In vitro evaluation of a novel fiducial marker for computed tomography and magnetic resonance imaging of soft tissues in small animals

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Objective—To construct and optimize a fiducial marker suitable for both CT and MRI.
Sample—Fiducial markers containing serial dilutions of iopamidol mixed with water.
Procedures—IV tubing sets were infused with serial dilutions (0% to 100%; increments of 10%) of iopamidol. Tubing ends were sealed; additional seals were added to create an equilateral triangle. A reference point was created by placing a crimp in 1 side. Markers were fixed to a gelatin soft tissue–attenuating phantom and evaluated by use of CT and MRI. For CT, simple linear regression analysis was used to assess the relationship between the percentage of marker contrast medium and quantitative variables, including marker attenuation, attenuation changes in the phantom, and beam-hardening artifact length. A subjective grading scheme for artifact creation on CT images and marker visibility on MRI images was used. Measurements were obtained by investigators who were unaware of the contents of each marker.

Results—Percentage of contrast medium in each marker was strongly correlated with marker attenuation ($r^2 = 0.96$), artifact length ($r^2 = 0.765$), and mean attenuation changes within the phantom ($r^2 = 0.826$) for CT. Subjective CT scores indicated that concentrations of contrast medium > 50% resulted in excessive artifacts. Markers with concentrations of iopamidol > 50% had poor subjective MRI visibility scores. No artifacts were seen on MRI.

Conclusions and Clinical Relevance—A marker containing a 10% solution of iodinated contrast medium mixed with water provided ideal contrast for both CT and MRI. (Am J Vet Res 2014;75:974–981)

The use of cross-sectional imaging modalities (eg, CT or MRI) is becoming increasingly commonplace in small animal veterinary practice. Advancements have allowed for faster image acquisition, improved image quality, and a better appreciation for 3-D structures. A common tool used with these imaging techniques in human medicine is a fiducial marker. A fiducial marker acts as a common reference point that links the location of the lesion on the CT or MRI images to the location of the lesion in the patient. Fiducial markers are typically used for surgical planning or radiotherapy.

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>HU</td>
<td>Hounsfield unit</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<td>STIR</td>
<td>Short tau inversion recovery</td>
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In veterinary medicine, the placement of a needle into the spinous process of a vertebra to guide a surgeon to the correct intervertebral disc space during decompressive surgery has been described anecdotally, although to the authors’ knowledge there are no peer-reviewed publications describing the use of a needle as a fiducial marker. Similarly, the use of bony landmarks or previously placed vascular clips can be considered a type of fiducial marker. In human medicine, fiducial markers can allow synchronization of the radiation beam with moving organs such as the lungs or heart, thereby decreasing target margins and doses to surrounding tissues. Similarly, the use of fiducial markers in a recent study on image-guided radiation therapy for partial breast irradiation allowed a decrease in planning target volume margins from 10 to 6 mm. Investigators in another study found that fiducial marker registration (superimposition of previous images onto intraoperative surgical anatomic structures) was possi-
ble during the preoperative planning process in patients that were to undergo perforator flap reconstructive procedures. In that same study, surface-landmark registration and laser surface matching registration both failed the registration process. Fiducial-based localization of the vertebral column in humans prior to neurosurgery is a standard protocol described in a recent report. Additional protocols that include fiducial-assisted preoperative imaging are becoming increasingly common for use in humans and have the potential to become similarly important in veterinary medicine.

The ideal fiducial marker should be simple to construct, inexpensive, and easily distinguishable from the surrounding tissues to which it is applied on images acquired by use of multiple advanced imaging modalities. Ability to use the marker with multiple imaging modalities is of particular interest because it is known that imaging modalities differ in their ability to detect various tissue types. Diagnostic and therapeutic protocols that use both MRI and CT are frequently encountered in the human literature, with software readily available to combine images obtained by use of each modality. Similar techniques are currently lacking in the veterinary literature, but it is reasonable to expect similar applications in the near future. Further considerations are that markers should not create imaging artifacts and should not disturb the relationships of surrounding tissues.

Iopamidol is an iodinated, nonionic, low-osmolarity contrast agent primarily used in angiography and excretory urography. It also has been commonly used for contrast myelography in dogs. Iodinated contrast agents such as iopamidol could be considered for use in a novel fiducial marker but offer potential challenges. Specifically, the production of beam-hardening artifact has been documented with iodinated contrast agents. Beam hardening occurs when low-energy photons are preferentially absorbed as a polychromatic x-ray passes through a material. This results in regions of streaking around the material in question, thereby obscuring images of the tissues adjacent to it, which is clearly an undesirable trait in a fiducial marker. Because MRI does not rely on x-ray attenuation, beam-hardening artifacts have not been reported with use of iodinated contrast agents for MRI. A variety of both positive and negative contrast materials have been evaluated for use with MRI. For example, vitamin E has been used to delineate laterality on MRI images but was found to be an inaccurate marker for surgical planning. Conveniently, the presence of water within a marker can act as a contrast agent and allow adequate MRI delineation for many commonly used pulse sequences, such as STIR or T2-weighted images. Theoretically, a marker could be made with the appropriate ratio of iodinated contrast agent to water, such that the marker would be easily seen on both CT and MRI pulse sequences while minimizing beam-hardening artifact on CT images. Such a marker could be sutured to the skin and used for surgical planning or radiotherapy.

To the authors’ knowledge, there are no published reports describing the construction of a cost-effective, consistent, and reliable fiducial marker that can be used for both CT and MRI in veterinary medicine. Therefore, the purpose of the study reported here was to design a marker that could easily be distinguished on both CT and MRI images while minimizing beam-hardening artifact on CT images. Our hypothesis was that the iodinated contrast medium in a marker meeting these criteria would be a low percentage of the solution.

**Materials and Methods**

**Fiducial markers**—Fiducial markers for the study were created from IV drip tubing (15 drops/mL) sets. These sets were chosen on the basis of their low cost and widespread availability. Sections (4 to 5 cm in length) were cut and used to make the markers. Serial dilutions (0% to 100%, intervals of 10%) of a commercially available iopamidol solution (300 mg of iodine/mL) were made by the addition of water. These solutions were infused into the lumen of the IV tubing. Ends of the IV tubing were then sealed together with a commercially available IV tubing sealer. The markers (n = 11) were then sealed in 2 additional places to create an equilateral triangle with 1.5-cm sides (Figure 1). Care was taken to prevent inclusion of air in the tubing. To provide a reference point in each marker, an additional crimp was created in 1 of the 3 sides with the IV sealer. In a clinical setting, this crimp would be used as a marker of directionality (eg, crimped side can be placed in a specific relationship to the patient).

**Imaging phantom**—An imaging phantom for soft tissue attenuation was created, similar to those described elsewhere. Briefly, 500-mL plastic bottles were filled with a gelatin mixture. Boiled water (480 mL) was added to 680 g of a commercially available powdered gelatin, which was followed by the addition of 480 mL of ice-cold water. The solution was thoroughly mixed and chilled for 12 hours at 4°C. Final gelatin concentration was approximately 8%. All mark-
ers were labeled for all imaging sequences with a number (1 through 11) by a CT technician; numbers were assigned by the technician by randomly drawing markers out of an envelope. Investigators who evaluated images were unaware of the contents of each marker used until after data collection.

**CT and MRI**—Each of the 11 markers was affixed in a random order onto the imaging phantom with 2-inch tape. Helical CT images of the phantom were acquired with a 64-detector helical CT scanner. Helical images were acquired as a volume with 0.5-mm voxels, 0.3-second rotation speed, 512 × 512 matrix, and 120 kVp with a tube current of 200 mA. Volume data were also allowed assessed because they were of clinical patients at our institution and allowed us to assess marker visibility on T2 pulse sequences (in which water is hyperintense) and T1 pulse sequences (in which water is not hyperintense).

**Evaluation of markers**—To evaluate each fiducial marker, objective and subjective data were collected. A board-certified veterinary radiologist (SN) and a resident in a small animal surgery training program (JLT) reviewed all image sequences; these investigators were unaware of the percentage of contrast medium in each fiducial marker. To measure beam-hardening artifact, CT images were evaluated in a soft tissue algorithm (window width, 120 HUs; window level, 40 HUs), with instructions that the investigators were allowed to change the window width and window level. To quantify visibility of the markers on CT images, marker attenuation was measured in HUs with the ROI tool of commercially available software. This tool allowed marker visibility to be objectively quantified; it allowed assessment of the relationship between marker attenuation and the percentage of contrast medium.

For each CT scan, attenuation of a baseline region (area 1 was closest to the marker and area 5 was most distant from the marker) was drawn sequentially below the marker along the longest streak artifact. The ROI closest to the marker was labeled as area 1, with subsequent ROIs (areas 2, 3, 4, and 5) labeled accordingly on the basis of their distance from the marker. Mean ± SD attenuation of each ROI was recorded. A higher SD of the selected ROIs indicated more heterogeneity in the attenuation of the phantom. Beam-hardening artifact was also assessed with these attenuation measurements and reported as the ratio in relation to attenuation measurements for the internal control region for each scan. By use of this method, higher ratios indicated a greater difference between marker and phantom attenuation. The longest artifact streak length was measured with the linear measurement tool of the software and plotted against its corresponding percentage of contrast medium.

Subjective scoring of CT artifacts was performed by a board-certified veterinary radiologist (SN). Artifact was defined as any discernible streaking or image distortion. All marker sequences were scored on a scale of 1 to 3 as follows: 1 = marker caused minimal or no artifact and would not be expected to affect image interpretation, 2 = marker caused moderate artifact that could affect image interpretation, and 3 = marker caused severe artifact that would likely affect image interpretation.

Because of a lack of validated qualification for MRI image intensity, a subjective scoring system was used to evaluate visibility of markers for MRI. Visibility was scored on a scale of 1 to 3 as follows: 1 = marker was easily differentiated from the phantom and would likely be clinically useful, 2 = marker could be differentiated maximum artifact was selected, 0.5-cm² ROIs were drawn sequentially in the phantom starting below the marker along the longest streak artifact (Figure 2). The ROI closest to the marker was labeled as area 1, with subsequent ROIs (areas 2, 3, 4, and 5) labeled accordingly on the basis of their distance from the marker. Mean ± SD attenuation of each ROI was recorded. A higher SD of the selected ROIs indicated more heterogeneity in the attenuation of the phantom. Beam-hardening artifact was also assessed with these attenuation measurements and reported as the ratio in relation to attenuation measurements for the internal control region for each scan. By use of this method, higher ratios indicated a greater difference between marker and phantom attenuation. The longest artifact streak length was measured with the linear measurement tool of the software and plotted against its corresponding percentage of contrast medium.

**Figure 2**—Transverse CT image (soft tissue algorithm [window width, 70 HU; window level, 90 HU]) of a representative fiducial marker containing 100% iodinated contrast medium and areas used for CT artifact quantification. An ROI (0.5 cm²) for each of 5 areas (area 1 was closest to the marker) was drawn sequentially below the marker along the longest streak artifact. Mean attenuation of each region was measured (HU) and used to quantify beam-hardening artifact. L = Left. R = Right.
from the phantom on most images but was difficult to differentiate from the phantom in at least 1 image, and 3 = marker was difficult to differentiate from the phantom and would be considered unreliable to use in a clinical setting.

**Statistical analysis**—All statistical analyses were performed with a statistical program. Simple linear regression was used to evaluate the relationship between percentage of contrast medium in a marker and individual fitted factors, including marker attenuation, marker attenuation-to-control attenuation ratio, streak length, and subjective scores for both CT and MRI. For relationships analyzed, coefficients of determination (ie, \( r^2 \)) were reported along with associated \( P \) values. When applicable, slope of the regression line was reported along with associated \( P \) values. Values of \( P < 0.05 \) were considered significant. Assumptions of simple linear regression including linearity and constant variance and normal distribution of the subpopulations were verified by use of residual plots (residuals against fitted values and normal probability plot of residuals).

**Results**

Eleven markers were created that contained various percentages of iopamidol (0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%). Mean CT control attenuation ranged from 83.7 to 84.9 HUs. No relationship was detected between control region attenuation and percentage of contrast medium in a marker (\( r^2 = -0.0072; \ P = 0.8 \)).

The percentage of contrast medium in each marker was correlated (\( r^2 = 0.96; \ P < 0.001 \)) with its corresponding CT attenuation measurements (Figure 3). Linear regression analysis revealed that for every 10% increase in contrast medium, marker attenuation increased by a mean ± SD of 60.3 ± 4.12 HUs (\( P < 0.001 \)). Ratios of the attenuation of each marker with that of the internal control region for the scans were calculated (Table 1). The marker with 0% iopamidol had no difference in attenuation (ratio, 0.86).

Areas closest to the marker and areas from the markers with the highest percentage of contrast medium had the largest differences in attenuation from the attenuation for the internal control regions (Figure 4). A positive correlation (\( r^2 = 0.826; \ P < 0.001 \)) was found between percentage of contrast medium in a marker and SD in area 1. It was found that all percentages of contrast medium in a marker tested, including the marker with 0% contrast medium, had some degree of x-ray attenuation in area 1. No difference in the attenuation of area 2 was seen for markers with 0%, 10%, and

![Figure 3](image-url) Linear regression analysis of the relationship between attenuation of each marker and percentage of contrast medium in a marker. Each circle represents a data point for a marker; the line represents an estimated regression line of marker attenuation for the percentage of contrast medium; and the gray-shaded area represents the standard error. There was a strong positive linear correlation (\( r^2 = 0.96; \ P < 0.001 \)) between the 2 variables.

### Table 1—Results of variables for each fiducial marker that contained a specific percentage of iopamidol mixed with water.

<table>
<thead>
<tr>
<th>Contrast medium (%)</th>
<th>CT score</th>
<th>MRI score*</th>
<th>Marker attenuation (HU)</th>
<th>Control region attenuation (HU)</th>
<th>Marker attenuation-to-control attenuation ratio</th>
<th>Artifact streak length (cm)</th>
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<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
<td>72.5</td>
<td>84.4</td>
<td>0.86</td>
<td>1.2</td>
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<td>83.8</td>
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<td>66.7</td>
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<td>100</td>
<td>3</td>
<td>3</td>
<td>6,670.1</td>
<td>83.9</td>
<td>79.5</td>
<td>5.2</td>
</tr>
</tbody>
</table>

*Results indicate scores for MRI STIR (T2 weighted).

Subjective CT score was assigned as follows: 1 = marker caused minimal or no artifact and would not be expected to affect image interpretation, 2 = marker caused moderate artifact that could affect image interpretation, and 3 = marker caused severe artifact that would likely affect image interpretation. Subjective MRI score was assigned as follows: 1 = marker was easily differentiated from the phantom and would likely be clinically useful, 2 = marker could be differentiated from the phantom on most images but was difficult to differentiate from the phantom in at least 1 image, and 3 = marker was difficult to differentiate from the phantom and would be considered unreliable to use in a clinical setting. A positive correlation was detected between percentage of contrast medium and subjective CT score, subjective MRI score, marker attenuation, and streak length. Ratios of approximately 1 indicate minimal difference between the marker and phantom, which indicate that they would be difficult to differentiate. Higher ratios indicate a greater difference in marker attenuation to phantom attenuation, which indicates the marker would easily be seen on CT images.
20% iopamidol. A positive correlation \( (r^2 = 0.765; P < 0.001) \) was also detected between the longest measured streak artifact and percentage of contrast medium in a marker (Figure 5).

Markers that contained a higher percentage of iodinated contrast medium typically had higher subjective CT scores (Table 1; Figure 6). A correlation \( (r^2 = 0.669; P = 0.002) \) was detected between subjective CT score and percentage of contrast medium in a marker. Analysis of subjective CT scores indicated that the lowest concentration of iopamidol tested (10%; mean attenuation, 1,065.5 HUs) was clearly and distinctly visible on CT images.

No significant artifacts were detected on MRI images. Analysis of subjective MRI scores indicated that markers with >50% iopamidol resulted in a less readily identifiable marker on STIR MRI pulse sequences (Table 1; Figure 6). There was a positive correlation \( (r^2 = 0.6; P = 0.005) \) between percentage of contrast medium in a marker and subjective MRI STIR score. Although the markers were visible on MRI T1 sequences, no correlation was detected between percentage of contrast medium in a marker and subjective MRI score.

**Discussion**

The specific objective of the present study was to construct a fiducial marker that was easily detected by both CT and MRI while minimizing beam-hardening artifact for CT. To assess the degree of CT contrast between the markers and normal soft tissues (ie, attenuation within established ranges), we measured attenuation of markers consisting of an increasing percentage of an iopamidol solution mixed with water. Marker attenuation was then compared with phantom attenuation and reported as a ratio. With a 10% iodinated contrast medium solution, marker attenuation was >10 times as high as that of the soft tissue phantom. This relationship continued in a linear manner for each incremental increase in iodinated contrast medium. Anatomically normal soft tissues (ie, attenuation within established ranges) have a mean attenuation of 40 to 60 HUs. Therefore, it was not surprising that the increase in attenuation of the 10% contrast medium marker (1,065.5 HUs) made it readily detectable on CT images with a contrast ratio of 12.7. Although markers with a higher percentage of iopamidol contrast medium yielded a higher marker attenuation-to-phantom at-

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**Figure 4**—Mean attenuation of areas 1 through 5 reported as the area attenuation-to-control attenuation ratio for fiducial markers that contained various percentages of iodinated contrast medium. Area 1 was located closest to each marker on the CT image and had the highest ratio for all markers, which indicated consistently more artifact in this area.

**Figure 5**—Linear regression analysis of the relationship between percentage of contrast medium in a fiducial marker and streak length on CT images. There was a positive correlation \( (r^2 = 0.765; P < 0.001) \) between streak length and percentage of contrast medium in each marker. See Figure 3 for remainder of key.

**Figure 6**—Transverse CT (A and B) and STIR MRI (C and D) images of markers containing 10% (A and C) and 90% (B and D) iodinated contrast medium. In panel A, the marker contained 10% contrast medium, was easily visible on CT images, and was assigned a subjective CT score of 1, which indicated little or no artifact. In panel B, the marker contained 90% contrast medium and was assigned a subjective CT score of 3 (was difficult to differentiate in at least 1 image). In panel C, the marker contained 90% contrast medium and was assigned a subjective MRI score of 3 (considered unreliable to use in a clinical setting).
tissue, which makes the diseased tissue hyperintense compared with that in surrounding healthy tissue. In vivo, diseased tissues typically have increased water content, compared with that in surrounding healthy tissues, which makes the diseased tissues hyperintense and conspicuous on T2 and T2-weighted STIR pulse sequences. Intravenous administration of gadolinium contrast medium can also cause these tissues to be hyperintense on T1 postcontrast MRI pulse sequences. In the present study, we used both T2-weighted STIR and T1 fat-saturated spin echo pulse sequences to assess the fiducial markers. The ability to visually identify the markers on MRI images was subjectively scored by a radiologist who was unaware of the marker contents (i.e., percentage of iodinated contrast medium). As expected, a higher water content in the markers (lower percentage of contrast medium) resulted in greater hyperintensity on T2-weighted STIR pulse sequences, which was reflected in the scoring system. Interestingly, the markers were also visible on the T1 fat-saturated images, possibly secondary to the plastic in the tubing. In general, the visibility scores were less on the T1 images, compared with scores for the STIR images, and there was no correlation between percentage of contrast medium in a marker and visibility on T1-weighted images. On the basis of these data, the markers with ≤ 50% iopamidol contrast medium were the best fiducial markers for MRI. Thus, combining the findings for MRI and CT, we concluded that the overall best fiducial marker for both imaging modalities was the marker that contained 10% iopamidol contrast medium.

To the authors’ knowledge, the study reported here is the first in which an easily constructed and effective marker compatible for both CT and MRI in veterinary medicine has been described. Reports on the use of other types of fiducial markers are limited in the veterinary literature. In recent studies, investigators have described the use of fiducial markers for canine brain biopsy, and inclusion of fiducial markers in image-guided radiation protocols in veterinary medicine is common. Authors of other studies have described the use of metal as a fiducial marker for CT. Although CT artifacts attributable to metal markers can be reduced by decreasing the size of a marker and by use of metal artifact–reducing algorithms, the properties of many solid metals make them inappropriate choices for MRI. The MRI susceptibility artifact is seen when ferrous metals cause disturbances in the local magnetic field, thereby shifting the position of the image. This artifact causes large regions of severe distortion of an image, which renders part or all of the image nondiagnostic. This artifact makes a metallic fiducial marker unsuitable for use in veterinary medicine and was avoided in the present study by the use of nonmetallic materials. Vitamin E capsules affixed to the skin with tape have been used as fiducial markers for MRI. Unfortunately, these markers have proven to be inaccurate for surgical planning, which is an intended use for fiducial markers. A fiducial marker should be easy to affix to the skin; hence the triangular shape of the markers used in the present study, which could easily be sutured to the skin. Furthermore, vitamin E is iso-attenuating to soft tissue on CT images and would not have adequate contrast for use as a fiducial marker for soft tissue CT images. To the author’s knowledge, vitamin E has not been described as a fiducial marker for soft tissue CT images.

The linear attenuation coefficient commonly described in x-ray physics refers to the degree of x-ray photon attenuation per unit of path length. This coefficient increases with increased electron density of a tissue. Compounds that have atoms with a high atomic number are considered more opaque on radiographs or more attenuating on CT images as a result of this phenomenon. Notable compounds frequently used as contrast medium in veterinary medicine include barium and iodine. In the present study, iopamidol was chosen because of its widespread availability and use as the primary iodinated contrast medium in our hospital. Iopamidol is an iodinated, nonionic compound, and it is used in both human and veterinary medicine. For purposes of the study reported here, the 100% iopamidol solution did not result in adequate marker intensity on MRI images and provided excessive artifact on CT images. To address this issue, water was used to dilute the iodinated solution until the most suitable concentration was identified.

One limitation of the present study was the in vitro nature of the technique. Although a gelatin phantom that mimicked soft tissues was used for simplicity and consistency, it is acknowledged that creation of artifacts and their assessment may differ slightly when evaluated on a heterogeneous soft tissue region of a clinical patient. A second limitation of the study was the reliance on a subjective scoring system as the only evaluation tool for MRI. This was justified because no validated measure of MRI intensity has been reported, to the authors’ knowledge.
We concluded that a fiducial marker can be easily created from infusion tubing filled with 10% iopamidol iodinated contrast medium and sealed into the shape of a triangle. We accepted our hypothesis because the ideal concentration of iodinated contrast medium was found to be a relatively low concentration (10%), which provided sufficient attenuation for visualization on both CT and MRI images without excessive beam-hardening artifact for CT. We propose that the technique described is a simple, cost-effective, and reasonable option for use when a surface fiducial marker is desired.

References

4. Hutchison MC, van Andel G, Bel A, et al. Intravesical markers for CT and MRI imaging without excessive beam-hardening artifact for CT. We propose that the technique described is a simple, cost-effective, and reasonable option for use when a surface fiducial marker is desired.

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