Breast Cyst Fluid Analysis Correlations with Speed of Sound Using Transmission Ultrasound

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Abstract

Rationale and Objective—The purpose of this work is to determine if the speed of sound value of a breast cyst can aid in the clinical management of breast masses. Breast macrocysts are defined as fluid-filled tissue masses greater than 1 cm in diameter and are thought to be aberrations of normal development and involution, often associated with apocrine metaplasia. The benign natural history of breast cysts is well known, and it is important to obtain high specificity in breast imaging to avoid unnecessary biopsies in women who have benign disease, particularly in those women with dense breast tissue. Transmission ultrasound is a tomographic imaging modality that generates high-resolution, 3D speed of sound maps that could be used to identify breast tissue types and act as a biomarker to differentiate lesions. We performed this study to investigate the microanatomy of macrocysts observed using transmission ultrasound, as well as assess the relationship of speed of sound to the physical and biochemical parameters of cyst fluids.

Materials and Methods—Cyst fluid samples were obtained from 37 patients as part of a case-collection study for ultrasound imaging of the breast. The speed of sound of each sample was measured using a Quantitative Transmission Ultrasound scanner in vivo. Electrolytes, protein, cholesterol, viscosity and specific gravity were also measured (in the aspirated cyst fluid) to assess their relationship to the speed of sound values obtained during breast imaging.

Results—We found positive correlations between viscosity and cholesterol (r = 0.71) and viscosity and total protein × cholesterol (r = 0.78). Additionally, we performed direct cell counts on cyst fluids and confirmed a positive correlation of number of cells with speed of sound (r = 0.74). The speed of sound of breast macrocysts, as observed using transmission ultrasound, correlated with the cytological features of intra-cystic cell clumps.

Conclusion—On the basis of our work with speed as a classifier, we propose a spectrum of breast macrocysts from fluid-filled to highly cellular. Our results suggest high speed cysts are mature macrocysts with high cell counts and many cellular clumps that correlate with cyst
microanatomy as seen by transmission ultrasound. Further studies are needed to confirm our findings and to assess the clinical value of speed of sound measurements in breast imaging using transmission ultrasound.

Introduction

Biology of breast cysts

The normal breast consists of segments (lobes) drained by collecting ducts. The segments consist of lobules composed of terminal ducts, acini and their supporting stroma (1). Breast macrocysts are reviewed in Hughes and are defined as fluid-filled tissue masses greater than 1 cm in diameter (2). They were first described in 1829 and are thought to be aberrations of normal development and involution, often associated with apocrine metaplasia (3, 4). Macrocyts are seen in 21% of women at post-mortem and in over 10,000 breast biopsies for benign disease 23% had macrocysts (5, 6). Cysts are manifestations of lobular involution and their origin from the breast lobule has been demonstrated using histochemical techniques (7). They are usually lined by a single layer of epithelium which have proteins only found in apocrine epithelium (8). Evidence that active secretion is responsible for cyst formation has come from analysis of cyst fluid (9). The benign natural history of breast cysts is well known with these women having a low incidental risk for cancer (6, 10). Thus, it is important to have high specificity in breast imaging to avoid unnecessary biopsies in women who have benign disease, particularly in those women with dense breast tissue (11, 12).

Transmission ultrasound

Quantitative Transmission Ultrasound (QT) is an imaging modality based on tomographic techniques extended to ultrasound. In such a system, images are generated using both reflection and transmission techniques. While transmission ultrasound has been investigated as an adjunct to mammography for quite some time, recent developments in hardware and imaging algorithms have enabled marked improvements in spatial resolution and clinical utility (13, 14). Physically, a transmitter and receiver pair is co-located with multiple reflection transducers with various focal lengths in a U-shaped arrangement (15–17). The transmitter emits a plane wave that is received by the receiver with multiple acquisitions at frequencies ranging from 300 kHz to 1.5 MHz as the U-channel is rotated 360 degrees around the subject. Once acquired, the projection information is reconstructed using nonlinear inverse scattering in 3D (18). The result of this reconstruction is a quantitative volume map of speed of sound (measured at 1.5 MHz), with units of meters per second (m/s). In reflection ultrasound imaging, each of the three reflection transducers (4 MHz center frequency) with different focal lengths are alternately fired between transmission measurements in a B-mode acquisition. The resulting images are compounded together and corrected for refraction using the speed map computed in the transmission phase. This compounding produces a nonquantitative image that is proportional to impedance mismatch, referred to simply as reflection units (RUs). The result of each scan is a 3D volume of speed and reflection. These image stacks are precisely co-registered since they are acquired at the same time, so they can be put together to form a 3D view of the object in the field of view (FOV). The imaging system can image human breast tissue anatomy with high spatial and
contrast resolution, while the speed of sound information corresponds well with tissue specificity (19–21).

**Mass visualization using transmission ultrasound**

We have previously shown that the speed of sound, as measured by QT ultrasound®, can both define tissue types and distinguish cystic from solid masses with high specificity in a clinical setting (21, 22). Traditional reflection ultrasound imaging of cysts reveals either an anechoic interior (no internal reflecting elements visible) for simple cysts or varying degrees of internal reflecting elements visible for complicated or complex cysts. When transmission ultrasound imaging these same “anechoic” cysts by reflection, there are variations in the internal speed of sound (from 1540 m/s to approximately 1575 m/s) with correlated small (~200 micron) foci of higher speed areas within the simple cyst. By making an accurate correlation with anatomy shown by transmission ultrasound, physicians can better interpret the type of breast mass visualized (fluid-filled cyst highly cellular cyst or solid mass). The current study was designed to aid in the interpretation of “low speed” simple cysts without high-speed foci and “high speed” simple cysts with high-speed foci.

**Cyst fluid analysis by others**

It is difficult to find studies of the physical properties of human breast cyst fluid (i.e., specific gravity or viscosity), but a number of studies measuring electrolytes, proteins and hormones can be found (8, 23–27). Variation in Na+, K+ and Na+/K+ ratios has been shown to correlate with typing by histologic examination of cells lining the cysts and correlate with a degenerative rather than secretory process (23). To our knowledge, no measurements of human breast cyst fluid that correlate with speed of sound have been published. The current prospective study was an extension of our retrospective investigation into the specificity of the speed of sound in transmission ultrasound of the breast for determining the presence of cystic or solid massa. Additionally, it was done to further clarify the radiologist’s interpretation of cysts with high speed foci observed by transmission ultrasound (22).

**Methods**

**Samples**

In this prospective study, we collected cyst fluid from patients in order to assess the relationship of speed of sound to the physical and biochemical parameters of the cyst fluids. All cyst fluid samples were obtained as part of a case-collection study at two academic institutions: George Washington University in Washington DC and Elizabeth Wende Cancer Center in Rochester, NY. The study was approved by the western IRB and is registered with ClinicalTrials.gov (https://clinicaltrials.gov/NCT02133417). Inclusion criteria consisted of any patient with an abnormality on their screening mammogram that was considered by the interpreting breast radiologist to be a mass. There were 207 cases collected: 38 fibroadenomas, 57 cysts, 46 cancers and 63 that turned out to be normal. However, not all patients with cysts agreed to have cyst aspiration and in some cases the collected fluid was not sufficient for a full analysis. Therefore, cyst fluids from thirty seven patients were available for analysis.
The following chemical tests were done on each cyst fluid sample: sodium, potassium, total protein and cholesterol. Specific gravity and viscosity were also performed on each sample. Thin preparations were made of the cyst fluid for cell counts and morphology. Speed of sound for each cystic mass was measured using the QT Viewer® workstation, a proprietary viewer that is a part of the QT Ultrasound Breast Scanner -1 (QT Ultrasound, Novato, CA). The viewer has been independently validated for accuracy of the speed measurement.

**Chemical Analysis**

All samples were appropriately preserved and shipped to Strong Memorial Hospital University of Rochester Medical Center (URMC) in Rochester, New York, for analysis. Most of the chemistry assays were performed on a Roche Cobas 8000 Modular Analytics System (28). Total protein was performed according to the in vitro test for the quantitative determination of total protein in human serum and plasma (29). Cholesterol was measured using the Cholesterol Gen.2 assay (30). Specific gravity was measured using the specific gravity test module (26). Viscosity was measured using the capillary method (31).

**Cytological Analysis**

Slide preparation of the cyst fluid was done using the Thin Prep® Processor (32). The Thin Prep® Processor processes all samples in a similar fashion and deposits cells from a similar sample size within a 20-mm circle on the microscope slide, permitting sample comparisons using uniform methods of cell deposition. Slide staining was done according to the method of Papanicolaou (33). Sodium and potassium analysis was done using standard ion-specific electrodes on the ISE Module of the Roche Cobas 8000 system (34). Cell counting on the thin-prep and stained slides was done in a semi-automated method, using open-source image analysis software DotCount v1.2 and ImageJ (NIH, Bethesda, MD) (35). For each slide, nine non-overlapping images were acquired, which covered over 80% of the sample area. The images were stitched before cell counting was performed. A representative single image is shown in Figure 1 below.

Parameters for the software were similar for all counting sessions and the scores results of the software were used for the analysis. All results of the chemical testing were provided by a spreadsheet from URMC and validated by an independent clinical studies team.

**Statistical and Correlation Analysis**

Correlation analysis was performed between speed of sound values and multiple chemical concentrations within the cyst aspirate. In addition, correlation analysis was performed between speed of sound and cell count performed on ThinPrep® slides. In all instances, “correlation” is defined as the Pearson Product-Moment correlation coefficient (r), which measures the strength of the linear relationship between two variables. We also tested whether speed of sound shows any relationship as a function of cyst size. We defined macrocysts as larger than 10 mm in diameter. We performed the non-parametric Mann-Whitney U Test to assess if speed of sound can be used as a predictor of cyst size. In addition, we performed linear discriminant analysis (LDA) with leave-one-out cross validation scheme to test if cysts can be classified as large or small based on the speed of
sound. All analysis was performed using JMP and Microsoft Excel software. Graphing was performed with OriginPro software.

Case selection and exclusion

A flow chart of the case selection process is shown in Figure 2. Cytological preparation was complete and available for visualization and cell counts on 82 cases from the case collection study. Out of these 82 cases, fluid for 37 cysts were collected for the study. 30 cysts had the entire panel of chemical tests done, but in 7 cases the amount of fluid for testing was not sufficient to do all of the tests. In order to look for a correlation between the speed of sound value of the cyst interior and the presence of punctate, high-speed foci within the cyst fluid, we selected cysts that were large enough to measure the speed of sound with statistical accuracy. For correlating speed of sound with cyst size, we selected a total of 52 cases based on the availability of size information and on the clarity of QT images to allow clear demarcation of the size of the cyst. Within these 52 cases, 41 cases were identified to have large macrocysts (size > 10 mm) and 11 cases were identified to have small cysts (size < 10 mm). For correlative analysis of speed of sound with cell counts, we selected 33 of these 52 cases of cyst samples. Cases were excluded due to the presence of large sized clumps of cells in the ThinPrep® slides and/or due to the presence of more than one slide for one study where we were unable to confirm the validity of the data point.

Results

Chemical Correlations

The chemical analysis results from 30 patient samples are shown in Table 1. Figure 3 summarizes the correlations between these various chemical parameters, and Table 2 shows the respective correlation coefficients for the scatterplots shown in Figure 3.

In general, we observed no strong meaningful correlations between the individual chemical parameters, except for sodium and potassium concentrations that are known to have a strong inverse relationship. We also observed a correlation between cholesterol and viscosity and between total protein × cholesterol and viscosity.

Speed of Sound Correlations

We observed a correlation between speed of sound in the cyst in vivo and the specific gravity of the aspirated cyst fluid, as shown in Fig. 4.

Because of the correlations observed between specific gravity and the product of protein and cholesterol (i.e. cell membrane components) and between specific gravity and speed, we reasoned that cell count could be a factor in the in vivo cyst speed. As shown in Figures 5 and 6, protein × cholesterol correlated with cyst cell count as did cell number and speed.

Figure 6 below shows a moderate to high linear relationship between speed of sound and the cell count (r = 0.74).
Cyst Classification

We observed that the larger macrocysts had on average a lower mean speed of sound value (1550.6 ± 8.7 m/s) in comparison to smaller cysts (1566.0 ± 22.2 m/s). The non-parametric Mann Whitney U test showed significant difference (p < 0.05) between the speed of sound values of the two groups based on size. The fluid in the larger macrocysts (over 10 mm diameter) had a non-homogeneous or “speckled” appearance (Figure 7), whereas the smaller cysts (< 10 mm diameter) had a more homogeneous appearance (Figure 8).

We then tested the ability of speed of sound to predict cyst size. As mentioned above, we used linear discriminant analysis with a leave-one-out cross validation scheme. The confusion matrix is shown below in Table 3. Overall, the classifier showed an accuracy of 80.3%.

As mentioned above, the cysts with larger clumps were excluded from the speed of sound versus cell count correlation. The reason for this exclusion is discussed below. However, speed of sound of cysts with clumps was found to be significantly different than cysts with regularly spread of cells. Specifically, the average speed of sound of cysts with regularly spread of cells was measured to be 1551.9 ± 12.7 m/s, whereas cysts with clumps were measured to be 1560.4 ± 15.4 m/s.

Discussion

In our chemical analysis of cyst fluid, we found positive correlations between viscosity and cholesterol (r = 0.71) and viscosity and total protein × cholesterol (r = 0.78). In our cytological analysis of breast cyst fluid, we showed a positive correlation between direct cell counts on cyst fluids and speed of sound. Furthermore, the speed of sound of breast macrocysts, as observed using transmission ultrasound, correlated with the cytological features of intra-cystic cell clumps.

Our results of cyst fluid Na+, K+ and Na+/K+ ratios are consistent with other studies (24–29), with 14 of the 30 samples having a high Na+/K+ ratio. The cholesterol levels in cyst fluid in this study (17.9 ± 6.8 mmol/L) are also consistent with other studies (15 ± 6 mmol/L) (27).

The correlation of cell count with speed of sound in macrocysts is consistent with the presence of cell number observed cytologically. This is not surprising given the high-fidelity nature and spatial resolution of transmission ultrasound, and its ability to provide quantitative measurements (19, 20). By way of theory, closely pack structures (such as cells in a suspension) exhibit higher effective refractive index (36). Hence, higher cell count result in higher value of refractive index, with a consequent increase in speed of sound within the cyst. We would like to highlight the importance of this finding since no other mesoscopic imaging modality, without the use of a contrast mechanism, has the ability to capture such quantitative variation as a function of cell count.

Very little is known about the speed of sound of human breast tissue subtypes. Also, any system for doing so must account for acoustic impedance, temperature and the fluid medium...
used to conduct the sound to allow comparison to other published values. Ophir examined the speed of sound in 10 human tissues in polyethylene glycol-ethanol-water solution at 21.5°C and compared the speed with their densities (37). Although there was no measurement of breast tissue, splenic tissue was the closest to our measurements for breast parenchyma. That study also found a correlation between density and speed with similar value ranges to our analysis. This is also in line with the fact that higher density usually results in higher refractive index, thereby increasing the speed of sound, as noted in our study.

In a previous publication, using a validated system and speed calculation methodology, we have measured the speed of sound in breast tissue subtypes (21). Using discriminant analysis, we have shown that speed of sound was the most important contributor towards the classification: with an accuracy rate of greater than 85% when distinguishing between the five tissue classes in the breast (glands, ducts, skin, connective tissue and fat). In the current study, we have extended this analysis to use LDA as a predictor of cyst size and type. When using speed of sound as a predictor of cyst size (to differentiate between two groups: cysts larger than 10 mm and cysts smaller than 10 mm), the classifier performed at an overall accuracy of greater than 80%. We then tested the ability of speed of sound to predict the type of cyst (regular versus clump). While the speed of sound values showed statistically significant difference, the discriminant analysis or logistic regression classifiers were not able to differentiate between the two classes with high accuracy; accuracy was calculated to be 60.1%.

When comparing the speed of sound in cysts with a ‘regular’ spread of cells in the ThinPrep® slides with those with cell ‘clumps,’ we must be careful in interpreting the results from slides with clumps. ThinPrep® machine collects the cells on the surface of a membrane with small pores (38). The pores are sufficiently small to aspirate the liquid from vial while trapping the cells on its surface. Debris material such as inflammatory cells, large clumps and contaminants can block the holes thus preventing the collection of enough epithelial cells (used for diagnostics) onto the filter membrane. Such blockage of the membrane can interfere with adequate collection consistency, which can greatly impact the variance of cell count in slides with clumps. Therefore, we did not include the cell count from slides with clumps in our speed of sound correlation. However, this did not prevent us from pooling together the speed of sound values from ‘clump’ to gather valuable information on the relationship of the number of cells with higher speed of sound. As noted above, there was significant difference in the speed of sound values of cysts with ‘regular’ spread of cells (i.e. fluid cysts) and in cysts showing clumping of cells.

Based on our work with speed of sound as a predictor, we propose the following classification of breast cysts based on speed of sound.
Limitations of the study

The main limitations of the study include the possibility of a bias due to the small number of cases studied. Although there were 37 cases with chemical analysis and 33 cases with cytologic analysis, the exclusions were for technical reasons (inadequate sample volume, very small cysts and ThinPrep® technical issues). Nevertheless, the statistical analysis confirmed the sample size was adequate. Since much of the data was visually analyzed, there is a risk of reader bias, however all cytological and image viewing was done blindly (without knowledge of any information from the non-visual datasets).

Conclusions

The speed of sound of breast macrocysts as observed using transmission ultrasound correlates well with biological and cytological features consistent with the spectrum of cystic masses observed clinically. Diagnostic breast radiologist guidelines would include that cysts with “high-speed” foci will have a higher speed of sound and that those foci are likely caused by clumps of cells. To our knowledge, this is the first study to correlate speed of sound as gathered from transmission ultrasound as a function of cyst type and size. Further studies are needed to confirm our findings and to determine the clinical value of speed of sound measurements in breast imaging using transmission ultrasound.

The clinical relevance of our work is that transmission ultrasound breast imaging will observe a spectrum of “low-speed” (1540 to 1569 m/s) cystic masses within the breast that can show (1) homogeneous interiors with low speed (fluid-filled cysts), (2) a homogeneous interior with high speed (cysts containing free-floating cells), and/or (3) a heterogeneous interior with many punctuate areas of high speed (cysts with large cell clumps). The present work will allow the breast imaging radiologists to have a basis for describing breast macrocysts and for following changes in these masses over time.

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Figure 1.
Case collection flow chart.
Figure 2.
Panel A (left) showing exfoliated cyst lining epithelial cells and panel B (right) showing clumps of exfoliated cyst epithelial cells.
Figure 3.
Correlation plots between the various chemical parameters. The data points correspond to the data in Table 1. In addition to the parameters in Table 1, the parameter of “Protein × Cholesterol” has also been included. Each of the plots includes 95% density ellipses as well, which means that the ellipses enclose approximately 95% of the points. The narrowness of the ellipses reflects the degree of correlation of the variables.
Figure 4.
Plot showing correlation between speed of sound as measured by QT and specific gravity of cyst fluid. The value of $r$ denotes the Pearson correlation coefficient.
Figure 5.
Plot of correlation between cell count and ‘protein × cholesterol’. The value of $r$ denotes the Pearson correlation coefficient.
Figure 6.
Plot of speed of sound as measured by QT as a function of cell count. The correlation coefficient ‘r’ was calculated to be 0.74.
Figure 7.
Sagittal view speed of sound image showing (with crosshairs) a macrocyst (cyst# 9 in Table 1) with an average speed value of 1554 m/s.
Figure 8.
Coronal view of a speed of sound image showing a macrocyst (~7 mm) with an average speed value of 1538 m/s (cyst#15 in Table 1).
| CYST | Sodium | Potassium | Na/K Ratio | Total Protein | Cholesterol | Viscosity | Sp. Gravity |
|------|--------|-----------|------------|---------------|-------------|-----------|-------------|
| 1    | 139    | 43        | 32.8       | 3.3           | 691         | 1.71      | 1.033       |
| 2    | 22     | 99.6      | 0.2        | 2.8           | 342         | 1.36      | 1.039       |
| 3    | 44     | 86.2      | 0.5        | 2.8           | 656         | 1.6       | 1.039       |
| 4    | 136    | 4.4       | 31.2       | 2.8           | 1369        | 2.92      | 1.035       |
| 5    | 24     | 98        | 0.2        | 1.7           | 489         | QNS       | 1.033       |
| 6    | <20    | 140.2     | <0.1       | 2.3           | 290         | 1.39      | 1.035       |
| 7    | 140    | 4         | 35         | 4.1           | 380         | 1.55      | 1.032       |
| 8    | 39     | 88.7      | 0.4        | 1.2           | 376         | 12        | 1.08        |
| 9    | 140    | 4.1       | 34.5       | 4.7           | 637         | 1.96      | 1.036       |
| 10   | <18    | 110.2     | <0.2       | 2.5           | 559         | QNS       | 1.038       |
| 11   | 65     | 77        | 0.8        | 1.7           | 766         | 2.43      | 1.033       |
| 12   | 24     | 107.1     | 0.2        | 1.3           | 563         | 1.17      | 1.08        |
| 13   | 22     | 94.1      | 0.2        | 1.7           | 695         | QNS       | 1.035       |
| 14   | 137    | 15.1      | 9.1        | 1.6           | 606         | QNS       | 1.08        |
| 15   | 85     | 65.7      | 1.3        | 2.8           | 810         | 1.61      | 1.04        |
| 16   | 47     | 108.9     | 0.4        | 2.1           | 442         | 1.34      | 1.035       |
| 17   | 137    | 3.9       | 34.6       | 3.4           | 499         | 1.23      | 1.031       |
| 18   | 24     | 87.9      | 0.3        | 1.9           | 841         | 2.49      | 1.038       |
| 19   | 136    | 3.9       | 34.7       | 2             | 861         | 1.89      | 1.028       |
| 20   | 31     | 117       | 0.3        | 1.4           | 653         | 1.28      | 1.032       |
| 21   | 23     | 119.7     | 0.2        | 2.4           | 886         | QNS       | 1.041       |
| 22   | 137    | 6         | 22.9       | 1.5           | 728         | 1.55      | 1.026       |
| 23   | 40     | 87.1      | 0.5        | 0.8           | 285         | 1.25      | 1.024       |
| 24   | 135    | 4.4       | 30.8       | 4.4           | 1369        | 7.97      | 1.042       |
| 25   | 106    | 38.1      | 2.8        | 1.9           | 744         | 1.37      | 1.08        |
| 26   | 94     | >54.6     | >1.7       | 2.1           | 968         | 3.12      | 1.036       |
| 27   | 83     | 63.9      | 1.3        | 2.4           | 665         | 2.52      | 1.033       |
| 28   | 49     | 91.3      | 0.5        | 2             | 805         | 3.64      | 1.034       |
| CYST | Sodium | Potassium | Na/K Ratio | Total Protein | Cholesterol | Viscosity | Sp. Gravity |
|------|--------|-----------|------------|--------------|-------------|----------|-------------|
| 29   | <20    | 130.9     | <0.2       | 2.1          | 820         | 3.8      | 1.035       |
| 30   | 140    | 14.8      | 9.4        | 1.4          | 883         | 2.62     | 1.03        |

Sodium/potassium = mmol/L; Protein = g/L; Cholesterol mg/dL; Viscosity = centipoise; Specific Gravity = gm per volume of cyst fluid/1gm per volume of water. QNS = the quantity provided was not sufficient to perform the analysis.
Table 2: Values of Pearson Product-Moment correlation coefficients ($r$) for scatterplots shown in Figure 3 and data in Table 1. An additional variable “protein × cholesterol” was added which is the product of protein and cholesterol.

| Sodium  | Potassium | Na/K ratio | Total Protein | Cholesterol | Viscosity | Sp. Gravity | Prot. x Chol. |
|---------|-----------|------------|---------------|-------------|-----------|-------------|--------------|
| 1.00    | -0.98     | -0.85      | 0.63          | -0.45       | -0.39     | 0.32        | -0.39        |
| Sodium  | Potassium | Na/K ratio | Total Protein | Cholesterol | Viscosity | Sp. Gravity | Prot. x Chol. |
| 0.44    | -0.26     | -0.26      | 0.55          | 0.54        | 0.35      | 0.79        | 0.54         |
| Sodium  | Potassium | Na/K ratio | Total Protein | Cholesterol | Viscosity | Sp. Gravity | Prot. x Chol. |
| 0.12    | -0.48     | -0.48      | 0.55          | 0.54        | 0.35      | 0.79        | 0.54         |
| Sodium  | Potassium | Na/K ratio | Total Protein | Cholesterol | Viscosity | Sp. Gravity | Prot. x Chol. |
| 0.47    | -0.26     | -0.26      | 0.55          | 0.54        | 0.35      | 0.79        | 0.54         |
| Sodium  | Potassium | Na/K ratio | Total Protein | Cholesterol | Viscosity | Sp. Gravity | Prot. x Chol. |
| 0.44    | -0.26     | -0.26      | 0.55          | 0.54        | 0.35      | 0.79        | 0.54         |
| Sodium  | Potassium | Na/K ratio | Total Protein | Cholesterol | Viscosity | Sp. Gravity | Prot. x Chol. |
| 0.12    | -0.48     | -0.48      | 0.55          | 0.54        | 0.35      | 0.79        | 0.54         |

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Table 3
Classification summary (confusion matrix) as generated by LDA using leave-one-out cross validation scheme.

| Actual group | Predicted group | Large cysts | Small cysts | Accuracy |
|--------------|-----------------|-------------|-------------|----------|
| Large cysts  |                 | 36          | 5           | 88 %     |
| Small cysts  |                 | 3           | 8           | 73 %     |