Adverse events following trigger point dry needling: a prospective survey of chartered physiotherapists

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Objectives: Trigger point dry needling (TrP-DN) is commonly used to treat persons with myofascial pain, but no studies currently exist investigating its safety. The aim of this study was to determine the incidence of Adverse Events (AEs) associated with the use of TrP-DN by a sample of physiotherapists in Ireland.

Methods: A prospective survey was undertaken consisting of two forms recording mild and significant AEs. Physiotherapists who had completed TrP-DN training with the David G Simons Academy (DGSA) were eligible to take part in the study. Data were collected over a ten-month period.

Results: In the study, 39 physiotherapists participated and 1463 (19.18%) mild AEs were reported in 7629 treatments with TrP-DN. No significant AEs were reported giving an estimated upper risk rate for significant AEs of less than or equal to (≤) 0.04%. Common AEs included bruising (7.55%), bleeding (4.65%), pain during treatment (3.01%), and pain after treatment (2.19%). Uncommon AEs were aggravation of symptoms (0.88%), drowsiness (0.26%), headache (0.14%), and nausea (0.13%). Rare AEs were fatigue (0.04%), altered emotions (0.04%), shaking, itching, claustrophobia, and numbness, all 0.01%.

Discussion: While mild AEs were very commonly reported in this study of TrP-DN, no significant AEs occurred. For the physiotherapists surveyed, TrP-DN appeared to be a safe treatment.

Keywords: Myofascial pain, Dry needling, Adverse events

Introduction

Trigger point dry needling (TrP-DN) is an invasive treatment approach whereby a solid filament needle is inserted into a myofascial trigger point (TrP) in a muscle.¹² A TrP consists of a hyperirritable spot in skeletal muscle, associated with a palpable nodule in a taut band. When compressed, TrPs may give rise to characteristic pain, tenderness, or motor dysfunction.³ Superficial dry needling (SDN) involves inserting the needle into the skin, fascia, and muscle overlying a TrP,⁴ whereas, with deep dry needling (DDN) the needle is inserted into the TrP with the aim of eliciting Local Twitch Responses (LTRs).⁵ Essential for obtaining therapeutic benefit with TrP-DN, LTRs are reflex spinal cord contractions of the muscle fibers in a taut band.⁵⁻⁸ Eliciting LTRs can reduce concentrations of nociceptive chemicals, such as substance P and calcitonin gene-related peptide, found in the immediate vicinity of active TrPs.⁹,¹⁰

Trigger point dry needling is commonly used in clinical practice by physiotherapists in conjunction with other physical therapy modalities.¹ In many countries, including Ireland, the United Kingdom, Canada, and Spain, TrP-DN has been recognized to fall within the scope of physiotherapy practice.¹ In fact, the term ‘intramuscular manual therapy’ is considered by some to be a more appropriate term for TrP-DN as this technique is closely associated with manual therapy.² Research is emerging supporting the use of TrP-DN for conditions such as back and neck pain,¹¹⁻¹³ shoulder pain,¹⁴ and upper quadrant myofascial pain.¹⁵ Furlan et al.¹⁶ conducted a systematic Cochrane meta-review of randomized controlled trials investigating acupuncture and TrP-DN for back pain. Trigger point dry needling was found to be a useful adjunct to other therapies in the treatment of persons with chronic low back pain. When used to treat individuals with temporomandibular pain and dysfunction, TrP-DN can also improve pain and movement.¹⁷⁻¹⁹ Non-invasive approaches, including TrP compression release and spray and stretch, are also used to treat TrPs.²⁰⁻²⁴

Trigger point dry needling is an invasive technique with potential for Adverse Events (AEs).

Searches of Pubmed, Medline, and CINAHL by the authors did not find any studies investigating AEs and TrP-DN beyond the level of case study.²⁵
Evidence on the safety of needling techniques comes primarily from prospective studies investigating AEs following acupuncture.\textsuperscript{26–31} Results from acupuncture AE studies cannot be extrapolated and applied to TrP-DN as it differs from acupuncture in the points treated and the method and depth of needle stimulation. As both involve the insertion of a solid filament needle, these studies do provide, however, potentially useful information about risks of needling therapies, similar to TrP-DN.

Witt \textit{et al.}\textsuperscript{30} carried out the largest prospective acupuncture study to date. Of the 229 233 patients who received 2.2 million acupuncture treatments, 8.6\% of patients \textit{(n=19 726)} experienced at least one AE. In this study, 24 377 AEs were reported, amounting to approximately one AE per 90 treatments (0.9\%). Most were mild, including bleeding, hematomas, and pain. More serious events did occur with two reported cases of pneumothorax.\textsuperscript{30} A prospective survey by White \textit{et al.}\textsuperscript{28} involving physiotherapists and doctors, reported 2178 AEs in 31 822 consultations, giving an AE rate of 7\%. The majority of these were considered minor AEs, including bleeding and bruising. Forty-three significant AEs were reported including one seizure, anxiety lasting 60 hours, cellulitis, and headache lasting 3 days. A significant event was defined as ‘unusual, novel, dangerous, significantly inconvenient or requiring further information’. The lowest rate of AEs found in a prospective acupuncture study was in a study by Yamashita \textit{et al.}\textsuperscript{26} whereby 94 mild AEs were reported in 65 482 acupuncture treatments (0.14\%). The higher rates of reactions to acupuncture found in the literature include 11.4\% (402 AEs in 3535 treatments) in a prospective acupuncture study by Ernst \textit{et al.}\textsuperscript{29} which were not classified into mild or significant; and 15\% in a prospective acupuncture study by MacPherson \textit{et al.}\textsuperscript{27} however the majority of these could be viewed as positive such as feeling relaxed, and feeling energized.

The acupuncture evidence, although useful, is not sufficient for ensuring the safety of patients undergoing TrP-DN due to the differences that exist between the two techniques. Trigger point dry needling, especially DDN, is performed with greater needle depth and involves manipulating the needle within the muscle to elicit multiple LTRs,\textsuperscript{1} whereas, with acupuncture, the needle commonly is inserted to the depth of the acupoint and manipulated gently until a dull ache called ‘deqi’ is achieved.\textsuperscript{32} The needle may then be left \textit{in situ} for as long as 15–20 minutes. Furthermore, the education of acupuncturists and physiotherapists using TrP-DN is considerably different.\textsuperscript{5} A specific study of AEs following TrP-DN was, therefore, deemed necessary. The aim of this study was to determine the incidence of AEs associated with the use of TrP-DN as practiced by a sample of physiotherapists with David G Simons Academy (DGSA) training in Ireland.

**Methods**

**Definition**

For the purposes of this study, an AE was defined as ‘any ill-effect, no matter how small, that is unintended and non-therapeutic’.\textsuperscript{33} This was chosen to include mild events and events that occurred through error.\textsuperscript{28} Based on severity, AEs were sub-classified as ‘significant’ or ‘mild’. The definitions for ‘significant’ and ‘mild’ events were adapted from those proposed by Carnes \textit{et al.}\textsuperscript{34} In the current study, a ‘mild’ AE was defined as short-term and non-serious, with no change in function, whereas the term, ‘significant’, was chosen to represent moderate or major AEs, described by Carnes \textit{et al.}\textsuperscript{34} as medium to long-term events that are serious, distressing and may require further treatment. In the study by Carnes \textit{et al.}\textsuperscript{34} specific time frames were not included in the final definitions of mild, moderate, or major AEs. However, the general consensus (>74\%) was that mild AEs lasted hours, moderate AEs lasted days and major AEs lasted weeks. These differed from the time frames discussed in a separate study considering AEs from the patient perspective.\textsuperscript{35} In that study, a mild AE was described as lasting from a matter of hours to 2 days by different participants. Moderate AEs could last from 1–5 days and major for more than 2 days. Due to these discrepancies in the literature and the multi-factorial nature of defining an AE,\textsuperscript{35} it was decided not to impose a strict time frame on distinguishing a mild AE from a significant one.

**Ethical approval**

Exemption from ethical approval was granted by the Human Research Ethics Committee of University College Dublin on 23 June 2011.

**Study design**

A prospective questionnaire design was used in this study to avoid recall error.

**Survey forms**

The questionnaire consisted of two forms, modified with permission from those used by White \textit{et al.}\textsuperscript{28} and a demographic data form. The forms were piloted by two physiotherapists for 2 weeks and subsequently, small changes were made.

Form A was used to record the number of TrP-DN treatments completed monthly and any mild AEs experienced. Specific headings for recording mild events included: bruising, bleeding, pain during treatment, pain after treatment, headache, and other mild AEs. This form was completed and returned monthly to the researchers. The form used to record
physiotherapists’ demographic data was returned with Form A following month one.

On a separate form (Form B) participants recorded any significant AEs. This could include: needling problems (e.g. forgotten needles, pneumothorax); systemic effects (e.g. fainting, vomiting); influence on symptoms (prolonged aggravation); or other significant events. Participants were asked to record the muscle being treated when the event occurred, the technique used, any necessary medical intervention, and the outcome. Form B was returned with Form A at the end of each month.

**Subjects**

In the study, 183 physiotherapists who had completed TrP-DN training with the DGSA were eligible to take part. Training with the DGSA in Ireland takes 64 hours and is available only to physiotherapists. This includes a two-day course on foundations of myofascial pain and MTrP palpation. Physiotherapists then complete two, three-day TrP-DN courses. DN 1 is concerned with needling safety as well as needling techniques for the upper and lower extremities. DN 2 is completed some months later with emphasis on the muscles of the trunk spine and pelvis. This model has been used extensively in Switzerland and other European countries.

**Recruitment**

Eligible physiotherapists were invited by email to take part in the study by one of the authors (JM). Potential participants were advised to email the principal investigator (SB) directly if they wished to volunteer for the study. Reminder emails were sent at two and four weeks to non-respondents.

**Distribution**

Following recruitment, packs were mailed to participants containing: an information leaflet, contact details of the researchers, nine copies of Forms A and B, a demographic data form and nine stamped addressed envelopes. Participants were informed that each respondent would be assigned a code for reporting and only the principal investigator (SB) would have access to the codes. Confidentiality was assured and participants informed that by volunteering for the study they were giving consent for data to be used for this purpose.

**Survey size**

The study aimed to identify any rare AEs, meaning a sample size of greater than 10,000 treatments was necessary. It was hoped to recruit a third of the 183 eligible physiotherapists (n = 61). Through discussion with physiotherapists, it seemed reasonable that participants would use TrP-DN 20 times per month. A time frame of 9 months was calculated as being required to record 10,000 treatments.

**Analysis**

Results were analyzed using Statistical Package for the Social Sciences 18 (SPSS). Descriptive statistics were used to calculate frequencies of various AEs and rates of occurrence per 100 treatments.

Adverse Events were classified based on how frequently they occurred, ranging from very common (more that once in ten treatments) to very rare (less than once in 10,000 treatments) following the European Commission’s (EC) recommended classification of AEs (Table 1). Spearman’s Rank Order Correlation (rho) coefficients were calculated to test for associations between participants’ age, experience, TrP-DN experience, choice of SDN over DDN, and number of TrP-DN treatments completed with their rate of AEs. The Mann–Whitney test was used to compare medians for the seven most common AEs of participants with particularly high rates of AEs and the remaining participants.

Where an AE does not occur in a certain number of treatments (n), Hanley’s Rule of Three states that the upper risk rate is at most, three in n (i.e. 3/n). This was used to estimate the upper risk rate of AEs that did not occur.

**Results**

In the study, 183 physiotherapists were invited to take part. Of these, 51 volunteered to participate and questionnaire packs were posted to all 51. Of the 51 volunteers, 39 returned at least one Form A giving a response rate of 76.47%. Demographic data (Table 2) were provided by 35 of the 39 participants (89.74%). Of the remaining four participants, one reported forgetting

<table>
<thead>
<tr>
<th>Very common</th>
<th>Common</th>
<th>Uncommon</th>
<th>Rare</th>
<th>Very rare</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1/10</td>
<td>1–10/100</td>
<td>1–10/1000</td>
<td>1–10/10,000</td>
<td>&lt;1/10,000</td>
</tr>
</tbody>
</table>

| Table 2 Demographic data for participating physiotherapists, n = 35 |
|-----------------|-----------------|-----------------|
| **Age**         | **Experience (years)** | **TrP-DN experience (months)** |
| Mean            | 34.03            | 10.29            | 23.74            |
| Standard deviation | 8.21            | 8.89            | 16.73            |
| Range           | 24–52            | 1–30            | 3–60             |
the form, the others did not respond to follow-up. The mean age of participants was 34 years (SD=8.21) with 30 females and five males taking part. The majority of participants worked in private practice (n=23, 65.7%), with four participants (11.42%) working within the Health Service Executive, which is the Public Health Sector in Ireland, and eight (22.86%) worked in both sectors. The respondents’ physiotherapy experience varied from 1–30 years (mean=10.29) and TrP-DN experience from 3–60 months (mean=23.74).

Data were collected from September 2011 until June 2012 with each respondent asked to participate for 9 months. In total, 273 Form A were returned, detailing 7629 TrP-DN treatments. The majority of treatments (82.7%, n=6312) used DDN, with the remainder (17.3%, n=1317) using SDN. Three reports were excluded from analysis as two did not record the number of treatments completed and one was a duplicate. The number of treatments completed per practitioner varied from 10 to 990 (mean=195, sd=204.16). In this study, 1463 AEs were recorded, giving a rate of 19.18 per 100 treatments. All AEs were reported on Form A and considered mild. No Form B was returned, therefore no significant AEs were reported. Using Hanley’s Rule of Three, the risk of these rare AEs can be estimated to be at worst 1/2543 treatments (≤0.04%).

Table 3 displays all mild AEs reported in the study. Data are presented in this table with rates per 100 treatments. The ‘Extreme Values’ column shows the highest recorded values for individual participants for each AE expressed as a rate per 100 treatments. Results are subsequently discussed using the guidelines suggested by the EC and categorized from common (1–10/100 treatments) to rare (1–10/10 000 treatments).

According to the EC, common AEs occur 1–10 times per 100 treatments. Four common AEs were recorded in the study. Bleeding was the most frequently reported AE, with 576 reported incidents, giving a rate of 7.55/100 treatments. Bruising was the second most frequently reported with 355 cases (4.65/100), followed by pain during treatment (n=230, 3.01/100), and pain after treatment (n=167, 2.19/100). Using the EC classification, 5 uncommon AEs were identified. These occur 1–10 times per 1000 treatments. Aggravation of symptoms occurred 67 times, giving a rate of 8.78 incidents per 1000 treatments (8.78/1000). This was followed by drowsiness (n=20, 2.62/1000), feeling faint (n=17, 2.23/1000), headache (n=11, 1.44/1000), and nausea (n=10, 1.31/1000).

Although the target of 10 000 treatments was not reached, an approximate rate for rare AEs was calculated based on the EC classification (occurs 1–10 times per 10 000 treatments). Patients experiencing fatigue or altered emotions were each recorded three times in 7629 treatments giving an estimated rate of 3.93/10 000 treatments. Each of the following AEs were recorded once: shaking, itching, claustrophobia, and numbness, by different physiotherapists giving an estimated rate for each of 1.31/10 000 treatments. Further information was provided for these rare AEs. The patient who was shaky recovered after 3 minutes. Itching was felt in the referral area of the gluteus medius for 2–3 minutes, which then dissipated. Numbness was experienced in the area of needling for 12 hours, a complete recovery ensued. Prone lying was the cause attributed to one patient experiencing claustrophobia during TrP-DN. The practitioner was unsure if TrP-DN was a contributing factor and changing the patient’s position relieved this.

A large range was noted in the rate of AEs recorded per participant. The mean rate of AEs per 100 treatments was 24.18 (sd=20.09) with figures ranging from 3.13 to 93.1. Analysis using the Kolmogorov–Smirnoff test revealed data were not normally distributed therefore non-parametric tests

<table>
<thead>
<tr>
<th>Event</th>
<th>Cases reported</th>
<th>Number per 100 treatments</th>
<th>Number (%) of physiotherapists reporting none</th>
<th>Extreme values recorded by individual practitioners per 100 treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding</td>
<td>576</td>
<td>7.55</td>
<td>4 (10.25)</td>
<td>32.23, 30</td>
</tr>
<tr>
<td>Bruising</td>
<td>355</td>
<td>4.65</td>
<td>3 (7.69)</td>
<td>26.09, 21.84</td>
</tr>
<tr>
<td>Pain during treatment</td>
<td>230</td>
<td>3.01</td>
<td>9 (23.08)</td>
<td>20.75, 20.69</td>
</tr>
<tr>
<td>Pain after treatment</td>
<td>167</td>
<td>2.19</td>
<td>14 (35.9)</td>
<td>20.69, 18.4</td>
</tr>
<tr>
<td>Aggravation</td>
<td>67</td>
<td>0.88</td>
<td>22 (56.41)</td>
<td>10.99, 5.75</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>20</td>
<td>0.26</td>
<td>32 (82.05)</td>
<td>4.44, 3.26</td>
</tr>
<tr>
<td>Feeling faint</td>
<td>17</td>
<td>0.22</td>
<td>28 (71.79)</td>
<td>4.17, 2.5</td>
</tr>
<tr>
<td>Headache</td>
<td>11</td>
<td>0.14</td>
<td>31 (79.49)</td>
<td>1.15, 1.1</td>
</tr>
<tr>
<td>Nausea</td>
<td>10</td>
<td>0.13</td>
<td>31 (79.49)</td>
<td>2.7, 2.22</td>
</tr>
<tr>
<td>Fatigue</td>
<td>3</td>
<td>0.04</td>
<td>37 (94.87)</td>
<td>1.77, 0.27</td>
</tr>
<tr>
<td>Emotional</td>
<td>3</td>
<td>0.04</td>
<td>37 (94.87)</td>
<td>1.77, 0.27</td>
</tr>
<tr>
<td>Shaky</td>
<td>1</td>
<td>0.01</td>
<td>38 (97.44)</td>
<td>3.03</td>
</tr>
<tr>
<td>Itching</td>
<td>1</td>
<td>0.01</td>
<td>38 (97.44)</td>
<td>0.47</td>
</tr>
<tr>
<td>Claustrophobia</td>
<td>1</td>
<td>0.01</td>
<td>38 (97.44)</td>
<td>0.16</td>
</tr>
<tr>
<td>Numbness</td>
<td>1</td>
<td>0.01</td>
<td>38 (97.44)</td>
<td>0.47</td>
</tr>
</tbody>
</table>
were chosen for analysis. Analysis using Spearman’s Rank Order Correlation (rho) revealed no significant correlation between the participant’s age (Correlation coefficient ($r_s=−0.113$, $P=0.520$), experience ($r_s=−0.175$, $P=0.316$), TrP-DN experience ($r_s=−0.121$, $P=0.487$), choice of SDN over DDN ($r_s=−0.027$, $P=0.878$), or number of TrP-DN treatments ($r_s=−0.164$, $P=0.346$) with the rate of AEs.

Six participants reported rates of AEs per 100 treatments that were greater than 1 sd above the mean (>44.27 AEs per 100 treatments) greater than 1 sd above the mean (>44.27 AEs per 100). The Mann–Whitney test was used to compare medians for the seven most common AEs between these six participants and the remaining 33 participants. Medians were significantly higher among the outliers for bleeding ($P=0.003$), bruising ($P=0.001$), and pain during treatment ($P=0.003$). Medians were higher for the remaining AEs but were not statistically significant for pain after treatment ($P=0.758$), aggravation ($P=0.154$), drowsiness ($P=0.898$), and feeling faint ($P=0.148$).

**Discussion**

In this study, AEs were reported in 19.18% ($n=1463$) of treatments using TrP-DN. Adverse Events would therefore be considered very common. All AEs reported were mild and no significant AEs were reported. This implies that the estimated risk of significant AEs using Hanley’s Rule of Three was $\leq0.04\%$ (3/7629). Therefore, in this study, the estimated rate of significant AEs can be considered, at worst, rare. Although no significant AEs occurred, the results should be interpreted in light of the sample size of the current study. Studies using greater numbers of treatments are needed to determine a more accurate rate of significant AEs.

When compared with similar prospective studies on acupuncture, the AE rate of 19.18% reported in this study appears high. Yamashita et al. reported a rate of 0.14%, followed by Witt et al. at 0.9%, White et al. at 7%, and Ernst et al. at 11.4%. Many factors may have contributed to the comparatively high rate observed in the current study. A different methodology was used by Witt et al., whereby AEs were reported by the patient. Patients view AEs differently from practitioners, with a change in function an important factor in whether a patient defines an event as adverse. This may mean under-reporting of mild AEs if function is unaffected. AE reporting by practitioners versus patients has not been investigated for physiotherapeutic modalities, but, in other disciplines differences have been found. In Yamashita’s study, AEs were only reported if the practitioner or patient felt it was a problem, which may account for the low rate of AEs in their study (0.14%).

The current study used a similar methodology to White et al., but that study reported a lower rate of AEs, 7%. Acupuncture and TrP-DN differ in the points treated and methods and depth of needle stimulation, and therefore are not directly comparable. It should be noted that there are many different schools of acupuncture with different treatment points and techniques. The manipulation of the needle with TrP-DN to elicit multiple LTRs is distinctly different from acupuncture where the needle is normally inserted to the depth of the acupoint and manipulated gently until a dull ache called ‘deqi’ is achieved. It is likely that compared with acupuncture, TrP-DN could lead to more local microtrauma resulting in bruising, bleeding, and pain. In the current study, however, no significant AEs were reported in 7629 treatments, giving an upper risk rate for significant AEs of $\leq0.04\%$. This compares favorably with 0.14% in the study by White et al., and 0.22% (AEs requiring further treatment) in the study by Witt et al. The estimated rate of significant AEs in this study ($\leq0.04\%$) is also much lower than that reported for some over-the-counter pain medications (aspirin, 18.7%; ibuprofen, 13.7%; and Paracetamol, 14.5%).

In the current study a large variation is seen in the rate of AEs reported per participant with figures ranging from 3.13–93.1/100 treatments with six of the 39 participants reporting particularly high rates of AEs. Among these six participants, rates of reporting of bruising ($P=0.003$), bleeding ($P=0.001$), and pain during treatment ($P=0.003$) were significantly higher compared with the other 33 participants. Participants were instructed to record any bruise as an AE, but the recording forms did not state how much bleeding or what level of pain constituted an AE. The definition of an AE was printed on all forms, but it is conceivable that different participants made interpretations as to what was meant by an AE. Varied rates of reporting could also arise due to differences in needling techniques or patient cohorts. The reasons for these differences are unknown as a follow-up of participants was not part of this study’s methodology. White et al. carried out a follow-up of participants with high rates of reporting and found that these participants had reported slight discomfort or a single drop of blood as an AE. Similar follow-up may be beneficial in future studies on TrP-DN. The definition used in the current study was chosen to be capable of identifying mild and significant events, however, the delineation between what constitutes an expected and acceptable consequence of treatment and what is adverse is unclear. A recent Delphi study introduced the term ‘not adverse’ for events that are mild and transient with no alteration in function, which were deemed by experts to be an acceptable consequence of treatment. When the patient perspective is considered,
mild pain with unaltered function may not be considered adverse.35 Further studies may use an alternative system of reporting to account for events considered ‘not adverse’. Problems can also arise due to the lack of consistency in the terms used for recording recording AEs. Calls have been made to standardize being made to standardize terminology.43 This variation in terminology makes comparisons between similar studies difficult.

There are a number of limitations to the current study. No significant AEs were reported, therefore, the risk of significant AEs could only be estimated using Hanley’s Rule of Three.38 This should be interpreted with caution as it is only an estimation, and further large-scale studies are indicated. Participants may have been reluctant to report events where negligence could be inferred, as participants were potentially identifiable. Future studies should consider the benefits of anonymous reporting. Some AEs may have been wrongly attributed to TrP-DN, as participants were not asked to judge causality, thus leading to possible over-reporting of mild AEs. This study was designed as a prospective study in an effort to obtain the most accurate results. However, as forms were returned at the end of each month, it is possible that participants completed the forms retrospectively at the end of each month rather than as each event occurred, introducing the possibility of inaccurate reporting.

Adverse Events can and do occur with needling therapies and when choosing a treatment approach, the risk of both mild and significant AEs must be discussed with patients.44 Clinicians should strive to maintain safety at all times and this paper provides practitioners using TrP-DN with a means of discussing the known risks in order to obtain informed consent.

Conclusion

Almost 20% of treatments with TrP-DN by the physiotherapists in this study resulted in a mild AE. Common AEs include bruising, bleeding, and pain. No significant AEs occurred and the estimated risk of significant AE was ≤0.04% by Hanley’s Rule of Three.38 This must be viewed in light of the scale of the study and further large-scale studies are warranted.

Acknowledgements

The authors wish to thank the participants in the study, whose contribution made this study possible. We are very grateful to Dr Adrian White for access to, and permission to modify his questionnaire.

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Journal of Manual and Manipulative Therapy 2013 VOL. 000 NO. 000 7