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13. ABSTRACT (Maximum 200 Words)

This study proposed to evaluate contrast-enhanced ultrasound imaging with a microbubble contrast agent in 300 subjects over a period of 3 years in order to improve the detection of prostate cancer. Although the grant period began 8/1/2001, patient recruitment actually commenced on 10/1/2001. Between 10/15/2001 and 7/31/2003 a total of 240 subjects provided informed consent. Laboratory blood tests (PSA) and ultrasound evaluations were completed on all 240 subjects (including the ultrasound interpretation worksheet of the primary reviewer). An independent blinded reader has completed review of the first 100 subjects. Pathological review for the presence and grade of cancer has been completed for all 240 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 preliminary analysis of the data from the first 201 subjects was incorporated into two abstracts that have been accepted for presentation at the 2003 annual meeting of the Radiological Society of North America (see appendix). Based upon the current rate of recruitment we expect to reach the target goal of 300 subjects by February 2004.

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INTRODUCTION:

The objective of this study is to utilize ultrasound imaging with intravenous infusion of a microbubble contrast agent to improve the detection of prostate cancer, and to identify those cancers which are clinically significant. Over a three year period, three hundred subjects with suspected cancer of the prostate are to be enrolled. These patients will be 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 will attempt to demonstrate that intermittent ultrasound imaging with a contrast agent 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:

Two hundred and forty subjects have been recruited into the study to date. Each subject has provided written informed consent and was evaluated with the required laboratory studies (PSA) prior to participation in the ultrasound contrast protocol. The examining physician (Dr. Ethan Halpern) has completed an ultrasound image interpretation worksheet for each of these subjects. Because of paperwork delays, patient recruitment began in October 2001, two month after the start date of the grant. However, recruitment has been progressing ahead of schedule, and this portion of the study is progressing on target.

#2 – Pathologic evaluation:

Specimens from all 240 subjects have been evaluated by standard pathologic evaluation. A pathology interpretation worksheet has been completed by our pathology consultant (Dr. Peter McCue). Approximately one-third of the subjects were found to have cancer. Among those subjects with cancer, targeted biopsy cores directed to the locations of maximum enhancement within the prostate have resulted in a cancer detection rate that is 50% higher than standard sextant cores. However, when considering all subjects (including those with cancer and those with no detected cancer), the degree of contrast enhancement did not discriminate those patients with cancer from those with negative biopsies. The interim statistical analysis of data from the pathological evaluation is summarized in section #4 below, and has been used to create the abstracts included in the appendix of this report.

For the evaluation of microvessel density, CD31 staining has been performed on tissue sections from the initial 40 subjects. Initial technical problems with the microvessel density counting system have been rectified. However, the automated counting process has required much more manual input that was initially expected. Consequently, the process has been more tedious than initially anticipated. Initial results with 63 biopsy specimens processed with CD31 stain demonstrated 52 specimens with sufficient tissue for microvessel density counting. 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.

Based on our current rate of microvessel density counting, it will not be possible for us to perform microvessel density counts for all 300 subjects. In order to achieve our stated goal of comparing microvessel density in benign versus malignant tissue in a timely fashion, we will concentrate our future 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. We expect to count microvessels for a total of 120 subjects (including

approximately 80 additional subjects with cancer). At present, we anticipate that a 6 month extension of the grant will be required to complete these microvessel density counts and analysis.

#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: was performed on the first 201 subjects. Cancer was detected in 252 biopsy cores from 67 of 201 subjects (33%). Cancer was found in 16.5% (124/753) of targeted cores versus 10.6% (128/1204) of sextant cores ($p < 0.01$). The diagnosis of cancer was discovered in 48 subjects by both targeted and sextant techniques, in 10 subjects by sextant biopsy alone and in 9 subjects by targeted biopsy alone (p-N.S.). With respect to the characterization of tissue as benign versus malignant, no statistically significant difference was found with different interscan delay times. The results of this analysis are presented in the three abstracts attached in the appendix. Based upon this analysis, an optimized ultrasound technique is suggested in the attached abstracts.

#5 – Consensus interpretations: blinded reviews of the ultrasound studies have been performed by a second reader (Dr. Stephen Strup) for the first 100 cases. Unfortunately, Dr. Strup left Thomas Jefferson University this summer. We have requested that the DOD allow us to substitute Dr. John Ramey as blinded reader so that we may complete the blinded reads and establish a consensus evaluation. This replacement of personnel was approved, and Dr. Ramey has begun to work as a blinded reader.

#6 – Publications: one abstract was accepted for presentation at the annual meeting of the mid-Atlantic section of the American Urological Association in October 2003. Two additional abstracts were accepted for presentation at the annual meeting of the Radiological Society of North America during November 2003. Copies of these abstracts are included in the appendix.

KEY RESEARCH ACCOMPLISHMENTS:

- Successful infusion of ultrasound contrast in 240 subjects
- Visible vascular enhancement within the prostate of all subjects studied to date
- Biopsy specimens targeted to vascular enhancement detected 85% (57/67) of subjects with cancer of the prostate.
- Targeted biopsy based upon contrast enhancement detected an additional 13% (9/67) of patients with cancer that would not have been detected with the conventional sextant biopsy protocol.

REPORTABLE OUTCOMES: three abstracts accepted for presentation (see attached):

1. Halpern EJ, Frauscher F, Strup SE, Ramey JR, Gomella LG. Comparison of Contrast-enhanced Targeted Biopsy of the Prostate to Modified Sextant Biopsy. Radiology: Proceedings of the 2003 meeting of the RSNA. Pg 666, Chicago, Ill. December 2003.

2. Halpern EJ, Frauscher F, Strup SE, Ramey JR, Gomella LG. Contrast Enhanced Imaging of the Prostate for Cancer Detection. Radiology: Proceedings of the 2003 meeting of the RSNA. Pg 665, Chicago, Ill. December 2003.

3. Halpern EJ, Strup SE, Ramey JR, Gomella LG. Comparison of Contrast-enhanced Targeted Biopsy of the Prostate to Modified Sextant Biopsy. Proceedings of the 61st annual meeting of the Mid-Atlantic Section of the AUA. Pg 114, Boca Raton, Fl. October 2003.

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. In our study, targeted biopsies of areas with increased blood flow detected approximately 85% of cancers found in our population. As noted in our initial report, most cancers that were not identified with the contrast-enhanced technique were located at the apex of the gland. In our final analysis we hope to determine whether the contrast-enhanced biopsy technique detects all "significant cancers", or whether the contrast technique should be augmented by "systematic" biopsy cores from the gland apex.

Appendix: Abstract accepted for presentation at the RSNA – 11/2003

Comparison of Contrast-enhanced Targeted Biopsy of the Prostate to Modified Sextant Biopsy

Purpose: Recent studies have demonstrated improved detection of prostate cancer with targeted biopsy using microbubble contrast agents. Nonetheless, systematic biopsy in a proscribed spatial distribution (ie. sextant biopsy) remains the standard of care. Our study compares cancer detection with a targeted biopsy approach versus a modified sextant biopsy distribution.

Methods and Materials: Two hundred and one subjects with an elevated PSA (≥4ng/ml) or abnormal digital rectal examination were evaluated by transrectal sonography during infusion of a microbubble contrast agent (Imagent; Alliance Pharmaceuticals). Sonography was performed with the Sonoline Elegra (Siemens Medical Systems) using 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 252 biopsy cores from 67 of 201 subjects (33%). Cancer was found in 16.5% (124/753) of targeted cores versus 10.6% (128/1204) of sextant cores ($p < 0.01$). The diagnosis of cancer was discovered in 48 subjects by both techniques, in 10 subjects by sextant biopsy alone and in 9 subjects by targeted biopsy alone (p-N.S.). The 10 subjects with cancer detected by sextant biopsy alone included 8 cancers at the gland apex, 1 in the mid-gland and 1 in the base. The 9 subjects with cancer detected by targeted biopsy alone included 6 cancers at the gland base, 2 in the mid-gland and 1 in the apex. While 40% (51/128) of positive sextant cores were obtained at the gland apex, only 18% (22/124) of positive targeted cores were obtained from the gland apex. Only 20% (149/753) of targeted biopsies were directed to the apex.

Conclusion: The cancer detection rate of contrast-enhanced targeted cores is significantly higher when compared to sextant cores. Furthermore, targeted biopsy detected an additional 13% (9/67) of cancers not found by the sextant approach. Nonetheless, targeted biopsy failed to detect 15% (10/67) of cancers, including 8 cancers at the apex of the prostate. 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.

Appendix: Abstract accepted for presentation at the RSNA – 11/2003

Contrast-enhanced Imaging of the Prostate for Cancer Detection

Purpose: Sonographic detection of prostate cancer is improved with contrast-enhanced targeted biopsy (Lancet 357:1849-1850, 2001; AJR 178:915-919, 2002; J Urol 167:1648-1652, 2002). We evaluated the discrimination of benign from malignant outer gland tissue during contrast-enhanced sonography of the prostate.

Methods and Materials: 201 subjects were evaluated by transrectal sonography during infusion of a microbubble contrast agent (Imagent; Alliance Pharmaceuticals). Contrast-enhanced imaging was performed with harmonic gray scale, color and power Doppler imaging. 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 on pre-contrast and post-contrast imaging. Sensitivity and specificity for detection of cancer were computed with a cutoff ≥ 3 as positive.

Results: Cancer was detected in 128 sextant cores from 58 of 201 subjects (29%). On pre-contrast imaging, gray scale, color and power Doppler imaging demonstrated a sensitivity/specificity of 48%/65%, 20%/93% and 24%/89% respectively. Contrast enhancement of prostatic parenchyma was demonstrated in every patient. Contrast-enhanced harmonic gray scale, color and power Doppler imaging demonstrated a sensitivity/specificity of 40%/84%, 70%/35% and 79%/31% respectively. Receiver operating characteristic analysis for pre-contrast imaging demonstrated areas under the curve (Az) of 0.59 for gray scale, 0.55 for color Doppler and 0.58 for power Doppler. Contrast-enhanced imaging demonstrated Az values of 0.64 for gray scale harmonic imaging, 0.55 for color Doppler and 0.58 for power Doppler. There was no significant difference between the Az values for pre-contrast and contrast-enhanced imaging. All imaging techniques with the exception of color Doppler were significantly better than random chance ($Az>0.5$ $p<0.05$).

Conclusion: Contrast-enhanced transrectal sonography does not improve sonographic discrimination between benign and malignant areas within the prostate outer gland. The sensitivity of color and power Doppler imaging is dramatically increased after contrast administration at the expense of reduced specificity. We suggest that contrast-enhanced targeted biopsy may result in increased cancer detection due to improved visibility of targeted sites even though it does not improve the discrimination between benign and malignant foci.

Appendix: Abstract accepted for presentation at the Mid-Atlantic AUA – 10/2003

Comparison of Contrast-enhanced Targeted Biopsy of the Prostate to Modified Sextant Biopsy

Introduction: We compared detection of prostate cancer with contrast enhanced targeted biopsy versus systematic sextant biopsy.

Methods: Two hundred and one subjects with an elevated PSA (≥4ng/ml) or abnormal digital rectal examination were evaluated by transrectal sonography during infusion of a microbubble contrast agent (Imagent). Up to four targeted biopsy cores were obtained from the sites of greatest contrast enhancement. Six additional biopsy cores were obtained in a modified sextant distribution.

Results: Cancer was detected in 252 cores from 67/201 subjects (33%). Cancer was found in 16.5% (124/753) of targeted cores versus 10.6% (128/1204) of sextant cores ($p < 0.01$). The diagnosis of cancer was discovered in 48 subjects by both techniques, in 10 subjects by sextant biopsy alone and in 9 subjects by targeted biopsy alone (p-N.S.). The 10 subjects with cancer detected by sextant biopsy alone included 8 cancers at the gland apex, 1 in the mid-gland and 1 in the base. The 9 subjects with cancer detected by targeted biopsy alone included 6 cancers at the gland base, 2 in the mid-gland and 1 in the apex. While 40% (51/128) of positive sextant cores were at the apex, only 18% (22/124) of positive targeted cores were from the apex. Only 20% (149/753) of targeted biopsies were directed to the apex.

Conclusion: The cancer detection rate of contrast-enhanced targeted cores is significantly higher compared to sextant cores. In order to maximize cancer detection and minimize the number of cores, we recommend a contrast-enhanced targeted biopsy strategy with additional cores at the apex of the prostate.