Hypothesis / aims of study
Botulinum-A Toxin (BTX-A), a potent inhibitor of acetylcholine release from motor nerve terminals, has been traditionally used to treat muscle spasticity. Recently, a direct modulation of the neurotoxin on the afferent C-fibers has been demonstrated, as it may inhibit the release of vesicular neurotransmitters as SP, glutamate, CGRP and ATP from C afferent terminals in isolated bladder tissue, and from the urothelium of spinal cord injured bladders (1). Actually there are no information about a possible mechanism of action of BTX-A involving Nerve Growth Factor (NGF) production and/or release into the bladder wall.
We sought to investigate the effects of BTX-A on visceral afferent nerve transmission by measuring NGF bladder tissue concentrations in patients affected by neurogenic detrusor overactivity before and after intravesical treatment with BTX-A. We also compared NGF bladder tissue content with clinical and urodynamic data.

Study design, materials and methods
Baseline evaluation.
23 spinal cord injured patients (SCI) underwent a history, physical examination, urinalyses and culture and urodynamics according to ICS standards (2). On urodynamics, uninhibited detrusor contractions (UDC) threshold, UDC maximum pressure, and maximum cystometric capacity were recorded. The number of daily episodes of incontinence was assessed by means of a voiding diary.

Cystoscopy and Treatment.
In the same session of treatment patients underwent a preliminary cystoscopy with bladder wall biopsy specimens; then they received BTX-A injections into the detrusor muscle (300 U of commercially available toxin, diluted in 30 ml normal saline, sparing the trigone), under cystoscopic control. The procedure was performed under mild anesthesia; after treatment a 16 Ch Foley indwelling catheter was routinely inserted for 24 hours. Patients were discharged after overnight observation.

Follow up.
Clinical evaluation, urodynamics and cystoscopy with bladder wall biopsies were repeated at 1 and 3 months follow up. Bladder wall biopsy specimens were taken from the opposite side of the bladder wall.

NGF measurement.
NGF levels were measured in tissue homogenate by means of enzyme linked immunosorbent assay (ELISA, Promega, Madison-WI). The sensitivity was 15.6 pg/ml of NGF. Data were expressed as ng/mg protein (mean of duplicate samples).

Results
At baseline, all patients presented with urinary incontinence and the mean number of daily episodes of incontinence was 4.6 ± 1.2; on urodynamics they were all affected by detrusor overactivity with detrusor sphincter dyssynergia. Mean value of NGF bladder tissue content was 141.8 ± 73.1 ng/mg. After treatment, a complete urinary continence was achieved by 18 patients at 1 and 3 months. In 5 patients a significant reduction in the number of daily episodes of incontinence was observed at 1 and 3 months (1.5 ± 1.6 and 1.3 ± 0.9 respectively, p<0.001). On urodynamics, we detected a significant increase in UDC threshold (p<0.001) and in maximum cystometric capacity (p<0.001), and a significant reduction in UDC maximum pressure (p <0.001) at 1 and 3 months after treatment, as compared to baseline. Furthermore, at the same time points we detected a significant reduction (p<0.02) in NGF bladder tissue content (see Table below).
<table>
<thead>
<tr>
<th></th>
<th>Baseline (mean ± SD)</th>
<th>1 month (mean ± SD)</th>
<th>3 months (mean ± SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDC threshold (ml)</td>
<td>165 ± 92.2</td>
<td>420.4 ± 105.6</td>
<td>445.5 ± 52</td>
<td>p &lt; 0.001</td>
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<tr>
<td>UDC max pressure (cmH₂O)</td>
<td>62.3 ± 21.8</td>
<td>17 ± 20.7</td>
<td>24.6 ± 32.5</td>
<td>p &lt; 0.001</td>
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<tr>
<td>Bladder capacity (ml)</td>
<td>246.3 ± 88.4</td>
<td>437.9 ± 78.2</td>
<td>459.2 ± 64.7</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>NGF (ng/mg)</td>
<td>141.8 ± 73.1</td>
<td>85.2 ± 41</td>
<td>78.7 ± 39</td>
<td>p &lt; 0.02</td>
</tr>
</tbody>
</table>

No local or systemic side effects were recorded during and after treatment.

Interpretation of results
To our knowledge this is the first study investigating the relationships between BTX-A intravesical treatment and the levels of NGF into the bladder wall of patients affected by neurogenic detrusor overactivity. The results in this study show that BTX-A intravesical administration in SCI patients induces a significant decrease in both detrusor overactivity and in NGF bladder tissue levels. The state of NGF deprivation in bladder tissues can result in a decreased hyperexcitability of C-fiber bladder afferents and, consequently, in a reduction of detrusor overactivity. Several neurotransmitters are retained to influence NGF bladder tissue levels, such as Acetilcholine, Noradrenaline and ATP, the latter normally released from the afferent C-terminals. One hypothesis is that BTX-A administered intravesically, can reduce the levels of NGF into the bladder wall by decreasing the levels of these neurotransmitters, i.e. ATP. Other investigations are necessary to elucidate the mechanisms underlying the effects of BTX-A on NGF production and/or release from the human bladder wall.

Concluding message
BTX-A intravesical administration induces a state of NGF deprivation in bladder tissue of patients with neurogenic detrusor overactivity, probably by interfering with NGF –dependent metabolism and/or production. Thus, BTX-A intravesical injections induce a decrease of the hyperexcitability of C-fiber bladder afferents and, consequently, of neurogenic detrusor overactivity.

References