Multiparametric high-resolution sonography with Color/Power-Doppler, elastography and contrast-enhanced sonography for an improved detection and characterization of breast tumors.

1. Introduction

The value of mammography in breast cancer screening and diagnosis is unquestioned. Yet mammography has certain limitations, especially in women with dense breasts, with a considerable decrease in sensitivity and specificity (1). Ultrasound (US) is an established adjunct to mammography for further characterization of mammographically detected breast lesions, for lesion detection in women with dense breasts (ACR 3 and 4) (2), and due to the lack of ionising radiation is the method of choice in younger women.

When using established criteria for the assessment of the probability of malignancy with B-mode US (2, 3), it has a high diagnostic performance with sensitivities ranging from 94 to 95.4% and specificities ranging from 83 to 87.4%) (4, 5). In B-mode breast US a malignant mass is typically round or irregularly shaped, vertically orientated to the skin, markedly hypoechoic and demonstrates a non circumscribed margin and posterior shadowing. Yet there are several benign or pre- cancerous lesions that demonstrate one or more of these characteristics, whereas malignant tumors do not always present with a typical morphology. In order to improve sonographic detection and especially characterization of breast lesions, other US examination modes and techniques apart from standard B-mode such as elastography, Color Doppler, contrast-enhanced and 3D imaging have been developed and are currently under investigation.

- Elastography measures the strain induced by probe compression on the breast. Based on the fact that breast cancer is in most cases stiffer than unaffected breast parenchyma, several studies have demonstrated an increase in specificity of conventional US by the addition of elastography (4-9).
- One of the hallmarks of malignant tumors is tumor neoangiogenesis. Therefore studying the vascularization features of breast tumors by the means of Color and Power Doppler US helps to differentiate breast cancer from benign lesions (9-12).
- Contrast-enhanced US assessing kinetic properties of breast tumors, especially with the use of second generation contrast agents, has been

investigated by several authors and shown promising results (13-19). Nevertheless different contrast agents and dosages have led to a certain degree of discordance among publications (16, 17).

- 3D imaging obtains reconstructions in the coronal plane and allows an improved depiction of the tumor interface, a valuable sonographic descriptor for differentiation of benign and malignant lesions (3, 20-22).

Cho et al (9) have shown that the addition of elastography and Color Doppler to B-mode US can lead to a higher accuracy and sensitivity. Yet up to now there are no published studies evaluating the possible benefit of a multiparametric approach by adding elastography, Color Doppler, contrast-enhanced and 3D studies to B-mode US.

1.1 State of the art

2D B-mode ultrasound has been established as an adjunct to mammography for both screening and diagnostic purposes in women with dense breast parenchyma, for further characterization of clinically or mammographically detected lesions, for follow up and for guiding interventional procedures (2, 23). Elastography, Color Doppler and contrast-enhanced sonography are applied for an improved differentiation of benign from malignant lesions, whereas 3D ultrasound mainly offers better conspicuity of lesions and their interface with normal surrounding tissue.

1.2 Clinical perspective and study aim

In this prospective study we will perform multiparametric high resolution ultrasound in patients with known suspicious breast lesions (BI-RADS 4 and 5). We will acquire morphologic by conventional B-mode and 3D US and functional information about the lesions by examination of tumor vascularity and structure as expressed by means of stiffness. It can be expected that with this multiparametric US imaging an increased sensitivity and especially specificity of US of the breast can be achieved.

2. Patients and Methods

Over a period of 24 months 120 patients with breast lesions categorized as BI-RADS 4 and 5 (3) will be included in this prospective study- that means patients with suspicious lesions, planned to undergo an image-guided biopsy or surgery. This number of patients is necessary for a hypothesized improvement of the AUC by 10% (from 0.8 to 0.9) with a type I error of 5% and a type II error of 20%. The recruitment of patients will take place immediately after a breast clinical or imaging examination (mammography, ultrasound or MRI of the breast) demonstrating a suspicious breast lesion as described previously. All patients will be examined with multiparametric ultrasound. Contrast-enhanced examination will be conducted subsequently to the non contrast-enhanced one. The whole examination time will be approximately 30 minutes. Histopathology with either image-guided biopsy or surgery will be used as the standard of reference. Both in situ and invasive carcinomas will be regarded as a malignant (positive) result whereas benign and precursor lesions will be regarded as a benign (negative) result. In case of an image-guided biopsy, this will be performed immediately after the sonographic examination, whereas in the cases of planned surgery, this will be performed according to the planning of the respective Departments of Surgery or Gynecology.

2.1 Inclusion criteria

- Clinical, mammographic, MR-tomographic and/or ultrasonographic verification of a suspicious breast lesion (BIRADS 4 and 5)
- Age > 18 years
- Written informed consent
- Histopathological verification of the lesions either by core biopsy or by surgical excision

2.2 Exclusion criteria

- Unstable or non-compliant patients
- Pregnant or lactating patients or patients with suspected pregnancy

- Known contraindication to the intravenous administration of US contrast agents
- Acute or chronic renal insufficiency
- Pre- or post-transplant patients

2.3 Imaging technique and evaluation

All patients will be examined using a CE certified Siemens Accuson S3000 ultrasound device, which is used for routine examinations in our department.

2.3.1 B-mode 2D/3D examination

2.3.1.1 Technique

For the B-mode examinations an 18L6HD linear transducer will be used. The settings that will be used are the following: Dynamic Range 75 dB, Custom Tissue Imaging 1, ASC 3, High Dynamic Tissue Contrast Enhancement, Margin 3, Persistence 3 and Space/Time encoding 3. For 3D imaging a data volume will be acquired and coronal plane images will be saved.

2.3.1.2 Image evaluation

Image evaluation of the B-mode ultrasound will be performed according to the revised ACR BI-RADS lexicon for ultrasound 5th edition, (3). Lesions will be evaluated for shape (oval, round or irregular), orientation (parallel or not), margin (circumscribed, microlobulated, indistinct, angular or spiculated), internal echogenicity (anechoic, hyperechoic, isoechoic, hypoechoic, heterogeneous or complex cystic and solid) and posterior acoustic features (none, enhancement, shadowing or combined pattern). Additionally on the coronal plane the presence or absence of retraction will be noted (22). The number and exact size of lesions in all 3 dimensions will be documented. Finally the likelihood of malignancy will be reported according to the ACR BI-RADS lexicon (3), with 4a = low suspicion for malignancy; 4b = moderate suspicion for malignancy; 4c = high suspicion for malignancy and 5 = highly suggestive of malignancy.

2.3.2 Elastography examination

2.3.2.1 Technique

For Acoustic Radiation Force Impulse (ARFI) elastography a 9L4 linear transducer and the “Virtual Touch IQ™” mode will be used, with following settings: Dynamic Range 65dB, Space/Time encoding 3, Spatial Compounding Off and Medium Dynamic Tissue Contrast Enhancement, whereas the velocity scale will be set from 0,5 to 10 m/sec. The images will be obtained by using a rectangular ROI including the lesion and at least 5 mm of normal surrounding tissue. Three 2 x 2 mm quantification ROIs will be placed in the lesion in order to measure the shear-wave velocity inside the lesion, whereas another equally big quantification ROI will be placed on fat tissue at the same depth to measure shear-wave velocity in fat tissue (24, 25).

2.3.2.2 Image evaluation

The shape (oval, round or irregular) and homogeneity (very, reasonably or not homogeneous) of the lesion as well as the elasticity ratio (lesion to fat elasticity) will be assessed, as has been proposed by Berg et al for shear-wave elastography (24). Using three ROIs placed in the lesion, the mean, maximum and minimum elasticity of the lesion (measured as shear-wave velocity in m/sec) as proposed by Berg et al and Bai et al (25)will be calculated (24).

2.3.3 Color/Power Doppler US examination

2.3.3.1 Technique

For Color/Power Doppler US examination a 18L6HD linear transducer will be used. The examination settings for the Color Doppler US examination will be as following: Dynamic Range 70dB, Low Flow, Smooth 3, Space/Time encoding 5, Persistence 2, Filter 0 and a PRF of 488. The settings for the Power Doppler examination will be the following: Dynamic Range 70dB, Low Flow, Smooth 2, Space/Time encoding 5, Persistence 2, Filter 1 and a PRF of 488. A ROI slightly bigger than the lesion will be used and cine clips of at least 5 sec per case will be saved.

2.3.3.2 Image evaluation

We will evaluate the number and distribution of vessels within and around the lesion according to the schemes proposed by Raza et al (12) and Moon et al (15). A score of 0-2 will be attributed as follows: 0= no visible vessels in the lesion; 1= one or two peripheral or central vessels; 2= three or more peripheral or central vessels or one or more penetrating vessels. Furthermore the morphology of the vessels will be assessed, as A= regular (uniform in caliber and course) or B= irregular (caliber irregularities, tortuous course) (26).

2.3.4 Contrast-enhanced examination

2.3.4.1Technique

The contrast agent used will be a CE certified standard US contrast agent approved for breast diagnostics by the Austrian Federal Office for Safety in Health Care (27), injected intravenously as a bolus at a dose of 0.03 ml (0,24 μl of sulphur hexafluoride microbubbles) per kg of body weight. Every injection will be followed by a flush with 5 ml of sodium chloride 9 mg/ml (0.9%) solution. The contrast-enhanced US examinations will be conducted using a 9L4 linear transducer and the following settings: Frame Rate 23 l/sec, Dynamic Range 80dB, Margin 3, Persistence 3, Space/Time encoding 3, MI 0,06 and MIF 0,05.

2.3.4.2 Image evaluation

For the evaluation of contrast-enhanced examinations the number and distribution as well as the morphology of vessels will be reevaluated, similarly as in the pre- contrast studies (12, 15, 26). Furthermore the degree of enhancement will be characterized as minimal (no additional vessels), moderate (one or two additional vessels) or marked (three or more additional vessels), as proposed by Moon et al (15). Finally dynamic features, i.e. Time To Peak (TTP) enhancement and the time until the elevated signal returns to baseline levels, will be noted (17). Any cases of prolonged (>5 min) enhancement will be recorded, as has been proposed by Jung et al (18).

2.4 Evaluation of multiparametric ultrasonographic data

After evaluation of US data for clinical purposes two radiologists blinded to the findings of the other imaging modalities or the histopathological diagnosis, will evaluate the multiparametric US data acquired according to the above mentioned criteria. Results of multiparametric US will be compared to the results of B-mode ultrasound.

2.5 Statistical evaluation

A McNemar test will be applied to the B-mode ultrasound alone and B-mode ultrasound with additional ultrasonographic examinations positive and negative results to determine statistical independence. ROC analysis will be performed to estimate the overall performance of B-mode ultrasound and multiparametric ultrasound. Using the histopathological data the numbers of true-negative (TN), true-positive (TP), false-negative (FN) and false-positive (FP) results for B-mode ultrasound and multiparametric US will be obtained and sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NVP) for both methods will be calculated.

3. Timetable

September 2015	Obtaining IRB approval
September 2015 to April 2017	Multiparametric ultrasound examinations according to protocol
May/June 2017	Statistical evaluation
July to September 2017	Preparation and submission of manuscripts

AMENDMENT TO THE STUDY PROTOCOL

Treatment of early breast cancer is usually surgical, comprising breast-conserving surgery for small, unifocal tumors, or mastectomy for larger or multicentric tumors. However, in women with locally aggressive disease or whose tumors display features that are predictive of early recurrence, neoadjuvant chemotherapy (NAC) is given to reduce the risk of recurrence. The early identification of chemotherapy responders and non-responders is crucial

in order to allow chemotherapy scheme modifications or changes to surgery or hormonal therapy, and thus provide optimal patient outcomes. Yet tumor response to NAC can be very different, resulting in fibrosis, concentric shrinking, fragmentation or change in the density of malignant tissue. All of these may affect the evaluation of residual tumor size (28).

Evaluation of tumor response to NAC is routinely done by using solely morphologic criteria (size reduction in mammography or B-mode ultrasound (US)), with US being more accurate than mammography in the evaluation of residual tumor size after NAC (28), showing a moderate correlation with the final histopathologic tumor size (29).

However, the potentials of a multiparametric (mp) US have not yet been explored. We hypothesize that a functional imaging of the breast using high-resolution mp US allows an improved monitoring of therapy-induced changes before morphologic changes are visible (e.g. tumor-shrinkage). Our approach focuses on the assessment of specific imaging biomarkers, which help to differentiate treatment responders from non-responders immediately after onset of NAC. The aim of this study-phase is to acquire mp US data with CP/PD, elastography and CEUS for an improved assessment of early treatment response.

For this reason, 40 patients with breast cancer undergoing NAC will be imaged before, during and after NAC/pre-surgery with mp US imaging of the breast. This number of patients is necessary for a hypothesized improvement of the AUC by 20% (from 0.7 to 0.9) with a type I error of 5% and a type II error of 20%. The participants to this part of the study will be recruited among the patients, already taking part in the first part of the study and planned to receive a NAC, according to the recommendations of the interdisciplinary tumor board.

Due to different underlying biochemical processes of the different US modalities, it can be assumed that the single modalities may have different time frames for the early detection of therapy-induced changes- yet literature data on this subject are sparse. To monitor therapy response, the patients will receive a baseline mp US examination (as already planned in the study protocol) and an additional mp US examinations on the 3rd day after the 1st cycle, in order to evaluate possible initial changes in tumor morphologic and functional parameters, induced by NAC, which might allow for an early

prediction of histologic response. Patients will be examined again in the week after the 2nd, 4th and 6th (last) cycles of NAC. In cases where 8 NAC cycles will be necessary, a further examination will be planned after the 8th cycle.
The image analysis will be conducted as described in the original study protocol.
Qualitative and quantitative imaging parameters will be correlated with histopathological tumor grade, microvascular density as an indicator of tumor-neoangiogenesis, hormone receptor status and other newer factors such as HER2-neu proliferative indices and gene expression profile before and after NAC.
For the assessment of treatment response, functional imaging data of the baseline examination and of the examinations after the 1st, 2nd, 4th and 6th (+/- 8th) cycle will be compared for therapy-induced changes of both lesion morphology and functional capabilities by two experienced radiologists independently.
The statistical evaluation will be performed as described in the original study protocol.

CHANGES IN THE STUDY PROTOCOL

- Additional mp US examinations after the first, second, fourth and sixth (and after the eighth, if 8 NAC cycles are necessary) cycle of NAC.
- Correlation of qualitative and quantitative imaging parameters with histopathological tumor grade, microvascular density, hormone receptor status, HER2-neu, proliferative indices and gene expression profile before and after NAC.

4. Literature

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