Frequency of Penetration of the Digital Flexor Tendon Sheath and Distal Interphalangeal Joint Using a Direct Endoscopic Approach to the Navicular Bursa in Horses

Justine Kane-Smyth, Sarah Elizabeth Taylor, Eugenio Cillán García, and Richard J.M. Reardon

The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, United Kingdom

Corresponding Author
Justine Kane-Smyth
The Philip Leverhulme Equine Hospital
The University of Liverpool
Neston
Cheshire CH64 7TE
United Kingdom
jks@liverpool.ac.uk

Submitted July 2014
Accepted June 2015
DOI:10.1111/vsu.12454

Objective: To evaluate the frequency of inadvertent penetration of the digital flexor tendon sheath (DFTS) and/or distal interphalangeal joint (DIPJ) when using a direct endoscopic approach to the navicular bursa, and to evaluate an alternate direct approach to the navicular bursa.

Study Design: Cadaveric study.

Sample Population: Equine cadaver limbs (n = 40 for direct; n = 12 for alternate approach).

Methods: Four surgeons performed the direct endoscopic approach to the navicular bursa on 10 limbs each. Frequencies of inadvertent synovial penetration and iatrogenic damage were compared between surgeons. Use of an alternate direct approach, adopting a straight parasagittal trajectory, was evaluated by 2 surgeons.

Results: Inadvertent synovial penetration occurred in 45% of limbs (DFTS 37.5%; DIPJ 17.5%; and both structures 10%). Successful bursa entry was achieved on the first attempt in 45% of limbs. Significant variation in frequency of inadvertent synovial penetration was observed between surgeons (range 10–80%). Inadvertent synovial penetration did not occur when using the alternate direct technique. Iatrogenic damage to navicular bone fibrocartilage and/or deep digital flexor tendon occurred in 55% of limbs using the direct endoscopic approach and in 0% of limbs using the alternate direct approach.

Conclusion: Because of the considerable risk of inadvertent penetration of the DFTS and/or the DIPJ when making a direct endoscopic approach to the navicular bursa, it is advisable to investigate for inadvertent penetration when treating navicular bursa sepsis using a direct approach. The alternate direct technique may reduce the risk of inadvertent penetration; however, the view within the bursa may be restricted.

Sepsis of the navicular bursa is typically encountered as a result of deep solar penetrating lesions, distal limb penetrating wounds, or less commonly associated with iatrogenic infection after bursa injection. Steckel et al.1 reviewed 50 cases of solar penetrating lesions and concluded that navicular bursa sepsis and its sequelae were the most frequent reasons for euthanasia in their cohort. Subsequent to that review, an endoscopic technique for examination of the navicular bursa using a direct approach through a portal just proximal to the ungual cartilage was developed and revolutionized the management and prognosis for horses with navicular bursa sepsis.2 This direct approach to the navicular bursa has also been employed for elective examination in cases with lameness localized to the region.3 A modified transthecal technique through the digital flexor tendon sheath (DFTS) has been described, but is typically reserved for elective navicular bursa endoscopy because of concerns regarding the potential for iatrogenic DFTS sepsis.3–5 The direct approach, therefore, remains the standard technique for treatment of navicular bursa sepsis for many surgeons.

Three previous studies have reported the potential for inadvertent synovial penetration of the DFTS and/or distal interphalangeal joint (DIPJ) during placement of the endoscope cannula using the direct approach.6–8 As a result, some authors have recommended lavage of the DFTS when endoscopy has been performed in the management of navicular bursa sepsis.8 The objectives of this study were to describe the frequency of inadvertent penetration of the DFTS and/or DIPJ during navicular endoscopy using the direct approach and to compare frequencies of inadvertent penetrations between different surgeons. In addition, this study describes an alternate direct approach to the navicular bursa. Knowledge of the level of the risk of inadvertent synovial penetration is essential in decreasing the potential for iatrogenic infection and minimizing the risk of clinical sequelae associated with iatrogenic bursitis.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Presented at the 23rd Annual Scientific Meeting of the ECVS Copenhagen, Denmark, July 2014.
penetration may help determine the need for DFTS and/or DIPJ lavage at the time of navicular endoscopy when treating septic bursitis. We hypothesized that the risk of inadvertent penetration of the DFTS or DIPJ would be >10%, that this frequency would vary between surgeons, and that the alternate direct approach would result in a lower frequency of inadvertent synovial structure penetration.

MATERIALS AND METHODS

Cadaver limbs were collected from horses at an abattoir. Specific details of age, sex, and breed were therefore not available. The cohort consisted of 8 limbs from ponies, 4 limbs from a draft-type horse, and the remainder were of Thoroughbred-type. Forelimbs were removed at mid-radius level and hindlimbs were removed mid-tibia. Limbs were frozen and stored at −20°C and subsequently thawed at room temperature for 24 hours before use.

Because the likely frequencies of DFTS and/or DIPJ penetration were unknown, a pilot study was performed by 2 surgeons using 8 cadaver limbs. Each of the 2 surgeons performed the procedure, as described below, on 2 forelimbs and 2 hindlimbs, with contralateral limb pairs divided between surgeons. The main study consisted of 20 paired forelimbs and 20 paired hindlimbs that were divided between the 4 surgeons, such that each surgeon performed the procedure on 5 forelimbs and 5 hindlimbs. Different surgeons performed the procedure on contralateral limbs and there was an even spread of left and right limbs between surgeons. The surgeon group consisted of 2 equine surgeons board-certified by the European College of Veterinary Surgeons (certified for 8 and 5 years), 1 residency-trained surgeon, and 1 surgical resident, each of whom were experienced in equine surgical endoscopic technique and the procedure in clinical cases. A 5 part test procedure was performed on each limb.

Pre-Endoscopy Positive Contrast Navicular Bursogram

The hair was clipped circumferentially from the distal limb and the described distal palmar/plantar approach to the navicular position was used by the same author to place a needle within the navicular bursa of each limb.6 Briefly, with the digit held in flexion, an 18 g 90 mm spinal needle with stylet in place was introduced between the heel bulbs, just proximal to the coronary band, and advanced sagittally, aiming for a point halfway between the most dorsal and palmar/plantar aspects of the coronary band and 0.5 cm distal to the coronary band, until the needle contacted navicular fibrocartilage. The navicular bursa was then maximally distended by the same author, with a mixture of tap water and 2 mL of iohexol (Omnipaque™ 300 mgI/mL, GE Healthcare AS, Oslo, Norway) using a 5 mL syringe until intrabursal pressure prevented further fluid ingress. A closed 3-way stopcock was attached to the needle and the needle left in situ for the rest of the procedure. A lateromedial radiograph was made to confirm correct needle placement and ensure there were no pre-existing communications with the DFTS and/or DIPJ. Limbs with pre-existing communications were excluded from the rest of the study.

Endoscope Placement

Described Direct Endoscopic Approach. Limbs were placed with the lateral aspect uppermost and the distal limb positioned neutrally to allow manipulation. Using the description by Wright et al.2 as a guide, a direct approach to the navicular bursa was made. A 5–7.5 mm skin incision was made using a #11 scalpel blade on the palmar/plantarolateral aspect of the limb, just proximal to the ungual cartilage, axial to the palmar/plantar digital neurovascular bundle, and abaxial to the lateral margin of the deep digital flexor tendon (DDFT). An endoscope cannula and blunt obturator assembly (Karl Storz GmBh, Tuttlingen, Germany) were introduced and advanced distally and axially toward the midsagittal aspect of the heel bulbs, aiming to enter the navicular bursa at the midpoint of the middle phalanx. Care was taken to ensure the cannula was dorsal to the DDFT before advancement, at which point the distal limb was held in extension. Fluid distension of the navicular bursa was achieved with ingress of tap water maintained with a 20 mL syringe via the previously placed spinal needle. A sudden loss of resistance and egress of fluid from the cannula occurred on entry to the navicular bursa. The obturator was replaced with a 4 mm 30° forward-oblique endoscope (Karl Storz GmBh) and navicular bursa distension with tap water was maintained using the previously placed spinal needle to enable examination. Successful entry of the cannula into the navicular bursa was confirmed by visualization of the spinal needle, the navicular bone fibrocartilage, dorsal surface of the DDFT, and the characteristic intervening synovial plicae that form the abaxial borders of the bursa. The number of attempts required to gain successful entry was recorded, with each redirection and advancement of the cannula and obturator counting as an additional attempt. Any evidence of iatrogenic damage to the navicular bone fibrocartilage or the DDFT, identified on endoscopic examination of the bursa, was also recorded.

Alternate Direct Endoscopic Approach. The results of testing indicated that one of the surgeons inadvertently penetrated the DFTS and/or DIPJ significantly less frequently than any of the others (see Results section). On review of that surgeon’s technique, it was apparent that a modification of the direct approach had been used.2 The endoscope was inserted from the described skin incision and advanced distally in a parasagittal plane, parallel to the DDFT, rather than directing it toward midline. To examine whether a lower frequency of DFTS and/or DIPJ penetration could be obtained using this alternate direct endoscope trajectory, 6 additional pairs of cadaver limbs were collected and divided between 2 of the other surgeons, such that contralateral limbs were divided between them. The test procedure (steps 1–5 as described above) was repeated, except that the endoscope was advanced
in a modified trajectory to aim distally along a straight parasagittal plane toward the lateral aspect of the navicular bursa.

Synovial Distension to Evaluate DFTS/DIPJ Involvement

After removal of the endoscope and cannula, the DFTS was distended with tap water, using an 18 g 40 mm needle, placed on the lateral aspect of the limb, just distal to the proximal sesamoid bone. The DIPJ was distended with tap water using an 18 g 40 mm needle placed in the dorsal pouch on dorsal midline 1 cm proximal to the coronary band. Distension of the DFTS or DIPJ was continued until intrathecal/infra-articular pressure prevented further ingress, or until egress was observed from either the endoscope portal skin incision or the needle in the navicular bursa. Fluid was allowed to drain passively from the needle until pressure in the DFTS or DIPJ had subsided before needle removal. All incidences of fluid egress, confirming iatrogenic penetration of the DFTS or DIPJ, were recorded.

Post-Endoscopy-Positive Contrast Navicular Bursogram

The skin incision was closed with a single cruciate suture using 1 nylon, apposing the skin edges under tension. The navicular bursa was injected via the preplaced spinal needle with approximately 4 mL iohexol and 4 mL tap water before making a lateromedial radiograph. Any egress from the skin incision was blotted with tissue paper and the incident recorded. Radiographs were reviewed and the location of contrast recorded. In cases where the dissemination of contrast material was not clearly identifiable from the first radiograph, an additional 4 mL iohexol and 4 mL tap water were injected and another lateromedial radiograph obtained.

Dissection

Superficial dissection was performed on each limb to examine the neurovascular bundle and ungual cartilage for evidence of iatrogenic trauma (e.g., transection incurred when making the portal or during placement of the cannula and obturator).

Statistical Analysis

To simplify analysis and interpretation, penetration of DFTS, DIPJ, and/or both combined was categorized as “any inadvertent penetration” and all iatrogenic damage to any structure was categorized as “any iatrogenic damage.” Limb number in test sequence was used as a proxy for surgeon experience and termed “test experience.” A power calculation was performed using the pilot inadvertent penetration frequencies (“any inadvertent penetration”) and a sample size calculator (Epi Info™ 7, Centers of Disease Control and Prevention, Atlanta, GA) using a confidence level (CI) of 95% (α = 0.05) and a power of 80% (β = 0.2). The results were used to determine the number of limbs required for each of the 4 surgeons in the main study.

Frequencies of “any inadvertent penetration,” “any iatrogenic damage,” and number of attempts required for successful entry to the navicular bursa were compared between surgeons and between forelimb and hindlimb using χ² tests, and with “test experience” using logistic regression analysis. In addition, logistic regression was used to examine the magnitude of difference in frequency of “any inadvertent penetration” between surgeons, using penetration frequency of surgeon A as the reference for comparison. Associations between frequencies of “any inadvertent penetration” and “any iatrogenic damage” as well as number of attempts were assessed using χ² tests. The association between frequency of “any iatrogenic damage” and number of attempts was assessed using a χ² test. A Fisher’s exact test was performed to determine whether a significant change in frequency of “any inadvertent penetration” had occurred for the surgeons using the alternate direct technique. Statistical analysis was carried out in Stata12™ (StataCorp LP, College Station, TX). Significance was set at P < .05.

RESULTS

Pilot

Inadvertent penetration of the DFTS occurred in 75% of limbs (6/8), of both the DFTS and DIPJ in 25% of limbs (2/8), and no inadvertent penetration occurred in 25% of limbs (2/8). Based on an expected inadvertent penetration frequency of between 25% and 75%, using a population size of 40, confidence limits of 5%, with 4 clusters (surgeons), it was calculated that for a 95% confidence level, 9 limbs would be required per cluster and for a 99% confidence level, 10 limbs would be required per cluster.

Main Study: DFTS and/or DIPJ Penetration (Table 1)

No pre-existing synovial communications between the navicular bursa and either the DFTS or DIPJ were identified on the bursograms performed before the test procedure. After navicular bursa endoscopy, communication between the endoscope portal and the DFTS and DIPJ occurred in 18/40 limbs (45%). Inadvertent synovial penetration was recognized as egress from the endoscopy portal in 16/18 limbs (89%) and on the positive contrast radiographs in all 18 limbs (100%). Structures were penetrated with the following frequencies: DFTS alone in 11 limbs (27.5%: 5 forelimbs and 6 hindlimbs); DIPJ alone in 3 limbs (7.5%: 1 forelimb and 2 hindlimbs); and both DFTS and DIPJ in 4 limbs (10%: 2 forelimbs and 2 hindlimbs). Frequency of “any inadvertent penetration” ranged from 10%–80% and varied significantly between surgeons (P = .008), with surgeons C and D significantly (P = .035 and .007, respectively) more likely to penetrate the DFTS and/or the DIPJ than surgeon A (odds ratio 13.5; 95% CI 1.2–152 and odds ratio 36; 95% CI 2.7–476,
Frequency of inadvertent synovial penetration in navicular bursa endoscopy

Kane-Smyth et al.

Table 1 Frequency of Synovial Structure Penetration Using the Described Direct Approach for Endoscope Placement

<table>
<thead>
<tr>
<th>Surgeon</th>
<th>Synovial Structure Penetrated</th>
<th>Logistic Regression Comparison for Penetration of DFTS and/or DIPJ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DFTS</td>
<td>DIPJ</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>7</td>
</tr>
</tbody>
</table>

n = 10 limbs/surgeon. *Surgeon A used as the reference value for determination of subsequent odds ratios. DFTS, digital flexor tendon sheath; DIPJ, distal interphalangeal joint; Any, DFTS and/or DIPJ; CI, confidence interval.

respectively). Frequency of “any inadvertent penetration” did not vary significantly between forelimbs and hindlimbs (P = 1.0), or with “test experience” (P = .659), number of attempts (P = .060), or occurrence of “any iatrogenic damage” (P = .949).

Number of Attempts Made for Successful Entry to the Navicular Bursa

Successful entry into the navicular bursa was achieved on the first advancement of the cannula and obturator assembly in 18/40 (45%) of limbs. Second and third attempts were necessary in 11 and 10 limbs, respectively, whereas 5 attempts were required in 1 limb. The number of attempts required did not significantly vary between surgeons (P = .728), between forelimbs and hindlimbs (P = .579), or with “test experience” (P = .056).

Iatrogenic Damage to Adjacent Structures (Tables 2 and 3)

Endoscopically identifiable iatrogenic damage to the navicular bone fibrocartilage and/or DDFT occurred in 22/40 limbs (55%). Damage to the DDFT was identified in 16 limbs (40%) and varied from mild superficial epitenon disruption (12 limbs) to penetrating lesions (2 limbs). Superficial navicular bone fibrocartilage lesions were observed in 12 limbs (30%). Lesions of both the DDFT and the navicular bone fibrocartilage were observed in 6 limbs (15%). Frequency of “any iatrogenic damage” differed significantly between the surgeons (P = .008), but did not significantly vary between forelimbs and hindlimbs (P = .525), or with “test experience” (P = .66), frequency of “any inadvertent penetration” (P = .949), or number of attempts (P = .68). Dissections of the endoscopic portal and proximal cannula tracts did not identify evidence of iatrogenic damage to the ungual cartilage or digital neurovascular bundle in any of the limbs.

Alternate Direct Endoscopic Approach

The frequency of “any inadvertent penetration” was significantly lower (P = .024) using the alternate direct approach compared to the described direct approach, with no evidence of either DFTS or DIPJ penetration identified in any of the 12 limbs where the alternate direct endoscopic approach was used. There was no evidence of iatrogenic damage to the DDFT or navicular bone fibrocartilage apparent in any of the 12 limbs. However, both surgeons concluded that there was subjectively less maneuverability of the endoscope and a reduced field of view using the alternate direct approach, compared with the described direct approach. In each limb, the endoscope entered the navicular bursa laterally, allowing visualization of this aspect from the proximal point of entry to the distal recess. Examination of the medial aspect of the bursa was limited, particularly proximally.

DISCUSSION

To the authors’ knowledge, this is the first study to quantify the risk of inadvertent penetration of the DFTS and/or DIPJ when using the direct endoscopic approach to the navicular bursa. This study demonstrates considerable risk of inadvertent DFTS (37.5%) and DIPJ penetration (17.5%). The authors were surprised by these high frequencies of inadvertent penetration (particularly of the DFTS), as it is our clinical experience that very few cases (<5%) treated for septic navicular bursitis subsequently develop DFTS or DIPJ sepsis. It is possible that inadvertent penetration does not result in patent communication, or that lavage of the navicular bursa results in clearance of sepsis, preventing its spread into surrounding synovial structures. We suggest that repeated withdrawal and replacement of contaminated instruments through the penetrated synovial structures along the instrument tract might allow transference of sepsis from the navicular bursa.
The very close apposition between the DFTS and navicular bursa has previously been suggested to account for DFTS penetration with the direct approach.\textsuperscript{8} The width of the distal extremity of the DFTS is reportedly highly variable and in some instances located dorsal or abaxial to the neurovascular bundle,\textsuperscript{11} suggesting that in some horses, it may not be possible to avoid penetration when using the digital neurovascular bundle as a landmark. More extensive dissection or DFTS endoscopy may have allowed identification of the sites of penetration in the current series.

A similar cadaveric study reported inadvertent DIPJ penetration in 31.2% of specimens and concluded that penetration was more likely when contact between the cannula and dorsal DDFT was not maintained during placement.\textsuperscript{8} This recommendation was employed by the surgeons in our study and may have contributed to the lower frequency (17.5%) observed. Differences in study design may also have influenced the outcome, as the DIPJ was distended before cannula insertion in the previous study, but not in our study.\textsuperscript{6} The navicular bursa was, however, distended before cannula placement in our study, potentially facilitating bursa entry. In another investigation, Rossignol and Perrin\textsuperscript{5} suggested that inadvertent DIPJ penetration occurred because of insufficient extension of the digit during advancement of the assembly. The surgeons in our study also observed this precaution, which potentially influenced the results.

Our study was performed by 4 surgeons, representing a range of experience, in order to replicate the clinical setting where surgeons of variable experience may perform the surgical technique. A smaller group of surgeons performing the procedure a greater number of times may have allowed each surgeon to improve their skill during the study, possibly influencing the results. While “test experience” was not significantly associated with the number of attempts required to successfully enter the bursa, an improving trend was observed, which may have reached significance if a greater number of limbs had been used per surgeon. Level of surgical experience was not well correlated with frequency of inadvertent penetration, suggesting interpretation of the technique description was perhaps more important than experience. Subjectively, the board-certified surgeons caused less iatrogenic damage, which may relate to clinical experience.

It had been expected that repeated attempts to enter the bursa would increase the likelihood of inadvertent penetration; however, this was not observed. The overall low number of attempts required may have influenced this outcome. Previous reports have suggested that distension of the navicular bursa before cannula placement is not necessary.\textsuperscript{5} However, the authors believe that distension facilitated entry to the navicular bursa and allowed confirmation of successful entry, and therefore recommend considering this technique in clinical cases.

Previous authors have reported high incidence of iatrogenic damage (DFTS 93.8% and navicular bone 75%) using the direct approach and attributed this to trauma caused by the open end of the cannula. It was suggested that the incidence may be reduced by introducing the obturator alone before placing the assembly.\textsuperscript{6} Despite omission of this precaution, the incidence of trauma identified endoscopically in our study was considerably lower (DFTS 40% and navicular bone 30%), although this may have been underestimated. Only superficial dissections were performed and iatrogenic trauma sustained to deeper structures may not have been apparent on endoscopic examination. The highest frequency of iatrogenic damage was incurred by the surgeon who achieved the lowest frequency of inadvertent penetration and was using the principles of the alternate direct approach to the navicular bursa. This might indicate that this approach increased the likelihood of iatrogenic trauma, although this was not appreciated during the subsequent investigation by 2 other surgeons. The reason for this difference is unknown, but is potentially related to slight variation in the orientation of the cannula and obturator during insertion. Further investigation of the alternate direct approach would be beneficial before recommending it for clinical cases. The subjectively more limited endoscopic field of view and maneuverability encountered when using the alternate direct approach may be considered an acceptable compromise to limit the likelihood of inadvertent penetration when employing this technique in cases of navicular bursa sepsis. As the restriction in view appeared to be primarily proximomedially within the bursa, it is possible that the approach would still be useful for access to sites of bursa penetration within the distal recess and particularly if combined with an equivalent portal from the medial side of the limb.

Performing the procedure on cadaveric limbs is not identical to performing it in live animals and the freeze-thaw process has been demonstrated to alter tissues.\textsuperscript{12} However, multiple other studies have used cadaver material to evaluate surgical techniques and the authors considered the procedure to be sufficiently similar to performing it in clinical cases to allow valid comparison.\textsuperscript{6,11,13}

### Table 3
Frequency of Inadvertent Synovial Penetration and Iatrogenic Damage Using the Described Direct Approach for Endoscope Placement

<table>
<thead>
<tr>
<th></th>
<th>No iatrogenic Damage</th>
<th>Iatrogenic Damage</th>
<th>DDFT Lesion</th>
<th>Fibrocartilage Lesion</th>
<th>DDFT and Fibrocartilage Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inadvertent penetration</td>
<td>10</td>
<td>12</td>
<td>10</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Inadvertent synovial penetration</td>
<td>8</td>
<td>10</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>DFTS penetration</td>
<td>7</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>DIPJ penetration</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>DFTS and DIPJ penetration</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

DFTS, digital flexor tendon sheath; DIPJ, distal interphalangeal joint; DDFT, deep digital flexor tendon.
The variation in cadaver limb size and type is a limitation of our study and may explain some of the differences observed in the measured outcomes. However, this variation was considered acceptable, as it may reflect the potentially mixed population treated for navicular bursa sepsis. The variation was accounted for when comparing surgeons by ensuring that contralateral limbs were examined by different surgeons.

There is considerable risk of inadvertent synovial penetration when using the described direct endoscopic approach to the navicular bursa. Although the consequences of such inadvertent penetration are unknown, examination of the DFTS and DIPJ for communication with the navicular bursa is advisable when treating septic bursitis. Lavage of these structures may be advisable if any evidence of communication is identified. For cases that do not respond as expected to navicular bursa lavage, consideration of DFTS and/or DIPJ sepsis is indicated. The examined alternate direct approach may reduce the risk of inadvertent synovial penetration; however, the field of view and endoscope maneuverability may be restricted. Further investigation of this technique is warranted.

**DISCLOSURE**

The authors declare no conflicts of interest related to this report.

**REFERENCES**


