Effect of scan plane and arthrography on visibility and inter-observer agreement of the equine distal sesamoidean impar ligament on magnetic resonance images

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Introduction: In magnetic resonance imaging (MRI) examinations, moderate to severe changes of the distal sesamoidean impar ligament (DSIL) were found in horses with lameness localised to their feet. Histological abnormalities were detected more commonly in lame horses. Due to its heterogeneity and small thickness, evaluation of the DSIL in MRI can be challenging. The aim of the study was to determine the optimal sequence and the ideal transverse perpendicular angle for visualisation of the DSIL before and after arthrography of the distal interphalangeal joint (DIPJ).

Material and methods: Twenty-five cadaver forelimbs were examined with low-field MRI. Sagittal, frontal and three different angled transverse planes were obtained before and after arthrography of the DIPJ. All planes were acquired in T1w (weighted) Gradient Recall Echo (GRE), T2*w GRE, T2w Fast Spin Echo (FSE), und Short Tau Inversion Recovery (STIR) FSE and visualisation of the DSIL was scored by two observers.

Results: Visualisation of the DSIL was best on sagittal T2w FSE and STIR FSE images. All transverse planes were inferior compared to sagittal sequences. After arthrography of the DIPJ, visualisation of the DSIL origin improved in sagittal T2w FSE sequences and agreement between observers increased for sagittal T2w FSE and STIR FSE images.

Conclusion: Sagittal T2w FSE and STIR FSE images allowed good visualisation of the DSIL in low field MRI. Visualisation of the DSIL did not improve for altered angled transverse sequences but increased with arthrography of the DIPJ. Subjective influence between different observers was found but decreased with DIPJ-arthrography.

Keywords: Horse; Foot; MRI; Ligament; Podotrochlear
**Introduction**

Since the introduction of magnetic resonance imaging (MRI) for evaluation of the equine distal limb, accuracy of detection of abnormalities has increased especially within the palmar foot area. Considering the podotrochlear apparatus, abnormalities of the navicular bone itself as well as changes of surrounding soft tissue structures, such as the deep digital flexor tendon, navicular bursa, collateral sesamoidean ligaments and the distal sesamoidean impar ligament (DSIL) were frequently identified [1-7].

In horses with lameness localised to the foot, low and high-field examinations of the foot found changes of the DSIL in 6 to 81% of the cases [2, 4-9]. High-field MR images showed good agreement with histological examinations for mild findings of the DSIL in sound horses [10]. In horses with lameness localised to the foot, histological abnormalities of the DSIL were found to be more common in lame horses compared to controls [6, 11], but agreement of high-field MRI with histology was only fair with high sensitivity and moderate specificity [12]. The latter could be due to the heterogeneous appearance and small dimensions of the DSIL making its evaluation challenging [9, 13, 14]. Additionally, it was supposed that the DSIL is often visible in just one transverse image in low field MRI [15]. None of the previously published studies have investigated the optimal angulation for transverse images or overall best imaging plane for visualisation of the DSIL. Arthrography of the distal interphalangeal joint (DIPJ) and bursography of the navicular bursa improved visualisation of some structures of podotrochlear apparatus, but the DSIL was not investigated in these studies [16, 17]. Therefore, the aim of this study was to determine the best plane and sequence as well as the optimal transverse angle for imaging the DISL in low-field MRI. Additionally, the influence of different observers and arthrography of the DIPJ on evaluation of the ligament was assessed. We hypothesized that transverse images in a specific plane and arthrography of the DIPJ would improve visualisation of the DSIL.
Material and Methods

Twenty-five front limbs of 13 horses were included in the study; nine horses were euthanized for a research project for different studies and not primarily for the current study (TV 96/13) and four horses were euthanized due to clinical reasons. Horses comprised of eight mares, three stallions and two geldings (age range from two to 26 years, median 15 years) of different breeds (three ponies, seven warmbloods, one draught horse and two Arabians).

Within six hours after euthanasia, limbs were disarticulated at the carpometacarpal joint and placed in a custom-made device to simulate a weight-bearing position. Examination was performed using a low-field MRI (Hallmarq EQ2 Scanner, Hallmarq Veterinary Imaging, Guildford, Surrey, Great Britain). The MRI-protocol consisted of sagittal, frontal and three different angled transvers images in T1w (weighted) Gradient Recall Echo (GRE), T2*w GRE, T2w Fast Spin Echo (FSE), and Short Tau Inversion Recovery (STIR) FSE sequences before and after injection of fluid into the DIPJ (Tab 1). Frontal images were acquired parallel to the facies flexoria of the navicular bone (FF). The three different angles of the transverse planes were: perpendicular to the FF (plane 1), parallel to the origin of the DSIL (plane 2) and orientated on a tangent through the dorsodistal aspect of the navicular bone and the palmarproximal aspect of the distal phalanx (plane 3) (Fig. 1). To avoid volume average artefacts, transverse images were carefully aligned between the distal aspect of the navicular bone and the palmar aspect of the distal phalanx, with one of the slices starting just distal to the navicular bone. After acquisition of the native scans, injection of the DIPJ with ten to 20 ml of fluid consisting of iodine-based contrast (Imeron 300, Fa. BIPSO GmbH, Singen, Germany) diluted 1:1 with saline was performed and the MRI protocol was repeated.

Evaluation of the MRI images was performed by two experienced radiologist (Associate of the European College of Veterinary Diagnostic Imaging (ECVDI) and resident ECVDI) using a DICOM viewer (Synedra View Personal, Synedra information technologies GmbH, Feldstraße 1/13, Innsbruck, Austria) using a four-grade modified scoring system [18]: A score of 0 was allocated if the DSIL was not visible. If the DSIL was poorly visualised, but detectable by its location and signal intensity a score of 1 was assigned. A score of 2
represented that the DSIL was clearly identified by its location, shape and signal intensity, but the margins were not clearly delineated. Score 3 indicated the DISL was well visualised and clearly delineated by location, shape, signal intensity, size and margins. Sequences were blinded and the ligament was divided in three zones, origin, body and insertion and each zone was graded separately before and after fluid application. The origin was defined as the distal aspect of the navicular bone including the proximal part of the ligament. The distal aspect of the ligament and the area of insertion of the ligament at the distal phalanx were graded as the insertional zone. For the body the main part of the ligament between the aforementioned areas was evaluated. The entire sequences in the specific plane and weighting were provided to the observers, which graded them independently once, unaware of the exact angle of the transverse images and the timepoint of acquisition (native vs after fluid application).

Statistical analysis was performed using SPSS 22 (IBM Deutschland GmbH, Ehningen, Germany). For comparison of visibility grades between the different sequences and time-points Friedman tests were used and if differences were found further analysis of the four highest rated sequences was done using the Wilcoxon-Test. P values < 0.05 were considered significant. For inter-observer agreement, Kappa coefficients were calculated and assessed according to Landis and Koch [19].
Results

The DSIL could be visualised as a primarily hypointense band running from the palmarodistal aspect of the navicular bone to the facies flexoria of the distal phalanx (Fig 2). However, two synovial structures, dorsal the DIPJ and palmar the navicular bursa, surround the ligament and synovial invaginations of both penetrate the ligament resulting in its more heterogenous appearance.

Overall, anatomical visualisation was poor (Fig 3-5). The only sequences, where images were rated by both observers and in all locations as grade 3 in at least two limbs, were sagittal T2w FSE und STIR FSE. Grade 3 was allocated for at least one leg by observer A in transverse STIR FSE plane 1 at the origin and at the body and by observer B in sagittal T1w GRE sequence for all three locations. In all other sequences no limb received a grade 3.

At each location and for each time point significant differences were found comparing all sequences using the Friedman test and the highest rated four sequences for each observer are stated below. The significances given are referring to the Wilcoxon test comparing only these four sequences.

1. Visualisation of the ligament in native images

1.1 Origin (Fig 3)

At the origin observer A graded sagittal T2w FSE sequences significantly better (p < 0.01) than all other sequences, with the exception of sagittal STIR FSE, which were evaluated as second-best sequence. Sagittal T2* GRE sequences were ranked tertiary followed by transversal T1w GRE in plane 2 and 3 as well as T2* GRE in plane 2. Sagittal STIR FSE images received the highest grades by observer B, followed by T2w FSE, T2* GRE and T1w GRE sagittal images, between these no significant differences were found.

1.2 Body (Fig 4)

For visualisation of the body, sagittal T2w FSE sequences were significantly better rated by observer A than other sequences, except sagittal STIR FSE images (p < 0.05). The latter
was ranked better than transverse STIR FSE in plane 1 and sagittal T2*w GRE images, but no significant difference were found. Observer B graded sagittal T2w FSE, followed by sagittal STIR FSE, T2*w GRE und T1w GRE images, highest for the visualisation of the body. Significant differences were only detected between sagittal T2w FSE and T1w GRE images (p < 0.05).

1.3 Insertion (Fig 5)

At the insertion of the DSIL, observer A graded sagittal T2w FSE significantly better than other sequences but sagittal STIR FSE, which were rated second best (p < 0.05). Transverse T1w GRE in plane 2 and 3 as well as transverse T2*w GRE in plane 2 were ranked equally third. Sagittal STIR FSE images, followed by sagittal T2w FSE, T2*w GRE und T1w GRE sequences were graded highest by observer B, but no significant differences were observed.

2. Comparison between native images and images after fluid application

2.1 Origin (Fig 3)

After injection of fluid in the DIPJ, observer A rated sagittal T2w FSE sequences superior to sagittal STIR FSE, sagittal T2*w GRE and transverse T2*w GRE in plane 1, for visualising the origin of the DSIL. Compared to the corresponding native sequences, all sequences were rated better with significant improvement noted in sagittal T2w FSE and T2*w GRE images (p < 0.05).

Just as for the native sequences, observer B graded sagittal T2w FSE images highest, followed by sagittal STIR FSE, T2*w GRE and T1w GRE sequences. However, only T2w FSE sequences showed significant improvement (p < 0.05).

2.2 Body (Fig 4)

According to the grading of observer A sagittal T2w FSE images were best for visualising the body of the DSIL after fluid injection. Sagittal STIR FSE sequences were ranked second
before transverse STIR FSE in plane 1 and sagittal T2*w GRE images. Compared to native images mild but not significant improvement was found. Observer B ranked sagittal T2w FSE images highest, followed by sagittal STIR FSE and T2*w GRE and transverse T2*w GRE in plane 2 sequences. All but the latter, were graded non-significantly lower than the native images.

2.3 Insertion (Fig 5)

For visualisation of the insertion of the DSIL, sagittal T2w FSE images were graded better than sagittal STIR FSE and T2*w GRE sequences by observer A. Frontal T2w FSE and transverse T2*w GRE in plane 2 images were ranked fourth. With exception of the latter, mild but non-significant improvement was observed compared to the native sequences. The four best sequences of observer B corresponded to the native sequences but in different order, sagittal T2w FSE, T2*w GRE, STIR FSE and T1w GRE images. All sequence but sagittal STIR FSE sequences showed mild but non-significant improvement.

3. Agreement between observers

For evaluating the agreement between observers only the best four sequences of each were evaluated.

3.1 Origin - native

Comparing the scoring of both of observers, sagittal T1w GRE images were rated significantly higher by observer B and transverse T1w GRE plane 2 and 3 as well as transverse T2*w GRE plane 2 higher by observer A (p < 0.001).

3.2 Body - native

Observer B rated sagittal T1w GRE und T2*w GRE images and observer A transverse STIR FSE plane 1 images significantly higher (p < 0.001).
3.3 Insertion - native

At the insertion of the DSIL, observer B rated sagittal T1w GRE and T2*w GRE sequences significantly higher (p < 0.001). Transverse T1w GRE plane 2 and plane 3 were graded significantly higher (p < 0.001) by observer A.

The overall two best sequences, sagittal T2w FSE und STIR FSE, showed fair agreement at all levels between both observers (κ= 0.29-0.38), except for the origin in sagittal T2w FSE images, where only slight agreement was found (κ= 0.12). (Tab 2). Agreement was excellent for transverse T2w FSE plane 3 (κ= 1.00). For the other sequences, no agreement was found between both observers.

4.1 Origin - After Injection of Fluid

Sagittal T1w GRE images were graded significantly higher by observer B and transverse T2*w GRE sequences were rated significantly better by observer A (p < 0.001).

4.2 Body - After Injection of Fluid

Just as for the native images observer B rated sagittal T2*w GRE images (p < 0.01) and observer A transverse STIR FSE plane 1 sequences significantly higher (p < 0.001). Transverse T2*w GRE plane 2 images were rated significantly higher by observer B (p < 0.001).

4.3 Insertion - After Injection of Fluid

Sagittal T1w GRE and T2*w GRE sequences were rated higher by observer B (p < 0.001) whereas observer A scored frontal T2w FSE images higher (p < 0.05)

After injection of fluid into the DIPJ, inter-observer agreement for the two highest graded sequences (sagittal T2w FSE and STIR FSE) was moderate for all levels in STIR FSE and
for the origin in T2w FSE images ($\kappa = 0.41-0.50$). T2w FSE images showed substantial agreement for the body ($\kappa = 0.62$) and fair agreement at the insertion of DSIL ($\kappa = 0.31$). For these sequences, agreement was higher compared to native images (Tab 2). Agreement between observers was moderate for transverse T2w FSE plane 3 ($\kappa = 0.75$) and decreased compared to plain images. For transverse T1w GRE plane 3 moderate ($\kappa = 0.47$) and for transverse T2*w GRE plane 1 fair agreement ($\kappa = 0.38$) was observed. No further agreement was found between both observers for any other sequence.

**Discussion**

Anatomical visualisation of the DSIL was poor and, contrary to our hypothesis, only poor to fair for most transverse images. In sagittal T2w FSE and STIR FSE sequences visualisation was fair to good and better than in transverse or frontal images. Additionally, besides some of the transverse sequences inter-observer values were better in sagittal T2w FSE and STIR FSE. Interestingly, even rated low for visualisation, transverse T2w FSE plane 3 images showed substantial agreement between both observers before fluid injection. This agreement should be interpreted with caution, as the visualisation was graded poor by both observers. Whilst this is in accordance to some studies [20, 21], other studies suggested frontal [22, 23] or transverse sequences [24] for the evaluation of the DSIL. However, in the current study frontal and transverse images were inferior compared to sagittal sequences and only included in the four best sequences by one observer after fluid injection. This could be due to the orientation of our images, which were parallel or perpendicular to the DSIL leading to including the ligament in only one slice. In high-field MRI, transverse images are recommended for optimal visualisation of the DSIL, however, increased slice thickness used in low-field MRI could have caused suboptimal visualisation of the DSIL in transverse images in the current study [14]. Due to the width of the slices used in the current study, not all parts of the ligament could be visualised in the frontal and transverse images. It should be noted, that the results of the current study in regards to visualisation of the ligament in these orientation are rather due to the physical
limitation than due to poor contrast in the images. Decreasing the slice thickness could have led to better visualisation of the ligament, however, in the current study settings of the sequences were in accordance to clinical protocols to investigate the visualisation in routinely used images. Nevertheless, the influence of decreasing the slice thickness in low-field MRI on the visualisation of the DSIL needs further investigation and is still speculative. Increase of field strength leads to higher image resolution resulting in better perceptibility of smaller structures in high-field MRI [7, 14, 15, 24-26]. The values of the thickness of the DSIL are stated with only up to 4mm; its length has not been measured, but is considered short leading to visualisation on possibly only one image in transverse planes [15]. Due to reduction of volume average artefacts, acquiring transverse images perpendicular to the DSIL should improve their visualisation compared to oblique images [27 -29]. However, in the current study transverse sequences, independent of their angulation, were found to be inferior for the visualisation of the DSIL compared to sagittal images.

Due to their lower signal to noise ratio compared to T1w GRE and T2*w GRE sequences, higher slice thicknesses have to be used for acquisition of T2w FSE- und STIR FSE images, nevertheless the latter was still found to be better for visualisation of the DSIL. The DSIL is bordered by two synovial structures, the DIPJ and the navicular bursa, which show in these sequences hyperintense signal compared to the hypointense signal of the ligament itself resulting in increased contrast [14, 30]. Additionally, STIR FSE sequences were excellent to visualise adhesions between the DDFT and DSIL [31]. However, these sequences are prone to motion artefacts causing possible decreased image quality in live horses. On T1w GRE images, fluid as well as ligaments have a hypointense signal resulting in low contrast between the DSIL and the surrounding synovial structures. Therefore, despite their thinner slice thickness these sequences were found to be less useful for visualisation of the DSIL in the current study.
Distension of the DIPJ could lead to better delineation of the hypointense ligament from the fluid filled synovial pouch. Previous studies have shown delineation of structures of the podotrochlear apparatus increased with saline arthrography of the DIPJ and podotrochlear bursography, however, the DSIL was not investigated [16, 17]. In the current study, injection of fluid into the DIPJ resulted in mild improvement of the visualisation in some of the sequences, such as sagittal T2w FSE und T2* GRE images. Nevertheless, observer B noted mild but non-significant reduction of visualisation of the body of DSIL in sagittal T1w GRE, T2w FSE and STIR FSE images as well as at the insertion in sagittal STIR FSE sequences. However, compared to native images inter-observer agreement was higher for saline arthrography of the DPJ, which could be due to better visualisation. This could lead to improved visualisation of the DISL in clinical cases with presence of DIPJ distension.

Gadolinium used as contrast agent in MRI improved visualisation of abnormalities including desmopathies of the DSIL after intravenous and intraarterial application [32, 33]. However, by using disarticulated limbs use of these application methods would have been challenging. Furthermore, the limbs were included in further studies evaluating the use of iodine-based contrast in assessing the soft tissue structures in computed tomography.

This study had some limitations. Evaluation of the images was done only for the visualisation of the DSIL and abnormalities were disregarded. However, the aim of the study was to investigate the visualisation of the DSIL comparing different sequences. The range of the age of the included horses was quite wide and no clinical examination was performed prior to euthanasia. In standing horses, pressure leads to compression of structures resulting in decreased visibility of some structures. Using limbs instead of live horses was one limitation of current study, however, by using a custom-made stand we were able to simulate closely the weight-bearing position. Additionally, only two observers graded the images and intra-observer agreement and therefore repeatability was not investigated.
In conclusion, on sagittal T2w FSE and STIR FSE sequences visualisation of the DSIL in low-field MRI was fair to good and better than in other sequences and poor to fair for most transverse sequences independent of their orientation. Therefore, the former should be used to evaluate the DSIL. Whilst no consistent improvement could be found for images with distension of the DIPJ, agreement between different observers was higher compared to native sequences and could improve visualisation of pathological changes of the DSIL. However, further studies evaluating this effect in detecting abnormalities of the DSIL are required.
References


**Table 1**: Details of the MRI sequences used for imaging of the distal sesamoidean impar ligament.

<table>
<thead>
<tr>
<th>Sequence and Orientation</th>
<th>TR (msec)</th>
<th>TE (msec)</th>
<th>Flip angle</th>
<th>Slice-thickness (mm)</th>
<th>FOV</th>
<th>Gap (mm)</th>
<th>Scan Time (min)</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1w GRE 3D</td>
<td>23</td>
<td>7</td>
<td>40</td>
<td>3</td>
<td>170x170</td>
<td>0</td>
<td>1:52</td>
<td>256 x 256</td>
</tr>
<tr>
<td>T2* GRE 3D</td>
<td>33</td>
<td>13</td>
<td>26</td>
<td>3</td>
<td>170x170</td>
<td>0</td>
<td>2:24</td>
<td>256 x 256</td>
</tr>
<tr>
<td>T2w FSE(2D)</td>
<td>2125</td>
<td>84</td>
<td>90</td>
<td>5</td>
<td>170x170</td>
<td>1</td>
<td>3:25</td>
<td>256 x 256</td>
</tr>
<tr>
<td>STIR FSE - (2D)</td>
<td>2910</td>
<td>27</td>
<td>90</td>
<td>5</td>
<td>170x170</td>
<td>1</td>
<td>3:18</td>
<td>256 x 256</td>
</tr>
<tr>
<td>STIR FSE (2D)</td>
<td>2700</td>
<td>27</td>
<td>90</td>
<td>5</td>
<td>170x170</td>
<td>1</td>
<td>3:18</td>
<td>256 x 256</td>
</tr>
<tr>
<td>STIR FSE + (2D)</td>
<td>3220</td>
<td>27</td>
<td>90</td>
<td>5</td>
<td>170x170</td>
<td>1</td>
<td>3:18</td>
<td>256 x 256</td>
</tr>
</tbody>
</table>

T1w: T1-weighted, T2w: T2-weighted; GRE: Gradient Recall Echo, FSE: Fast Spin Echo, STIR: Short Tau Inversion Recovery, 2D: two-dimensional, 3D: three-dimensional, TR: Repetition Time, TE: Echo Time, FOV: Field of View, msec: Millisecond; mm: Millimetre; min: Minute
Table 2: Observer-agreement (weighted Kappa) of the two best sequences before and after fluid injection (Landis and Koch 1977): Bold numbers represent values after fluid injection.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Origin</th>
<th>Body</th>
<th>Insertion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sag. T2w FSE native</td>
<td>0.12</td>
<td>0.36</td>
<td>0.29</td>
</tr>
<tr>
<td>Sag. T2w FSE post</td>
<td><strong>0.47</strong></td>
<td><strong>0.62</strong></td>
<td><strong>0.31</strong></td>
</tr>
<tr>
<td>Sag. STIR native</td>
<td>0.38</td>
<td>0.29</td>
<td>0.34</td>
</tr>
<tr>
<td>Sag. STIR post</td>
<td><strong>0.41</strong></td>
<td><strong>0.5</strong></td>
<td><strong>0.44</strong></td>
</tr>
</tbody>
</table>

Sag: Sagittal, T2w FSE: T2 weighted Fast Spin Echo; STIR: Short Tau Inversion Recovery; post: after injection of fluid; <0: Poor agreement; 0-0.20 slight agreement; 0.21-0.40: fair agreement; 0.41-0.60: moderate agreement; 0.61-0.80: substantial agreement; 0.81-1.00: almost perfect agreement.
Fig. 1: Sagittal T1weighted 3D Gradient Recall Echo magnetic resonance image of a hoof. The red lines indicate the three different transverse planes for imaging of the distal sesamoidean impar ligament. Plane 1: Orientated perpendicular to the facies flexoria of the navicular bone; Plane 2: Orientated parallel to the origin of the distal sesamoidean impar ligament; Plane 3: Tangent between the dorsodistal aspect of the navicular bone and the palmaroproximal aspect of the distal phalanx.

Fig 2: Sagittal images of one limb before (A-D) and after fluid injection (E-H), in T1weighted (w) Gradient Recall Echo (GRE) (A,E); T2*w GRE (B,F), T2w Fast Spin Echo (FSE) (C,G) and Short Tau Inversion Recovery (STIR) FSE (D,H). In T1w GRE sequences, both observers graded the body with a score of 1 in native images and a score of 0 in images after fluid injection. The body was scored by both observers in native T2*w GRE sequences with 1 and after fluid injection with grade 1 by observer a and grade 2 by observer B. Both observers graded the body in native T2w FSE and STIR images with a score of 2. After fluid injection both observers scored the T2w FSE sequences with a grade of 3, and the STIR sequences were graded by observer A as 3 and by observer B as 2.

Fig 3: Mean score of the different sequences for visualisation of the origin of the distal sesamoidean impar ligament in magnetic resonance imaging. T1: T1 weighted (w) Gradient Recall Echo (GRE); T2*: T2*w GRE, T2: T2w Fast Spin Echo and STIR: Short Tau Inversion Recovery. SAG: sagittal, FRO: frontal, TRA1: transverse plane 1, TRA2: transverse plane 2, TRA3: transverse plane 3, Obs: Observer. Native: before fluid injection, Post: after fluid injection
Fig 4: Mean score of the different sequences for visualisation of the body of the distal sesamoidean impar ligament in magnetic resonance imaging. T1: T1 weighted (w) Gradient Recall Echo (GRE); T2*: T2*w GRE, T2: T2w Fast Spin Echo and STIR: Short Tau Inversion Recovery. SAG: sagittal, FRO: frontal, TRA1: transverse plane 1, TRA2: transverse plane 2, TRA3: transverse plane 3, Obs: Observer. Native: before fluid injection, Post: after fluid injection

Fig 5: Mean score of the different sequences for visualisation of the origin of the insertion sesamoidean impar ligament in magnetic resonance imaging. T1: T1 weighted (w) Gradient Recall Echo (GRE); T2*: T2*w GRE, T2: T2w Fast Spin Echo and STIR: Short Tau Inversion Recovery. SAG: sagittal, FRO: frontal, TRA1: transverse plane 1, TRA2: transverse plane 2, TRA3: transverse plane 3, Obs: Observer. Native: before fluid injection, Post: after fluid injection