



Cognitive Performance After Facial Botulinum Toxin Treatment in a Cohort of Neurologic Patients: An Exploratory Study

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Cognition and facial botulinum treatment

Cognitive performance after facial botulinum toxin treatment in a cohort of neurological patients – an exploratory study

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NCT02179450

1 **Cognitive performance after facial botulinum toxin treatment in a cohort of**
2 **neurological patients – an exploratory study**

3
4
5 **Abstract**

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7
8 **Objective:** To investigate higher cognitive functions after changes of the mimicry by facial
9 botulinum toxin injections, we tested verbal and non-verbal reasoning in patients with
10 blepharospasm or hemifacial spasm before and after their long-term botulinum toxin
11 treatment. **Design:** Explorative, non-randomized, clinical trial. **Setting:** Patients: ambulatory
12 care. Healthy control: general community. **Participants:** Volunteer sample. Patients: 21
13 patients with blepharospasm or hemifacial spasm – facial botulinum toxin injections.
14 Controls: 30 patients with cervical dystonia – cervical botulinum toxin injections - and 33
15 healthy subjects. **Intervention:** The two groups receiving injections were tested before and
16 three weeks after their treatment. Healthy subjects received no injections. **Main Outcome**
17 **Measures:** Verbal and non-verbal reasoning scores. **Results:** The key unexpected finding is
18 that patients who receive facial BTX injections perform significantly worse in non-verbal
19 reasoning tasks, when compared to healthy control ($p=0.022$). There was no significant
20 difference in the baseline reasoning scores and at follow up for verbal reasoning between the
21 three groups. There was no correlation between toxin dose and reasoning scores (verbal:
22 $p=0.132$, non-verbal: $p=0.294$). **Conclusion:** Because of potential confounders, the results do
23 not allow any conclusion on causality yet. Further research is needed to confirm our findings.
24
25 **Keywords:** Cognition, botulinum toxin, facial muscles

26

27

28 **Abbreviations**

29

30

31 ANCOVA analyses of covariance

32 ANOVA analyses of variance

33 BEB blepharospasm

34 BEB/HS blepharospasm or hemifacial spasm

35 BTX botulinum toxin

36 CI confidence interval

37 CD cervical dystonia

38 df degrees of freedom

39 HS hemifacial spasm

40 IBM International Business Machines Corporation

41 rsfMRI resting state functional MRI

42 SPSS Statistical Package for the Social Sciences

43

44

45 There is a well-established link between the mechanics of one's own facial expressions and the

46 ability to perceive the emotions of others.¹ This link includes facial 'mimicry', or the automatic

47 response of analogous muscles when observing the facial expressions of others. One strand of

48 this research is that aesthetic botulinum toxin (BTX) therapy of the corresponding facial

49 muscles leads to a delayed processing of emotions, either positive or negative.^{2,3} This effect is

50 typically explained by the well investigated "facial feedback hypothesis": that emotions are not

51 only expressed by activation of certain facial muscles, but also that activation of certain facial
52 muscles induces the corresponding emotion.¹ The underlying mechanism remains unclear, but
53 functional MRI data suggest a range of neural circuits, such as emotion-linked activation of the
54 amygdala⁴ and insula⁵, and facial motor-linked activations of the inferior frontal gyrus. The
55 reports also give further evidence that emotion and cognition may often be closely intertwined.⁶
56 ⁷ Areas of activation include perception-linked systems such as the primary visual cortex and
57 the inferior temporal cortex, memory-related regions such as the hippocampus, and the
58 orbitofrontal cortex and prefrontal cortex.⁷

59
60 BTX has a large spectrum of applications in the rehabilitation of acute and chronic diseases.⁸
61 Amongst others, it is the first line therapy in BEB (blepharospasm) and HS (hemifacial spasm).
62 Here, small doses are injected into facial muscles, such as the orbicularis oculi, corrugator and
63 procerus muscle.⁹ The botulinum effect is reversible, and the therapy is typically repeated every
64 three to four months. Since the increasing public awareness of the link between facial palsy and
65 emotion, neurological patients receiving long-term botulinum toxin treatment were concerned
66 about a probable affection of their cognitive function. To date, there has been no systematic
67 study of cognition after BTX induced facial palsy. However, BTX induced plasticity of brain
68 structures, namely the motor cortex in primary dystonia, has been described previously.¹⁰ Thus,
69 the extension to unaffected motor-cortical areas in cervical dystonia, i.e. the hand region, has
70 also been reported.¹¹ By analogy, we assume, that apart from emotional processing, also
71 cognitive domains could be affected by BTX induced facial palsy. Given the fact that
72 experiencing emotion and understanding emotion in language use the same neural systems²,
73 this might include networks, which are part of the reading process.¹²

74

75 The purpose of the present, exploratory study was to assess cognitive function in neurological
76 patients receiving long-term facial BTX therapy (BEB and HS), before and after BTX
77 treatment. As screening tests, we chose a verbal reasoning task to cover the language domain
78 and added a non-verbal reasoning task to extend the spectrum of cognitive domains. As one
79 form of control, we assessed healthy subjects, who received no injections. We also investigated
80 patients with cervical dystonia, who generally receive higher doses. Their injections are limited
81 to cervical muscles, with no effect on the facial musculature.¹³

82

83

84 **Materials and Methods**

85

86

87 **Participants**

88

89

90 Patients with the clinical diagnosis of BEB, HS or CD were recruited from the BTX outpatient
91 clinic for movement disorders at a Clinical Department of Neurology of a University Hospital.
92 All patients included in this study were pre-treated with all common BTX preparations and
93 reported good treatment response. To evaluate a potential correlation of BTX dose and
94 reasoning scores, the equivalent unit ratio of the preparations onabotulinumtoxinA : abobotulinumtoxinA was
95 calculated 1: 3.¹⁴

96

97 Healthy control subjects were recruited amongst patient companions, from geriatric facilities
98 and through public announcements. Participants aged 18 to 85 were eligible. Participants with
99 known neurological or psychiatric comorbidities were excluded.

100

101

102 **Procedure**

103

104

105 We investigated two groups:

106

107 • Patients with blepharospasm or hemifacial spasm (BEB/HS) - BTX treatment of facial
108 muscles;

109

110 • patients with cervical dystonia (CD) - BTX treatment of cervical muscles, no treatment
111 of facial muscles;

112

113 • healthy subjects – no BTX treatment, to exclude effects of repeated testing.

114

115 All participants performed a baseline assessment of reasoning measures, which was conducted
116 by three investigators. Afterwards, all patients received their regular long-term BTX treatment.

117

118 BTX injections were applied by muscle palpation at the known anatomical landmarks without
119 further technical aids. Dose finding and all injection schemes were individualized (see
120 Table1for the list of included muscles).

121

122 Table 1. List of included muscles

123

124 After three weeks, a different investigator, who was blinded to the baseline test results, repeated
125 the cognitive assessment of each participant.

126

127

128 **Cognitive assessment**

129

130

131 Verbal reasoning was assessed with the Verbal Analogies subtest of the Intelligence Structure
132 Test 2000-R.¹⁵ Here 20 tasks are presented, each task consisting of three words. In these tasks,
133 a relation exists between the first and the second word, and a similar relation can be applied
134 between the third word, and one of five alternatives.

135

136 Nonverbal reasoning was measured using the Matrices subtest of the Intelligence Structure Test
137 2000-R.¹⁵ This subtest consists of 20 tasks, each showing a two-by-two matrix with three
138 different figures, which are located based on a rule. The task is to detect the rule, and choose
139 the correct missing figure from five alternatives. Overall scores were built by aggregating the
140 correct answers for Verbal Analogies and Matrices.

141

142

143 Standard protocol approval and patient consents

144

145

146 The study was approved by the local ethical committee and has been registered at
147 ClinicalTrials.gov. All participants gave their informed consent for inclusion before they

148 participated in the study. The study was conducted in accordance with the Declaration of
149 Helsinki, and the protocol was approved by the local ethical committee.

150

151

152 Statistical analysis

153

154

155 Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences)
156 software (IBM, International Business Machines Corporation, SPSS Statistics, Version 20).¹⁶

157 The desired number of at least 30 patients was based on the central limit theorem.¹⁷ Equivalence

158 of basic sample characteristics (age, gender, educational level) between patients (i.e. BEB/HS

159 and CD) and healthy controls was analysed using an ANOVA (analyses of variance) and Chi

160 square tests. Furthermore, analyses of variance were conducted to compare nonverbal as well

161 as verbal reasoning scores of patients with CD, BEB/HS and healthy controls at baseline. The

162 analyzed variables were normally distributed (verbal reasoning - baseline: $\chi^2=3.10$, $df=2$,

163 $p=0.213$; non-verbal reasoning - baseline: $\chi^2=2.29$, $df=2$, $p=0.319$; verbal reasoning – follow-

164 up: $\chi^2=0.615$, $df=2$, $p=0.735$; non-verbal reasoning – follow up: $\chi^2=1.78$, $df=2$, $p=0.411$).

165 Differences in verbal and nonverbal reasoning before and after BTX-treatment, as well as the

166 presence of a potential learning effect, was analysed using two-way mixed ANCOVA (analyses

167 of covariance) with the three groups (CD, BEB/HS and the healthy controls) as the between-

168 subjects factor, time (pre- and post-treatment) as the within-subjects factor, overall treatment

169 time and dose as covariates. Relationships between dose and verbal or nonverbal reasoning was

170 analysed using Pearson correlation. The significance level was set at $p<0.05$. Post hoc power

171 analyses for the used within-between factorial design ($f = 0.25$, $\alpha = 0.05$, sample size = 84,

172 groups 3, measurements = 2) revealed a power of 0.99.

173

174

175 **Results**

176

177

178 Participants

179

180

181 In the injection groups, a total of 169 patients were screened for participation, 88 patients
182 were eligible and willing to participate. Thirty-seven patients did not complete baseline
183 and were excluded from further analyses (see Table 2 for details of drop out).

184

185 Table 2. Details of drop out

186

187 Finally, a total of 84 subjects participated in the present study. Fifty-one (60.7%) received
188 BTX injections – 21 (41.2%; BEB: n=14, HS: n=7) patients with BEB/HS and 30
189 (58.8%) patients with CD - and 33 (39.3%) were healthy subjects. The desired number of
190 at least 30 patients in the BEB/HS group could not be recruited, as some of those patients
191 refused to perform a neuropsychological test, for example when they realized that they
192 would be tested in their cognitive, reading and/or arithmetic competence, or when they
193 realized that some more study-related appointments were necessary. Participants that
194 refused to perform neuropsychological tests were not included in the present study. For
195 clinical details of all participants at baseline assessment, see Table 3.

196

197 Table 3. Clinical details of all participants at baseline assessment

198

199 The patient group (i.e. BEB/HS and CD) consisted of 30 women (59%) and 21 men (41%). The
200 age of patients ranged from 26 to 78 years (mean=59.7, SD=12.24). 34 of the patients (66.7%)
201 had terminated their education at the end of compulsory schooling. 17 of the patients (33.3%)
202 held a college or university degree.

203

204 The healthy controls consisted of 22 women (67%) and 11 men (33%), ranging in age from 29
205 to 81 years (mean=61.3, SD=11.90). Sixteen of the controls (49%) had compulsory schooling
206 as their highest educational level, with 17 (51%) with college or university degrees.

207

208 There were no significant differences between the three groups regarding age ($F(2,81)=1.865$,
209 $p=0.161$), gender ($\chi^2=1.454$, $df=2$, $p=0.483$) and educational level ($\chi^2=3.085$, $df=2$, $p=0.214$).

210 In addition, the number of subjects per group ($\chi^2=2.79$, $df=2$, $p=0.248$), BTX treatment duration
211 ($\chi^2=7.57$, $df=3$, $p=0.056$), and the applied BTX preparations ($\chi^2=5.84$, $df=3$, $p=0.119$) were
212 equally distributed in all three groups. BTX equivalent dosage was not equally distributed
213 ($\chi^2=37.63$, $df=4$, $p<0.0001$), but the dosage did not correlate with verbal and non-verbal
214 reasoning scores (see below).

215

216

217 Baseline assessment of non-verbal and verbal reasoning

218

219

220 There were no statistically significant differences in scores of nonverbal as well as verbal
221 reasoning before BTX-treatment. An analysis of variance showed no significant differences
222 ($F(2,81)=2.14$, $p=0.124$) between CD (mean=7.77, SD=3.52, 95%CI=5.82-9.82), BEB/HS

223 (mean=6.26, SD=2.72, 95%CI=4.67-7.78) and control subjects (mean=7.83, SD=2.85,
224 95%CI=6.1-9.52) with respect to scores of nonverbal reasoning at baseline. There were also no
225 statistically significant differences at baseline with respect to scores of verbal reasoning
226 ($F(2,80)=2.92$, $p=0.060$) between CD (mean=7.7, SD=3.15, 95%CI=6.03-10.17), BEB/HS
227 (mean=6.32, SD=3.11, 95%CI=4.6-7.85) and control subjects (mean=8.66, SD=3.42,
228 95%CI=6.5-10.11).

229

230

231 Differences in reasoning scores: pre- and post-treatment

232

233

234 Non-verbal reasoning (Fig 1)

235

236

237 The scores of control subjects improved (mean=9.23, SD=3.62, 95%CI=7.1-11.17), those of
238 CD slightly improved (mean=8.07, SD=3.97, 95%CI=5.86-10.62). and those of BEB/HS
239 slightly decreased (BEB/HS: mean=6.21, SD=3.36, 95%CI=4.25-7.95).

240

241 Figure 1. Non-verbal reasoning scores.

242

243 A mixed design ANCOVA revealed no significant main effect of time [$F(1,74)=0.86$, $p=0.357$,
244 $\eta^2=0.011$], but a significant main effect of group [$F(2,74)=3.34$, $p=0.041$, $\eta^2=0.083$] with
245 respect to nonverbal reasoning, indicating no differences between pre-and post-treatment
246 evaluation but differences between the three groups. The covariates dose [$F(1,74)=0.12$,

247 $p=0.731$, $\eta^2=0.002$] and overall treatment time [$F(1,74)=0.22$, $p=0.641$, $\eta^2=0.003$] showed no
248 significant impact.

249

250 Pairwise comparisons showed a significant difference between healthy controls and BEB/HS
251 ($p=0.022$), with the lowest mean difference in non-verbal abilities regarding pre- and post-
252 treatment evaluation in BEB/HS. No difference was shown between healthy controls and CD
253 ($p=0.794$) and between the two patient groups ($p=0.197$).

254

255 The interaction effect of time and group was not significant [$F(2,74)=1.23$, $p=0.297$, $\eta^2=0.032$]
256 indicating no differences in the mean change in nonverbal reasoning scores between patient
257 groups and healthy controls and no learning effect occurred regarding non-verbal reasoning.
258 There were also no significant interaction effects of time and dose [$F(1,74)=0.11$, $p=0.743$,
259 $\eta^2=0.001$] as well as time and overall treatment time [$F(1,74)=0.15$, $p=0.696$, $\eta^2=0.002$].

260

261

262 Verbal reasoning (Fig 2)

263

264

265 The scores of CD improved (mean=8.77, SD=4.01, 95%CI=7.13-11.76), those of controls
266 (mean=8.34, SD=3.38, 95%CI=5.74-9.8) and BEB/HS (mean=6.0, SD=3.38, 95%CI=3.98-
267 7.63) slightly decreased.

268

269 Fig 2. Verbal reasoning scores.

270

271 With respect to verbal reasoning a mixed design ANCOVA revealed no significant main effect
272 of time [$F(1,73)=0.37$, $p=0.546$, $\eta^2=0.005$] but a significant main effect of group [$F(2,73)=3.37$,
273 $p=0.040$, $\eta^2=0.084$], indicating no differences between pre-and post- treatment evaluation but
274 differences between the three groups. The covariates dose [$F(1,73)=0.07$, $p=0.793$, $\eta^2=0.001$]
275 and overall treatment time [$F(1,73)=0.85$, $p=0.359$, $\eta^2=0.012$] did not have a significant impact.
276

277 Pairwise comparisons showed a borderline significance suggesting a noticeable difference
278 between healthy controls and BEB/HS ($p=0.051$) as well as borderline significant differences
279 between the two patient groups ($p=0.067$), with the lowest mean difference in verbal abilities
280 regarding pre- and post- treatment evaluation in BEB/HS. No difference was shown between
281 healthy controls and CD ($p=0.669$).

282
283 The interaction effect of time and group was not significant [$F(2,73)=1.12$, $p=0.331$, $\eta^2=0.030$],
284 indicating that the mean changes in verbal reasoning scores did not differ between both patient
285 groups and healthy controls and no learning effect occurred regarding verbal reasoning.
286 Furthermore, there are no significant interactions between time and dose [$F(2,73)=0.13$,
287 $p=0.718$, $\eta^2=0.002$] as well as overall treatment time [$F(2,73)=0.02$, $p=0.893$, $\eta^2=0.000$].
288

289

290 Relationship between BTX dose und verbal or nonverbal reasoning

291
292

293 There was no significant correlation between BTX dose and verbal reasoning (Pearson
294 correlation, $r(49)=0.22$, $p=0.132$) as well as nonverbal reasoning scores (Pearson correlation,
295 $r(49)=0.153$, $p=0.294$) after treatment.

296

297

298 **Discussion**

299

300

301 The main finding of this exploratory study was a significant difference of non-verbal reasoning
302 scores in the patient group who received BTX treatment of their facial muscles (BEB/HS),
303 when compared to healthy controls. In passing, we note that there was also a noticeable
304 difference of the post-treatment verbal reasoning scores, but they failed to reach significance
305 ($p=0.051$).

306

307 These preliminary results are in several respects surprising, given that the treatment might have
308 been predicted to *address* potential physical impairments, and thus *improve* test scores. For
309 example, an improvement of the visual sustained attention span might have been expected, after
310 clinical improvement of the BEB symptoms (as has been described previously).¹⁸ A related
311 issue would be an expected improvement in the performance of written tests, given that patients
312 report difficulties reading, which also tends to improve after their botulinum treatment. Indeed,
313 when the effect of the BTX treatment wears off, patients tend to again complain about those
314 difficulties. This was also reflected in the higher number of patients who refused to participate
315 in this study, as a result of these impairments. Cognitive impairment after facial BTX injections
316 in the treatment of neurological disorders has never been reported.^{19, 20} However, discrete
317 impairment of cognitive performance has been described as a non-motor syndrome of BEB.²¹
318 Two-thirds of patients in the facial injection group suffered from BEB. We cannot exclude that
319 the pathophysiological alterations due to the non-motor syndrome might be a potential
320 confounding factor. However, cognitive disturbances as non-motor symptoms have also been

321 described in cervical dystonia.²² Therefore, one might expect a similar performance. But in the
322 case of verbal scores, the cervical injection cohort *improved* with a borderline significance
323 ($p=0.067$), when compared to the facial injection group.

324

325 There is little data referring to neural correlates of the two specific aspects of reasoning, which
326 were investigated in the present study.²³ Verbal analytic reasoning has been correlated with
327 rsfMRI (resting state fMRI) data and has been related with brain regions for integration (i.e. the
328 angular and supramarginal gyrus), hypothesis testing, cognitive control (i.e. inferior frontal
329 gyrus) and response selection (i.e. dorsal anterior cingulate cortex). Non-verbal reasoning
330 scores were non-significantly associated with the left occipital- and right anterior temporal lobe,
331 and right frontoinsula cortex, respectively.²³

332

333 One account might be a more direct link between facial muscles and these cognitive networks.
334 In this context, the role of the corrugator muscle in several emotional and non-emotional facial
335 expressions has been reported.²⁴ Amongst others, the corrugator muscle is activated during
336 concentration, and plays an important role in communication and interaction, including when
337 accompanying or emphasizing elements of speech.²⁵ A recent investigation measured motor
338 activity of grip strength during verbal processing, and found a context sensitive increase during
339 processing of “action words”.²⁶

340

341 An alternative approach might be that the BTX induced facial palsy leads to a delay in emotion-
342 related responses, which is well-established.² There is an increasing awareness of the ways that
343 emotion might interact with cognitive processes – such as perception, attention, memory and
344 decision making.⁷ However, emotion-linked domains like memory -i.e. emotion based learning

345 ²⁷, or planning and decision-making⁷ were not covered by the present reasoning tasks. Further
346 investigations might profit from additional objective tests of these cognitive domains.

347

348 Regardless of cause, our unexpected findings illustrate the importance of careful monitoring
349 during a regular rehabilitative treatment of chronic diseases, even though this treatment is well-
350 established. In addition, further research might consider tests of the cognitive performance
351 during the rehabilitation of facial palsies of other origin, e.g., Bell's palsy.

352

353 Notably, we found no indications for a dose dependent effect on the reasoning scores. It is also
354 notable that performance of the control patients with cervical muscle injections (CD) did not
355 differ from healthy controls at any time point, even though those patients received high
356 cumulative doses (Table 3). This might be a relevant issue for other BTX applications with high
357 cumulative doses in neurological rehabilitation, namely spasticity. These data also support the
358 assumption that there is no direct effect of BTX due to a questionable retrograde transport or
359 systemic distribution.^{28, 29}

360

361

362 Study limitations

363

364

365 At this point, - and as the major limitation of this study- these preliminary results do not allow
366 a causality of facial palsy and cognitive performance; thus, the interpretation of the present data
367 remains highly speculative and cannot be generalized. The data needs to be confirmed by trials
368 involving a larger sample size and additional control groups to adjust for confounding factors,
369 such as a selection bias.

370

371 Here, we did not perform sham-injections as placebo control. One reason for this were ethical
372 considerations, as BTX is the first line therapy and very effective in dystonia.^{9,13} Furthermore,
373 unblinding appears highly probable. We suggest the additional investigation of subjects
374 receiving cosmetic facial BTX injections.

375

376 These appear to be conventional clinical samples, with normal baseline intelligence
377 measurements of the patient and control groups. Novelty does not seem to be a relevant
378 confounding variable, as there were no BTX “naive” patients included, and both patient groups
379 have a mean treatment duration of almost six years, as part of a regular cycle of treatments.

380

381 We did not perform a follow up evaluation to clarify if the reduced performance is temporary
382 and completely reversible. Therefore, future research should address the sustainability of the
383 results after the paralysis of facial muscles wears off.

384

385

386 **Conclusions**

387

388

389 The unexpected key finding is that patients who receive facial BTX injections appear to
390 perform significantly worse in non-verbal (and as a trend, verbal) reasoning tasks. However,
391 these are preliminary results of an exploratory study with potential confounders. Thus, at this
392 point, BTX induced facial palsy and cognitive performance cannot be related, and therefore
393 the interpretation of these results remains highly speculative. It is clear that the findings

394 should be backed by further controlled investigations and illustrate the importance of careful
 395 monitoring during a well-established, rehabilitative treatment of chronic diseases.

396

397

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399

400

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466

467 **Figure legends**

468

469

470 **Fig 1. Non-verbal reasoning scores.** Estimated marginal mean scores and 95% CI of
471 non-verbal reasoning tests absolved by patients and controls at baseline and three weeks
472 after BTX-treatment (controls received no injections). Abbreviation: BEB:
473 blepharospasm; BoNT: botulinum neurotoxin; HS: hemifacial spasm.

474

475 **Figure 2. Verbal reasoning scores.** Estimated marginal mean scores and 95% CI of
476 verbal reasoning tests absolved by patients and controls at baseline and three weeks after
477 BTX-treatment (controls received no injections). Abbreviation: BEB: blepharospasm;
478 BoNT: botulinum neurotoxin; HS: hemifacial spasm.

1 **Cognitive performance after facial botulinum toxin treatment in a cohort of**
2 **neurological patients – an exploratory study**

3
4
5 **Abstract**

6
7
8 **Objective:** To investigate higher cognitive functions after changes of the mimicry by facial
9 botulinum toxin injections, we tested verbal and non-verbal reasoning in patients with
10 blepharospasm or hemifacial spasm before and after their long-term botulinum toxin
11 treatment. **Design:** Explorative, non-randomized, clinical trial. **Setting:** Patients: ambulatory
12 care. Healthy control: general community. **Participants:** Volunteer sample. Patients: 21 of 38
13 eligible patients with blepharospasm or hemifacial spasm – facial botulinum toxin injections.
14 Controls: 30 of 50 eligible patients with cervical dystonia – cervical botulinum toxin
15 injections - and 33 healthy subjects. **Intervention:** The two groups receiving injections were
16 tested before and three weeks after their treatment. Healthy subjects received no injections.
17 **Main Outcome Measures:** Verbal and non-verbal reasoning scores. **Results:** The key
18 unexpected finding is that patients who receive facial BTX injections perform significantly
19 worse in non-verbal reasoning tasks, when compared to healthy control (p=0.022). There was
20 no significant difference in the baseline reasoning scores and at follow up for verbal reasoning
21 between the three groups. There was no correlation between toxin dose and reasoning scores
22 (verbal: p=0.132, non-verbal: p=0.294). **Conclusion:** Because of potential confounders, the
23 results do not allow any robust conclusion on causality yet. Further research is needed to
24 confirm our findings.

25

26 **Keywords:** Cognition, botulinum toxin, facial muscles

27

28

29 **Abbreviations**

30

31

32 ANCOVA analyses of covariance

33 ANOVA analyses of variance

34 **BEB** blepharospasm

35 BEB/HS blepharospasm or hemifacial spasm

36 BTX botulinum toxin

37 CI confidence interval

38 CD cervical dystonia

39 df degrees of freedom

40 **HS** hemifacial spasm

41 IBM International Business Machines Corporation

42 rsfMRI resting state functional MRI

43 SPSS Statistical Package for the Social Sciences

44

45

46 There is a well-established link between the mechanics of one's own facial expressions and the

47 ability to perceive the emotions of others. ¹ This link includes facial 'mimicry', or the automatic

48 response of analogous muscles when observing the facial expressions of others. One strand of

49 this research is that aesthetic botulinum toxin (BTX) therapy of the corresponding facial

50 muscles leads to a delayed processing of emotions, either positive or negative. ^{2,3} This effect is

51 typically explained by the well investigated “facial feedback hypothesis”: that emotions are not
52 only expressed by activation of certain facial muscles, but also that activation of certain facial
53 muscles induces the corresponding emotion.¹ The underlying mechanism remains unclear, but
54 functional MRI data suggest a range of neural circuits, such as emotion-linked activation of the
55 amygdala⁴ and insula⁵, and facial motor-linked activations of the inferior frontal gyrus. The
56 reports also give further evidence that emotion and cognition may often be closely intertwined.⁶
57 ⁷ Areas of activation include perception-linked systems such as the primary visual cortex and
58 the inferior temporal cortex, memory-related regions such as the hippocampus, and the
59 orbitofrontal cortex and prefrontal cortex.⁷

60
61 BTX has a large spectrum of applications in the rehabilitation of acute and chronic diseases.⁸
62 Amongst others, it is the first line therapy in **BEB (blepharospasm)** and **HS (hemifacial spasm)**.
63 Here, small doses are injected into facial muscles, such as the orbicularis oculi, corrugator and
64 procerus muscle.⁹ The botulinum effect is reversible, and the therapy is typically repeated every
65 three to four months. Since the increasing public awareness of the link between facial palsy and
66 emotion, neurological patients receiving long-term botulinum toxin treatment were concerned
67 about a probable affection of their cognitive function. To date, there has been no systematic
68 study **of the effect of cognition after** BTX induced facial palsy. However, BTX induced
69 plasticity of brain structures, namely the motor cortex in primary dystonia, has been described
70 previously.¹⁰ Thus, the extension to unaffected motor-cortical areas in cervical dystonia, i.e. the
71 hand region, has also been reported.¹¹ By analogy, we assume, that apart from emotional
72 processing, also cognitive domains could be affected by BTX induced facial palsy. Given the
73 fact that experiencing emotion and understanding emotion in language use the same neural
74 systems², this might include networks, which are part of the reading process.¹²

75

76 The purpose of the present, exploratory study was to assess cognitive function in neurological
77 patients receiving long-term facial BTX therapy (**BEB** and **HS**), before and after BTX
78 treatment. As screening tests, we chose a verbal reasoning task to cover the language domain
79 and added a non-verbal reasoning task to extend the spectrum of cognitive domains. As one
80 form of control, we ~~also~~ assessed healthy subjects, who received no injections. We also
81 investigated patients with cervical dystonia, who generally receive higher doses. Their
82 injections are limited to cervical muscles, with no effect on the facial musculature.¹³

83

84

85 **Materials and Methods**

86

87

88 **Participants**

89

90

91 Patients with the clinical diagnosis of **BEB, HS** or **CD** were recruited from the BTX outpatient
92 clinic for movement disorders at a Clinical Department of Neurology of a University Hospital.
93 All patients included in this study were pre-treated with all common BTX preparations and
94 reported good treatment response. To evaluate a potential correlation of BTX dose and
95 reasoning scores, the equivalent unit ratio of the preparations on-/inco-BTX : abo-BTX was
96 calculated 1: 3.¹⁴ ~~Co-medication has been routinely assessed and documented by the treating~~
97 ~~neurologist in the patient's file and constant during study period.~~

98

99 Healthy control subjects were recruited amongst patient companions, from geriatric facilities
100 and through public announcements. Participants aged 18 to 85 were eligible. Participants with
101 known neurological or psychiatric comorbidities were excluded.

102

103

104 **Procedure**

105

106

107 We investigated two groups:

108

109 • Patients with blepharospasm or hemifacial spasm (BEB/HS) - BTX treatment of facial
110 muscles;

111

112 • patients with cervical dystonia (CD) - BTX treatment of cervical muscles, no treatment
113 of facial muscles;

114

115 • healthy subjects – no BTX treatment, to exclude effects of repeated testing.

116

117 All participants performed a baseline assessment of reasoning measures, which was conducted
118 by three investigators. Afterwards, all patients received their regular long-term BTX treatment.

119

120 BTX injections were applied by muscle palpation at the known anatomical landmarks without
121 further technical aids. Dose finding and all injection schemes were individualized (see
122 **Table 1 for the list of included muscles**).

123

124 Table 1. List of included muscles

125

126 ~~of BEB and HS involved orbicularis oculi, palpebral part of the orbicularis oculi, corrugator~~
127 ~~supercilii, levator anguli oris, depressor anguli oris and platysma muscle. Injection scheme of~~
128 ~~CD involved sternocleidomastoid, splenius capitis, semispinalis capitis, trapezius and levator~~
129 ~~scapulae muscle.~~

130

131 After three weeks, a different investigator, who was blinded to the baseline test results, repeated
132 the cognitive assessment of each participant.

133

134

135 **Cognitive assessment**

136

137

138 Verbal reasoning was assessed with the Verbal Analogies subtest of the Intelligence Structure
139 Test 2000-R.¹⁵ Here 20 tasks are presented, each task consisting of three words. In these tasks,
140 a relation exists between the first and the second word, and a similar relation can be applied
141 between the third word, and one of five alternatives.

142

143 Nonverbal reasoning was measured using the Matrices subtest of the Intelligence Structure Test
144 2000-R.¹⁵ This subtest consists of 20 tasks, each showing a two-by-two matrix with three
145 different figures, which are located based on a rule. The task is to detect the rule, and choose
146 the correct missing figure from five alternatives. Overall scores were built by aggregating the
147 correct answers for Verbal Analogies and Matrices.

148

149

150 Standard protocol approval and patient consents

151

152

153 The study was approved by the local ethical committee and has been registered at
154 ClinicalTrials.gov. All participants gave their informed consent for inclusion before they
155 participated in the study. The study was conducted in accordance with the Declaration of
156 Helsinki, and the protocol was approved by the local ethical committee.

157

158

159 Statistical analysis

160

161

162 Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences)
163 software (IBM, International Business Machines Corporation, SPSS Statistics, Version 20).¹⁶

164 The desired number of at least 30 patients was based on the central limit theorem.¹⁷ Equivalence
165 of basic sample characteristics (age, gender, educational level) between patients (i.e. BEB/HS
166 and CD) and healthy controls was analysed using an ANOVA (analyses of variance) and Chi
167 square tests. Furthermore, analyses of variance were conducted to compare nonverbal as well
168 as verbal reasoning scores of patients with CD, BEB/HS and healthy controls at baseline. The
169 analyzed variables were normally distributed (verbal reasoning - baseline: $\chi^2=3.10$, $df=2$,
170 $p=0.213$; non-verbal reasoning - baseline: $\chi^2=2.29$, $df=2$, $p=0.319$; verbal reasoning – follow-
171 up: $\chi^2=0.615$, $df=2$, $p=0.735$; non-verbal reasoning – follow up: $\chi^2=1.78$, $df=2$, $p=0.411$).
172 Differences in verbal and nonverbal reasoning before and after BTX-treatment, as well as the
173 presence of a potential learning effect, was analysed using two-way mixed ANCOVA (analyses

174 of covariance) with the three groups (CD, BEB/HS and the healthy controls) as the between-
175 subjects factor, time (pre- and post-treatment) as the within-subjects factor, overall treatment
176 time and dose as covariates. Relationships between dose and verbal or nonverbal reasoning was
177 analysed using Pearson correlation. The significance level was set at $p < 0.05$. Post hoc power
178 analyses for the used within-between factorial design ($f = 0.25$, $\alpha = 0.05$, sample size = 84,
179 groups 3, measurements = 2) revealed a power of 0.99.

180

181

182 **Results**

183

184

185 Participants

186

187

188 In the injection groups, a total of 169 patients were screened for participation, 88 patients
189 were eligible and willing to participate. Thirty-seven patients did not complete baseline
190 and were excluded from further analyses (see Table 2 for details of drop out). **BEB:**

191 **n=13/27, 48.1%; HS: n=4/11, 36.4%; CD: n=20/50, 40%**

192

193 **Table 2. Details of drop out**

194

195 **Physical handicap (BEB:n=3/13, 23.1%; CD:n=1/20, 5%), fear of a bad test performance**
196 **(BEB:n=1/13, 7.7%), lack of time (CD:n=3/20, 15%), language barrier (BEB:n=1/13,**
197 **7.7%; CD:n=1/20, 5%) or no specific reason (BEB:n=8/13, 61.5%; hemifacial**
198 **spasm:n=2/4, 50%; CD:n=14/20, 70%) have been reported as reasons for drop out.**

199 Finally, a total of 84 subjects participated in the present study. Fifty-one (60.7%) received
200 BTX injections – 21 (41.2%; **BEB**: n=14, **HS**: n=7) patients with BEB/HS and 30
201 (58.8%) patients with CD - and 33 (39.3%) were healthy subjects. The desired number of
202 at least 30 patients in the BEB/HS group could not be recruited, as some of those patients
203 refused to perform a neuropsychological test, for example when they realized that they
204 would be tested in their cognitive, reading and/or arithmetic competence, or when they
205 realized that some more study-related appointments were necessary. Participants that
206 refused to perform neuropsychological tests were not included in the present study. For
207 clinical details of all participants at baseline assessment, see **Table 3**.

208

209 **Table 3**. Clinical details of all participants at baseline assessment

210

211 The patient group (i.e. BEB/HS and CD) consisted of 30 women (59%) and 21 men (41%). The
212 age of patients ranged from 26 to 78 years (mean=59.7, SD=12.24). 34 of the patients (66.7%)
213 had terminated their education at the end of compulsory schooling. 17 of the patients (33.3%)
214 held a college or university degree.

215

216 The healthy controls consisted of 22 women (67%) and 11 men (33%), ranging in age from 29
217 to 81 years (mean=61.3, SD=11.90). Sixteen of the controls (49%) had compulsory schooling
218 as their highest educational level, with 17 (51%) with college or university degrees.

219

220 There were no significant differences between the three groups regarding age ($F(2,81)=1.865$,
221 $p=0.161$), gender ($\chi^2=1.454$, $df=2$, $p=0.483$) and educational level ($\chi^2=3.085$, $df=2$, $p=0.214$).
222 In addition, the number of subjects per group ($\chi^2=2.79$, $df=2$, $p=0.248$), BTX treatment duration
223 ($\chi^2=7.57$, $df=3$, $p=0.056$), and the applied BTX preparations ($\chi^2=5.84$, $df=3$, $p=0.119$) were

224 equally distributed in all three groups. BTX equivalent dosage was not equally distributed
225 ($\chi^2=37,63$, $df=4$, $p<0.0001$), but the dosage did not correlate with verbal and non-verbal
226 reasoning scores (see below).

227

228

229 Baseline assessment of non-verbal and verbal reasoning

230

231

232 There were no statistically significant differences in scores of nonverbal as well as verbal
233 reasoning before BTX-treatment. An analysis of variance showed no significant differences
234 ($F(2,81)=2.14$, $p=0.124$) between CD (mean=7.77, SD=3.52, 95%CI=5.82-9.82), BEB/HS
235 (mean=6.26, SD=2.72, 95%CI=4.67-7.78) and control subjects (mean=7.83, SD=2.85,
236 95%CI=6.1-9.52) with respect to scores of nonverbal reasoning at baseline. There were also no
237 statistically significant differences at baseline with respect to scores of verbal reasoning
238 ($F(2,80)=2.92$, $p=0.060$) between CD (mean=7.7, SD=3.15, 95%CI=6.03-10.17), BEB/HS
239 (mean=6.32, SD=3.11, 95%CI=4.6-7.85) and control subjects (mean=8.66, SD=3.42,
240 95%CI=6.5-10.11).

241

242

243 Differences in reasoning scores: pre- and post-treatment

244

245

246 Non-verbal reasoning (Fig 1)

247

248

249 The scores of control subjects improved (mean=9.23, SD=3.62, 95%CI=7.1-11.17), those of
250 CD slightly improved (mean=8.07, SD=3.97, 95%CI=5.86-10.62). and those of BEB/HS
251 slightly decreased (BEB/HS: mean=6.21, SD=3.36, 95%CI=4.25-7.95).

252

253 Figure 1. Non-verbal reasoning scores.

254

255 A mixed design ANCOVA revealed no significant main effect of time [$F(1,74)=0.86$, $p=0.357$,
256 $\eta^2=0.011$], but a significant main effect of group [$F(2,74)=3.34$, $p=0.041$, $\eta^2=0.083$] with
257 respect to nonverbal reasoning, indicating no differences between pre-and post-treatment
258 evaluation but differences between the three groups. The covariates dose [$F(1,74)=0.12$,
259 $p=0.731$, $\eta^2=0.002$] and overall treatment time [$F(1,74)=0.22$, $p=0.641$, $\eta^2=0.003$] showed no
260 significant impact.

261

262 Pairwise comparisons showed a significant difference between healthy controls and BEB/HS
263 ($p=0.022$), with the lowest mean difference in non-verbal abilities regarding pre- and post-
264 treatment evaluation in BEB/HS. No difference was shown between healthy controls and CD
265 ($p=0.794$) and between the two patient groups ($p=0.197$).

266

267 The interaction effect of time and group was not significant [$F(2,74)=1.23$, $p=0.297$, $\eta^2=0.032$]
268 indicating no differences in the mean change in nonverbal reasoning scores between patient
269 groups and healthy controls and no learning effect occurred regarding non-verbal reasoning.
270 There were also no significant interaction effects of time and dose [$F(1,74)=0.11$, $p=0.743$,
271 $\eta^2=0.001$] as well as time and overall treatment time [$F(1,74)=0.15$, $p=0.696$, $\eta^2=0.002$].

272

273

274 Verbal reasoning (Fig 2)

275

276

277 The scores of CD improved (mean=8.77, SD=4.01, 95%CI=7.13-11.76), those of controls
278 (mean=8.34, SD=3.38, 95%CI=5.74-9.8) and BEB/HS (mean=6.0, SD=3.38, 95%CI=3.98-
279 7.63) slightly decreased.

280

281 Fig 2. Verbal reasoning scores.

282

283 With respect to verbal reasoning a mixed design ANCOVA revealed no significant main effect
284 of time [$F(1,73)=0.37$, $p=0.546$, $\eta^2=0.005$] but a significant main effect of group [$F(2,73)=3.37$,
285 $p=0.040$, $\eta^2=0.084$], indicating no differences between pre- and post- treatment evaluation but
286 differences between the three groups. The covariates dose [$F(1,73)=0.07$, $p=0.793$, $\eta^2=0.001$]
287 and overall treatment time [$F(1,73)=0.85$, $p=0.359$, $\eta^2=0.012$] did not have a significant impact.

288

289 Pairwise comparisons showed a borderline significance suggesting a noticeable difference
290 between healthy controls and BEB/HS ($p=0.051$) as well as borderline significant differences
291 between the two patient groups ($p=0.067$), with the lowest mean difference in verbal abilities
292 regarding pre- and post- treatment evaluation in BEB/HS. No difference was shown between
293 healthy controls and CD ($p=0.669$).

294

295 The interaction effect of time and group was not significant [$F(2,73)=1.12$, $p=0.331$, $\eta^2=0.030$],
296 indicating that the mean changes in verbal reasoning scores did not differ between both patient
297 groups and healthy controls and no learning effect occurred regarding verbal reasoning.

298 Furthermore, there are no significant interactions between time and dose [$F(2,73)=0.13$,
299 $p=0.718$, $\eta^2=0.002$] as well as overall treatment time [$F(2,73)=0.02$, $p=0.893$, $\eta^2=0.000$].

300

301

302 Relationship between BTX dose und verbal or nonverbal reasoning

303

304

305 There was no significant correlation between BTX dose and verbal reasoning (Pearson
306 correlation, $r(49)=0.22$, $p=0.132$) as well as nonverbal reasoning scores (Pearson correlation,
307 $r(49)=0.153$, $p=0.294$) after treatment.

308

309

310 **Discussion**

311

312

313 The main finding of this exploratory study was a significant difference of non-verbal reasoning
314 scores in the patient group who received BTX treatment of their facial muscles (BEB/HS),
315 when compared to healthy controls. In passing, we note that there was also a noticeable
316 difference of the post-treatment verbal reasoning scores, but they failed to reach significance
317 ($p=0.051$).

318

319 These preliminary results are in several respects surprising, given that the treatment might have
320 been predicted to *address* potential physical impairments, and thus *improve* test scores. For
321 example, an improvement of the visual sustained attention span might have been expected, after
322 clinical improvement of the BEB symptoms (as has been described previously).¹⁸ A related

323 issue would be an expected improvement in the performance of written tests, given that patients
324 report difficulties reading, which also tends to improve after their botulinum treatment. Indeed,
325 when the effect of the BTX treatment wears off, patients tend to again complain about those
326 difficulties. This was also reflected in the higher number of patients who refused to participate
327 in this study, as a result of these impairments. Cognitive impairment after facial BTX injections
328 in the treatment of neurological disorders has never been reported.^{19, 20} However, discrete
329 impairment of cognitive performance has been described as a non-motor syndrome of BEB.²¹
330 Two-thirds of patients in the facial injection group suffered from BEB. We cannot exclude that
331 the pathophysiological alterations due to the non-motor syndrome might be a potential
332 confounding factor. However, cognitive disturbances as non-motor symptoms have also been
333 described in cervical dystonia.²² Therefore, one might expect a similar performance. But in the
334 case of verbal scores, the cervical injection cohort *improved* with a borderline significance
335 ($p=0.067$), when compared to the facial injection group.

336

337 There is little data referring to neural correlates of the two specific aspects of reasoning, which
338 were investigated in the present study.²³ Verbal analytic reasoning has been correlated with
339 rsfMRI (resting state fMRI) data and has been related with brain regions for integration (i.e. the
340 angular and supramarginal gyrus), hypothesis testing, cognitive control (i.e. inferior frontal
341 gyrus) and response selection (i.e. dorsal anterior cingulate cortex). Non-verbal reasoning
342 scores were non-significantly associated with the left occipital- and right anterior temporal lobe,
343 and right frontoinsula cortex, respectively.²³

344

345 One account might be a more direct link between facial muscles and these cognitive networks.
346 In this context, the role of the corrugator muscle in several emotional and non-emotional facial
347 expressions has been reported.²⁴ Amongst others, the corrugator muscle is activated during

348 concentration, and plays an important role in communication and interaction, including when
349 accompanying or emphasizing elements of speech.²⁵ A recent investigation measured motor
350 activity of grip strength during verbal processing, and found a context sensitive increase during
351 processing of “action words”.²⁶

352

353 An alternative approach might be that the BTX induced facial palsy leads to a delay in emotion-
354 related responses, which is well-established.² There is an increasing awareness of the ways that
355 emotion might interact with cognitive processes – such as perception, attention, memory and
356 decision making.⁷ However, emotion-linked domains like memory -i.e. emotion based learning
357 ²⁷, or planning and decision-making⁷ were not covered by the present reasoning tasks. Further
358 investigations might profit from additional objective tests of these cognitive domains.

359

360 Regardless of cause, our unexpected findings illustrate the importance of careful monitoring
361 during a regular rehabilitative treatment of chronic diseases, even though this treatment is well-
362 established. In addition, further research might consider tests of the cognitive performance
363 during the rehabilitation of facial palsies of other origin, e.g., Bell’s palsy.

364

365 Notably, we found no indications for a dose dependent effect on the reasoning scores. It is also
366 notable that performance of the control patients with cervical muscle injections (CD) did not
367 differ from healthy controls at any time point, even though those patients received high
368 cumulative doses (Table 3). This might be a relevant issue for other BTX applications with high
369 cumulative doses in neurological rehabilitation, namely spasticity. These data also support the
370 assumption that there is no direct effect of BTX due to a questionable retrograde transport or
371 systemic distribution.^{28, 29}

372

373

374 Study limitations

375

376

377 At this point, - and as the major limitation of this study- these preliminary results do not allow
378 a causality of facial palsy and cognitive performance; thus, the interpretation of the present data
379 remains highly speculative and cannot be generalized. The data needs to be confirmed by trials
380 involving a larger sample size and additional control groups to adjust for confounding factors,
381 such as a selection bias.

382

383 Here, we did not perform sham-injections as placebo control. One reason for this were ethical
384 considerations, as BTX is the first line therapy and very effective in dystonia.^{9, 13} Furthermore,
385 unblinding appears highly probable. We suggest the additional investigation of subjects
386 receiving cosmetic facial BTX injections.

387

388 These appear to be conventional clinical samples, with normal baseline intelligence
389 measurements of the patient and control groups. Novelty does not seem to be a relevant
390 confounding variable, as there were no BTX “naive” patients included, and both patient groups
391 have a mean treatment duration of almost six years, as part of a regular cycle of treatments.

392

393 We did not perform a follow up evaluation to clarify if the reduced performance is temporary
394 and completely reversible. Therefore, future research should address the sustainability of the
395 results after the paralysis of facial muscles wears off.

396

397

398 **Conclusions**

399

400

401 The unexpected key finding is that patients who receive facial BTX injections appear to
 402 perform significantly worse in non-verbal (and as a trend, verbal) reasoning tasks. However,
 403 these are preliminary results of an exploratory study with potential confounders. Thus, at this
 404 point, BTX induced facial palsy and cognitive performance cannot be related, and therefore
 405 the interpretation of these results remains highly speculative. It is clear that the findings
 406 should be backed by further controlled investigations and illustrate the importance of careful
 407 monitoring during a well-established, rehabilitative treatment of chronic diseases.

408

409

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412

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478

479 **Figure legends**

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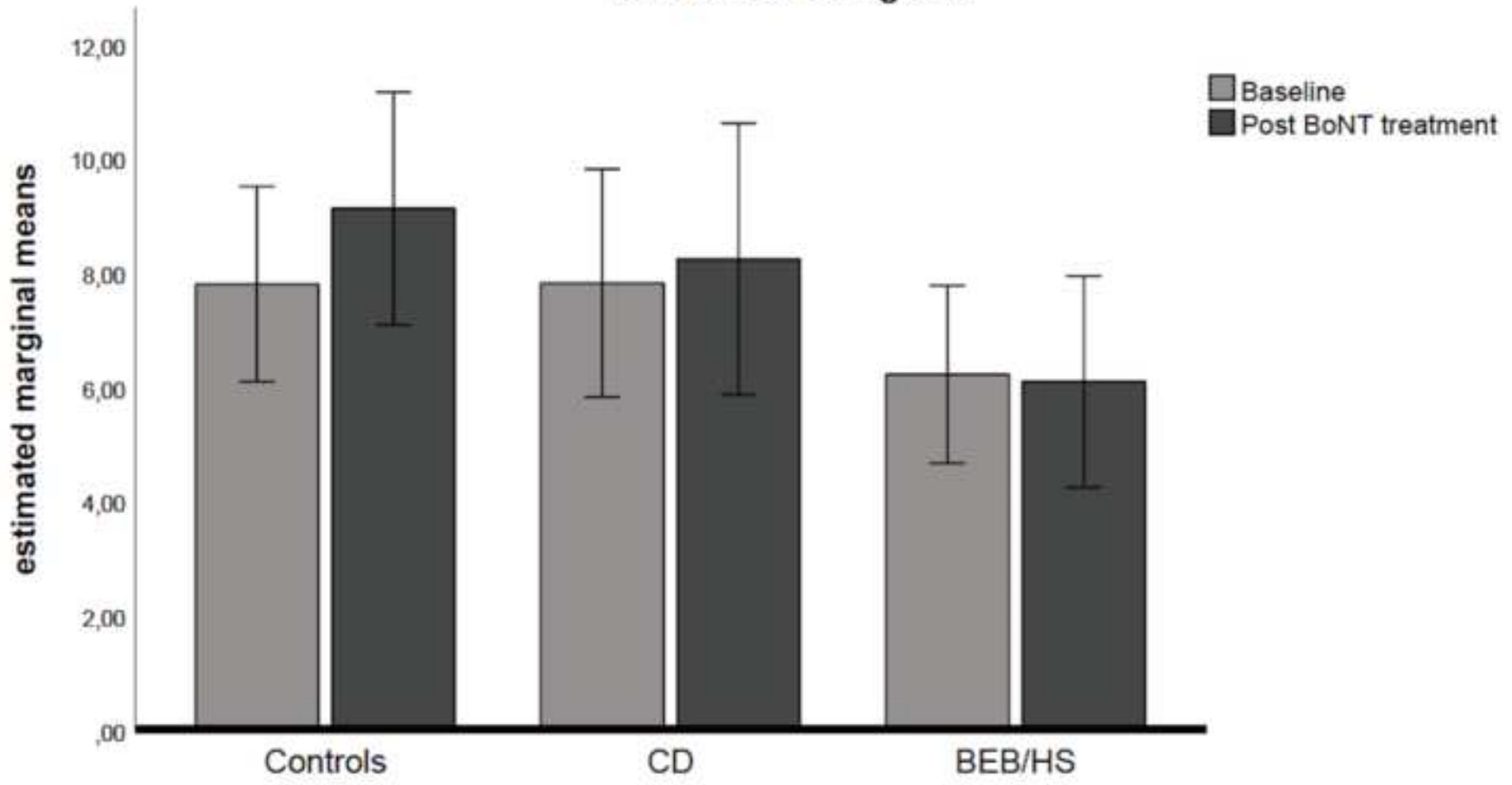
481

482 **Fig 1. Non-verbal reasoning scores.** Estimated marginal mean scores and 95% CI of
483 non-verbal reasoning tests absolved by patients and controls at baseline and three weeks
484 after BTX-treatment (controls received no injections). Abbreviation: BEB:
485 blepharospasm; BoNT: botulinum neurotoxin; HS: hemifacial spasm.

486

487 **Figure 2. Verbal reasoning scores.** Estimated marginal mean scores and 95% CI of
488 verbal reasoning tests absolved by patients and controls at baseline and three weeks after
489 BTX-treatment (controls received no injections). Abbreviation: BEB: blepharospasm;
490 BoNT: botulinum neurotoxin; HS: hemifacial spasm.

non-verbal intelligence



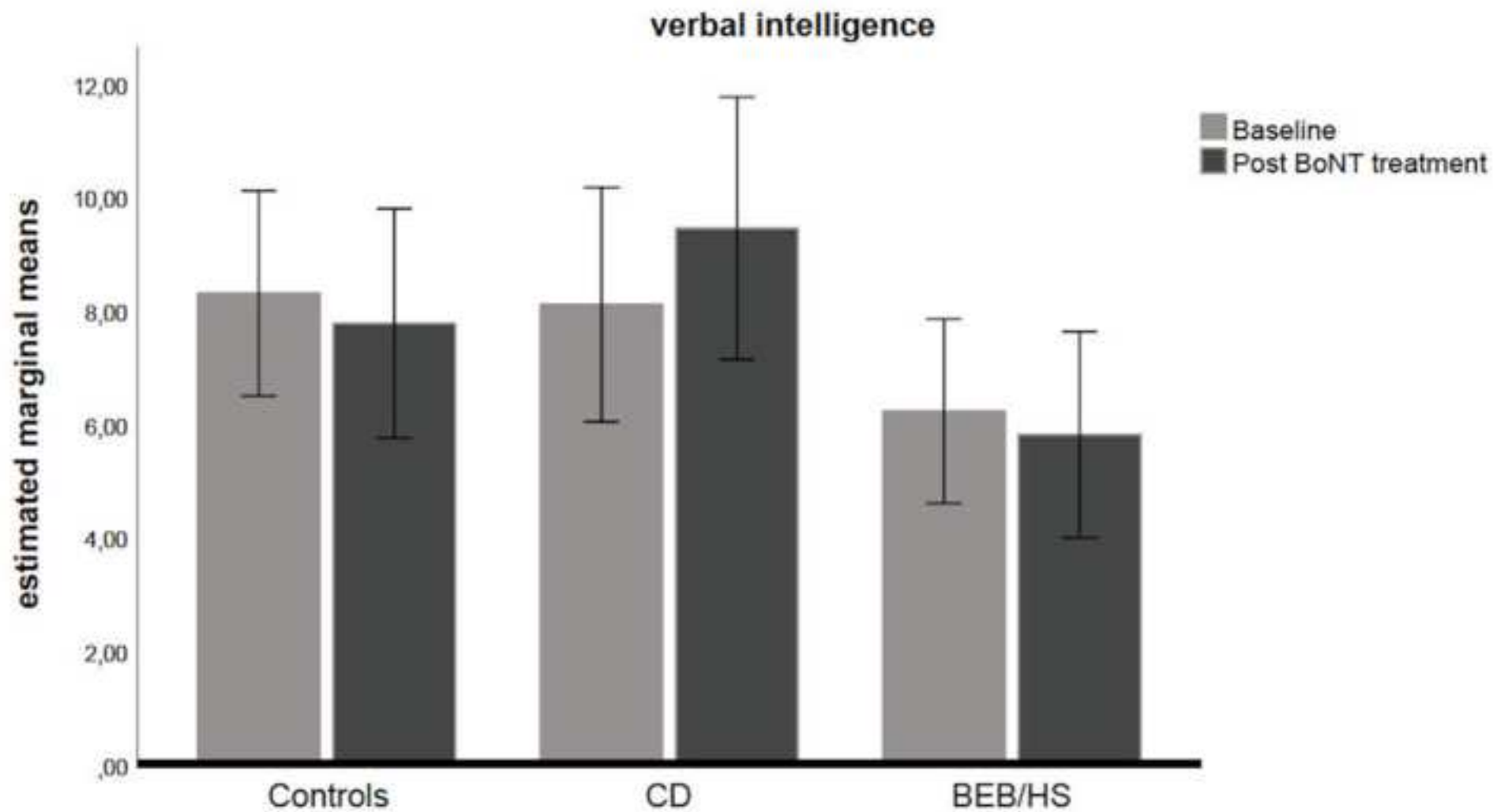


Table 1. List of included muscles

BEB/HS	CD
Orbicularis oculi	Sternocleidomastoid
Palpebral part of the orbicularis oculi	Splenius capitis
Corrugator supercilii	Semispinalis capitis
Levator anguli oris	Trapezius
Depressor anguli oris	Levator scapulae
Platysma	

Table 2. Details of drop out

	BEB	HS	CD
Total number of drop out	13/27 (48.1)*	4/11 (36.4)*	20/50 (40)*
Physical handicap	3 (23.1)†	-	1 (5) †
Fear of a bad test performance	1 (7.7) †	-	-
Lack of time	-	-	3 (15) †
Language barrier	1 (7.7) †	-	1 (5) †
No specific reason	8 (61.5) †	2 (50) †	14 (70)†

*Total number of drop out /number of eligible patients (% of number of eligible patients).

†Number of patients (% of total number of drop out)

Table 3. Clinical details of all participants at baseline assessment.

	BEB/HS	CD	HC
Number of subjects	21 (25.0)*	30 (35.7)*	33 (39.3)*
Age	63.4a (11.9)†	57.1a (12)†	61.3a (11.9)†
Male	7 (33.3)*	14 (46.7)*	11 (33.3)*
Female	14 (66.7)*	16 (53.3)*	22 (66.7)*
BTX treatment duration†	5.8a (5.96)†	8a (6.7)†	-
< 3 years	11 (52.4)*	6 (20)*	
3 to 5 years	7 (33.3)*	11 (36.7)*	
6 to 8 years	1 (4.8)*	2 (6.7)*	
> 8 years	2 (9.5)*	11 (36.7)*	
BTX preparation applied			-
Abo-BTX A‡	7 (33.3)*	19 (63.4)*	
Inco-BTX A§	5 (23.8)*	4 (13.3)*	
Ona-BTX A	9 (42.9)*	6 (20.0)*	
Rima-BTX B¶	-	1 (3.3)*	
BTX equivalent dosage	55.2MU (37.2)†	236.5MU (94.3)†	-
< 100	17 (81.0)*	-	
100 to 200	4 (19.0)*	17 (56.7)*	
201 to 300	-	7 (23.3)*	
301 to 400	-	5 (16.7)*	
> 400	-	1 (3.3)*	

*Number of subjects (%), †Mean (SD), ‡Dysport® (Ipsen), §Xeomin® (Merz), ||Botox® (Allergan), ¶NeuroBloc® (USWorldMed); Abbreviations: a=years, MU=Mouse Units



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STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2-3
Objectives	3	State specific objectives, including any prespecified hypotheses	3-4
Methods			
Study design	4	Present key elements of study design early in the paper	3-4;5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (b) For matched studies, give matching criteria and number of exposed and unexposed	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) If applicable, explain how loss to follow-up was addressed (e) Describe any sensitivity analyses	7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	8, Table2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	8-9, Table3
Outcome data	15*	Report numbers of outcome events or summary measures over time	8

Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9-12
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-12
Discussion			
Key results	18	Summarise key results with reference to study objectives	13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14;15-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	n.a.

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.



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