Author’s response to reviews

Title: The role of the paravertebral muscles in adolescent idiopathic scoliosis evaluated by temporary paralysis.

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Author’s response to reviews:

Dear professor Grivas and reviewers

Thank you for the possibility to revise this manuscript. The reviewers has provided me with excellent comments, which has led to a through revision - especially the introduction and discussion – and moreover, I have had to be more precise in scope of this article and other aspects of this article. I have extracted text from the manuscript to highlight the answers to the reviews comments. This is put in with bold text in this letter and additional text in the manuscript is highlighted in red. I have added line numbers, so it can be located in the manuscript, but you’ll have to excuse me, if this in not completely correct, since the revisions were done consecutively and small changes in wording and line numbers might have been changed throughout the revision.

The changes are clearer to evaluate in the submitted word document.

Yours sincerely

Christian Wong

SCOL-D-17-00007

The role of the paravertebral muscles in idiopathic scoliosis evaluated by temporary paralysis.
christian wong, PhD, MD; kasper gosvig; stig sonne-holm

Scoliosis and Spinal Disorders

Dear Dr wong,

Your manuscript "The role of the paravertebral muscles in idiopathic scoliosis evaluated by temporary paralysis." (SCOL-D-17-00007) has been assessed by our reviewers. Although it is of interest, we are unable to consider it for publication in its current form. The reviewers have raised a number of points which we believe would improve the manuscript and may allow a revised version to be published in Scoliosis and Spinal Disorders.

Their reports, together with any other comments, are below. Please also take a moment to check our website at http://scol.edmgr.com/ for any additional comments that were saved as attachments.

If you are able to fully address these points, we would encourage you to submit a revised manuscript to Scoliosis and Spinal Disorders. Once you have made the necessary corrections, please submit online at:

http://scol.edmgr.com/

If you have forgotten your username or password please use the "Send Login Details" link to get your login information. For security reasons, your password will be reset.

Please include a point-by-point response within the 'Response to Reviewers' box in the submission system and highlight (with 'tracked changes'/coloured/underlines/highlighted text) all changes made when revising the manuscript. Please ensure you describe additional experiments that were carried out and include a detailed rebuttal of any criticisms or requested revisions that you disagreed with. Please also ensure that your revised manuscript conforms to the journal style, which can be found in the Submission Guidelines on the journal homepage.

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Please be aware that we may investigate, or ask your institute to investigate, any unauthorised attempts to change authorship or discrepancies in authorship between the submitted and revised versions of your manuscript.

The due date for submitting the revised version of your article is 11 Jul 2017.

I look forward to receiving your revised manuscript soon.

Best wishes,

Theodoros B. Grivas, MD, PhD
Scoliosis and Spinal Disorders
https://scoliosisjournal.biomedcentral.com/

Reviewer reports:
Reviewer #1: Dear Authors,

There are some grammatical and spelling errors that should be corrected.
This is corrected.

Furthermore the patient group includes 9 patients with different therapy strategies (physiotherapy, bracing), which were maintained through the study and could affect the results. For e.g. how many hours or days before X-ray control did the patients remove the brace?
As the reviewer stated; treatment otherwise was maintained, since we were interested in seeing the effect of the temporary effect of the paralysis alone.

In regards to omitting the brace, this was done routinely 24 hours before radiological examination, which has the standard policy of our clinic for all x-rays for scoliosis.

This is now added in the methods section, by stating………

The radiographic acquisitions were performed by the same staff in a uniform, systematic manner, where the brace was omitted for 24 hours before the acquisition…(1104-106)

There are also patients with main thoracic curves (patients 3, 4 and 7). What do we expect to achieve by injecting ITB at the compensatory lumbar curve? I believe it would be better if only patients with a main lumbar curve were included in this study.

We agree with the reviewer, that primary lumbar curves would benefit the most for this injection treatment. In retrospect this study should have been performed with injection in the lumbar spine, in for example in the psoas major and quadratus lumborum in the primary lumbar scoliosis

This is now added in the discussion…

These changes were as expected better in the lumbar region, since the primary effect is in the lumbar region, thus having subsequent less change in the thoracic region as seen in figure 2. (1152-153)

and

Moreover, three patients had main thoracic curves (patients 3, 4 and 7), and we would expect lesser effect (as in fact seen) than if all patients had main lumbar curves. (l 166-168)

and

In retrospect, the ITB should have been performed either on the convex side or bilaterally, and this could be undertaken in a future study, if another study using ITB in humans was to be undertaken. Our suggestion would be to focus on primary lumbar curves, since the radiological effects were more pronounced in this region. Also, the multifidi and quadratus lumborum muscles has been examined as potential scoliogenic muscles and could be of interest for future studies [10,26]. (1192-197)

We have also elaborated on and clarified our purpose in the introduction, for example…
However, to our knowledge ITB for AIS has not been investigated, and would seem to be ideal for examining the role of the muscles in AIS, since it provides temporary muscle paralysis without long-term side effects or complications in therapeutic doses in otherwise healthy humans [19].

And

The purpose was to examine if ITB would induce a change in curvature in AIS. This could clarify if the spinal muscles play in the pathological process of the AIS; whether the spinal muscles would in fact induce the spinal deformity of AIS by the muscle forces/pull of the PM, thus having a scoliogenic effect, which would be seen by a regression of the AIS after paralysis..(l82-85)

The introduction and discussion section has been revised thoroughly.

Moreover, an additional explanatory figure has been added. (added to figure 1)

In addition to these, there is an article about lumbar curves and muscle affection, which should be mentioned in the discussion (Scoliosis Spinal Disord. 2016 Oct. Idiopathic and normal lateral lumbar curves: muscle effects interpreted by 12th rib length asymmetry with pathomechanic implications for lumbar idiopathic scoliosis. Grivas et al.)

This article is indeed very interesting, which I was not aware of and is now included in our article. We have adapted the syntax of this article, since it seems more appropriate in regards to describe the ‘pathomechanic’ role of the muscles as ‘scoliogenic’ or ‘counteracting scoliosis’

Large parts of the introduction have been re-written and the essential change are….

This important question of the ‘pathomechanic role’ of paravertebral muscles is still debated [10], but the evidence for the specific role as scoliogenic (inducing scoliosis) or counteracting scoliosis in human is still circumstantial [2,3,9-13]. Recently, Grivas et al. (2016) examined this for the Quadratus lumborum muscle by comparing the length of the 12th rib in a group of children with right lumbar idiopathic scoliosis and straight spines; he suggested, that stimulation of the paravertebral muscles should be performed to determine the ‘pathomechanic role’ in future studies [10]. In this study we examined if the hypnotized ‘pathomechanic role’ of the Psoas Major (PM) of the Iliopsoas muscle is scoliogenic - not by stimulation, but by paralysis. (l.43-51)

Yours sincerely,

E. Christodoulou
Reviewer #2: The role of para-psinal muscles in the etiology or natural history of idiopathic scoliosis has been discussed for many years. Various studies have investigated different properties of para-spinal muscles in IS patients including histology, biomechanics, EMG and morphology but still they are unable to conclude whether the changes observed in para-spinal muscles indicate a progressive or corrective effect to IS. This study attempted to understand the role of para-spinal muscle by paralyzing one group of muscle and observe the response of IS. The reviewer found this method innovative.

After reading the manuscript, questions and comments based on the study design and the presentation style are summarized as follows.

1. Keywords are missing in the Abstract part.
   This is included

2. Botulinum toxin A (BTA) was injected to patients' iliopsoas muscle on the concave side of the curve as described in Abstract and Methods (Line 88). What was the reason to put the focus in this muscle group? The rationale or the importance of iliopsoas muscle should be thoroughly elaborated in Introduction or Discussion.

   We have elaborated on and clarified our purpose in the introduction, where large parts has been re-written and moreover an explanatory figure has been added...

The argument for targeting Psoas major is

The PM muscle is interesting for examining the ‘pathomechanic role’ for AIS; Bruggi et al. (2014) found an interrelationship between the paravertebral muscle Iliopsoas and AIS, where muscle in isometric contraction had a corrective effect of the scoliotic curve [14]. In addition, a volumetric asymmetry of the PM has also been demonstrated in patients with degenerative AIS, where hypertrophy of 6.3 % on the convex side was concluded to be associated with the scoliosis [12]. Yet another study was unable to demonstrate, that this difference had a significant effect in either maximal voluntary isometric contraction force between healthy girls scoliosis (161.4 N) and girls with scoliosis (144.3 N) or in strength of the paravertebral muscle on either side of the scoliosis [15].(151-59)

And furthermore...

The hypnotized scoliogenic role of the PM muscle would be that of initiating or maintaining a lumbar scoliotic curvature by muscle contraction. The PM would act by performing a lateral pull
in the upper part of the lumbar spine into a concave scoliotic curvature, thus creating a convex thoracic curve in the thoracic and thoracolumbar scoliosis. This is illustrated in figure 1. (l66-70)

And

The PM muscle would seem an ideal case in which to examine and clarify this specific scoliogenic effect, since it is of such a strength/magnitude/size, that temporary paralysis would affect the scoliotic curves when recorded radiographically, and at the same time would be attainable for safe percutaneous injection treatment. (l70-73)

And

The purpose was to examine if ITB would induce a change in curvature in AIS. This could clarify if the spinal muscles play in the pathological process of the AIS; whether the spinal muscles would in fact induce the spinal deformity of AIS by the muscle forces/pull of the PM, thus having a scoliogenic effect, which would be seen by a regression of the AIS after paralysis. (l82-85)

3. It was noted that in many places of this manuscript, the presentation is inconsistent with the common presentation in most journal articles as in Line 30 "idiopathic adolescent scoliosis", Line 53 "prossessus transversi" (should be transverse process), Line 100 "Nash and Moe's classification", Line 109 the citation of equipment's company, Line 131 "corset" (should be brace) and Line 156 "5 to ten degrees".

This has been changed accordingly. However, in regards to citation of a company name, I cannot find this. Would that be in reference to SPSS (version 22)? I would think, that an interested reader would be able to replicate the statistical analysis, thus being interested in knowing the exact analysis using the exact same software; thus has been maintained in the manuscript.

4. In Line 40, reference 7 is missing from the reference list.

Thank you; reference 7 was placed in conjunction with reference 6, and is now moved so it is clear and easy to locate the reference

5. In Line 48, "trophy" means hypotrophy or atrophy?

This has been clarified…
In addition, a volumetric asymmetry of the PM has also been demonstrated in patients with degenerative AIS, where hypertrophy of 6.3% on the convex side was concluded to be associated with the scoliosis [12]. (I53-56)

6. In Line 49 and 50, the difference of muscle size asymmetry was not presented in reference 14.

This has been clarified for reference 14…

Yet another study was unable to demonstrate, that this difference had a significant effect in either maximal voluntary isometric contraction force between healthy girls scoliosis (161.4 N) and girls with scoliosis (144.3 N) or in strength of the paravertebral muscle on either side of the scoliosis [16]. (I56-59)

This has been clarified for reference 11…

Also, a volumetric asymmetry of the PM has also been demonstrated in patients with degenerative AIS, where hypertrophy of 6.3% on the convex side was concluded to be associated with the scoliosis [11]. (I53-56)

7. In Line 55, the reference paper was describing psoas major but not iliopsoas.

This has been changed accordingly (I59)

8. In Line 56, the muscle attachment site should be femur instead of hip.

This has been changed accordingly (I64)

9. In Line 58, what does "hypnotized role" means?

This has been elaborated on in the introduction and discussion, for example:

The hypnotized scoliogenic role of the PM muscle would be that of initiating or maintaining a lumbar scoliotic curvature by muscle contraction. The PM would act by performing a lateral pull in the upper part of the lumbar spine into a concave scoliotic curvature, thus creating a convex thoracic curve in the thoracic and thoracolumbar scoliosis. This is illustrated in figure 1. (I66-70)

And
The PM muscle would seem an ideal case in which to examine and clarify this specific scoliogenic effect, since it is of such a strength/magnitude/size, that temporary paralysis would affect the scoliotic curves when recorded radiographically, and at the same time would be attainable for safe percutaneous injection treatment. (170-73)

And

The purpose was to examine if ITB would induce a change in curvature in AIS. This could clarify if the spinal muscles play in the pathological process of the AIS; whether the spinal muscles would in fact induce the spinal deformity of AIS by the muscle forces/pull of the PM, thus having a scoliogenic effect, which would be seen by a regression of the AIS after paralysis. (182-85)

10. From Line 58 to Line 61, if this statement "the hypnotized role of … secondary and compensatory" is a hypothesis of iliopsoas muscle activities being associated with IS lumbar curvature the statement, should this study only focused on IS patients with major lumbar curve (type 5 or 6 in Lenke classification)?

The author agrees with the reviewer. Moreover, bilateral and/or injection treatment on the convex side might have given clearer and more pronounced radiological results. Alas, the nature of this study do not allow us to replicate this study on primary lumbar curves for ethical reasons, since another study treating neuromuscular scoliosis with this treatment had an occurrence of one death*. However, this was unrelated to the treatment, but still this dis-encouraged us for further injection treatment in this study


What kind of curve type to be included should be explained in Methods. Also the statement is better to be supported by reference(s).

Since we agree with the reviewer, we have found it more appropriate to include this in the discussion section as seen below…

In this study, the role of the PM muscle in humans would be scoliogenic, that maintains AIS, but this conclusion can probably not be extrapolated to all of the paravertebral muscles in general. However, this is to our knowledge the first study to directly influence the paravertebral muscles by immediate temporary paralysis in humans in order to examine the role in AIS, and in our view being an important step for the further exploration and understanding of the etiology of AIS. We
would recommend to examine this by stimulation instead of paralysis for future studies as suggested by Grivas et al. (2016) [10]. Our above-described radiographic changes may be seen as mimicking a ‘wavy pattern’ as described earlier [4], where slight changes in level and size occurred as a response to the almost immediate paralysis of the PM muscle. However, if bilateral paralysis were performed instead of unilateral, this might have resulted in larger changes and have shed light on the role of the paravertebral muscles even further, but bilateral paralysis had to be omitted for safety reasons to minimize botulinum toxin dosage for the patients to prevent systemic spread, and ethical approval was only for unilateral treatment. Moreover, studies using electromyography and/or magnetic resonance imaging for muscle volume and muscle quality (fatty infiltration) indicate that the spinal muscles are significantly stronger and larger on the convex side at the apex of the curve of the scoliosis [9,11,24,25]; this would indicate that ITB of the paravertebral muscles would have a correcting effect, when injected on the convex side. At initiation of this study, we evaluated, that the muscle contraction/pull of PM on the concave side of the lumbar curve of the thoracolumbar scoliosis in fact brought about the deformity as seen in figure 1. In retrospect, the ITB should have been performed either on the convex side or bilaterally, and this could be undertaken in a future study, if another study using ITB in humans was to be undertaken. Our suggestion would be to focus on primary lumbar curves, since the radiological effects were more pronounced in this region. Also, the multifidi and quadratus lumborum muscles has been examined as potential scoliogenic muscles and could be of interest for future studies [10,26] (l174-197) Moreover, since injection therapy is largely ‘uncharted territory’, we find it hard to substantiate the recommendation of this experimental ‘treatment’ of the psoas major in the lumbar spine with a reference – except from our own findings of this study.

11. In Line 71, the authors cited reference 17 to support the positive effect of ITB in neuromuscular scoliosis. However, it seemed to have contradicted with the conclusion of the original reference.

As the reviewer correctly point out, the results of the two studies are contradictory, for this reason we have only stated that studies examined …the corrective and clinical efficacy (l76-77)

I would rather not changes this since this study was performed before the dis-encouraging results of ‘The effect of botulinum toxin A injections in the spine muscles for cerebral palsy scoliosis.’ and the next sentence would be meaningless, namely

However, to our knowledge ITB for AIS has not been investigated, and would seem to be ideal for examining the role of the muscles in AIS, since it provides temporary muscle paralysis without long-term side effects or complications in therapeutic doses in otherwise healthy humans [19]. (l77-80)
Inappropriate use of citation was found in a number of places. For example, reference 20 in Line 92 was a commentary on ultrasound guided injection which a reference which can serve as a guideline is suggested for Methods.

We acknowledge this and have replaced this reference with a review of the importance of using ultrasound and electric stimulation (1347-348)

Similar problems were also found in reference 19 in Line 98,
We acknowledge this and have removed this reference from the manuscript at 198.
reference 20 and 21 in Line 156.
We acknowledge this and have removed these references, and have replaced them with the appropriate reference 19. Reference 32 has been removed from the manuscript.

12. In Line 83, the authors mentioned the recruited patients were being treated for AIS and later reported in Line 125 that the treatments were maintained throughout the study. How to eliminate the effect of treatment in these patients from the effect of BTA?

As the reviewer state correctly; treatment otherwise was maintained, since we were interested in seen the effect of the temporary effect of the paralysis alone. This has been clarified in the conclusion;

The radiological changes are prescribed to the induced muscular paralysis due to the short follow-up of six weeks, since all prior treatment was maintained and no other clinical events occurred in the patient’s life. (1157-160)

13. Were there any exclusion criteria for this study?
Yes, this has been added to the manuscript…
Exclusion criteria’s were hypersensitivity or allergy to botulinum toxin A, ongoing infection at the injection sites and prior ITB within the last 6 months. (191-93)

14. In Line 88, the authors stated the injections were made on the psoas in concave side of the curve. More elaborations should be given to support this choice in the Introduction. Also, it
is better for the authors to explain the mechanism of psoas in concave side in regulating IS curvature in Discussion.

This has been elaborated on in the introduction and discussion; I kindly refer the reviewer to his own question 9, 10 and 14.

15. In Line 91, the use of "electric needle guided identification" is mentioned. It may be better to cite related references or insert images, so that readers not in the field of anesthesiology can have better understanding.

We acknowledge this and have replaced this reference with a review of the importance of using ultrasound and electric stimulation (reference 19)

16. In Line 102, it was noted that 3 experienced doctors carried out measurements on radiographs for this study. However the authors didn't mention weather the results presented in this paper were an average of the 3 doctor's or not.

All measurements were performed in a similar manner and blinded by three experienced doctors separately, and the average results were used for further analyses. (1110-111)

If the results in table 2.1 were mean values, SD should be included as well. Was there standardization between doctors during the measurement?

The SD to the mean values of the Cobb’s angle is now added, and there was standardization between the doctors. However, the ‘average SD’ was on the high side of 9.1 of the average Cobb’s angle measurements

We have added this in the discussion…

Additionally, our intra-observer variation for Cobb’s angle was high (average 9.1 degrees) in spite of we tried to minimize measuring error by using three blinded experienced doctors and achieve higher accuracy in our radiological recordings by a standardized standing radiographic protocol. For these reasons nonparametric statistical analysis of Wilcoxon signed-rank and one sample binomial test were used, in which the clinical cut off value were not included.

17. In Line 109 and Line 162 the name of statistical test "Wilcoxon's rang sign test" is incorrect.

This has been changed accordingly to: Wilcoxon’s signed-rank test (1118)
18. In Line 111, the use of one sample binomial test is mentioned. However, what was the test value used in the analysis. Also the level for statistical significance should be noted.

This has been changed accordingly to: …and one sample binomial test for change in levels of apex vertebrae and levels of curve measurements and Nash and Moe's classification (significance level 0.05); if the Nash and Moe’s classification, level of measurement of Cobb’s angle or apex vertebrae changed with one, it was considered a change (+1), otherwise no effect (0). The statistical analyses were performed as in a previous similar study [17].

19. In Line 116, what is off label medicine referring to? If it means "the medication is being used in a manner not specified in the FDA's approved packaging label, or insert", then what was the reason for using it?

The treatment was used in our institution for many years for neuromuscular scoliosis; we evaluated this treatment as in the study; Christian Wong, Søren Anker Pedersen, Kasper Gosvig, Billy Kristensen and Stig Sonne-Holm. The effect of botulinum toxin A injections in the spine muscles for cerebral palsy scoliosis. Spine (Phila Pa 1976). 2015 Dec;40(23):E1205-11. Today this treatment is omitted in our institution both for neuromuscular and adolescent idiopathic scoliosis after the results of this study and the for the results of the above mentioned study.

We understand the reviewers question and concern. This is the reason for elaboration on the ethical concerns of this study:

The ethical motivation to perform such a study in humans should be discussed. First of all, our primary ethical motivation for initiating this experimental study was to discover a potential effective corrective or clinical beneficial treatment for AIS. This should be performed strenuously protocolated experimental and monitored study as in this study. The window for effective ITB treatment for AIS would be in the small curve AIS as a supplement our current conservative treatment of bracing. This might have been able to alleviate adolescent girls with AIS, since currently it is strenuous and with low compliance to follow [25-27]. From this point of view, it would seem inappropriate not to look for alternate treatment strategies, and it certainly would be attractive to find an alternative treatment or to complement the current conventional treatment. This was our motivation for initiating this study; namely to investigate if using ITB to treat AIS would lead to improvement of curve and stop curve progression for affected adolescent girls. This radiological corrective effect were plausible since we supposedly addressed the culprit of the potential pathology, namely the PM muscle of the back. (199-201)

And

We would recommend to examine this by stimulation instead of paralysis for future studies as suggested by Grivas et al. (2016) [10].(178-188)
20. In Line 142, the authors mentioned a non-significant thoracic and significant lumbar derotation were observed. However which parameter indicated the change in rotation, this result was not mentioned in Results.

This would be the measurements of Nash and Moe’s classification in the thoracic and lumbar region as seen in table 2.2 with a significance level of 0.201 and 0.023, respectively.

This has been clarified in the discussion by adding;

… a non-significant thoracic and significant lumbar derotation (changes in Nash and Moe’s classification)…(l150-151)

21. In Line 182, the authors claimed ITB treatment might be able to alleviate AIS in girls. Why only girls?

This is now changed to humans (l204 and l210). Initially girls was put in, since number of girls, who get adolescent idiopathic scoliosis, are more than boys.

In Line 189, the authors summarized no convincing radiological corrective effect but wrote significant corrective effect in Abstract. This appears contradicting.

This is now clarified in the discussion. We found radiological changes, that were statistical significant, but not to a level, that seemed clinical beneficial. This is now rephrased in the discussion:

However, we did not find radiological corrective effect or patient-reported benefits to a convincing clinical level in our population of patients with AIS – even though radiological correction of significant magnitudes were achieved.

23. In Line 191, the abbreviation "TS" was not mentioned in previous section.

This is now changed from TS (test subjects) to patients

24. In general, the authors should address more on the reasons for a general spinal curvature change after paralyzing muscle in the concave side. This result can be compared with previous studies on other para-spinal muscles properties to answer the hypothesis in Introduction. Instead, the Discussion focused and repeated similar arguments made in Introduction. More reference should be included as well.
We have elaborated on this in the conclusion (l186-197) and added new 5 references in this section (9,11,24,25,26)

25. Detailed proofreading should be done on the presentation format and language. Some sentences and use of words were confusing to readers. Many statements lacked linkage with the rest of the paragraph. This problem is very obvious in Discussion.
Especially the introduction and discussion has been thoroughly revised.

26. In table 1, the author presented patients characteristics. What was the patients' age when recruited in this study?
This has been added to table 1

The reviewer is curious to know why patient 1 and 2, who already had other medical conditions and still being recruited as subjects?

Patient 1 and 2 were not diagnosed with their disorders at the time of inclusion and injection treatment. They were considered as otherwise healthy except for their AIS at the time.

Would the procedures in this study induce stress or any physical harm to them?
No, this has been rephrased in the results section;

Two patients reported temporary soreness at the injection site, which regressed within days, and no other serious adverse events occurred during the study, except for one patient was injected in erector spine and quadratus lumborum as well as the PM. No other major medical or orthopedic surgical events at the time of and after termination of the study; the subsequent spinal surgeries took placed years after injection treatment.(l140-144)

The review suggest to change all indication with common English terms such as left, right, pre and post in table 2.1 for easier interpretation.

We see the point of the reviewer, and the terms pre and post are in table 2.1. Since there are right and left-‘handed’ scoliosis in this study, I would suggest to keep the terms concave and convex, since it might confuse even more to explain this impediment of different scoliosis ‘going to the right and left’ and then change to the direction of….

27. Consistent formatting of table 2.1 and table 2.2 is suggested.
28. In table 2.1, some patients showed increase of Cobb angle after the injection. Were the measurements affected by inter-rater reliability?

The measurements of the primary parameters could have been affected by our interrater bias of the measurements. We have acknowledged this in the manuscript, since and this is a bias for this study. Instead we looked at the trend in the measurements using Wilcoxon signed-rank test; was there an improvement or a deterioration? This is shown for the average data in the manuscript, but the almost the same trend in measurement in regards to regression and progression was found for the three raters individually. This indicated for us, that the trend was relevant in spite of the SD. This is not included in the manuscript, since we found that it was represented by using Wilcoxon’s signed-rank test.

We have written this in the discussion…

A methodological obstacle of this study was to find an adequate way of evaluating our radiographic results. In clinical practice, a Cobb’s angle of at least in between five to ten degrees would be a cut off value of clinical radiographic change [19]. The diurnal variation in Cobb’s angle for AIS is 5 degrees and the inter and intra-observer variation are 7.2 degrees and 4.9 degrees, respectively [22]. In this study we would expect subtle smaller radiographic changes, due to ITB induce only partial reduction of muscle function [23,24], and seen in this perspective we would not expect to detect radiological changes as high as clinical cut off values [17]. Moreover, three patients had main thoracic curves (patients 3, 4 and 7), and we would expect lesser effect (as in fact seen) than if all patients had main lumbar curves. Additionally, our intra-observer variation for Cobb’s angle was high (average 9.1 degrees) in spite of we tried to minimize measuring error by using three blinded experienced doctors and achieve higher accuracy in our radiological recordings by a standardized standing radiographic protocol. For these reasons nonparametric statistical analysis of Wilcoxon signed-rank and one sample binomial test were used, in which the clinical cut off value were not included.

This was not explained clearly in Discussion. Also the mean Cobb angle and SD before and after injection should be indicated in the tables.

This is now included in the manuscript in the discussion section.

29. For figure 2, it would be clearer to the readers to see the change after the injection by comparing the radiographs before and after injection in parallel.
An example of pre and post injection radiographs has been added for one of the included patients in figure 2.

30. In table 3, the meanings of TCorI and TCorT are not very clear

This is now changed to IniBra and TermBra as for initiation of brace treatment and termination of brace treatment

and why did patient 5 receive a negative number for months of treatment (TCorI and TInj)?

This is computer related mistake in the syntax of the date, when the scoliosis was detected. This is now corrected in table 3 (injection after 1 month and brace after 6 months).

Why patient 4 was injected with more BTA than specified in Methods?

This is a ‘typo’. I have revisited the ‘surgical journal’ to make sure. The patient received 100 ie botox, and this is corrected in table 3.

Also why patient 9 was injected in other muscle groups not targeted in this study?

This was an adverse event, where the patient 9 was actually treated as the first patient. This was performed by the treating physician by his regular regime (which included the psoas major) where he misunderstood the research protocol. We have included this patient, since this regime also included the psoas major, but also had quadratus lumborum and erector spina injected.

This is now included in the manuscript

Two patients reported temporary soreness at the injection site, which regressed within days, and no other serious adverse events occurred during the study, except for one patient was injected in erector spine and quadratus lumborum as well as the PM (l143-145)

The patient, who received multiple injections, was one of the patients with soreness.

31. The authors recorded some patient underwent subsequent spinal corrective surgery and one patient developed into eating disorder in many years after the study has completed. Was there any linkage between the two events?

I cannot say that I am an expert in eating disorders. The eating disorder was mentioned to give a comprehensive description of the demography of the patients; the eating disorder postponed surgery for this particular patient, hence occurred prior to the planned spinal surgery and long after injection treatment; my conclusion would be that this was unrelated to the scoliosis and
either of the treatments of brace and injection. In the context of this study we were not allowed/at liberty to revisit now and explore further psychological charts.