseline and one-week, one-month and six-months values). BTX-A (left) vs. placebo (right).

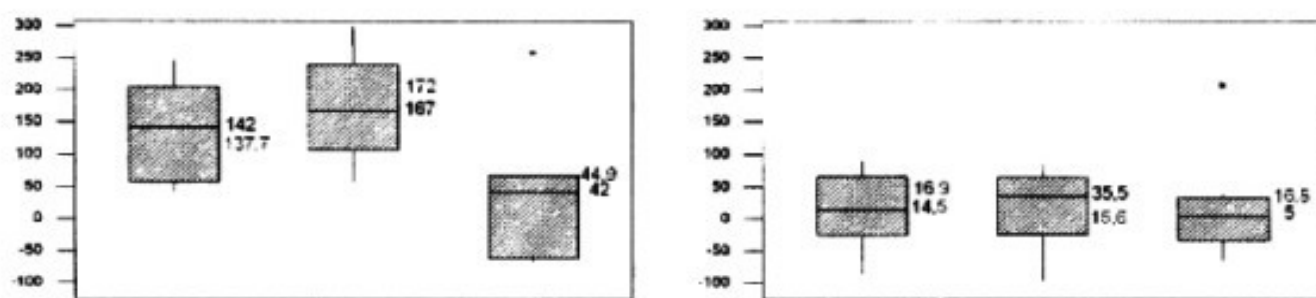
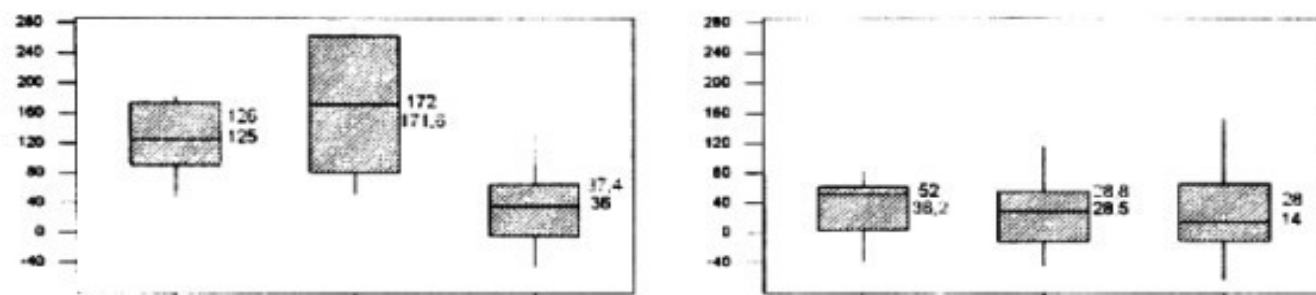


Figure 14. Boxplot. Right masseter EMG activity during clenching on cotton rolls (difference between baseline and one-week, one-month and six-months values). BTX-A (left) vs. placebo (right).



**Table 1 - Permutation test. Differences in EMG values between baseline and one week, one month and six months (BTX-A vs. Placebo). Significance set at  $p < 0.05$**

EMG values	Corrected p-values		
	Difference between baseline and one-week values	Difference between baseline and one-month values	Difference between baseline and six-months values
Right Anterior Temporalis at clenching on cotton rolls	0.02255	0.03315	n.s.
Left Masseter at clenching on cotton rolls	0.03315	0.01050	n.s.
Right Masseter at clenching on cotton rolls	0.03000	0.03885	n.s.

n.s. = not significant

**Table 2 - Permutation test. Differences in symptoms between baseline and one week, one month and six months (BTX-A vs. Placebo). Significance set at  $p < 0.05$**

Signs	Corrected p-values		
	Difference between baseline and one-week values	Difference between baseline and one-month values	Difference between baseline and six-months values
Pain at chewing	n.s.	n.s.	0.02300
Efficacy	n.s.	n.s.	0.01155

n.s. = not significant

### **References**

Finos L, Salmaso L (2006) Weighted methods controlling the multiplicity when the number of variables is much higher than the number of observations. *Journal of Nonparametric Statistics*, 18, 245-261.

Pesarin F (2001) Multivariate permutation tests with applications to biostatistics. *Wiley, Chichester*.