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PRINCIPAL INVESTIGATOR: Ethan Joseph Halpern, M.D.

CONTRACTING ORGANIZATION: Thomas Jefferson University
Philadelphia, Pennsylvania 19107

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Intermittent Ultrasound Imaging of Prostate Cancer

Ethan Joseph Halpern, M.D.

Thomas Jefferson University
Philadelphia, Pennsylvania 19107
E-mail: ethan.halpern@jefferson.edu

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

This study proposed to evaluate contrast-enhanced ultrasound imaging with a microbubble contrast agent in 300 subjects in order to improve the detection of prostate cancer. Between October 2001 and January 2004 a total of 301 subjects were enrolled. Laboratory blood tests (PSA) and ultrasound evaluations were completed on all 301 subjects (including the primary ultrasound interpretation worksheet). Independent blinded readers have reviewed the first 241 subjects. Pathological review for the presence and grade of cancer has been completed for all 301 subjects. CD31 staining for micro-vessel density has been performed on the first 40 subjects. All available data has been entered into a computer database using an Excel spreadsheet. A statistical analysis of the prostate cancer detection data from all 301 subjects was incorporated into two abstracts that have been accepted for presentation at the 2004 annual meeting of the Radiological Society of North America (see appendix). A preliminary microvessel density analysis was presented at the annual meeting of the American Institute of Ultrasound in Medicine (see appendix). A one year no cost extension has been requested to allow completion of the micro-vessel density analysis as well as the blinded review of the final 60 subjects.
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INTRODUCTION:

Numerous studies have demonstrated an increased number of very small blood vessels in prostate cancers, as well as an association between the number of these vessels and aggressiveness of disease. The present study is designed to visualize these vessels with ultrasound during intravenous infusion of a microbubble contrast agent. The objective of this study is to utilize contrast-enhanced ultrasound imaging to improve the detection of prostate cancer, in order to identify those cancers which are clinically significant.

The study protocol includes enrollment of three hundred subjects with suspected cancer of the prostate. Each subject is imaged with conventional and intermittent ultrasound both before and after administration of the contrast agent. Based upon a comparison of ultrasound findings with biopsy results, this study is designed to demonstrate that contrast-enhanced intermittent ultrasound imaging of the prostate results in improved detection of prostate cancer. Furthermore, ultrasound findings with the contrast agent are to be correlated with microvessel density, Gleason score and PSA in order to determine whether intermittent imaging can selectively identify clinically significant cancers.
BODY:

Statement of Work tasks:

#1 - Ultrasound contrast studies:

Patient recruitment was completed in January 2004. A total of 301 subjects provided written informed consent, were evaluated with the required laboratory studies (PSA) and participated in the ultrasound contrast protocol. The examining physician (Dr. Ethan Halpern) has completed an ultrasound image interpretation worksheet for each of these subjects. This portion of the study was completed as scheduled.

#2 – Pathologic evaluation:

Prostate biopsy specimens were obtained from all 301 subjects and were evaluated by standard pathologic evaluation. A pathology interpretation worksheet has been completed by our pathology consultant (Dr. Peter McCue).

For the evaluation of microvessel density, CD31 staining has been performed on tissue sections from 40 subjects, but stained specimens have been evaluated for only 13 subjects. Due to a variety of technical difficulties beyond our control, the analysis process for microvessel density has taken longer than expected. Microvessel density assessment is performed on a complex computer-based histomorphology system. A computer failure last year resulted in loss of the vessel counting software and made the entire system inaccessible for almost five months. An alternate source of funding was identified to repair the computer system and purchase new vessel counting software. During this time, the graduate student’s effort was used to repair the system and reinstall the programs, as well as to work on image files that were captured prior to the computer failure. The computer system is now repaired. However, the graduate student who was initially working on the project resigned and it took several months to find a replacement. Although we have now restarted the process of evaluating microvessel density, the automated counting process requires more manual input and time than was initially expected. Consequently, the process has been more tedious than initially anticipated.

Based on our current rate of microvessel density counting, it will not be possible for us to perform microvessel density counts for all 104 subjects with cancer. In order to achieve our stated goal of comparing microvessel density in benign versus malignant tissue, we will continue to concentrate our efforts to count microvessel density in those patients with a pathological diagnosis of cancer. Since each subject with cancer will contribute slides with both benign and malignant tissue, we will be able to use this data to compare microvessel density in benign and malignant tissue. A one-year no-cost extension of the current grant that has been requested. Microvessel density analysis will continue during this extension period.

#3 – Database entry:

A database has been established. All ultrasound, laboratory and pathology data available to date have been entered into the database by the research coordinator.
#4 – Interim statistical evaluation:

The interim evaluation was reported in the previous annual report. Several abstracts reporting the interim analysis were presented at the annual meeting of the Radiological Society of North America in 2003. These abstracts were included in the previous annual report. References to these abstracts are provided in the reference section below. Statistical analysis of the currently available data is presented in #6 below and in the abstracts included in the appendix.

#5 – Blinded reader & consensus interpretations:

In addition to the observations recorded by the primary reader, independent observations by a blinded observer have been completed on the first 241 subjects.

The initial blinded reader for this study (Dr. Strup) performed blinded readings on the first 100 subjects, but resigned his position at Thomas Jefferson University over one year ago. Several months elapsed before a replacement blinded reader (Dr. Ramey) was approved by the U.S. Army Medical Research and Materiel Command. Although Dr. Strup is an experienced urologist and has performed many biopsies, he did not have prior experience with contrast-enhanced ultrasound. Dr. Ramey is a more junior urologist, but did undergo substantial training with contrast-enhanced ultrasound. For training purposes Drs. Halpern and Ramey jointly reviewed many of the initial 100 cases read by Dr. Strup.

The second blinded reader has reviewed videotapes from an additional 141 subjects. As mentioned above, a no cost extension of the current grant has been requested. This extension will provide time for the second blinded reader to complete the final 60 subjects.

Interobserver agreement in the assessment of ultrasound findings between the primary reader (Dr. Halpern) and the blinded readers (Dr. Ramey & Dr. Strup) have been computed with a quadratic weighted kappa. The value of kappa ranges from 0 (no agreement) to 1 (perfect agreement). The kappa values are reported in the table below:

<table>
<thead>
<tr>
<th>Ultrasound Imaging Technique</th>
<th>Kappa: Halpern-Strup</th>
<th>Kappa: Halpern-Ramey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-contrast gray scale (baseline)</td>
<td>0.19</td>
<td>0.33</td>
</tr>
<tr>
<td>Pre-contrast color Doppler (baseline)</td>
<td>0.28</td>
<td>0.48</td>
</tr>
<tr>
<td>Pre-contrast power Doppler (baseline)</td>
<td>0.28</td>
<td>0.50</td>
</tr>
<tr>
<td>Post-contrast gray scale harmonic imaging</td>
<td>0.17</td>
<td>0.51</td>
</tr>
<tr>
<td>Intermittent gray scale harmonic imaging (0.2s)</td>
<td>0.18</td>
<td>0.46</td>
</tr>
<tr>
<td>Intermittent gray scale harmonic imaging (0.5s)</td>
<td>0.19</td>
<td>0.47</td>
</tr>
<tr>
<td>Intermittent gray scale harmonic imaging (1.0s)</td>
<td>0.16</td>
<td>0.46</td>
</tr>
<tr>
<td>Intermittent gray scale harmonic imaging (2.0s)</td>
<td>0.07</td>
<td>0.33</td>
</tr>
<tr>
<td>Contrast-enhanced color Doppler</td>
<td>0.12</td>
<td>0.50</td>
</tr>
<tr>
<td>Contrast enhanced power Doppler</td>
<td>0.09</td>
<td>0.56</td>
</tr>
</tbody>
</table>
It is clear from the kappa values in this table that interobserver agreement between Drs. Halpern and Ramey is much better than the agreement between Drs. Halpern and Strup. The most likely explanation for this difference is related to the additional training that Dr. Ramey received in the interpretation of contrast-enhanced imaging. Nonetheless, the agreement between Drs. Halpern and Ramey is not perfect. Re-review of discrepant cases suggests that differences between the readers are related, in part, to subjective differences in evaluation of the level of enhancement. However, another important source of interobserver discrepancy is difference in interpretation as to location within the prostate. When reviewing the study on videotape, the blinded reviewer is often less certain of the image location than the primary examining physician who has a hand on the ultrasound probe. Since the primary reader (the examining physician) was also responsible for performance of the prostate biopsy procedure, the readings provided by the primary reader correspond more closely to the ultrasound findings at each biopsy site. Based upon these considerations, we have decided to use the ultrasound interpretation provided by the primary reader for statistical analysis with the pathological findings.

#6 – Analysis & Publications:

For the reasons described above, statistical analysis of the ultrasound findings has been completed based upon the interpretation of the primary reader. Slightly over one-third of the subjects were found to have cancer on pathological evaluation. Malignant tissue was detected in 363 biopsy cores from 104 of 301 subjects (35%), including 15.5% (175/1133) of targeted cores and 10.4% (188/1806) of sextant cores (p < 0.01). Among subjects with cancer, targeted cores directed to the locations of maximum enhancement within the prostate were twice as likely to return a positive biopsy (OR = 2.0, p < 0.001). Targeted biopsy based upon contrast enhancement detected an additional 11 patients with cancer that were not detected with the conventional sextant biopsy protocol. Among 21 patients with cancer that was not found by targeted biopsy, 17 of these subjects had malignant cores on sextant biopsy of the gland apex.

In order to evaluate the ability of contrast-enhanced ultrasound to discriminate between benign and malignant tissue, a clustered ROC analysis of ultrasound findings was compared to pathological diagnosis on the sextant biopsy specimens. Cancer was detected in 188 sextant cores from 93 of 301 subjects (31%). Clustered ROC analysis demonstrated the following values for area under the curve:

<table>
<thead>
<tr>
<th>Ultrasound Imaging Technique</th>
<th>Area under the ROC curve (Az)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-contrast gray scale (baseline)</td>
<td>0.58</td>
</tr>
<tr>
<td>Pre-contrast color Doppler (baseline)</td>
<td>0.53</td>
</tr>
<tr>
<td>Pre-contrast power Doppler (baseline)</td>
<td>0.58</td>
</tr>
<tr>
<td>Post-contrast gray scale harmonic imaging</td>
<td>0.62</td>
</tr>
<tr>
<td>Intermittent gray scale harmonic imaging (0.2s)</td>
<td>0.64</td>
</tr>
<tr>
<td>Intermittent gray scale harmonic imaging (0.5s)</td>
<td>0.63</td>
</tr>
<tr>
<td>Intermittent gray scale harmonic imaging (1.0s)</td>
<td>0.65</td>
</tr>
<tr>
<td>Intermittent gray scale harmonic imaging (2.0s)</td>
<td>0.61</td>
</tr>
<tr>
<td>Contrast-enhanced color Doppler</td>
<td>0.60</td>
</tr>
<tr>
<td>Contrast enhanced power Doppler</td>
<td>0.62</td>
</tr>
</tbody>
</table>
With respect to the characterization of tissue as benign versus malignant, no significant differences were found among the three methods of baseline imaging without contrast material. A statistically significant benefit was found for all methods of post-contrast intermittent harmonic imaging over baseline gray scale and Doppler imaging ($p < 0.05$). No significant difference in ROC area was observed with contrast-enhanced imaging at different interscan delay times. No single intermittent delay time was superior for characterization of malignant sites. Furthermore, there was no significant difference in the detection of malignancy between continuous and intermittent contrast-enhanced imaging. Although there was a statistically significant improvement in the characterization of tissue as benign versus malignant with contrast-enhanced imaging, the relatively low ROC areas ($< 0.65$) suggest that contrast enhanced sonography cannot definitively differentiate benign from malignant tissue without biopsy confirmation.

Microvessel Density Analysis:

Initial results of our microvessel density assessment were presented at the 48th annual meeting of the American Institute of Ultrasound in Medicine (June 2004 – Phoenix, AZ – see abstract in appendix). These results included 63 biopsy specimens from 7 subjects processed with CD31 stain. Eight of the specimens (15%) contained malignant tissue. A strong correlation was found between the presence of cancer and microvessel density (area under the ROC curve = 0.82). In this small data set, microvessel density correlated significantly with contrast-enhancement during harmonic gray scale imaging ($r=0.33$, $p<0.025$), but not with contrast-enhanced color and power Doppler imaging.

At the present time, microvessel density analysis has been completed for 13 subjects, including 6 with prostate cancer. A total of 121 biopsy cores were stained in these 13 subjects, but only 94 had sufficient tissue for microvessel density determination. Malignant tissue was present in 16/94 cores (17%). The mean microvessel density in the 16 malignant cores was 67.6 vessels/mm with a standard deviation of 78. The mean microvessel density in the 78 benign cores was 32.4 vessels/mm with a standard deviation of 26.5. The difference in microvessel density between malignant and benign cores was statistically significant ($p = 0.0017$), though there was substantial overlap between the microvessel density of malignant and benign cores. A statistically significant correlation was present between microvessel density and the level of enhancement observed with contrast-enhanced gray scale harmonic imaging ($r = 0.25$, $p = 0.015$).

Publications (see complete references under reportable outcomes):

Two abstracts describing the pathological results and ultrasound correlation on all 301 subjects have been accepted for presentation at the 2004 annual meeting of the Radiological Society of North America. (see appendix). An additional abstract describing the microvessel density findings has been presented at the 48th annual meeting of the American Institute of Ultrasound in Medicine (see appendix). As mentioned above, a one-year no-cost extension has been requested. A full manuscript describing the final results of this study should be completed during the extension period.
KEY RESEARCH ACCOMPLISHMENTS:

- Successful infusion of ultrasound contrast in 301 subjects with ultrasound guided biopsy.
- Targeted cores, based upon ultrasound findings with contrast-enhanced imaging, detected the presence of prostate cancer twice as frequently as non-targeted cores (OR = 2.0, p < 0.001).
- Contrast-enhanced intermittent harmonic imaging provided a statistically significant (p < 0.05) improvement in discrimination between benign and malignant areas of the prostate outer gland.
- Targeted biopsy based upon contrast enhancement detected an additional 11 patients with cancer that would not have been detected with the conventional sextant biopsy protocol.
- Microvessel density was found to be greater in malignant than in normal prostate tissues (p = 0.0017);
- Microvessel density correlated with the enhancement seen on contrast-enhanced ultrasound imaging.

REPORTABLE OUTCOMES: Three new abstracts accepted for presentation (see appendix).


CONCLUSIONS:

Intravenous infusion of a microbubble contrast agent provides sonographically visible enhancement of the prostate. This enhancement can be used to guide biopsy of the prostate into areas of increased vascular flow. Among subjects with cancer, targeted cores were twice as likely to return a positive biopsy (OR = 2.0, p < 0.001). With respect to the characterization of tissue as benign versus malignant, a statistically significant benefit was found for all methods of post-contrast intermittent harmonic imaging over baseline gray scale and Doppler imaging (p < 0.05). Targeted biopsy of the prostate based upon contrast-enhanced imaging will identify cancers that are not detected by conventional sextant biopsy. However, targeted biopsy will also miss cancers that might be detected by a systematic sextant biopsy. As noted in our prior report, most cancers that were not identified with the targeted contrast-enhanced technique were located at the apex of the gland. In order to maximize cancer detection, we therefore recommend a contrast-enhanced targeted biopsy strategy with additional systematic cores distributed to the apex of the prostate.

REFERENCES:


Previously reported abstracts:


APPENDICES:

Accepted for presentation in Dec ‘04 to the Radiological Society of North America

Abstract ID: 4403704
Submission Type: Scientific Papers

Ethan Halpern
Thomas Jefferson University Hospital

Phone: 215-955-5345
Fax: 215-955-8549,215-955-8549
E-Mail: Ethan.Halpern@jefferson.edu

PROSTATE CANCER DETECTION WITH TARGETED BIOPSY DURING CONTRAST ENHANCED SONOGRAPHY
E J Halpern (P); F Frauscher; J R Ramey; P McCue; L G Gomella

PURPOSE
To evaluate cancer detection with a contrast-enhanced targeted biopsy approach compared with a modified sextant biopsy distribution.

METHOD AND MATERIALS
Three hundred and one subjects with an elevated PSA (above 4ng/ml) or abnormal digital rectal examination were evaluated by transrectal sonography during infusion of a microbubble contrast agent (Imagent; Imcor). Sonography was performed with a 6.5MHz end-fire transducer. Up to four targeted biopsy cores were obtained from the sites of greatest enhancement in the outer gland during contrast-enhanced imaging. Six additional outer gland biopsy cores were obtained in a modified sextant distribution.

RESULTS
Cancer was detected in 363 biopsy cores from 104 of 301 subjects (35%), including 15.5% (175/1133) of targeted cores and 10.4% (188/1806) of sextant cores (p < 0.01). Among subjects with cancer, targeted cores were twice as likely to return a positive biopsy (OR = 2.0, p < 0.001). Cancer was discovered in 72 subjects by both techniques, in 21 subjects by sextant biopsy alone and in 11 subjects by targeted biopsy alone (p = 0.08). The 21 subjects with cancer detected by sextant biopsy alone included 5 positive cores at the gland base, 7 in the mid-gland and 17 in the apex. The 11 subjects with cancer detected by targeted biopsy alone included 8 positive cores at the gland base, 4 in the mid-gland and 3 in the apex. While 38% (72/188) of positive sextant cores were obtained at the gland apex, only 17% (30/175) of positive targeted cores were obtained from the gland apex. Only 21% (233/1133) of targeted biopsies were directed to the apex.

CONCLUSIONS
The cancer detection rate of contrast-enhanced targeted cores is significantly higher when compared to a modified sextant approach. Although targeted biopsy detected 11% (11/104) of cancers not found by the sextant approach, targeted biopsy failed to detect 20% (21/104) of cancers. The low proportion of targeted biopsy cores at the apex suggests that contrast enhancement is less efficacious at the apex. In order to maximize cancer detection and minimize the number of biopsy cores, we recommend a contrast-enhanced targeted biopsy strategy with additional cores at the apex of the prostate.
Accepted for presentation in Dec '04 to the Radiological Society of North America

Abstract ID: 4403687
Submission Type: Scientific Papers

Ethan Halpern
Thomas Jefferson University Hospital

Phone: 215-955-5345
Fax: 215-955-8549,215-955-8549
E-Mail: Ethan.Halpern@jefferson.edu

DETECTION OF PROSTATE CANCER WITH CONTRAST ENHANCED SONOGRAPHY USING HARMONIC GRAY SCALE, COLOR DOPPLER AND POWER DOPPLER IMAGING
E J Halpern (P); J R Ramey; F Frauscher; P McCue; L G Gomella

PURPOSE
To evaluate the discrimination of benign from malignant prostate outer gland tissue during contrast-enhanced sonography.

METHOD AND MATERIALS
301 subjects with an elevated PSA or abnormal digital rectal examination were evaluated with transrectal sonography during infusion of a microbubble contrast agent (Imagent; Imcor). Baseline imaging was performed with conventional gray scale, color and power Doppler. Contrast-enhanced imaging was performed with harmonic gray scale, including continuous harmonic imaging (CHI) and intermittent harmonic imaging (IHI) with interscan delay times of 0.2s, 0.5s, 1.0s, 2.0s, as well as with continuous color and power Doppler. Six biopsy cores were obtained in a modified sextant distribution with one core from the most suspicious area in each sextant. A sextant with no suspicious area was sampled with a laterally directed core. Each biopsy site was prospectively rated for suspicion of cancer on a 1-5 scale with each imaging technique. In order to compensate for clustering of data within each subject, clustered ROC analysis was performed.

RESULTS
Cancer was detected in 188 sextant cores from 93 of 301 subjects (31%). Clustered ROC analysis demonstrated the following values for area under the curve, Az: pre-contrast gray scale – 0.58, pre-contrast color Doppler – 0.53, pre-contrast power Doppler – 0.58, CHI – 0.62, IHI (0.2s) – 0.64, IHI (0.5s) – 0.63, IHI (1.0s) – 0.65, IHI (2.0s) – 0.61, contrast-enhanced color Doppler – 0.60, contrast enhanced power Doppler – 0.62. A statistically significant benefit was found for IHI over baseline gray scale and Doppler imaging (p < 0.05).

CONCLUSIONS
Contrast-enhanced transrectal sonography with IHI provides a statistically significant improvement in discrimination between benign and malignant areas of the prostate outer gland. However, as evidenced by relatively low ROC areas, contrast enhanced sonography cannot definitively differentiate benign from malignant tissue without biopsy confirmation.
Presented June '04: 48th annual meeting of the American Institute of Ultrasound in Medicine

MICROVESSEL DENSITY AND CONTRAST ENHANCED TRUS FOR PROSTATE CANCER DIAGNOSIS

AU: F. Forsberg, B. Kuruvilla, Douglas Elwood, E. J. Halpern

Department of Radiology, Thomas Jefferson University, Philadelphia, PA 19107.

Objective: To evaluate microvessel density (MVD) measurements and contrast enhanced transrectal ultrasound imaging (TRUS), using the ultrasound contrast agent Imagent® (Alliance Pharmaceutical Corp., San Diego, CA), for prostate cancer detection.

Methods: Seven men scheduled for TRUS guided prostate biopsies (random sextant biopsies and up to 4 additional contrast directed biopsies) were evaluated. TRUS was performed after infusion of Imagent (approximate dose 0.31 mg/(kg min)) in grayscale phase inversion harmonic imaging (PIHI), color Doppler imaging (CDI) and power Doppler imaging (PDI) modes using a Sonoline Elegra scanner (Siemens Medical Systems, Issaquah, WA). The enhancement and the suspicion of cancer at each biopsy site were assessed prospectively on a 5-point scale. All biopsy specimens were assessed for cancer with standard H&E stain and for MVD with an endothelial cell marker stain (CD31). MVD was determined using an SMZ-10A microscope (magnification 100x; Nikon, Melville, NY) and Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The area under the receiver operating characteristic (ROC) curve for MVD was computed using histopathology as the gold standard. Linear regression was used to correlate MVD with enhancement and suspicion of cancer for all imaging modes.

Results: Of the 63 biopsy specimens, 52 had sufficient tissue for MVD determination. Eight (15 %) contained malignant prostate tissue. The area under the ROC curve for the diagnosis of prostate cancer with MVD was 0.82. There was a statistically significant difference between benign and malignant MVDs (mean values: 35.8 and 106.4 vessels/µm², respectively; p<0.002). MVD correlated significantly with PIHI enhancement (r=0.33; p<0.025) but not with CDI and PDI enhancement (r<0.16; p>0.1). The suspicion of cancer assessed with all 3 TRUS modes correlated significantly with MVD (r>0.40; p<0.005).

Conclusions: MVD is greater in malignant than in normal prostate tissues. MVD correlates with the enhancement seen on grayscale PIHI TRUS of Imagent and, thus, can assist in the diagnosis of prostate cancer.