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Endoscopic Imaging and Size Estimation of Colorectal Adenomas in the Multiple Intestinal Neoplasia Mouse

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Abstract

Background—The scientific potential of animal models of carcinogenesis has not been fully realized due to our limited ability to monitor tumor growth in vivo.

Objective—To develop an endoscopy-based protocol for the accurate estimation of adenoma size in vivo from images obtained during colonoscopy.

Design—Compare estimates of lesion size acquired during endoscopy with those obtained from MRI and at necropsy.

Setting—Small animal imaging facility.

Subjects—Apc^{+/Min}-FCCC mice that develop multiple colorectal adenomas.

Methods—Mice received colonoscopic examinations using a rigid endoscope, and high-resolution images of colon adenomas were captured using a charge coupled device camera. Lesion size was estimated by comparing the dimensions of the adenoma relative to a reference rod using a novel geometric construction. The resulting areas were compared with estimates from MRI scans and validated at necropsy.

Main Outcome and Measurements—Cross-sectional area of colon adenomas.

Results—The cross-sectional area of 20 adenomas was measured in vivo during colonoscopy and compared to the size as measured at necropsy, yielding a Pearson's correlation coefficient of 0.94 (p-value = 6.52×10^{-9}). Assessment of interoperator variability, using measurements from 11 adenomas, yielded a Pearson's correlation coefficient of 0.85 (p-value = 4.35×10^{-3}) and demonstrated excellent reproducibility.

Limitations—Can view only the distal colon; and endoscopic measurements are 2-dimensional.

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Conclusions—An endoscopic method for the reliable measurement of colorectal adenomas in vivo has been established. Application of this technique to mouse models of colon carcinogenesis will provide unique insight into the dynamics of adenoma growth.

The advent of both powerful small animal imaging techniques and a diverse array of genetically defined mouse models of human cancer have provided a unique opportunity to establish novel preclinical protocols for monitoring tumor growth in vivo. Numerous reports now document the use of magnetic resonance imaging (MRI) to image spontaneous tumors of the colon, brain, prostate, stomach and breast.^{1–5} Although a number of additional image-based techniques have been established for the detection of colorectal adenomas and cancers in humans, including virtual colonoscopy, double-contrast barium enemas, computed tomography (CT) and positron emission tomography (PET), their routine use in rodents has been hindered by the need for the miniaturization of complex equipment, lack of portability and cost. Colonoscopy remains the most widely used tool for screening patients at increased risk for colorectal cancer,⁶ providing high sensitivity and specificity for lesion detection and surveillance. In addition, the procedure is well tolerated, low risk, relatively inexpensive and, unlike PET and CT, does not require highly specialized, automated instrumentation.

For more than a decade, the $Apc^{+/Min}$ mouse model has been used extensively to evaluate the efficacy of chemopreventive agents against colon cancer.⁷ However, only a limited number of groups have demonstrated their success in obtaining images of the mouse colon using microendoscopes^{2,4,8} and microcomputed tomography colonography.⁹ Following early efforts by Huang et al,⁸ Becker and colleagues² published a detailed protocol for capturing high-resolution images of the mouse colon using a rigid endoscope. In parallel with this development, Funovics and colleagues^{3,4} have developed a multichannel fluorescence endoscope for the detection of exogenous fluorophores in lesions of the mouse colon. Use of the conventional $Apc^{+/Min}$ mouse for the majority of these studies has been problematic due to the predominance of small intestinal adenomas and few if any colorectal adenomas in these animals.¹⁰ This deficiency has precluded the establishment of a reliable method for quantifying lesion size in vivo.

A unique strain of mice ($Apc^{+/Min-FCCC}$) has been established by this group, which spontaneously develops multiple colorectal adenomas;¹¹ thus representing an ideal system in which to establish endoscopic protocols for the detection of colorectal lesions. Use of the $Apc^{+/Min-FCCC}$ mouse strain facilitated the first detection of colon adenomas by MRI.¹² MRI represents a noninvasive method for adenoma detection, the acquisition of high-resolution images of anatomical structures, and a means for accurate serial measurement of solid tumor volumes in animal models.^{12–14} Although attempts have been made to grade colon adenomas based on their size relative to the circumference of the colon,² accurate measurement of the area/volume of colon lesions in vivo has not been reported to date.

The goal of the present study was to develop an endoscopy-based protocol for the accurate estimation of adenoma size in vivo from images obtained during colonoscopic examinations. While the methods are similar in principle to those currently in use for human colonoscopy, such as open biopsy forceps or linear probe methods,^{15–18} the highly restricted geometry of the mouse colon and the large size of the adenomas relative to the diameter of even the fully insufflated lumen of the colon make it necessary to employ a modified technique involving a geometrical construction of the measuring probe. A strong correlation of the resulting size estimates with those obtained by MRI and at necropsy supports the future use of endoscopy to monitor adenoma growth and response to therapeutic intervention in animal models over time.

MATERIALS AND METHODS

Animals

Male and female $Apc^{+/Min-FCCC}$ mice (60–100 days of age, $n = 32$) were obtained from a breeding colony at Fox Chase Cancer Center (FCCC), Philadelphia, PA, and maintained with free access to autoclavable PMI rodent breeder chow 5013 (PMI Nutrition International, Richmond, IN) and water. All animals were subjected to colonoscopy for lesion identification and size estimation. If an adenoma greater than 1.5 mm in diameter was detected during colonoscopy, the mouse also received an MRI scan on the following day. All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at FCCC, and mice were euthanized by CO₂ asphyxiation per IACUC guidelines.

Colonoscopy

Bowel preparation—In order to clear the bowel of fecal material, solid food was removed from each cage 24 hours prior to colonoscopy, and drinking water was replaced with a solution of 20% glucose (w/v) in water. At the time of colonoscopy, mice were anesthetized with 2% isoflurane in oxygen, and 0.3 ml of commercial Fleet's enema solution was introduced into the lumen of the colon via a 2 French polyurethane catheter. Mice were allowed to regain consciousness and void their bowels before being anesthetized for the colonoscopy procedure.

Examination—Colonoscopies were performed using a rigid bore endoscope with a 0-degree viewing angle and 1.5 mm outer diameter (Gradient Lens Corp., Rochester, NY). The scope remained stationary while the mouse was manipulated with a positioning stage that allowed precise positioning of the mouse relative to the endoscope tip in all directions and orientations (Fig. 1). The positioning stage consisted of a calibrated XY stage (Sherline Products Inc., Vista, CA), a vertical translation platform with 360-degree rotation (Thorlabs Inc., Newton, NJ), and a dual axis goniometer (OptoSigma, Inc., Santa Ana, CA). The endoscope was coupled to a QImaging Micropublisher 3.3 digital camera (QImaging Corp., Surrey, BC, Canada), chosen for the small pixel size of the sensor (3.3 μ m) and thermoelectric cooling of the charge coupled device (CCD). In vivo illumination was provided by a 350 watt xenon lamp (Medit Inc., Manitoba, Canada). Animals were kept under anesthesia (2% isoflurane in oxygen) and a heat lamp for the duration of the exam (10–15 minutes). The colon was insufflated with phosphate buffered saline (PBS) through the endoscopic sheath, and the degree of the abdominal distention was monitored to prevent distress. The endoscope was inserted 3 cm into the mouse anally and gradually withdrawn over time. The location of each identified adenoma was recorded based on the extent to which the endoscope was inserted using gradations on the instrument.

Size estimation and geometric construction—A method for the quantitative and reproducible estimation of lesion size has been developed, which involves placing an object of known size in close proximity with the adenoma and using it as a reference for estimating adenoma size in the resulting images. Specifically, a flexible metal rod (the biopsy forceps supplied by the sheath manufacturer) (1 mm diameter) is placed in the biopsy channel of the endoscope sheath and inserted until it is visible in the field of view of the endoscope, adjacent to the lesion (Fig. 2). The positioning stage facilitates placement of the mouse colon and reference object at a precise orientation relative to the endoscope tip. Using software written in-house in the IDL programming language (ITT Visual Information Solutions, Boulder, CO), a geometric construction, based on estimating the position of the cylindrical rod in the image relative to the adenoma, permits quantitative estimation of adenoma size. Since the endoscope views the adenoma from a single direction, it is only possible to estimate the height, width, and cross-sectional area (not volume) of the adenoma.

In order to estimate lesion size quantitatively, a measurement grid must be constructed on the image. The operator first chooses a set of points in the 2-dimensional image that defines the reference rod in a 3-dimensional space. (The rod is modeled as a cylindrical object in the field of view.) The edges of the cylinder are then marked manually by selecting two pixels on either side of the rod. The lines formed by these points are extended to a vanishing point (VP) (Fig. 2), and a line that runs precisely in the center between the two edge lines is calculated by software and displayed on the image. Given that the rod is held by the sheath at approximately the same angle for each image, concentric circles on the rod are represented by ellipses of eccentricity 2 in the image. The operator next estimates the point on the center line that is in close proximity with a groove on the reference rod (point "A"), and then marks another point (point "B") on the edge of the rod along the same groove. The point on the center line closest to point A is calculated by software. The ellipse representing a circle on the reference rod is defined uniquely by points A and B, its eccentricity, and the fact that the minor axis of the ellipse is parallel to the center of the rod.

Lesion size can be calculated from the image by choosing a point on the rod (ellipse) that is the same distance from the scope lens as the adenoma. An ellipse of eccentricity 2, representing a circle on the axis of the rod, was calculated by software and drawn on the image. The minor axis of the ellipse is fixed parallel to the center line of the rod. The points of intersection of the ellipse with the edges of the reference rod are calculated as the ratio of the distance from reference point "A" to the image vanishing point (A_{VP}) with that of adenoma point "T" (T_{VP}), and the distance of point "B" to the vanishing point (B_{VP}). Point "C" is, therefore, a point along the rod's edge, whose distance to the vanishing point is given as $C_{VP} = B_{VP} * (T_{VP}/A_{VP})$. The distance across the rod (perpendicular to the center line at point C) gives the 1 mm reference distance for calibrating the size of objects in contact with the rod at that point. The longest dimension of the adenoma in the image is taken as the width (W), and the size of the adenoma in the perpendicular direction is taken as the height (H). The cross-sectional area is measured by outlining the edges of the adenoma manually, or by assuming that the adenoma cross section is elliptical in shape and computed as $area = (W \times H) \times \pi/4$.

MRI

The procedure for acquiring MRI data sets from $Apc^{+/Min-FCCC}$ mice has been described in detail previously.¹² Briefly, mice were anesthetized (2% isofluorane in oxygen) and a sealed 2 French polyurethane tube (Access Technologies, Skokie, IL) containing 4 mM Gd-DTPA was inserted into the colon of the mouse to serve as an identifiable marker on the resulting MRI data sets. Following an intramuscular injection (200 μ l) of Gd-DTPA (Magnevist, Berlex Laboratories, Hamilton, NJ) diluted 1:10 in 1X PBS, each mouse was placed in a vertical wide-bore magnet with a field strength of 7 Tesla and a Bruker DRX300 console and microimaging accessory.

Scout images were obtained in coronal and axial orientations and used to create a high-resolution isotropic data set with a 3-dimensional gradient echo pulse sequence ($256 \times 256 \times 64$ matrix) and a voxel length of 0.133 mm, $T_E = 3.3$ msec, $T_R = 0.2$ sec. Once preliminary scans were completed to optimize positioning, isofluorane was reduced to 0.5% for the duration of the scan (30 minutes) and respiration was monitored using a respiratory sensor. Lesion volume was measured with a 3-dimensional planimetry technique, using software written in-house in the IDL programming language (ITT Visual Information Solutions). This method has been described in detail in a previous publication by this group.¹² Briefly, the sections from the 3-dimensional data set were displayed in one of three orthogonal dimensions (axial, coronal, or sagittal), and the adenoma was outlined manually using a combination of views. Cross-sectional area of the adenoma was estimated from MRI data sets by displaying a slice in the axial orientation (approximating the view of the distal colon one would get through the

endoscope) through the lesion, and manually measuring the height and width of the adenoma in pixels. Measurements were then converted to centimeters from the known field of view and dimensions of the data set. Area of the adenoma was computed using the formula $\text{area} = (W \times H) \times \pi/4$.

Histopathologic evaluation

Following completion of the imaging procedures, mice were euthanized by CO₂ asphyxiation. The colons were excised, opened lengthwise and rinsed with PBS. Gross lesions that had been imaged by either MRI or endoscopy were identified based on their distance from the anus. The height (H) and width (W) of each lesion were measured using calipers, and the adenoma cross-sectional area was computed again from $A = (W \times H) \times \pi/4$. The adenomas were excised subsequently and fixed in 10% neutral buffered formalin for 24 hours. Paraffin-embedded sections were stained with hematoxylin and eosin and subjected to histopathologic review. An adenoma was defined as a circumscribed neoplasm composed of tubular and/or villous structures and lined with dysplastic epithelium. All colorectal adenomas included in the present study were confirmed histopathologically.

Statistical analyses

The Pearson's correlation coefficient and the paired t-test were used for all statistical analyses. These included a comparison of adenoma measurements obtained from endoscopy or MRI with caliper measurements of gross lesions obtained at the time of sacrifice, a comparison of endoscopy and MRI measurements as well as an assessment of interoperator variability.

For each comparison, the extent of agreement between paired observations was quantified by computing the Pearson's correlation coefficient (ρ), the associated p-value and the 95% confidence interval for testing the null hypothesis that $\rho = 0$. The Bonferroni correction was applied to account for the testing of multiple hypotheses and statistical significance was determined using a 5% cut-off. Additionally, the null hypothesis that the mean difference between any 2 groups being compared is zero versus the 2-sided alternative was tested at the 5% significance level. Rejecting the null hypothesis would, therefore, indicate agreement between the 2 groups. The corresponding 95% confidence interval for the mean difference was also computed.

RESULTS

Animals

Thirty-two Apc^{+/Min-FCCC} mice were examined by colonoscopy. Adenomas were detected within the distal region of the colon that was accessible to the endoscope (3 cm from the anus) in 66% (21/32) of the animals. High-quality colonoscopic images of the adenomas were acquired from 95% (20/21) of the tumor-bearing animals. Thirty-eight percent (8/21) of the tumor-bearing mice had colorectal adenomas greater than 1.5 mm in diameter and received MRI scans on the day following colonoscopy.

Colonoscopy

A total of 175 colonoscopic examinations were performed on 32 mice during the course of this study. Colonoscopies were approximately 15 minutes in duration and were well tolerated by Apc^{+/Min-FCCC} mice. Only 2.9% of the colonoscopies (5/175) resulted in the death of the mouse within the first 24 hours following the procedure (3 died during the procedure and 2 died the next day). It should be noted that the health of Apc^{+/Min-FCCC} mice is compromised by severe anemia. The most common procedural complication was excessive colon insufflation leading to respiratory distress. Although one can obtain images of excellent quality using air or CO₂

insufflation,^{2,14} use of a minimal amount of PBS for insufflation was optimal for reducing animal mortality. In addition, due to surface tension at the gas-fluid interface, it was difficult to image the adenomas and measuring probe following gas insufflation. Although the suspended particles in the fluid in the colon tended to scatter and absorb light, the resulting images were of sufficiently high quality to perform the measurements of interest.

Colonoscopic images of representative colon adenomas are presented in Fig. 3. Adenomas as small as 0.5 mm in height and 1.0 mm in diameter were readily detected by colonoscopy. Tumor-bearing animals possessed 1–5 adenomas per mouse, with the majority located 1–2.5 cm from the anus. Adenomas within 0.5 cm of the anus were difficult to image. Animals with either multiple colorectal adenomas in close proximity or one adenoma ≥ 5 mm in diameter could not be screened, since advancement of the scope past these large adenomas was not possible.

MRI

In Fig. 4, a colonoscopic image of an Apc^{+/Min-FCCC} mouse colon adenoma is compared with both an MRI scan and a histological section of the same lesion stained with hematoxylin and eosin. The MRI scan has been cropped from an axial section through an isotropic 3-dimensional data set to present a view that approximates the orientation of a colonoscopic exam. Our version of MRI software did not support the 3-dimensional reconstruction of the surface of the adenoma (Bruker Paravision 3.0.2). The lesion was readily identified as a colorectal adenoma because it was located directly adjacent to the Gd-DTPA-filled reference tube (bright spot on image) that was inserted into the lumen of the colon and surrounded by the intestinal wall. The vascularized stalk of the polypoid adenoma is clearly visible in the histological section.

Validation of adenoma size estimates obtained by colonoscopy

Lesion size estimates from colonoscopic exams (20 adenomas) were validated by comparing in vivo measurements with those made at necropsy. Size estimates (adenoma cross-sectional area as measured during endoscopy or volume as determined by MRI) were compared with the same calculations based on caliper measurements at necropsy. In a subset of animals (10 adenomas), adenoma size was measured by colonoscopy, MRI and at necropsy. Adenomas ranged in cross-sectional area from 0.9–6.8 mm². The measurements at necropsy were made by observers blinded to the results from colonoscopy, and were performed immediately following animal sacrifice, and prior to formalin fixation. A strong correlation was observed between the cross-sectional area of a lesion estimated from colonoscopic images and that based on caliper measurements at necropsy (Pearson's correlation coefficient $\rho = 0.94$; Bonferroni corrected p-value = 6.52×10^{-9} , 95% CI: (0.84, 0.97)) (Fig. 5). Comparison of lesion volume as calculated from MRI scans with the estimated volume of the gross lesion at necropsy yielded a correlation of 0.92 (corrected p-value = 1.29×10^{-4} , 95% CI: (0.73, 0.98)). Unlike MRI, where lesions can be measured in 3 dimensions and volume calculated, colonoscopy supports the acquisition of lesion measurements in only 2 dimensions. For this reason, a direct comparison of lesion size as determined by the 2 technologies was made by computing a cross-sectional area from axial sections of the MRI scans and correlating it with similar measurements obtained at the time of colonoscopy. Comparison of the cross-sectional area of a colon adenoma as computed from MRI scans and colonoscopic images yielded a correlation coefficient of $\rho = 0.81$ (corrected p-value = 3.73×10^{-2} , 95% CI: (0.33, 0.96)); a value similar to that obtained when comparing area from the MRI scan vs. necropsy ($\rho = 0.87$; corrected p-value = 1.02×10^{-2} , 95% CI: (0.50, 0.97)).

To address interoperator variability, the images of 11 adenomas were measured and lesion size calculated by 2 independent operators who were blinded to each other's results. Comparison of the resulting data sets from each operator yielded a Pearson's correlation coefficient of 0.85

(corrected p -value = 4.35×10^{-3} , 95% CI: (0.52,0.96)), indicating that the measurement technique is reproducible. For each comparison outlined above, the Bonferroni corrected p -value satisfied the 5% cut-off for determining statistical significance. Also, in each case, the null hypothesis that the mean difference between the pairs is zero was rejected using the paired t -test. This is evidenced by the corresponding 95% confidence intervals shown in Table 1.

DISCUSSION

A novel method has been established for the reliable imaging and size estimation of murine colorectal adenomas *in vivo*. This protocol is highly advantageous in that: 1) adenomas can be observed *in vivo*, 2) adenoma growth profiles can be potentially established from longitudinal images from the same animal, rather than as discrete measures collected from unique animals over time and 3) the response of individual adenomas to therapeutic intervention can be potentially monitored serially using adenoma size as a surrogate. Quantitative assessment of murine adenoma size *in vivo* is anticipated to yield invaluable information that has not been available previously.

The strong correlation observed between quantitative estimates of lesion size obtained from colonoscopy or MRI vs. necropsy indicates that the measurements obtained by endoscopy are reliable and accurate. The correlation between colonoscopy and necropsy measurements (0.90) is comparable to that obtained by Hofstad and colleagues¹⁷ when computerized analyses were employed to compare polyp diameter in photos with that determined *ex vivo*. As in a clinical setting, the imaging modalities utilized in the present study yield complementary data. MRI supports the acquisition of high-resolution images of the mouse colon. Even at the highest resolution practical for these studies (isotropic voxel size of approximately 100 μ), reliable detection of adenomas smaller than 1.5 mm in diameter is difficult. This is due to the complex geometry of the colon and our limited ability to distinguish small, flat lesions from the colon wall. In contrast, adenomas greater than or equal to 2 mm in diameter can be detected readily and measured precisely. However, there was a tendency to underestimate the size of larger lesions during colonoscopic exams (Fig. 5). This finding is consistent with that of others,^{18–20} who observed a consistent underestimation of the diameter of human polyps by endoscopy. In the present study, the most precise measurements of adenoma size were obtained for lesions with a cross-sectional area of 1–5 mm².

Endoscopic examinations of the colon are preferred frequently over MRI scans due to their high throughput and portability. These procedures permit the detection of smaller adenomas; and by implementing the technical developments reported here, individual adenoma growth patterns can be determined from quantitative estimates of lesion size. However, unlike MRI, where volumetric measurements of lesion size can be extrapolated directly from scans, measurements obtained from endoscopic images are restricted to 2 dimensions.

The image analysis of adenomas that arise spontaneously in mice presents significant challenges. First, continuous surveillance is required since adenomas can arise at any time during the life span of the animal. Second, both the time to adenoma development and the growth characteristics of adenomas can be very heterogeneous within a single mouse strain. Third, in strains where multiple adenomas form within a given target organ, it is essential to accurately identify the same lesion during repetitive exams and monitor the growth of each over time on an individual basis. Fourth, application of this technology to the area of cancer prevention is extremely challenging since the primary focus is on detecting lesions as early as possible, thus very small adenomas. Determination of the length of time that a specific therapeutic intervention delays the onset of adenoma formation is also of critical importance. Lastly, now that adenomas can be measured reliably from endoscopic images, algorithms must be developed to model adenoma growth over time. The latter is anticipated to significantly

enhance our understanding of the response of adenomas to therapeutic intervention. It should be noted that the number and size of colon adenomas, as determined from endoscopic images, were the primary endpoints of the landmark trial that led to the FDA approval of the cyclooxygenase 2 inhibitor celecoxib as a chemopreventive agent for patients with familial adenomatous polyposis.²¹

It is imperative that the present methods differ from those used commonly in humans (linear probe or open forceps) because of the restricted geometry of the mouse colon. Our method does have some methodological issues similar to those of human exams.¹⁶ The probe will not always be aligned in the same orientation, parts of the adenoma may be obscured by mucosal folds, and the adenoma may be measured at different distances from the endoscope tip. As presented, measurements of adenoma size from different observers are consistent and reproducible. The relatively restricted nature of the probe and colon geometry in the mouse gives the operator comparatively little flexibility with respect to the alignment of the endoscope tip, measuring probe, and the adenoma.

In summary, data resulting from the established endoscopic protocol for the identification and measurement of colorectal adenomas in mice are anticipated to provide novel insight into the dynamics of adenoma growth in vivo. This technology when coupled with recent advances in gene expression profiling provides a powerful approach for the comprehensive assessment of the molecular basis of associated alterations in gross morphology and/or growth kinetics.

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Figure 1. Rigid bore endoscope (Gradient Lens Corp.), camera, and mouse positioning stage. The anesthetized mouse is restrained in a supine position and the endoscope is inserted anally. The camera and endoscope are held stationary while all positional adjustments can be made in 6 directions using controls on the positioning stage.

Figure 2. Geometric construction used to estimate adenoma size. Measurements were obtained using a flexible metal rod of known diameter (1 mm) as a reference. Point “T” is the point on the forceps that is the same distance from the endoscope tip as the adenoma. Points “A,” “B,” and “C” define the geometry of the forceps in the image, upon which the scale of the image is based. The point labeled “VP” represents the extension of the edges of the reference rod to a vanishing point. The height “H” and width “W” of the adenoma are marked with white lines.

Figure 3. Range of size of detectable adenomas. A) A relatively small adenoma, with a height of 0.5 mm and a width of 1.25 mm. B) A larger adenoma, with a height and width of 5 mm.

B

Figure 4. Comparison of the morphology of a representative colon adenoma in an Apc⁺/Min-FCCC mouse using different diagnostic techniques. The arrows represent the scale bar for each respective panel, with all equivalent in length to the diameter of the adenoma (3.2 mm). A) An image obtained from a colonoscopic examination. B) A hematoxylin and eosin-stained histological section. C) A slice in the axial plane from an MRI data set. The adenoma is indicated by the yellow arrow.

Figure 5.

Scatterplot of colonoscopy estimates of adenoma size with measurements obtained at necropsy using calipers. Values represent cross-sectional area. (Colonoscopy is limited to estimating size in 2 dimensions.)

TABLE 1

Confidence Intervals (95%) for statistical comparisons

Comparison	95% Confidence Interval*
Endoscopy vs. necropsy (area)	(−0.30, 0.60)
MRI vs. necropsy (volume)	(−1.02, 2.28)
MRI vs. endoscopy (area)	(−0.47, 0.93)
MRI vs. necropsy (area)	(−0.42, 0.67)
Operator 1 vs. Operator 2 (area)	(−0.45, 0.51)

*
Based on the mean difference using the paired t statistic