RODRIGO MENEZES JALES

Importância da ultrassonografia na predição de malignidade e sua correlação com os fenótipos Luminal, Her 2 *overexpression* e Triplo Negativo nos nódulos de mama classificados na categoria BI-RADS® US 4

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Importância da ultrassonografia na predição de malignidade e sua correlação com os fenótipos Luminal, Her 2 *overexpression* e Triplo Negativo nos nódulos de mama classificados na categoria BI-RADS® US 4

RODRIGO MENEZES JALES

Tese de Doutorado apresentada ao programa de Pós-Graduação em Tocoginecologia, da Faculdade de Ciências Médicas da Universidade Estadual de Campinas para obtenção do titulo de Doutor em Ciências da Saúde, Área de Concentração Oncologia Ginecológica e Mamária, sob orientação da Profª. Drª. Sophie Françoise Mauricette Derchain.

Campinas, 2012

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Banca examinadora:
Sophie Françoise Mauricette Derchain [Orientador]
Francisco Mauad Filho
Simone Elias
Aarão Mendes Pinto Neto
Cassia Raquel Teatin Juliato

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BANCA EXAMINADORA DA TESE DE DOUTORADO

Aluno: RODRIGO MENEZES JALES

Orientadora: Profª. Drª. SOPHIE FRANÇOISE MAURICETTE DERCHAIN

Membros:

1.
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3.
4.
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Curso de Pós-Graduação em Tocoginecologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas

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Dedico este trabalho...

Aos meus filhos Pedro e Antônio que,
como eu, possam realizar seus sonhos.

À Karla,
marinheira, companheira, amiga, esposa, mulher.

Aos meus pais Maria Helena e Alfredo,
por tudo.

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Sumário

Símbolos, Siglas e Abreviaturas

ACR – *American College of Radiology*

AUC – *Area Under Curve* / Área Sob a Curva

BI-RADS® – *Breast Imaging and Reporting Data System*

BI-RADS®-MG – *Breast Imaging and Reporting Data System Mammography*

BI-RADS®-US – *Breast Imaging and Reporting Data System Ultrasound*

BMP – Bitmap

HM-CAISM – Hospital da Mulher Prof.Dr. José Aristodemo Pinotti -Centro de Atenção Integral à Saúde da Mulher

CEP – Comitê de Ética em Pesquisa

DCIS – *Ductal Carcinoma in situ*

e.g. – *exempli gratia / for example*

EGFR – *Epidermal Growth Factor Receptor*

Her2 – *Human Epidermal growth factor Receptor 2*

i.e. – *that is*

IC – *Interval Confidence*

IDC – *Invasive Ductal Carcinoma*

IHC – *Immunohistochemistry*

ILC – *Invasive Lobular Carcinoma*

MG – Mamografia / *Mammography*

MHz – Megahertz

mm – Milímetro(s)

OR – *Odds Ratio*

p – *Significance*

PACS – *Picture Archiving and Communication System*

PASH – *Pseudoangiomatous Stromal Hiperplasia*

PPV – *Predictive Positive Value*

RE / ***ER*** – Receptor de Estrógeno / *Estrogen Receptor*

REF – *Reference Variable*

RNM / **MRI** – Ressonância Nuclear Magnética / *Magnetic Nuclear Resonance*

ROC – *Receiver Operator Curve*

RP / **PR** – Receptor de Progesterona / *Progesterone Receptor*

SD – *Standard Desviation*

US – Ultrassonografia / *Ultrasound*

USA – *United States of America*

Resumo

Objetivo: Avaliar a importância da ultrassonografia na predição de malignidade e sua correlação com os fenótipos Luminal, Her 2 *overexpression* e Triplo Negativo nos nódulos de mama classificados na categoria BI-RADS® US 4. **Objetivo Artigo 1:** avaliar se a medida ultrassonográfica do diâmetro dos cistos pode contribuir com a predição de malignidade em um tipo específico de nódulos complexos classificados na categoria BI-RADS®-US 4. **Objetivo Artigo 2:** avaliar as características ultrassonográficas de nódulos mamários classificados na categoria BI-RADS®-US 4 associadas aos fenótipos Luminal, HER2 *overexpression* e Triplo Negativo. **Sujeitos e métodos:** No primeiro artigo foram incluídos em um estudo de corte transversal 48 casos de nódulos com características ultrassonográficas sugestivas de benignidade, entretanto apresentando no seu interior pelo menos um componente cístico. Todos os nódulos foram biopsiados (25 biópsias de fragmento; 23 biópsias de fragmento seguidas de biópsia excisional). O exame anatomopatológico classificou 12/48 (25%) casos como malignos. O maior diâmetro do nódulo, o maior diâmetro do cisto e o padrão de vascularização ao Doppler foram avaliados na predição de malignidade. No segundo artigo, foram selecionados em um estudo de corte transversal 327 nódulos classificados nas categorias BI-RADS®-US 4a, 4b e 4c. Todos os nódulos foram biopsiados. Os resultados

anatomopatológicos foram classificados em benigno 195 (60%) ou maligno 132 (40%). Os nódulos malignos foram então agrupados em três subtipos fenotípicos: Luminal, Her 2 overexpression e Triplo Negativo. As características ultrassonográficas dos nódulos foram comparadas com a categorização fenotípica. **Resultados:** no primeiro artigo, o padrão da vascularização[presente na lesão (p=1) ou presente imediatamente adjacente à lesão (p=0,46)] não esteve relacionado com a malignidade, enquanto os maiores diâmetros do nódulo e do cisto apresentaram uma relação significativa com a malignidade (p=0,02 e p<0,001, respectivamente). No segundo artigo, as subcategorias BI-RADS®-US 4a, 4b e 4c não se relacionaram claramente aos fenótipos Luminal, Her2 overexpression ou Triplo Negativo. Entretanto, margens espiculadas, margens indistintas, halo ecogênico e reforço acústico posterior relacionaram-se significativamente com o fenótipo Luminal. Além disso, margens circunscritas e atenuação das ondas de ultrassom relacionaram-se positivamente com o fenótipo Triplo Negativo. Nenhuma característica ecográfica associou-se ao fenótipo Her2 *overexpression*. **Conclusões:** O primeiro artigo traz o conceito inédito de que o diâmetro máximo do cisto é um bom preditor de malignidade em nódulos complexos que, exceto pela presença de um ou mais cistos, seriam classificados como provavelmente benignos (BI-RADS®-US 3). O segundo artigo está em concordância com o conhecimento atual de que existe associação entre variáveis ultrassonográficas como margens, halo ecogênico e características acústicas posteriores e os subtipos fenotípicos Luminal e Triplo Negativo. Entretanto, na amostra avaliada, essa associação não se manifestou claramente na subcategorização BI-RADS®-US 4a, 4b e 4c.

Summary

Objective: To evaluate the importance of ultrasound in predicting malignancy and its correlation with the phenotypes Luminal, Her 2 overexpression and Triple Negative in breast masses classified as BI-RADS®-US 4. Article 1: To assess whether cyst diameter might contribute to the prediction of malignancy in complex breast masses. Article 2: To assess the sonographic characteristics of BI-RADS®-US 4 breast masses in the Luminal, Triple Negative and HER2 phenotypes. **Methods**: In the first article, in a cross-sectional study, we identified 48 breast masses that had sonographic features suggestive of benignity, but presenting at least one cystic component. All breast masses were biopsied (25 core-needle; 23 core-needle and excision). Subsequent histologic analysis was performed and 12/48 (25%) malignancies were identified. Different sonographic measurements (largest diameter of the mass and cyst, vascular pattern) were assessed for the detection of malignancy. In the second article, in a cross-sectional study, we selected 327 masses classified in subcategories BI-RADS®-US 4a, 4b and 4c. All masses were biopsied. The pathologic results were classified as benign 195 (60%) or malignant 132 (40%). The malignant masses were further grouped into three phenotypic subtypes: Luminal, Her 2 overexpression and

Triple Negative. We then compared the sonographic features of the malignant lesions according to the phenotypic status of the masses. **Results:** In the first article, among sonographic features, vascular pattern [(present in the lesion (p=1.0) or present immediately adjacent to the lesion (p=0.46)] was not associated with malignancy, whereas the largest mass and cyst dimension had a significantly positive correlation (p=0.02 and p<0.001, respectively) with tumor malignancy. In the second article, the subcategories BI-RADS®-US 4a, 4b and 4c were not clearly related to the phenotypes Luminal, Her2 overexpression or Triple Negative. However, spiculated margins, indistinct margins, echogenic halo, and posterior acoustic shadowing were significantly correlated with the Luminal phenotype. Moreover, circumscribed margins and attenuation were positively related to the Triple Negative phenotype. No sonographic variable was associated with Her2 overexpression phenotype. **Conclusions:** The first article presents the new concept that cyst diameter is a good predictor of malignancy in complex breast tumors which, except for the presence of the anechoic formation, would otherwise be rendered as probably benign (BI-RADS® 3). The second article is in agreement with current knowledge that there is an association between ultrasound features as margins, posterior acoustic features and lesion boundary and phenotypic subtypes Luminal and Triple Negative. In our sample, this association was not clearly expressed in the subcategorization BI-RADS®-US 4a, 4b and 4c.

1. Introdução

O câncer de mama é a principal causa de morte por câncer entre as mulheres no mundo, sendo responsável por 458.400 mortes em 2008 (1). No Brasil a estimativa mais recente aponta 11.735 mortes por câncer de mama em 2008, sendo esta também a principal causa de morte por câncer em mulheres desde a década de 90 (2) Além disso, a cada ano, quase um milhão e meio de mulheres são diagnosticadas com câncer de mama em todo o mundo (1). No Brasil foram estimados 49.240 casos novos em 2010, com profundos impactos emocionais e econômicos (2, 3).

Entre 1980 e o final da década de 1990, a taxa de incidência do câncer de mama nos países ocidentais aumentou aproximadamente 30%. As razões para essa elevação não estão completamente esclarecidas mas provavelmente refletem mudanças nos padrões de reprodução, prevalência de obesidade e sedentarismo, maior aderência das mulheres ao tabagismo e às políticas de rastreamento ao câncer de mama (4, 5, 6, 7, 8).

O rastreamento do câncer de mama tem a finalidade de identificar lesões pequenas, ainda restritas à mama, que presumivelmente respondem melhor às modalidades de tratamento disponíveis (7). Diferentes métodos de imagem como a mamografia (MG), a ressonância nuclear magnética (RNM) e a ultrassonografia (US), além do exame clínico das mamas, podem detectar lesões assintomáticas (9). Entre os métodos de imagem, revisões sistemáticas de estudos randomizados atribuem ao rastreamento mamográfico uma redução na mortalidade ao redor de 15%, tornando a mamografia o método de imagem padrão para o rastreamento (10). Evidências bem fundamentadas sugerem que a recomendação do autoexame das mamas pelas mulheres não reduz a mortalidade por câncer de mama (9).

Dados recentes referentes à última década revelam redução da mortalidade por câncer de mama em países desenvolvidos, sobretudo nos EUA e na Europa Ocidental (8,11, 12, 13). A participação do rastreamento mamográfico nessa redução é controversa, pois essa conquista também estaria associada à diminuição de fatores de risco, como a redução no uso de terapia hormonal combinada para a menopausa e melhores opções de tratamento (8, 14,15).

Por outro lado, o rastreamento mamográfico apresenta efeitos indesejáveis como exames complementares e biópsias desnecessárias, relacionados a resultados falsos positivos e danos associados ao tratamento de cânceres que nunca se tornariam palpáveis ou mesmo que se tornassem palpáveis não encurtariam a vida da mulher. Evidências recentes sugerem que até 50% dos cânceres de mama diagnosticados por métodos de imagem apresentam crescimento lento e

não progridem para formas letais de metástases a distância, o que é definido como *overdiagnosis* (7, 10, 16).

O *Breast Imaging and Reporting Data System* (BI-RADS®) é uma publicação do Colégio Americano de Radiologia que tem como objetivo homogeneizar a confecção dos laudos, reduzindo a confusão na interpretação das imagens mamárias, além de facilitar a monitorização dos resultados (17). A primeira edição, publicada em 1992, referia-se apenas à mamografia (BI-RADS®-MG). Desde então o BI-RADS® foi aceito, com pequenas variações, pela maioria das organizações relacionadas ao câncer de mama e pela maior parte dos radiologistas em todo o mundo (18). A edição mais recente, a quarta, foi publicada em 2003 e trouxe, pela primeira vez, conceitos relacionados à ultrassonografia (BI-RADS®-US) (17).

A ultrassonografia mamária apresenta alta especificidade na avaliação dos nódulos mamários, podendo acrescentar ao estudo mamográfico informações quanto à textura, orientação, limites e margens (19). Textura anecoica ou hiperecogênica, orientação paralela, limites abruptos e margens circunscritas são relacionados a nódulos benignos. Orientação não paralela, halo ecogênico, margens microlobuladas, anguladas, indistintas ou espiculadas são características de nódulos malignos (17). Assim, a quarta edição do BI-RADS® recomenda que os nódulos ultrassonográficos sejam classificados, de acordo com suas características morfológicas, em uma das seguintes categorias: BI-RADS®-US 0 (necessária complementação com outros exames), BI-RADS®-US 1 (exame negativo), BI-RADS®-US 2 (exame benigno), BI-RADS®-US 3 (exame provavelmente benigno), BI-RADS®-US 4a (exame com baixa suspeita de malignidade), BI-RADS®-US

4b (exame com suspeita intermediária de malignidade), BI-RADS®-US 4c (exame com suspeita moderada de malignidade), BI-RADS®-US 5 (exame com alta suspeita de malignidade) ou BI-RADS®-US 6 (exame com malignidade previamente confirmada). Nódulos ovais, hipoecóicos, circunscritos, com orientação paralela e limites abruptos apresentam risco de malignidade <2%, devem ser classificadas na categoria BI-RADS® 3 e podem ser acompanhados sem biópsia. A categoria BI-RADS® 5 representa nódulos com todas as características ultrassonográficas suspeitas, apresentam risco de malignidade >95% e devem ser biopsiados (17). A categoria BI-RADS®-US 4 representa um grupo bastante heterogêneo de lesões, com diferentes aspectos de imagem e risco de malignidade variável entre 3% e 94% (17). Assim, a categoria BI-RADS® 4 responde pela maior parte das indicações de biópsia (20).

O BI-RADS®-US estabelece que nódulos com componentes sólidos e císticos devem ser classificados como complexos, incluidos na categoria BI-RADS® 4, e não devem ser confundidos com cistos complicados ou microcistos coalescentes, os quais, se não palpáveis, devem ser classificados na categoria BI-RADS® 3. Cistos complicados são caracterizados por serem preenchidos por líquido espesso, cujo aspecto ultrassonográfico é caracterizado por debris em suspensão ou depósito de sedimentos, que se movem à mudança de decúbito (21). Microcistos coalescentes são definidos como um grupamento de microcistos, medindo até 3mm de diâmetro, separados por finas septações, medindo até 0,5mm, sem um único componente sólido identificável (17).

Dessa maneira, nódulos com todas as características ultrassonográficas de benignidade, ou seja, ovais, circunscritos, paralelos, com limites abruptos, mas apresentando áreas císticas no seu interior, devem ser classificados na categoria BI-RADS® 4. Imagens císticas no interior de nódulos com características benignas são um achado relativamente comum; entretanto o seu significado continua incerto (22).

Em concordância com estudos prévios, os nódulos complexos, ou seja com textura sólida e cística, são classificados em quatro tipos, de acordo com a proporção do componente cístico, desde imagens císticas septadas ou com paredes espessas (tipo I) até nódulos predominantemente sólidos com áreas císticas centrais ou periféricas (tipo IV) (23). A prevalência de malignidade é maior em nódulos predominantemente sólidos, variando desde 18%, em uma série de 38 nódulos, até 62% em uma amostra de 53 casos (23, 24, 25). Entretanto, nesses estudos, características fundamentais dos nódulos como margens, forma, orientação e limites não foram consideradas. Além disso, esses estudos não avaliaram se características das áreas císticas, como o seu maior diâmetro, apresentam algum valor preditivo para malignidade.

Além de apresentar valor na predição da malignidade, alguns achados de imagem, inclusive ultrassonográficos, podem estar relacionados com o comportamento biológico dos tumores e, consequentemente, com marcadores moleculares de prognóstico (26, 27, 28, 29, 30). O câncer de mama é um grupo heterogêneo de doenças, com marcante heterogeneidade morfológica, histopatológica e molecular. A heterogeneidade molecular entre as apresentações do câncer de mama geralmente cursa com diferentes apresentações clínicas,

prognósticos e respostas a esquemas terapêuticos (31). A determinação de subtipos genéticos do câncer de mama, seus correspondentes fenotípicos e a sua correlação com diferentes padrões de mortalidade têm sido amplamente aceitos, independentemente de outros marcadores clínicos e patológicos e da terapia sistêmica recebida (32, 33).

Geralmente há uma classificação hierarquizada dos fenótipos relacionados ao câncer de mama. Assim, os tumores são inicialmente avaliados quanto aos receptores de estrógeno (RE) e progesterona (RP). Os tumores que expressam algum desses receptores são classificados como Luminais, enquanto os tumores que não apresentam estes receptores são Luminais negativos. Os tumores Luminais apresentam prognóstico favorável em relação aos Luminais negativos e respondem à terapia com antiestrogênicos (34, 35). Os tumores são então classificados em relação à expressão do *Human Epidermal growth factor Receptor 2* (Her 2). Assim, os tumores Luminais negativos com positividade para o Her2 são definidos como Her2 *overexpreesion* e apresentam prognóstico desfavorável, mas respondem ao tratamento com bloqueadores da atividade do Her2, como o anticorpo monoclonal Traztuzumab (36, 37, 38). Tumores Luminais negativos, sem expressão Her 2, são classificados como Triplo Negativo e apresentam prognóstico desfavorável, com risco aumentado para metástases viscerais e no sistema nervoso central, além de não apresentarem resposta à terapia adjuvante com antiestrogênicos ou ao anticorpo monoclonal anti-Her2 (36, 38, 39).

Cada subtipo fenotípico especificado poderia ainda ser subdividido de acordo com a expressão de marcadores basais, como o *Epidermal Growth Factor*

Receptor (EGFR) e as citoqueratinas 5/6, que são importantes do ponto de vista da mortalidade, mas não são utilizados na rotina assistencial (32, 40). Além disso, ainda há uma discordância evidente na correlação entre os subtipos fenotípicos, embasada nos marcadores imuno-histoquímicos, e os subtipos baseados na expressão genética e mesmo para a determinação da positividade dos RE, RP e Her2 (31, 41).

O desenvolvimento de um esquema de classificação que incorpore achados de imagem e subtipos fenotípicos poderia predizer mais adequadamente o prognóstico e a resposta a regimes terapêuticos específicos, facilitando a tomada de decisões (27). A relação entre alguns aspectos de imagens e subtipos fenotípicos já é conhecida. Dessa maneira, margens espiculadas já foram relacionadas ao fenótipo Luminal e margens circunscritas, ao fenótipo Triplo Negativo (26, 29, 30, 42). Entretanto, ainda permanece desconhecida se a relação entre aspectos específicos de imagem e subtipos fenotípicos são suficientes para determinar a classificação BI-RADS® dos nódulos mamários. Atualmente a classificação BI-RADS® facilita a tomada de decisões em relação ao risco de malignidade; entretanto não sugere o comportamento biológico do tumor, caso a malignidade seja confirmada. Esse conceito favoreceria a identificação de lesões relacionadas a maior letalidade, antes da confirmação histológica.

Esse estudo visa à determinação de conceitos, até onde se sabe, inéditos, aplicáveis à ultrassonografia mamária, mais especificamente em relação aos nódulos classificáveis na categoria BI-RADS®US 4. Foi avaliada a utilidade da ultrassonografia na predição de malignidade em uma categoria específica de nódulos complexos, homogêneos em relação à forma, margens, limites e orientação,

que a não ser pela presença de pelo menos um componente cístico, seriam classificados como provavelmente benignos (BI-RADS®US 3). Foi analisado o valor da maior dimensão do maior componente cístico identificável pela ultrassonografia no interior desses nódulos na predição de malignidade. Essa informação poderá ser utilizada na indicação de biópsias relacionadas ao tipo específico de nódulo avaliado, o que poderia reduzir o número de biópsias desnecessárias. Além disso, foi avaliada a correlação entre as subcategorias BI-RADS®US 4a, 4b e 4c e os fenótipos relacionados ao prognóstico do câncer de mama: Luminal, Her 2 *overexpression* e Triplo negativo. Esse conceito pode contribuir para o reconhecimento de categorias de imagens relacionadas a tumores agressivos, o que poderá ajudar a diminuir o diagnóstico de neoplasias sem importância clínica ou agilizar o diagnóstico de neoplasias com comportamento biológico agressivo.

2. Objetivos

2.1. Objetivo Geral

Avaliar a importância da ultrassonografia na predição de malignidade e sua correlação com os Fenótipos Luminal, Her 2 *overexpression* e Triplo Negativo nos nódulos de mama classificados na categoria BI-RADS® -US 4.

2.2. Objetivos Específicos

- Avaliar se a medida ultrassonográfica do diâmetro dos cistos pode contribuir com a predição de malignidade em nódulos complexos.
- Avaliar as características ultrassonográficas de nódulos mamários classificados na categoria BI-RADS® -US 4 associadas aos fenótipos Luminal, HER2 *overexpression* e Triplo Negativo.

3. Publicações

Artigo 1 – **Complex breast masses; assessment of malignant potential based on cystic diameter**

Artigo 2 – **Sonographic features of BI-RADS®-US 4 breast masses in Luminal, HER2 overexpression and Triple Negative phenotypes**

3.1. Artigo 1

> Date: Fri, 21 Oct 2011 10:13:30 -0400
> From: jum@aium.org
> To: rodrigoj@hotmail.com
> Subject: Ms. 11-0616 Version 5 Complex breast masses; assessment of malignant potential based on cystic diameter.
>
> 11-0616 Version 5 "Complex breast masses; assessment of malignant potential based on cystic diameter."
>
> Dear Dr Jales:
>
> Your manuscript has been accepted for publication in the Journal of Ultrasound in Medicine. Your manuscript will appear in the journal in approximately 5 months.
>
> You will receive your manuscript proofs by e-mail approximately 3 weeks prior to the publication date. During copyediting, some wording changes may be made; however, there will be no alteration of scientific content. When you receive your page proofs, please read, correct, and return them as soon as possible so that publication will not be delayed.
>
> A disclosure form must be completed by all authors and e-mailed to jum@aium.org. To obtain this form, go to
>
> http://www.aium.org/publications/jum/authors.aspx.
>
> Thank you for submitting this interesting work to the Journal of Ultrasound in Medicine.
>
> Sincerely,
>
> Levon Nazarian, MD, Editor-in-Chief
> Journal of Ultrasound in Medicine
> American Institute of Ultrasound in Medicine
> 14750 Sweitzer Lane, Suite 100
> Laurel, Maryland 20707-5906
> USA
> jum@aium.org

Complex breast masses; assessment of malignant potential based on cystic diameter

Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil

Authors / *scientific address*

Rodrigo Menezes Jales, MD, Msc: *Department of Obstetrics and Gynecology Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Luis Otávio Sarian MD, PhD: *Department of Obstetrics and Gynecology Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Cleisson Fábio Andrioli Peralta MD, PhD: *Department of Obstetrics and Gynecology Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Renato Torresan MD, PhD: *Department of Obstetrics and Gynecology Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Emílio Francisco Marussi MD, PhD: *Department of Obstetrics and Gynecology Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Beatriz Regina Alvares, MD, PhD: *Department of Radiology, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Sophie Derchain MD, PhD: *Department of Obstetrics and Gynecology Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Full mailing and e-mail address of corresponding author

Sophie Derchain

E-mail: derchain@fcm.unicamp.br

Mailing Address: Departmento de Ginecologia e Obstetricia, Faculdade de Ciências Médicas, caixa postal 6111, Universidade Estadual de Campinas – UNICAMP

CEP 13083-970, Campinas, SP, Brasil.

telephone number: 55 19 3521-9500

Abstract

Objective: to assess whether cyst diameter might contribute to the prediction of malignancy in complex breast masses. **Methods:** In this cross-sectional study, we identified 48 breast masses that had sonographic features suggestive of benign breast lesions (oval shape, circumscribed margins, parallel axis, abrupt limits). However, these masses were categorized as BI-RADS® 4 due to the presence of at least one cyst (complex echogenicity). All breast masses were biopsied (25 core-needle; 23 core-needle and excision). Subsequent histologic analysis was performed and 12/48 (25%) malignancies were identified. Mammographic features were reviewed. Different sonographic measurements (largest diameter of the mass and cyst, vascular pattern) were assessed for the detection of malignancy. **Results:** Among sonographic features, vascular pattern, i.e. the detection of blood flow (present in the lesion ($p>0.99$) or present immediately adjacent to the lesion ($p=0.46$)) was not associated with malignancy, whereas the largest mass and cyst dimension had a significantly positive correlation ($p=0.02$ and $p<0.001$, respectively) with tumor malignancy. In ROC analysis, the point with the highest sum of sensitivity and specificity corresponded to maximum cyst diameter=8mm (sensitivity=67%; specificity=86%). The positive and negative predictive values at that cut-off point were 61% and 86%, respectively. The area under the curve (AUC) was 0.772. In our series, all masses with cyst <3mm in diameter (7 cases) were benign and all masses with cyst >13mm in diameter (4 cases) were malignant. **Conclusions:** Cyst diameter is a good predictor of malignancy in complex breast masses which, except for the presence of the internal cyst(s), would be otherwise rendered as BI-RADS® 3.

Key words: breast ultrasound, breast cancer, complex masses, circumscribed masses, BI-RADS.

Introduction

On ultrasonographic examination, a hypoechoic, oval, circumscribed, parallel (wider-than-tall) breast mass, with an abrupt interface is probably benign.[1] The Breast Imaging Reporting and Data System (BI-RADS®) classifies this type of mass as BI-RADS® category 3 based on the assumption that there is less than a 2% probability of malignancy (American College of Radiology (ACR).[2] Cysts contained within these masses are a relatively common finding, even though the clinical significance of these cystic foci remains uncertain.[3] The BI-RADS® assigns complex cysts with echogenic/ solid and anechoic/cystic components to category 4, thus warranting histologic analysis, regardless of mass shape, margin, axis orientation, lesion boundary and ultrasonographic characteristics of the cystic components.[2]

Furthermore, BI-RADS® established that complex breast masses on ultrasound should not be mistaken with both complicated cysts and clustered microcysts which, when not palpable, may be rendered as probably benign and allotted to the BI-RADS® 3 category and managed for short-interval follow-up.[4] Complicated cysts are characterized by homogeneous low-level internal echoes and may have a layered appearance. These cysts may contain brightly echogenic foci that scintillate as they shift. Fluid-debris levels may also shift with changes in patient position.[4] Clustered microcysts are described as a cluster of tiny (individually measuring <3 mm in diameter) anechoic foci, with thin (< 0.5 mm) septations with no discrete solid component.[2] In addition, ultrasonograhy can depict if a complex cyst has at least a 50% cystic portion (intracystic mass) or is predominantly solid and has at least a 50% solid component (complex mass).[4]

According to previous studies, complex cysts are classified according to the proportion of solid component. Thus, breast masses are classified into four types,

ranging from thick outer walls and/or thick internal septa (Type 1) to predominantly solid masses (complex breast masses) with eccentric or central cystic foci (Type 4). The prevalence of malignancy is higher in predominantly solid type 4 masses, varying widely among different studies from 18% in a series of 38 women to 62% in another sample of 53 masses.[5,6,7] However, in those studies only complex breast masses with eccentric or central cystic foci were reported and major sonographic features, e.g. shape, margins and axis orientation, were not taken into account. Most importantly, those studies did not assess whether cyst characteristics, such as maximum diameter, had a predictive value for malignancy.

In the present study, we examined a series of complex breast masses that otherwise would have been classified as probably benign, except for the presence of at least one cyst. Our objective was to examine whether cyst size could predict the risk of malignancy in a homogeneous study sample with regard to confounding factors, e.g. shape, axis orientation and margins, and to assess if the presence of a discrete cystic component should categorize the otherwise probably benign lesion as a complex mass.

Methods

This study was approved by our institutional review board and all participants signed an informed consent term.

Study design

In this cross-sectional study, we consecutively examined a series of 1549 women referred for breast sonography due to various medical reasons, e.g. ultrasound follow-up of breast masses previously categorized as BI-RADS® 3 (464/1549 (30%)),

evaluation of palpable masses (340/1549 (22%)), ultrasound screening in high-risk patients with mammographically dense breasts (263/1549 (17%)) and ultrasound evaluation of masses categorized as BI-RADS® 0 by mammography (186/1549 (12%)). Among the cases in which abnormalities were detected, we further selected 48/1549 (3%) cases with oval, circumscribed, parallel (wider-than-tall) breast mass with an abrupt interface, containing at least one cystic component (complex echogenicity), that otherwise would have been classified as probably benign. All women were examined in the Ultrasound Division of the *Hospital José Aristodemo Pinotti* (HM-CAISM) from March 2009 to March 2011.

Ultrasonography was performed with a 12MHz linear-array transducer (Accuvix V 10, Medison CO, Korea), and examinations were recorded in Bitmap (BMP) format. Breast sonographic findings included: echogenicity, margins, shape, axis orientation, lesion boundary, presence of internal cystic areas and cyst diameters. The BI-RADS® lexicon defines that oval masses may have up to 3 macrolobulations. Breast masses exhibiting more than 3 macrolobulations were considered to have an irregular shape and therefore were not included in the sample.[2] The largest cyst diameter was the largest length identified within the largest cystic foci (Figure 1A). There was no restriction to size or number of cysts, since the solid component predominated (Figure 1B). Vascular pattern was assessed on Power Doppler imaging. Posterior acoustic features were not taken into account for categorization.

Mammography was performed in 33/48 cases. Women younger than 40 years who received benign histologic diagnoses did not undergo mammography (15/48 cases). The majority of patients undergoing mammography (30/33 cases) were categorized as BI-RADS® 0 (oval, circumscribed masses (25/30 cases), with obscured margins (5/30 cases).

In 2/33 cases, the mammogram result was negative (BI-RADS®-MG 1), i.e. the ultrasound image corresponding to the mass was not identified. On one mammogram, a focal area of asymmetry, categorized as BI-RADS® 3, matched to the sonographic findings.

All masses were biopsied using core-needle biopsy techniques performed under US guidance, antisepsis and local anesthesia. A median number of 4 (3–8) tissue samples were obtained using an automated biopsy gun with a 14-gauge needle (Bard Magnum; (Bard Biopsy Systems, Tempe, Arizona, USA). An experienced pathologist performed histologic analysis. Complete surgical excision was recommended after core-needle biopsy in 23/48 (48%) cases. Indications for excision were positivity for malignancy on core-needle biopsy in 12/23 (52%) cases, patient decision to completely remove the lesion in 10/23 (43%) cases, and pathologist request (one case) for additional pathology material following a diagnosis of sclerosing adenosis on core-needle biopsy (the final pathologic diagnosis was complex fibroadenoma after complete excision).

Statistical analysis

All calculations were performed with a software designed by the R Project for Statistical Computing.[8] Statistical significance was set at 95% (p=0.05). We compared mean cyst dimension and mean mass dimension in benign and malignant breast masses using the Student's t-test. The presence of vascularity in or adjacent to the lesion was compared by Fisher's exact test. A graphical display of the Receiver Operator Characteristics (ROC) curve was generated to assess the diagnostic potential of cyst dimension in malignancy. Patients with benign and malignant masses were compared, according to age by the Mann-Whitney U-test and according to menopausal status and

family history of breast cancer by Fisher's exact test. The key clinical and sonographic features of complex breast masses are displayed in Table 1.

Results

The key clinical and sonographic features of complex breast masses (oval, circumscribed, parallel, abrupt limits, containing at least one anechoic image) are displayed in Table 1. Women who presented with malignant masses were significantly older (61$\pm$17.9 years) than their counterparts who had benign disease (37.5$\pm$12.4 years) ($p<0.001$) and were more frequently postmenopausal ($p=0.005$). Having a positive family history of breast cancer ($p=0.55$) or a palpable lesion ($p=0.46$) were features not associated with malignancy. Among the sonographic features, vascular pattern was not associated with malignancy (present in the lesion ($p>0.99$) or immediately adjacent to the lesion ($p=0.46$)); whereas largest mass dimension ($p=0.02$) and largest cyst dimension had a significantly positive correlation ($p=0.02$) with malignancy. All masses with cyst < 3mm in diameter (7 cases) were benign and all masses with cyst > 13mm in diameter (4 cases) were malignant ($p<0.01$) (Figures 2, 3, 4, 5 and 6).

Table 2 depicts the histologic diagnoses of oval, circumscribed, parallel, abrupt limits, breast masses, containing in their interior at least one anechoic image. Of the 48 complex masses, 29 (60%) were diagnosed as fibroadenoamas, whereas the most prevalent diagnosis in the "malignant" group was invasive ductal carcinoma [6/12 (50%) cases]. Overall, 36/48 (75%) cases were considered benign and 12/48 (25%) cases were diagnosed as malignant. The 25 women diagnosed with a benign mass after core-needle biopsy are currently receiving clinical follow-up, along with annual routine US and mammography, according to the patient's age and breast density. To date, the follow-up

time ranges from 6 to 30 months and none of the women have had any additional changes warranting excisional biopsies.

Figure 7 depicts the ROC curve for the largest cyst diameter as a predictor of breast malignancy. The point with the highest sum of sensitivity and specificity corresponds to maximum cyst diameter=8mm (sensitivity=67%; specificity=86%, positive predictive value=61%, negative predictive value = 86%, area under the curve (AUC) = 0.772).

Discussion

This study demonstrated that the diameter of a cyst contained within a breast mass diagnosed as probably benign on ultrasound, strongly correlates with a pathologic diagnosis of malignancy. In addition, in our series all masses with a cyst < 3mm in diameter were benign and all masses with cyst > 13mm in diameter were malignant (Table 2). Although our sample was relatively small, masses were homogeneous in terms of margins (circumscribed), shape (oval), axis orientation (parallel) and lesion boundary (abrupt limits). Our study was based on a sample of more than 1500 breast sonograms. Of the total number of examinations, only 3% had the desired features. In other smaller and heterogeneous samples studied, the malignancy rate of circumscribed masses was lower (9%) than in our study (25%). However, in our study the malignancy rate of complex masses (defined as cystic masses with a predominantly solid component) was similar to the results of other study (18%).[9]

In the present study, half (6/12) of the malignant masses were invasive ductal carcinomas (IDC), the most common histologic type. This may explain the high prevalence rate of IDC in our sample, although sonographic characteristics were unusual.

In general, these lesions are irregular or round, spiculated and not parallel (taller-than-wide) masses detected by ultrasound. It is important to know that all lesions were categorized as high-grade in our sample and on sonography may have circumscribed margins due to a rapid growth pattern.[10] Colloid and medullary carcinomas usually have sonographic features suggestive of benign breast lesions, but these neoplasms are rare in comparison to invasive ductal carcinomas[11].

Concerning benign lesions, complex fibroadenomas tend to be smaller and more frequent in older women when compared to simple fibroadenomas. These findings are related to the time elapsed since the beginning of cellular abnormalities inherent to fibroadenoma formation and the regression of mass cellularity over the years.[12] Although there are striking pathological differences between complex and simple fibroadenomas, the sonographic and mammographic features of these lesions generally overlap.[12] Because of the high proportion of complex fibroadenomas in our sample (Table2), given the age distribution of the patients, we suspect that the presence of anechoic formation in these masses may be associated with complex fibroadenomas. This is important because the relative risk of invasive breast carcinoma among women with complex fibroadenomas is 3.1 (95% CI: 1.9 – 5.1) when compared to the general population.[13]In the present study, we found one case of complex fibroadenoma containing a focal area of invasive lobular carcinoma. This was not entirely unexpected, considering the prevalence of roughly 2% malignancy rate among complex fibroadenomas.[12]

In our sample, only one patient received a diagnosis of phyllodes tumor. On ultrasonography, these tumors generally manifest themselves as oval, circumscribed masses. Cystic components are not the rule in such cases.[14]However, anechoic areas are more common in phyllodes tumors than in fibroadenomas.[15]Therefore the prevalence of

phyllodes tumor was expected to be somewhat higher in our sample. It should be emphasized that it may be difficult for the pathologist to distinguish fibroadenomas from phyllodes tumors, especially when examining small tissue samples.[16]In our study, this situation occurred in 25 core-needle biopsies. It was likely that more phyllodes tumors could have been found if excision had been performed in all cases.

Of the clinical variables scrutinized, only patient age and menopausal status were significantly associated with malignancy. This was hardly surprising since it is well-known that the likelihood of a woman having malignant breast tumor increases with advancing age.[17] Furthermore, 1/12(8%) women diagnosed with a malignant tumor reported having a close family member with breast cancer. In our sample, the prevalence of family history of breast cancer in this particular type of mass was consistent with the prevalence of expected positive family history of breast cancer in breast cancer patients.[18,19] Another important clinical finding in our study was that 3/12 of malignant tumors were nonpalpable masses. This finding reinforces the concept that oval, circumscribed, parallel, abrupt limits masses, containing cystic areas, should be assigned to BI-RADS® 4 category, regardless of palpability.

The vast majority of breast masses (20/25 (80%)) were assigned to BI-RADS® 0 category on mammography. Ultrasonography is of great importance in these cases to characterize mass texture, according to BI-RADS® recommendations.[2]

Previous attempts at determining this relationship were fraught with problems, since samples of breast masses were widely heterogeneous in terms of shape, margins, axis orientation and lesion boundary. Thus, in a recent study, complex masses with a maximum diameter of 20 mm or larger, with no circumscribed margins, or with a mammographic finding of suspected malignancy had a high probability of malignancy.

However, this analysis refers to an entire sample and it is uncertain whether these results would apply to specific complex breast masses.[7] In our data, mammographic findings were not good predictors of malignancy when a specified complex breast mass was analyzed. We have now overcome this weakness by focusing on a sample of completely homogeneous masses. Our data clearly indicate that sonographers, as well as general practitioners, must be aware of the importance of cyst diameter in a breast mass that would probably be benign on ultrasound, especially if cyst formation exceeds 3mm in its largest diameter.

Our study has some limitations. The results refer to a relatively small sample of 48 cases, which is insufficient to adequately describe lesions with a low prevalence rate such as a BI-RADS® 3. Thus, we cannot generalize the findings in our sample for routine diagnostic workup for breast cancer. An oval, circumscribed, parallel, abrupt limits mass with a cystic component <3mm should not be assigned to BI-RADS® 3 category. These masses should still be categorized as BI-RADS® 4 and biopsy should be performed. However, we suggest that masses containing cysts <3 mm should be categorized as BI-RADS® 4a, while masses with larger cysts should be categorized as BI-RADS® 4b. Another limitation was that we failed to assess the multivariable relationship between cyst size and size of the mass as a predictor of malignancy. Nevertheless, this study provides the initial data to support further studies.

Conclusions

Cyst diameter is a good predictor of malignancy in complex breast masses that would otherwise be categorized as BI-RADS® 3, except for the presence of a cyst. In our series, all masses with a maximum cyst diameter < 3mm (7 cases) were benign and all masses with cyst > 13mm in diameter (4 cases) were malignant.

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Tables

Table 1. Comparison of key clinical and sonographic features of benign and malignant oval, circumscribed, parallel, with abrupt limits, harboring at least one anechoic image in its interior masses

Characteristic	Final pathological diagnosis		Significance
	Benign	**Malignant**	
Clinical features	**Mean/SD**	**Mean/SD**	
Age	37.5/12.4	61/17.9	<0.001 φ
	n(%)	**n (%)**	
Postmenopausal	5/36 (14)	7/12 (58)	0.005*
Family history of breast cancer	4/36 (11)	1/12 (8)	0.55*
Palpable lesiom	24/36 (67)	9/12 (75)	0.46*
Sonographic features	**Mean/SD**	**Mean/SD**	
Largest mass dimension (mm)	25.6/13.6	37.2/17.4	0.02 #
Largest cyst dimension (mm)	5/ 2.5	10.8/ 5.8	<0.001 #
	n (%)	**n (%)**	
< 3mm	7 (100)	0	
3 – 7mm	24 (86)	4 (14)	
8 – 13mm	5 (55)	4 (45)	
> 13mm	0	4 (100)	
Vascularity			
present in lesion	22/36 (61)	8/12 (67)	>0.99*
present immediately adjacent to lesion	8/36 (22)	4/12 (33)	0.46*

∗ Fisher's Exact Test φ Mann-Whitney U-test
Student's T-test SD = Standard Deviation

Table 2. Pathological diagnoses of the, oval, circumscribed, parallel, with abrupt limits, harboring at least one anechoic image in its interior breast masses

Benign	n	Malignant	n
Fibroadenoma simplex	14	Invasive ductal carcinoma	6
Complex Fibroadenoma	12	Colloid adenocarcinoma	3
Hyalinized Fibroadenomas	3	Invasive ductal carcinoma associated with papilliferous carcinoma	2
Pseudoangiomatous Stromal Hyperplasia	1	Foci of Invasive lobular carcinoma in a complex fibroadenoma	1
Phylloid tumor	1		
Plasma cell mastitis	1		
Others	4		
Total	36	Total	12

core biopsies: 25 cases; core and excision biopsies: 23 cases.

Figures and Legends

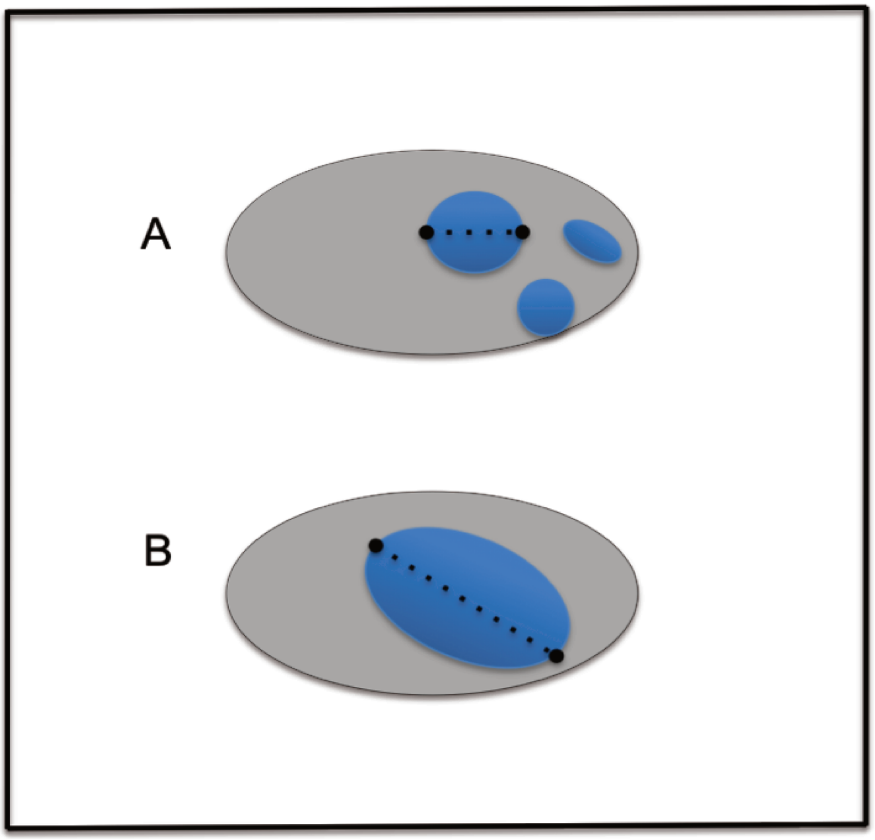

Figure 1. Demonstration of the cystic component measurement. The largest cyst diameter was the largest length identified within the largest cystic foci (1A). There was no restriction to size or number of cysts, since the solid component predominated (1 B).

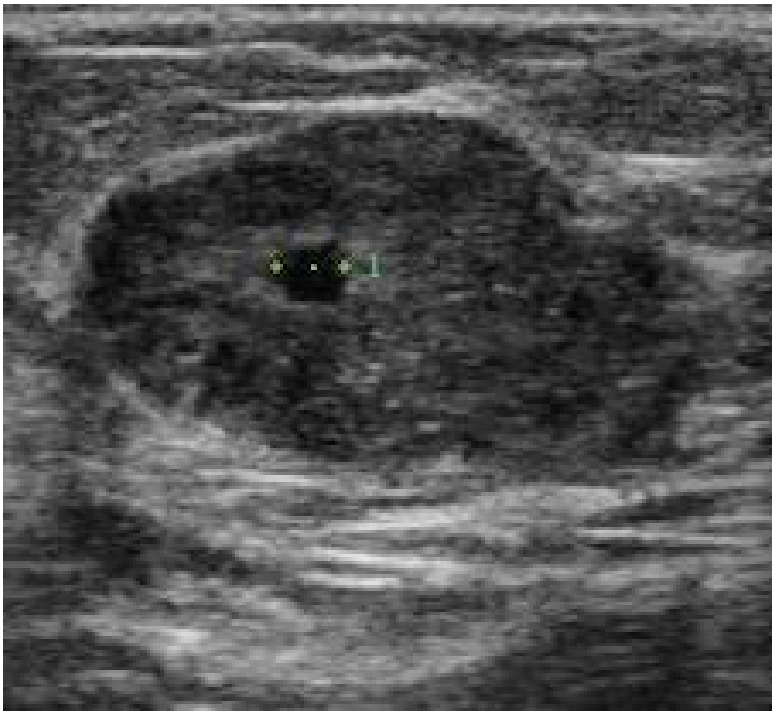

Figure 2. Fibroadenoma in a 43-year-old woman with a palpable mass. Ultrasound image shows the oval, macrolobulated, circumscribed, parallel, with abrupt limits breast mass, with an anechoic formation of 2mm (mass diameters: 21x12 mm).

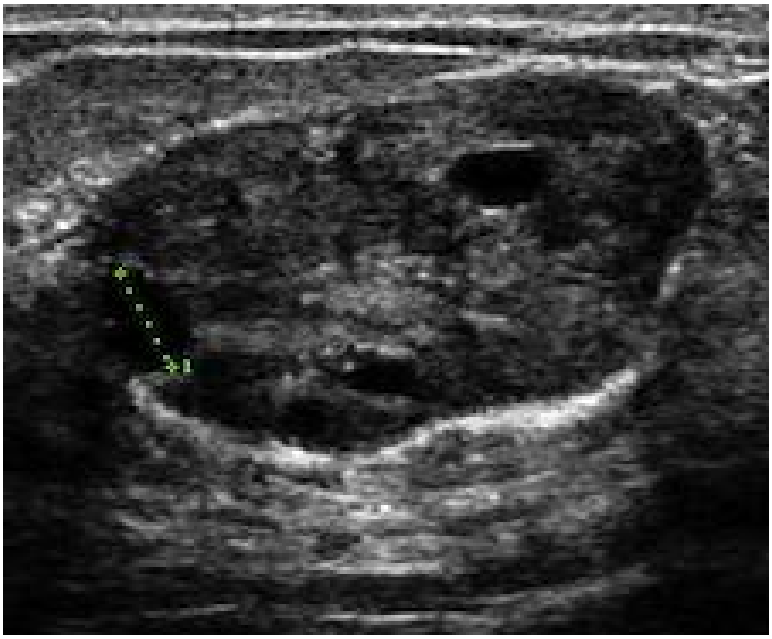

Figure 3. Fibroadenoma in a 20-year-old woman with a palpable mass. Ultrasound image shows the oval, circumscribed, parallel, with abrupt limits breast mass, with an anechoic formation of 5mm (mass diameters: 27x15mm).

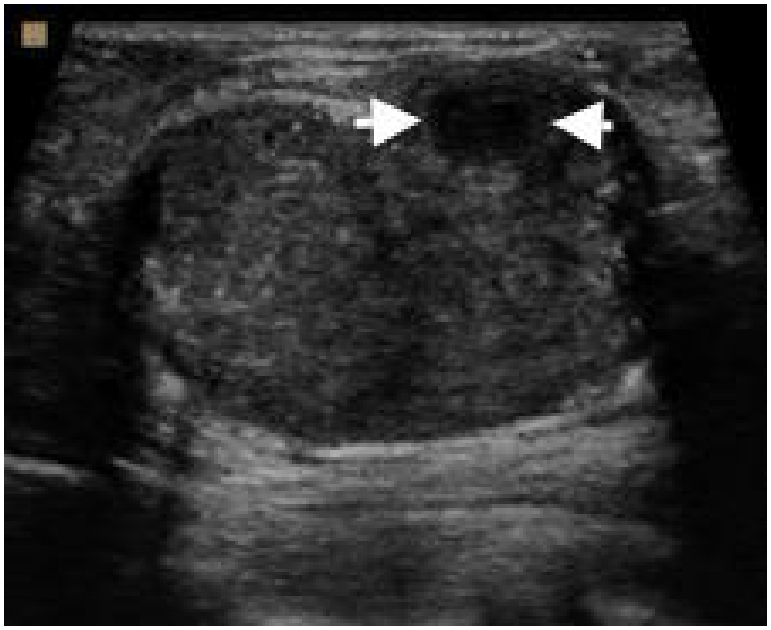

Figure 4. Phylloid tumor in a 29-year-old woman with a palpable mass. Ultrasound image shows the oval, macrolobulated, circumscribed, parallel, with abrupt limits breast mass, with an anechoic formation of 6mm (mass diameters: 34x27mm).

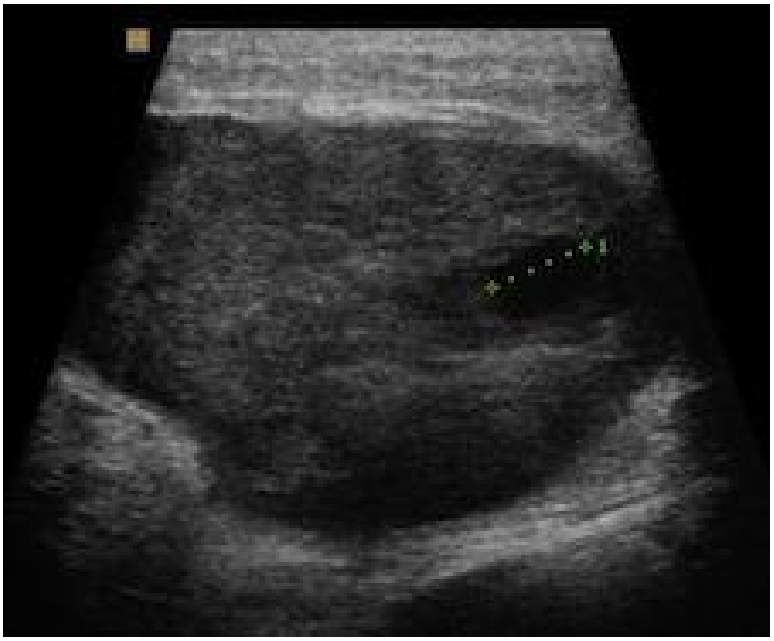

Figure 5. Invasive Ductal Carcinoma with necrotic areas in a 49-year-old woman with a palpable mass. Ultrasound image shows the oval, macrolobulated, circumscribed, parallel, with abrupt limits breast mass, with an anechoic formation of 8mm (mass diameters: 58x46mm).

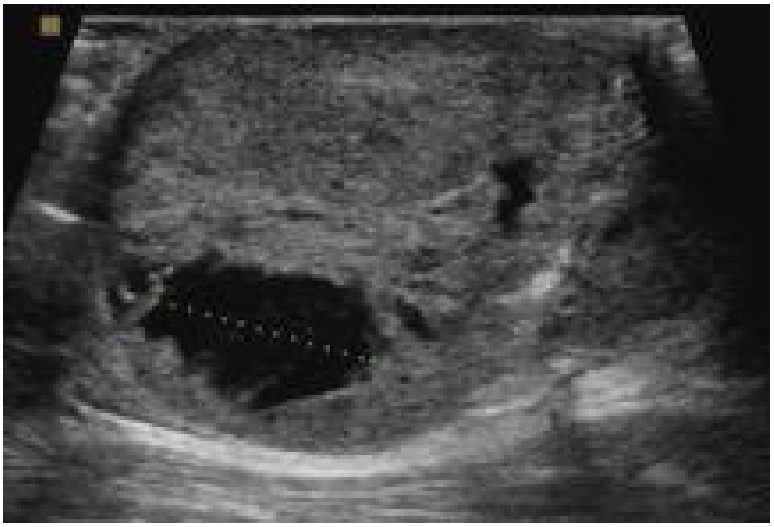

Figure 6. Invasive Ductal associated with Papilliferous Carcinoma in a 84-years-old woman with a palpable mass. Ultrasound image shows the oval, circumscribed, parallel, with abrupt limits breast mass, with an anechoic formation of 14mm (mass diameters: 46x27mm).

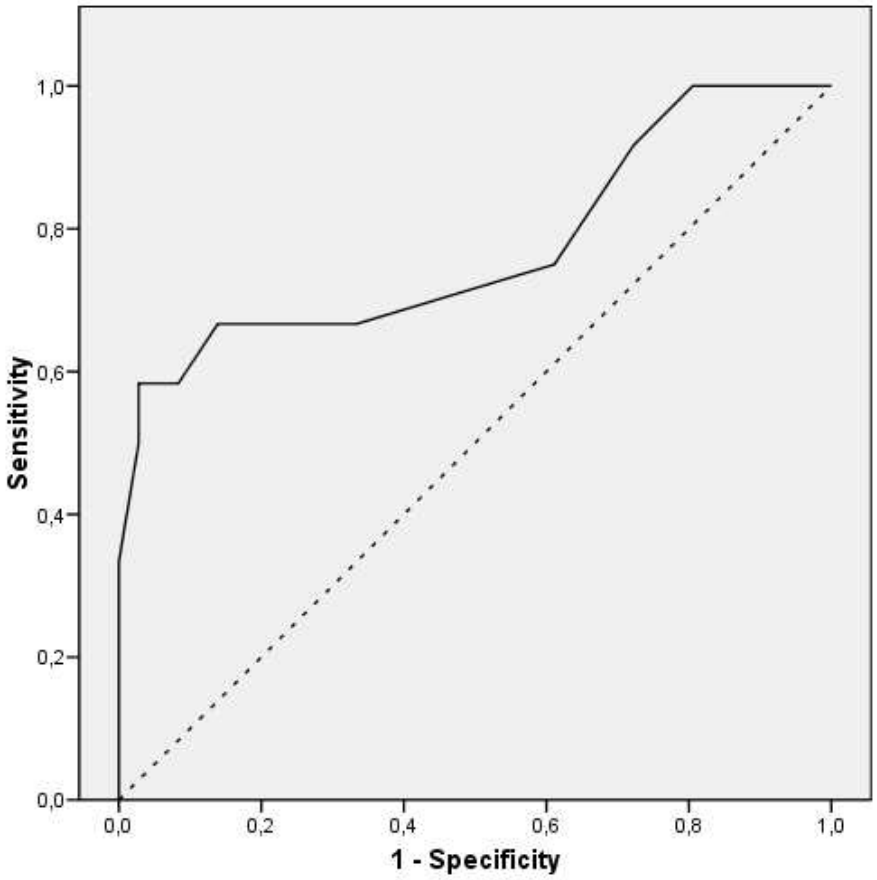

Figure 7. *Receiver Operator Characteristic (ROC) curve depiction of* largest cyst diameter as predictor of breast malignancy.

3.2. Artigo 2

Sonographic features of BI-RADS®-US 4 breast masses in Luminal, HER2 overexpression and Triple Negative phenotypes

Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil

Authors / *scientific address*

Rodrigo Menezes Jales, MD, Msc: *Department of Obstetrics and Gynecology Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Luis Otávio Sarian MD, PhD: *Department of Obstetrics and Gynecology Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Hélio Sebastião Amâncio de Camargo Jr. MD: *CDE Medical Director, Campinas, São Paulo, Brazil*

Cleisson Fábio Andrioli Peralta MD, PhD: *Department of Obstetrics and Gynecology Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Renato Torresan MD, PhD: *Department of Obstetrics and Gynecology Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Emílio Francisco Marussi MD, PhD: *Department of Obstetrics and Gynecology Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Sophie Derchain MD, PhD: *Department of Obstetrics and Gynecology Faculty of Medical Sciences, State University of Campinas – Unicamp, Campinas, São Paulo, Brazil*

Full mailing and e-mail address of corresponding author

Sophie Derchain

E-mail: derchain@fcm.unicamp.br

Mailing Address: Departmento de Ginecologia e Obstetricia, Faculdade de Ciências Médicas, caixa postal 6111, Universidade Estadual de Campinas – UNICAMP

CEP 13083-970, Campinas, SP, Brasil.

telephone number: 55 19 3521-9500

Abstract

Purpose: To assess sonographic characteristics of BI-RADS®-US category 4 breast masses in luminal, HER2 overexpressing and triple-negative phenotypes. **Methods:** In this cross-sectional study, we selected 327 sonographic breast masses classified as BI-RADS®-US subcategories 4a, 4b and 4c. Histologic examination of all masses confirmed 132 (40%) malignant or 195 (60%) benign tumors. We estimated the positive predictive value for BI-RADS®-US subcategories 4a, 4b and 4c. The agreement between three observers was calculated with kappa statistics. Malignant lesions were then grouped into three phenotypic subtypes: luminal, HER2 overexpressing and triple-negative phenotype categories using previously published Immunohistochemical methods. We compared sonographic features of the malignant lesions to tumor phenotype status. **Results:** The positive predictive values for subcategories 4a, 4b and 4c of the 327 BI-RADS®-US 4 masses were 16%, 43% and 84%, respectively. There was moderate agreement between the three observers (kappa=0.62) in the BI-RADS® -US 4 subcategorization. BI-RADS®-US subcategories 4a, 4b and 4c were not clearly related to luminal, triple-negative and HER2 overexpressing phenotypes. The luminal phenotype was positively associated with the following sonographic features: spiculated margin (OR=6.5; 95%CI=1.7 to 23.6), indistinct margin (OR=17.2; 95%CI=1.9 to 149), echogenic halo (OR=3.8; 95%CI=1.05 to 13.6). The luminal phenotype was negatively associated with enhancement (OR=0.3; 95%CI=0.1 to 0.7). Triple-negative phenotype was negatively associated with spiculated margin (OR=0.13; 95%CI=0.02 to 0.8) and shadowing (OR=0.02; 95%CI=0.01 to 0.4). The HER2 phenotype was not associated with any of the

sonographic features. **Conclusion:** Although some sonographic features were related to luminal and triple-negative phenotypes, BI-RADS®-US subcategories 4a, 4b and 4c were not clearly related to these molecular markers.

Keywords: breast cancer, breast ultrasound, prognostic molecular markers, BI-RADS®-US.

Introduction

Breast cancer is a group of diseases characterized by its morphologic, histopathological and molecular heterogeneity. Molecular dissimilarities between breast cancer types often produce different clinical presentations, prognoses and response to various treatments.[1] Gene expression profiling has recently been more widely used to define molecular phenotypes of breast cancer. It is now accepted that these phenotypic subtypes behave differently with specific patterns of mortality over time. These characteristics are independent of other clinicopathologic prognostic markers and systemic therapy received.[2,3]

The effects of mammography screening on the reduction of breast cancer mortality are currently under debate.[4,5,6] Recent evidence suggests that a substantial number of breast cancers diagnosed by imaging methods grow slowly and do not progress to lethal, metastatic disease.[7,8] Thus, more important than diagnosing breast cancer is identifying tumor characteristics that impact survival.[7,8]

It has been demonstrated that breast ultrasound (US) has a high degree of accuracy in the differentiation between benign and malignant lesions.[9] Moreover, a growing body of evidence emerged suggesting that the predictive molecular profiling of breast malignancies also correlates with some sonographic, mammographic and magnetic resonance imaging (MRI) findings.[10-14] However, how well the BI-RADS®-US categorization of breast lesions correlates with the molecular profile of breast cancer remains largely unknown. To that end, we thoroughly assessed the sonographic features of a large set of malignant breast lesions previously ranked as BI-RADS®-US 4. Post hoc

phenotyping of breast lesions into the luminal, HER2 overexpressing and triple-negative categories was carried out. Then we evaluated whether US features in relation to phenotypes were discrete enough to assign lesions into different BI-RADS®-US subcategories (4a, 4b or 4c).

Patients and Methods

This study was approved by our institutional review board (N 031/2009) and all participants signed an informed consent form.

In this cross-sectional study, we consecutively examined a series of 1212 women referred for breast US due to diverse medical reasons: ultrasound follow-up of masses previously categorized as BI-RADS®-US 3 (29%), evaluation of palpable masses (25%), sonographic screening of high-risk patients with dense breasts (16%), ultrasound evaluation of masses categorized as BI-RADS® 0 by mammography (11.5%) and other indications (18.5%). All women attended the ultrasonography division from March 2009 to December 2010. Sonography was performed with a 12MHz linear-array transducer (10 V Accuvix, CO Madison, Korea), and examinations were recorded in Bitmap (BMP) format. Among these patients, 327 were diagnosed with BI-RADS®-US 4 breast masses exclusively by ultrasound. According to the BI-RADS®, the category 4 is reserved for findings that do not have the classic appearance of malignancy but have a wide range of probability of malignance that is greater than those in category 3.[15] For purpose of this study, to be classified as BI-RADS® 4, a mass presenting several suspicious characteristics should had at least one benign feature or one minor suspicious finding.

All sonographic Bitmap (BMP) images were independently reviewed by three physicians who had great expertise in breast imaging and were blinded to pathology results. These experts further subdivided the BMP images into BI-RADS®-US subcategories 4a, 4b and 4c (Table 1). We evaluated interobserver agreement for subcategory assessment. The criteria used in our institution and replicated in this study for BI-RADS®-US 4 categorization were: BI-RADS®-US 4a: palpable mass with probably benign sonographic features (hypoechoic, oval, circumscribed, and parallel); probably benign sonographic features except for suspicious Doppler signals; probably benign sonographic features except for irregular shape; probably benign sonographic features except for nonparallel orientation; probably benign sonographic features except for microlobulated margin. BI-RADS®-US 4b: complex masses and masses with characteristics consistent with BI-RADS®-US category 4 that cannot be placed into subcategories 4a or 4c. BI-RADS®-US category 4c: masses exhibiting angled or indistinct margins, with any shape or orientation; spiculated mass with parallel orientation.

All masses were biopsied using core biopsy techniques alone (103 cases [31%]), complete surgical excision alone (39 cases [12%]) or core biopsy followed by surgical excision (185 cases [57%]), according to routine clinical practice in the breast surgery service. Core biopsy technique was performed under sonographic guidance, antisepsis and local anesthesia using an automated biopsy gun with a 14-gauge needle (Bard Biopsy Systems, Tempe, Arizona, USA). A pathologist that had great expertise in breast pathology performed the histologic examination.

Histologic results were classified as malignant (132 cases [40%]) or benign (195 cases [60%]) (Table 2). In malignant cases, clinical information was collected from medical records and included age, menopausal status, family history of breast cancer, palpable lesion, pathological diagnosis, tumor stage, nodal stage, presence of metastasis, pathological stage, estrogen receptor (ER), progesterone receptor (PR) and Human Epidermal growth factor receptor 2 (HER2).

Assessment of molecular phenotypes

To make our results reproducible in daily practice, we used immunohistochemical markers applied in routine care, i.e. ER, PR and HER2. Assessment was made by using standard immunohistochemical methods (Dako, Glostrup, Denmark). Tumors with >10% nuclei staining positive for ER or PR were defined as ER+ and PR+, respectively (Figures 2b and 2c).[2,12] Absent (score 0) or weak (score 1+) HER2 membrane immunostaining were considered HER2- (Figure 3d). Moderate (score 2+) or strong (score 3+) HER2 membrane immunostaining were considered HER2+.[12,16] We then separated breast masses into 3 major groups: luminal (favorable prognosis, responsive to hormonal blockade), HER2 overexpressing (unfavorable prognosis, responsive to Trastuzumab) and triple-negative (unfavorable prognosis, unresponsive to adjuvant therapy) phenotypes.[16-20] Luminal tumors either express ER or PR.[2] The HER2 overexpressing subtype exhibits ER - and HER2 + phenotypes. [16] Finally, triple-negative breast cancers are negative for ER, PR and HER2 (Figure 1). [17,21]

Statistical analysis

All calculations were performed with SPSS version 15 (SPSS Inc., Chicago IL). Statistical significance was set at 5% ($p<0.05$). Interobserver agreement on recategorization of BI-RADS®-US 4 masses into subcategories 4a, 4b and 4c was calculated with kappa statistics. We estimated the positive predictive value for BI-RADS®-US subcategories 4a, 4b and 4c (Table 1). The distribution of malignant cases into BI-RADS®-US subcategories 4a, 4b and 4c were compared in relation to menopausal status, family history of breast cancer, clinical presentation of the lesion on palpation (palpable or nonpalpable), main pathological features and pathological stage using Fisher's Exact test. For continuous non-parametric variables such as age, comparisons were performed using the Kruskal-Wallis H test (Tables 3 and 4). We calculated the odds ratios with 95% confidence intervals (95%CI) for expression of the different tumors markers in BI-RADS®-US 4a, 4b and 4c lesions using multivariate logistic regression analysis (Tables 5 and 6).

Results

The positive predictive values (PPV) for subcategories 4a, 4b and 4c of the 327 BI-RADS®-US 4 masses were 16%, 43% and 84%, respectively (Table1). There was moderate agreement between the three observers (kappa=0.62) in the BI-RADS®-US 4 subcategorization.

BI-RADS®-US subcategories 4a, 4b and 4c were not positively related to any of the studied clinical variables: age (p=0.32), menopausal status (p=0.36),

family history of breast cancer (p=0.49) and palpable lesion (p=0.31) (Table 3). In addition, BI-RADS®-US subcategories 4a, 4b and 4c did not correlate positively with pathological diagnoses: tumor size (T) (p=0.57), lymph node status (N) (p=0.95), metastasis (p=0.77) and final pathological stage (p=0.79) (Table 4). BI-RADS®-US subcategories 4a, 4b and 4c were not positively related to the majority of histologic types: invasive ductal carcinoma (IDC) (p=1), ductal carcinoma *in situ* (DCIS) (p=0.94) and IDC + DCIS (p=0.18). However, mucinous carcinoma was significantly associated with BI-RADS®-US 4a and 4b categories (p=0.48) (Table 4).

Among the malignant masses categorized as BI-RADS®-US 4, 89/132 (67%) were luminal. Among the nonluminal masses, 27/132 (20%) were triple-negative and 16/132 (12%) were HER2 overexpressing lesions. Ultrasound findings related to the luminal phenotype were: spiculated margins (OR=6.5; 95%CI=1.7 - 23.6), indistinct margins (OR=17.2; 95% CI= 1.9 – 149) (Figure 2a), echogenic halo (OR= 3.78; 95% CI=1.05 – 13.6) and enhancement (OR=0.3; 95%CI=0.15 – 0.7). On the other hand, variables related to the triple-negative phenotype were: spiculated margin (OR=0.13; 95% CI=0.02 - 0.8) and shadowing (OR=0.02; 95%CI= 0.01 – 0.4). None of the sonographic variables was positively related to HER2 phenotype (Table 5). Concerning the subcategorization of BI-RADS®-US category 4, the luminal phenotype (OR=0.3; 95% CI=0.09-0.9) was more frequently assigned to BI-RADS®-US 4a than 4b (Table 6).

Discussion

In our study, we detected that the final BI-RADS®-US 4 subcategories of breast lesions were not clearly related to the luminal, HER2 overexpressing or triple-negative molecular phenotypes. Luminal phenotype tumors were more frequently assigned to BI-RADS®-US 4a rather than 4b subcategory. However, this finding may have been fortuitous. According to our criteria, subcategory 4b comprises masses that have intermediate characteristics between groups 4a and 4c and masses with complex echotexture, unrelated to any of the phenotypes.

The results of this study were closely aligned with those of previous reports, corroborating the concept that the biological diversity of breast cancers may manifest itself in imaging features that may be predictive of current molecular phenotypes.[11] In our study, spiculated margins occurred more frequently in masses with luminal phenotype and less frequently in triple-negative phenotypes. This was not unexpected, since spiculated margins have been previously linked to ER and PR positivity and longer survival.[22] Also, it has been reported that triple-negative cancers are more likely to display circumscribed margins on US.[10,13] Finally, enhancement was less frequent in the luminal phenotype and posterior acoustic shadowing was less frequent in triple-negative tumors in our study sample. A previous study evaluating triple-negative tumors obtained similar results.[13] On the other hand, a hypothesis concerning increased tumor blood flow in HER2 positive tumors has been supported by MRI data. This was not confirmed in our study, in which mass vascularity was assessed by color Doppler.[23]

To the best of our knowledge, our study is the first to describe an association between lesion boundary and luminal phenotype. The explanation for a correlation between margins, posterior acoustic features, lesion boundary and biological behavior is derived from tumor pathological features. It is understood that both desmoplasia (likely responsible for an echogenic halo and posterior acoustic shadowing) and spiculated margins represent a slowly developing host response to tumor. The tumor is walled off from the surrounding tissues with fibrosis and elastosis in an attempt to keep it from spreading. Therefore, these sonographic features are associated with slow-growing lesions.[24] Indistinct margins, usually identified in infiltrating tumors with little desmoplastic response were also highly related to luminal phenotype in our data. However, the occurrence of indistinct margins is also associated with partially spiculated margins in slowly growing lesions and also correlates with desmoplastic reaction masses (Figure 2a). In contrast, breast masses that have a faster growth rate are more cellular and elicit inflammatory reactions with lymphocyte and/or plasma cell invasion. That is why these masses tend to be circumscribed, with abrupt limits and also associated with posterior acoustic enhancement (Figure 3a).[24]

We restricted our analyses to BI-RADS®-US category 4 tumors because this US category encompasses a very heterogeneous group of masses, with different imaging characteristics and malignancy risk ranging from 2% to 95%.[15] Most indications of breast biopsies fall into this category.[25,26] The latest edition of BI-RADS® published in 2003 encourages the subdivision of category 4 lesions into 4a (low suspicion for malignancy), 4b (intermediate suspicion for

malignancy) and 4c (moderate, but not classic for malignancy) groups. The reason for this subcategorization is to facilitate decision-making by both the physician and patient, because the risk of malignancy differs according to varying imaging aspects.[27] We used well-defined criteria for subcategorizing BI-RADS®-US category 4, resulting in appropriate scaling of the positive predictive value (PPV) (Table 1), as well as moderate agreement between three observers (kappa=0.62). Previous studies have reported that lesions classified into subcategories 4a, 4b and 4c have the worst interobserver agreement between the BI-RADS®-US categories. Even among experienced observers, there was only a fair degree of interobserver agreement.[28-30] This probably occurred because lesions have not been clearly described by BI-RADS®-US and various institutions had to define their most appropriate criteria by using internal audits.[15,31] Although our criteria for subcategorizing BI-RADS®-US 4 was reproducible and masses were properly classified in terms of PPV, these subcategories were not related to tumor aggressiveness. Circumscribed margins were significantly associated with a triple-negative phenotype. Nevertheless, subcategory 4a, which encompasses circumscribed masses, was not positively related to a triple-negative phenotype. According to our criteria, the BI-RADS®-US subcategory 4a is heterogeneous in terms of prognosis because it also includes US features such as microlobulated margins, irregular shape, not parallel to skin orientation and suspicious Doppler signals.

To our knowledge, our study is the first to evaluate the relationship between BI-RADS®-US 4 subcategories and main prognostic markers. Furthermore, we controlled for possible confounders such as patient age,

menopausal status, pathological diagnosis, family history of breast cancer, palpability and pathologic stage, which were homogeneous in BI-RADS®-US subcategories 4. However, there were some limitations to our study. First, mammographic findings were not considered in the subcategorization of BI-RADS®-US category 4, which is not usually done in routine practice. Only breast masses were evaluated. However, mammographic evaluation of these masses would probably make a modest contribution to final BI-RADS®-US assessment category, since ultrasound has a high level of performance for this assessment. Another important limitation is that we did not categorize molecular subtypes according to basal markers, such as epidermal growth factor receptor (EGFR) and cytokeratin 5/6. These basal markers are important for survival prediction, but are not routinely used.[2,32] Actually, there is still considerable disagreement to the mapping of IHC subtypes onto the subtypes based on gene expression and even to ER, PR and HER2 categorization.[1,2,33,34]

Further studies on the relationship between imaging features and the biological behavior of breast tumors may prove to be useful for BI-RADS® classification. It would be important if the BI-RADS® provided information on the prevalence of malignancy and tumor aggressiveness. In combination, these elements might supply the breast surgeon with more information than information about malignancy risk alone. Thus, a spiculated mass should be placed into a high PPV category, as BI-RADS® 4c or 5, but also assigned to a less aggressive category, such as category “L”. In contrast, a circumscribed mass should be placed into a low PPV category, such as BI-RADS® 3 or 4a, but assigned to a highly aggressive category such as category ”H”. Thus, an

improvement in the diagnosis of breast cancer types that may progress to lethal, metastatic disease can take place.

Conclusion

Although sonographic features such as margins, lesion boundaries and posterior acoustic features of breast masses were positively related to luminal and triple-negative phenotypes, BI-RADS®-US subcategories 4a, 4b and 4c were not clearly related to these predictive markers.

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Table 1. Prevalence of malignancy in tumors according to the BI-RADS®-US categories 4a, 4b and 4c

	BI-RADS®-US Category							
	4a		**4b**		**4c**		**4**	
	n	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Malign	20	(16)	58	(43)	54	(84)	132	(40)
Benign	107	(84)	78	(57)	10	(16)	195	(60)
Total	127	(100)	136	(100)	64	(100)	327	(100)
PPV	16%		43%		84%		40%	

PPV=Positive Predictive Value

Table 2. Pathological diagnoses of the 335 breast masses classified as BI-RADS®-US 4

Benign	n	(%)	Malignant	n	(%)
Simple Fibroadenoma	58	(30)	IDC	81	(61)
Hyalinized Fibroadenoma	28	(14)	IDC + DCIS	28	(21)
Complex Fibroadenoma	29	(15)	Mucinous Carcinoma	7	(5)
Papilloma	15	(8)	DCIS	6	(5)
Myxoid Fibroadenoma	10	(5)	Invasive Lobular Carcinoma	3	(2)
Benign Phyllodes	9	(5)	Invasive Papillary Carcinoma	2	(2)
PASH	6	(3)			
Others	40	(20)	Others	5	(4)
Total	195	(100)	Total	132	(100)

IDC = Invasive Ductal Carcinoma. DCIS = Ductal Carcinoma *in situ*.
PASH = Pseudoangiomatous Stromal Hyperplasia

Table 3. Comparison of key clinical variables and BI-RADS®-US 4a, 4b and 4c malignant masses

	BI-RADS®-US Category				
	US 4	**4A**	**4B**	**4C**	**p**
Clinical features	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Age	53.6 (13.7)	54.2 (12.6)	55.2 (14.8)	51.6 (12.8)	0.32*
	N (%)	**N (%)**	**N (%)**	**N (%)**	**p**
Menopause status					0.36†
Premenopausal	56 (42)	6 (70)	24 (41)	26 (48)	
Postmenopausal	71 (54)	14 (30)	30 (52)	27 (50)	
Unknown	5 (4)	0	4 (7)	1 (2)	
Family history of breast cancer					0.49†
Yes	32 (24)	5 (25)	13 (22)	4 (9)	
No	96 (73)	14 (70)	42 (73)	40 (91)	
Unknown	4 (3)	1 (5)	3 (5)	0	
Palpable lesion					0.31†
Yes	106 (80)	15 (75)	50 (86)	41 (76)	
No	26 (20)	5 (25)	8 (14)	13 (24)	
Total	132	20	58	54	

* Kruskal-Wallis H test. †Fisher's Exact Test.

Table 4. Comparison of key histological diagnosis, pathological stage and BI-RADS®-US 4a, 4b and 4c malignant masses

Clinical features	BI-RADS®-US Category				p
	US 4	4A	4B	4C	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
	N (%)	N (%)	N (%)	N (%)	
Pathological diagnosis					
IDC	81 (62)	13 (65)	35 (61)	33 (61)	1.0†
DCIS	6 (4)	1(5)	3 (5)	2 (4)	0.94†
IDC + DCIS	28 (21)	3 (15)	9 (15)	16 (30)	0.18†
Mucinous Carcinoma	7 (5)	2 (10)	5 (9)	0	0.48†
Others	10 (8)	1 (5)	6 (10)	3 (5)	
T Stage					0.57†
T1	49 (37)	10 (50)	18 (31)	21 (39)	
T2	44 (33)	7 (35)	18 (31)	19 (35)	
T3	17 (13)	2 (10)	9 (16)	6 (11)	
T4	22 (17)	1 (5)	13 (22)	8 (15)	
Nodal Stage					0.95†
positive	70 (53)	10 (50)	31 (53)	29 (54)	
negative	62 (47)	10 (50)	27 (47)	25 (46)	
Metastasis					0.77†
yes	7 (5)	0 (0)	4 (7)	3 (6)	
no	125 (95)	20(100)	54 (93)	51 (94)	
Stage					0.79†
0 (in situ)	9 (7)	1 (5)	5 (9)	3 (5)	
I	28 (21)	6 (30)	9 (15)	13 (24)	
II	41 (31)	8 (40)	18 (31)	15 (28)	
III	47 (36)	5 (25)	22 (38)	20 (37)	
IV	7 (5)	0 (0)	4 (7)	3 (6)	
Total	132	20	58	54	

* Kruskal-Wallis H test. †Fisher's Exact Test.
IDC = Invasive Ductal Carcinoma. DCIS = Ductal Carcinoma *in situ* ILC = Invasive Lobular Carcinoma
SD = Standard Deviation

Table 5. Frequency distribution of sonographic findings by molecular profiling in BI-RADS®-US 4 malignant masses

Ultrasound Findings	Molecular profiling								
	Luminal (ER + or PR+)			**Her2 Overexpression (PR- /ER- / Her2 +)**			**Triple Negative (PR- /ER- / Her2 -)**		
	N+(%)	**OR**	**95% CI**	**N+(%)**	**OR**	**95% CI**	**N+(%)**	**OR**	**95% CI**
Mass shape									
Oval	55(62)	REF	–	11(13)	REF	–	15(18)	REF	–
round	3(60)	0.9	0.1 - 5.6	1(20)	1.6	0.2 – 15.8	1(20)	1.12	0.1 – 10.7
irregular	31(66)	1.2	0.5 – 2.4	4(9)	0.6	0.19 – 2.1	11(24)	1.45	0.6 – 3.4
Mass margin									
Circumscribed	13(45)	REF	–	5(18)	REF	–	9(33)	REF	–
microlobulated	33(57)	1.6	0.6 – 3.9	8(14)	0.7	0.2 – 2.5	14(25)	0.67	0.2 – 1.8
angular	8(61)	1.9	0.5 – 7.4	0(0)	0.2	0.01 – 3.5	3(27)	0.75	0.1 – 3.5
indistinct	14(93)	17.2	1.9 – 149	1(7)	0.3	0.03 – 2.9	0	0.06	0 – 1.2
spiculated	21(84)	6.5	1.7 – 23.6	2(9)	0.4	0.07 – 2.4	1(4)	0.13	0.02 – 0.8
Mass orientation									
Parallel	72(64)	REF	–	14(13)	REF	–	21(20)	REF	–
taller	17(63)	0.9	0.4 – 2.3	2(8)	0.6	0.1 – 2.7	6(24)	1.29	0.4 – 3.6
Echo									
Hypoechoic	81(66)	REF	–	13(11)	REF	–	21(18)	1	–
complex	8(44)	0.4	0.1 – 1.1	3(18)	1.7	0.4 – 6.6	6(35)	2.44	0.8 – 7.3
Lesion boundary									
abrupt interface	72(60)	REF	–	15(13)	REF	–	26(23)	REF	–
echogenic halo	17(85)	3.8	1.05 – 13.6	1(6)	0.4	0.05 – 3.4	1(6)	0.21	0.03 – 1.7
Posterior acoustic features									
none	57(70)	REF	–	9(12)	REF	–	9(60)	REF	–
shadowing	13(87)	2.7	0.5 – 13	1(7)	0.5	0.07 – 4.8	0	0.02	0.01 – 0.4
enhancement	16(44)	0.3	0.15 – 0.7	6(17)	1.5	0.5 – 4.6	13(37)	0.39	0.1 – 1.3
combined pattern	3(37)	0.2	0.06 – 1.1	0	0.4	0.02 – 7.7	5(62)	1.11	0.2 – 6.4
Vascularity									
Absent	33(67)	REF	–	3(7)	REF	–	7(16)	REF	–
present in lesion	45(58)	0.6	0.3 – 1.4	13(17)	3	0.8 – 11.1	18(24)	1.74	0.6 – 4.5
present adjacent to lesion	39(66)	0.9	0.4 – 2.1	6(10)	1.6	0.4 – 6.8	14(24)	1.73	0.6 – 4.7

OR = Odds Ratios CI = confidence interval REF = Reference variable

Table 6. Frequency distribution of BI-RADS®-US 4a, 4b and 4c malignant masses by molecular profiling

BI-RADS®-US Categories	Molecular profiling								
	Luminal Phenotype (ER + or PR+)			Her2 Overexpression (PR- /ER- / Her2 +)			Triple Negative (PR- /ER- / Her2 -)		
	N+(%)	OR	95% CI	N+(%)	OR	95% CI	N+(%)	OR	95% CI
B4a (REF)	16 (76)	REF	–	0	REF	–	3 (15)	REF	–
B4b	29 (48)	0.3	0.09 – 0.9	12 (21)	11.0	0.6 – 195	17 (29)	2.3	0.6 – 9.1
B4c	44 (74)	0.9	0.3 – 2.9	4 (7)	3.6	0.2 – 71	7 (13)	0.8	0.2 – 3.6
Total	89			16			27		

OR = Odds Ratios
CI = confidence interval
REF = Reference variable

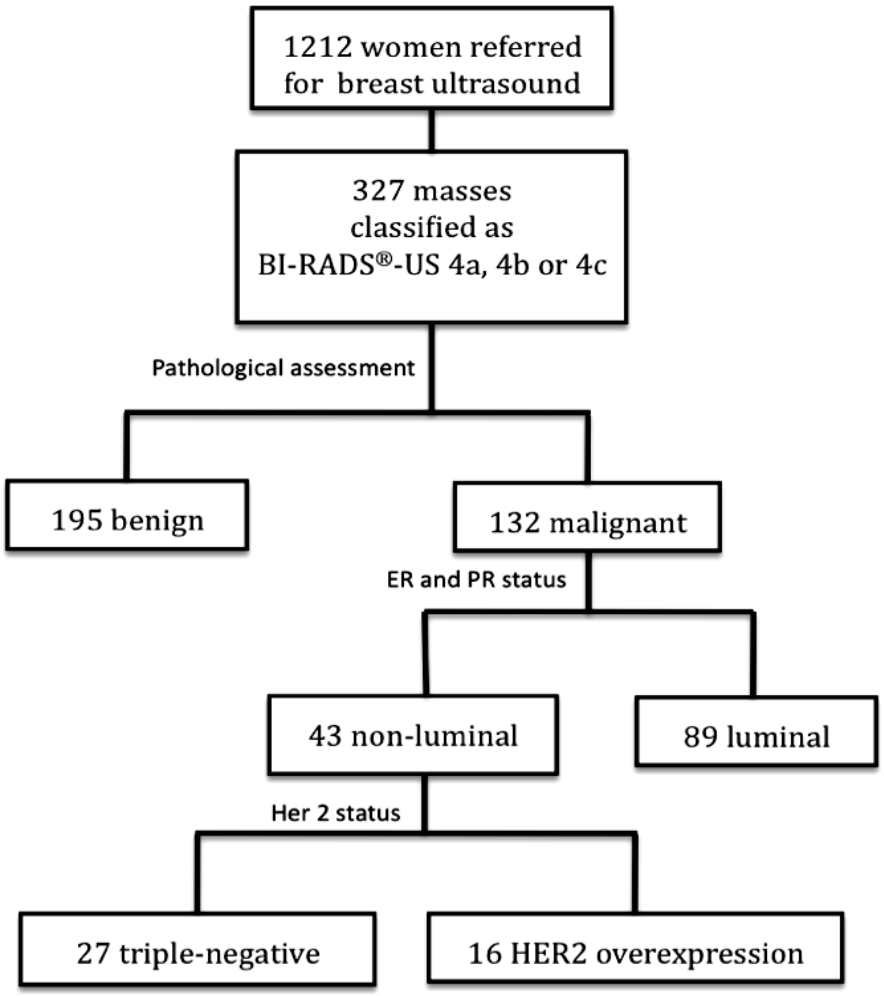
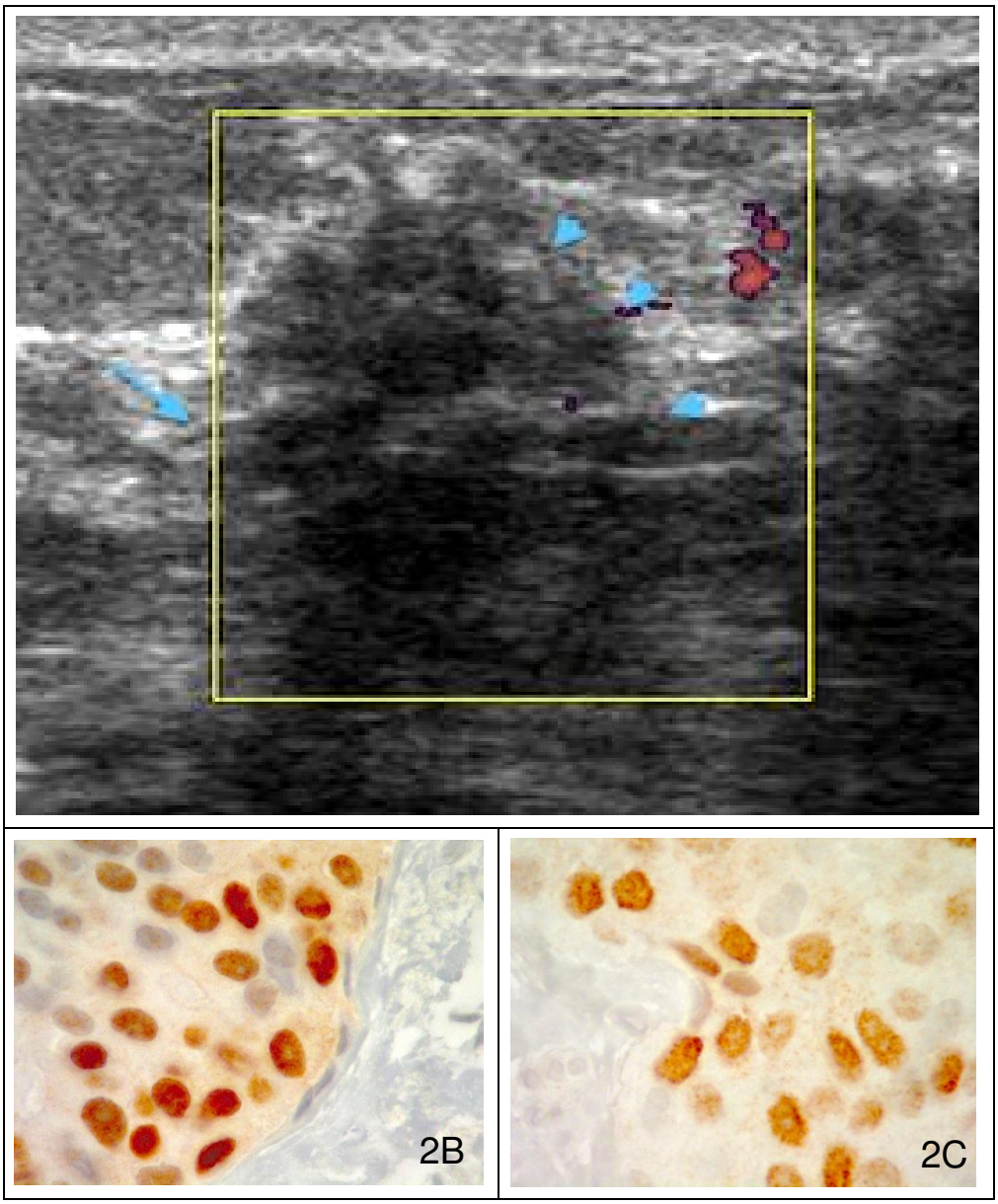
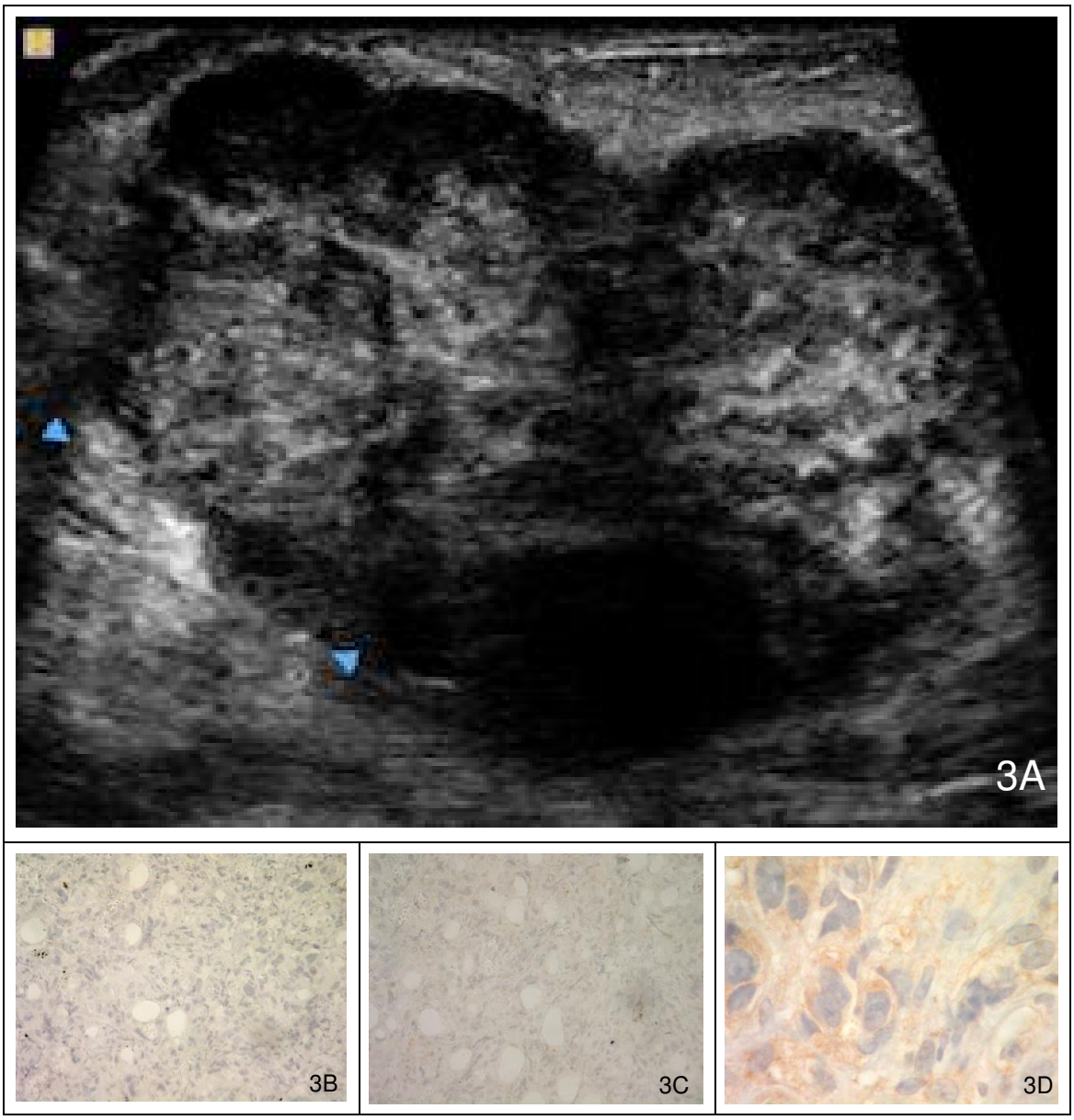


Figure 1. Flowchart showing the selection of cases.



Figures 2a, 2b and 2c. 45-year-old woman with a palpable breast mass. Ultrasound image shows the predominant indistinct (arrow head), also presenting spiculated margins (arrow), with discrete echogenic halo and shadowing mass, classified as BI-RADS®-US 4c (Figure 2a). Pathologic diagnosis: Invasive ductal carcinoma associated with multiple foci of ductal carcinoma *in situ* of solid and cribriform types (luminal phenotype). Positive reaction for ER (Figure 2b) and PR (Figure 2c) was observed as strong brown-black, fine, intranuclear granules (original magnification [100X]).



Figures 3a, 3b, 3c and 3d. 62-year-old woman with a palpable mass. Ultrasound image shows the circumscribed, abrupt interface, with enhancement immediately deep to the lesion (arrow head) mass, classified as BI-RADS®-US 4a (Figure 3a). Pathologic diagnosis: invasive ductal carcinoma (triple-negative phenotype). Negative reaction for ER (Figure 3b) and PR (Figure 3c) was observed as no brown-black, fine, intranuclear granules (original magnification [40x]). Negative reaction for HER2 (Figure 3d) was observed as a faint/barely perceptible membranous reactivity (score 1). Cytoplasmic staining should be ignored (original magnification [100X]).

4. Discussão

Este estudo trouxe novos conceitos relacionados à avaliação ultrassonográfica de nódulos mamários, sobretudo aos classificados na categoria BI-RADS® US 4. Assim, no primeiro artigo, demonstrou-se que o diâmetro de um cisto contido em um nódulo de mama com as demais características ultrassonográficas referentes às margens, à forma, aos limites e à orientação, classificáveis como provavelmente benignas, correlaciona-se fortemente com o diagnóstico anatomopatológico de malignidade. Assim, na amostra deste estudo, todos os nódulos contendo cistos com diâmetro <3mm eram benignos e todos os nódulos contendo cistos >13mm eram malignos.

Até onde sabemos, este é o primeiro estudo a descrever a relação entre a dimensão do cisto e malignidade. Comparando estes resultados com os de outros estudos relacionados a amostras menores e heterogêneas, a prevalência de malignidade nesta amostra (25%) foi maior do que a prevalência de malignidade em nódulos circunscritos (9%) e similar em relação a nódulos complexos predominantemente sólidos (18%) descrita em outros estudos, o que justifica a classificação desse grupo de lesões na categoria BI-RADS® 4 (23, 43).

Apesar da associação evidente entre a dimensão dos cistos e malignidade, a amostra avaliada, de 48 casos, apesar de ser originada de uma série de mais de 1500 exames ultrassonográficos, não é suficiente para generalizar os seus resultados. Assim, com base neste estudo, não se pode sugerir que nódulos com aspecto ultrassonográfico benigno, mas contendo cistos <3mm, sejam classificados na categoria BI-RADS®US 3. Entretanto, podemos sugerir e adotar na Seção de Imagem Mamária do HM-CAISM que esses nódulos sejam classificados na categoria BI-RADS® US 4a, enquanto nódulos com cistos ≥3mm sejam classificados na categoria BI-RADS® US 4b. Essas subcategorias apresentam valores preditivos positivos diferentes, o que ajudará na interpretação dos resultados dos exames de imagem pelo médico mastologista.

Além disso, no segundo artigo, observa-se que a subclassificação de nódulos mamários nas categorias BI-RADS®-US 4a, 4b e 4c não está claramente relacionada aos fenótipos Luminal, Her2 *overexpression* e Triplo negativo. Apesar do fenótipo Luminal ter sido mais frequentemente relacionado à categoria BI-RADS®-US 4a do que à categoria BI-RADS®-US 4b, este achado deve ter sido fortuito. De acordo com os critérios de classificação utilizados na amostra, a subcategoria BI-RADS®-US 4b foi constituída por nódulos complexos, que isoladamente não se relacionaram a um único fenótipo, além de nódulos com características ultrassonográficas com suspeita intermediária entre as subcategorias BI-RADS®-US 4a e 4c.

Por outro lado, os achados referentes à associação entre subtipos fenotípicos e variáveis ultrassonográficas estão alinhados com os de estudos

prévios, corroborando o conceito de que a diversidade biológica dos cânceres de mama está relacionada com diferentes características de imagem (27,30). Assim, no presente estudo, margens espiculadas e indistintas ocorreram mais frequentemente no fenótipo Luminal. Isso era esperado, já que margens espiculadas foram relacionadas anteriormente aos RE e RP e a maiores taxas de sobrevida (42). Além disso, o fenótipo Triplo Negativo, como neste estudo, foi previamente relacionado a margens circunscritas (26). Na amostra do presente estudo, a atenuação das ondas de US esteve mais relacionada ao fenótipo Luminal e menos relacionada ao Triplo Negativo. Uma publicação recente, avaliando tumores Triplo Negativos, obteve os mesmos resultados (29). Por outro lado, este é, até onde se sabe, o primeiro estudo a descrever uma associação entre o halo ecogênico e o fenótipo Luminal. Uma explicação para a correlação entre margens, limites, características acústicas posteriores e o comportamento biológico pode derivar das características anatomopatológicas do tumor. Tanto a reação inflamatória desmoplásica, responsável pelo halo ecogênico e pela atenuação das ondas de ultrassom, quanto as espiculações podem ser entendidas como uma lenta resposta inflamatória da paciente ao tumor, associada à fibrose e à elastose, em uma tentativa de bloquear a disseminação do tumor. Esse processo é mais frequente em tumores com crescimento lento, menos agressivos, relacionados a fenótipos associados ao melhor prognóstico, como o Luminal (44). Nódulos com crescimento rápido, com comportamento biológico mais agressivo, tendem a ter margens circunscritas, com limites abruptos, e estar vinculados a respostas inflamatórias associadas a linfócitos e plasmócitos (44). Por fim, estudos analisando exames de RNM sugerem relação entre o aumento do fluxo sanguíneo tumoral

e o fenótipo Her2 (45). Entretanto, essa correlação não foi confirmada neste estudo, no qual a vascularização foi examinada pelo método Doppler.

Para a execução deste estudo, foram desenvolvidos e instituídos na Seção de Imagem Mamária do HM-CAISM critérios bem definidos para a subcategorização dos nódulos de mama nas categorias BI-RADS®-US 4a , 4b e 4c. Quando aplicados na amostra estudada de 327 nódulos, independentemente, por três médicos da seção de imagem do HM-CAISM, obteve-se um adequado escalonamento dos valores preditivos positivos nas categorias BI-RADS®-US 4a (16%), 4b (43%) e 4c (85%). A concordância entre os três observadores foi moderada (Kappa = 0,62). Estudos prévios descrevem que as lesões classificadas nas subcategorias BI-RADS®-US 4 apresentam a pior concordância interobservadores entre as categorias BI-RADS®, mesmo entre observadores experientes, para os quais a concordância foi descrita apenas como razoável (46, 47, 48). Essa divergência deve-se provavelmente ao fato de não terem sido descritos claramente pelo BI-RADS® os critérios que devem ser utilizados na subclassificação BI-RADS® 4. As diferentes instituições devem definir os seus critérios por meio das auditorias internas dos seus resultados (17, 49).

Uma limitação importante deste estudo refere-se ao fato de que os achados mamográficos não foram utilizados na classificação das lesões analisadas, o que não é a prática usual. Entretanto, apenas nódulos foram avaliados e a mamografia provavelmente contribuiria pouco na subclassificação BI-RADS®, uma vez que a ultrassonografia apresenta excelente performance nessa avaliação, pois suas margens não são obscurecidas pelo parênquima adjacente. Essa

falha não deverá mais ocorrer em novos estudos, já que recentemente as imagens mamográficas estão disponíveis em todo o hospital através da rede *Picture Archiving and Communication System* (PACS), que é uma eficiente tecnologia relacionada ao arquivamento e ao acesso a imagens médicas de alta resolução.

Após a conclusão do presente estudo, foi mantida, na Seção de Imagem Mamária do HM-CAISM, a linha de pesquisa associada à seleção de nódulos que apresentam as características estudadas no primeiro artigo, ou seja, características ultrassonográficas de benignidade associadas a cistos no seu interior. Este estudo tem como objetivo reunir uma amostra superior a 200 nódulos, o que levará cerca de dois anos, para que novas publicações possam trazer dados mais abrangentes quanto à orientação da conduta em relação a esses nódulos, o que pode diminuir a taxa de biópsias desnecessárias. Outra linha de pesquisa que será mantida refere-se à correlação dos aspectos de imagem com o comportamento biológico dos tumores, o que poderá gerar conceitos que possam contribuir para a redução do diagnóstico de lesões sem significado clínico e agilizar o diagnóstico de neoplasias com comportamento biológico agressivo (30). A categoria 4 do BI-RADS, sendo a mais heterogênea e relacionada ao maior número de biópsias negativas, provavelmente será a maior beneficiada por esses novos conceitos (20).

5. Conclusões

- **Artigo 1:** O diâmetro dos cistos é um bom preditor de malignidade em nódulos complexos que, exceto pela presença do componente cístico, seriam categorizados como BI-RADS® 3. Na presente amostra, todos os nódulos com cistos <3mm (7 casos) eram benignos e todos os nódulos com diâmetros máximos dos cistos >13mm (4 casos) eram malignos.

- **Artigo 2:** Embora as características ultrassonográficas como margens, limites, e características acústicas posteriores estiveram positivamente relacionadas aos fenótipos Luminal e Triplo negativo, as subcategorias BI-RADS® US 4a, 4b e 4c não estiveram claramente relacionadas a nenhum dos marcadores preditivos.

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7. Anexos

7.1. Anexo 1 – Termo de Consentimento Livre e Esclarecido

Eu, _____ estou sendo convidada a participar de uma pesquisa no CAISM sobre a utilização do exame de ultra-sonografia ou ecografia (exame realizado aplicando-se um gel e um aparelho na pele da mama) na avaliação dos nódulos suspeitos de mama. Para participar da pesquisa eu preciso ser acompanhada no ambulatório de patologia mamária do CAISM, realizar um exame de ultra-sonografia no CAISM e apresentar, nesse exame, um nódulo de mama com suspeita baixa ou média de ser maligno. A intenção do estudo é avaliar a opinião de dois médicos diferentes sobre a chance do nódulo de mama ser maligno. Esse conhecimento irá facilitar a indicação e a avaliação dos resultados de biópsias pelo médico que pede os exames de Ultrassonografia.

O meu exame de Ultrassonografia será realizado da mesma maneira caso eu participe da pesquisa ou não. Esse exame é feito no setor de ultra-sonografia do CAISM, que fica no andar térreo. Esse exame não dói nem faz mal à saúde. O exame é realizado passando gel e um aparelho sobre a pele da mama. Durante o exame eu ficarei deitada com as mãos para cima durante cerca de 15 minutos.

Todos os nódulos suspeitos de mama devem ser biopsiados, independentemente da pesquisa. Dependendo do tipo do meu nódulo, ele poderá ser biopsiado durante o exame de ultra-sonografia, pelo médico no ambulatório ou no centro cirúrgico. A maneira mais fácil e mais adequada de se biopsiar os nódulos da mama é, na maioria das vezes, através do exame de ultrassonografia. Caso haja a indicação de fazer uma biópsia do meu nódulo através da ultrassonografia, eu poderei ser submetida à biópsia na mesma

hora do exame, independentemente da minha participação na pesquisa. A biópsia é realizada com anestesia local. O anestésico tira praticamente toda a sensação de dor, mas não tira a sensação da pressão, que pode incomodar um pouco. Também pode haver um pouco de sangramento, que passa depois de comprimir um pouco o local.

Assim, esta pesquisa não vai mudar em nada o meu tratamento. Os possíveis benefícios que esta pesquisa poderá trazer ao tratamento do câncer de mama só poderão ser usados depois que a pesquisa termine e seus resultados sejam estudados, o que levará pelo menos 1 ano.

Fui informada que, se eu não quiser participar dessa pesquisa ou desistir de fazer parte dela a qualquer momento (mesmo depois de fazer o exame de ultrassonografia) o meu tratamento no CAISM não será modificado e eu serei tratada do mesmo modo.

Os meus dados pessoais serão mantidos em segredo.

Qualquer dúvida que eu tenha, agora ou mais tarde, sobre a pesquisa poderá ser esclarecida pelo Dr. Rodrigo Jales, médico responsável pela pesquisa, pelo telefone (019) ***3521-9500.*** *Se eu quiser, eu também posso tirar dúvidas sobre a pesquisa com o Dr Emílio, chefe do setor de ultrassonografia, pelo telefone* ***3521-9533****. Também posso entrar em contato com o setor responsável por pesquisas nos hospitais da UNICAMP pelo telefone* ***(019) 3521-8936.***

O título da Pesquisa é: Avaliação do valor preditivo positivo da Ultrassonografia mamária na subclassificação da categoria 4 do BIRADS-US.

Assinando este documento, eu concordo em participar desta pesquisa.

*Nome:*_____*HC:*_____

*RG:*_____*Idade:*_____

*Endereço: (rua/av)*_____*no.*_____

*Bairro:*_____*Cidade:*_____*UF:*_____

Para participar da pesquisa, assine aqui o seu nome

*Campinas,*_____*de*_____*de 2011*

7.2. Anexo 2 – Paracer da Comissão de Pesquisa DTG/CAISM

UNICAIUIP

Comissão de Pesquisa do DTG / CAISM

Campinas, 3 de dezembro de 2008

Protocolo n°: 059/2008

O protocolo de pesquisa *"Valor da ultra-sonografia mamaria na subclassificação da categoria 4 do BIRADS-US, sua variação interobservador e comparação com a subclassificação mamográfica"* do pesquisador Rodrigo Menezes Jales sob a orientação da Profa. Dra. Sophie Françoise Mauricette Derchain e co-orientação do Prof. Dr. Renato Zocchio Torresan foi aprovado pela Comissão de Pesquisa do DTG/CAISM em 02/12/2008.

Atenciosamente,

Profa. Dra. Ellen Hardy
Presidenta

Rua Alexander Flemming, n.°101 - Cidade Universitária Zeferino Vaz - Campinas-SP
Fone: (19) 3521-9402
comissaopesquisa@caism.unicamp.br

7.3. Anexo 3 – Paracer do Comitê de Ética em Pesquisa

FACULDADE DE CIÊNCIAS MÉDICAS
COMITÊ DE ÉTICA EM PESQUISA

MP

(?) www.fcm.unicamp.br/pesquisa/etica/index.html

CEP, 17/02/09.
(Grupo III)

PARECER CEP: N° 031/2009 (Este n° deve ser citado nas correspondências referente a este projeto)
CAAE: 0022.0.146.000-09

I - IDENTIFICAÇÃO:

PROJETO: **"AVALIAÇÃO DO VALOR PREDITIVO POSITIVO DA SONOGRAFIA MAMARIA NA SUBCLASSIFICAÇÃO ULTRA-DA BIRADS-US".**
PESQUISADOR RESPONSÁVEL: Rodrigo Menezes Jales
INSTITUIÇÃO: CAISM/UNICAMP
APRESENTAÇÃO AO CEP: 03/02/2009
APRESENTAR RELATÓRIO EM: 17/02/10 (O formulário encontra-se no *site* acima)

II - OBJETIVOS

Avaliar o valor preditivo positivo da Ultra-sonografia mamaria na subclassificação da categoria 4 do BIRADS-US, avaliar sua variação intra e interobservador e comparar seu desempenho com a Mamografia isoladamente ou como método associado à Ultra-sonografia.

III - SUMÁRIO

Será realizado um estudo de validação de teste diagnóstico com cerca de 210 mulheres com nódulo palpável ou Mamográfico de mama que sejam classificados pela Ultra-sonografia na categoria BIRADS 4, acompanhadas no Ambulatório de Patologia Mamaria do CAISM, utilizando como padrão ouro o resultado histológico obtido por biópsia de fragmento ou excisional. Análise dos dados: Serão calculados os valores preditivos das subcategorias BIRADS 4 A, B e C Ultra-sonográficos e Mamográficos, quando aplicável. Será avaliada as variações intra e interobservador da classificação Ultra-sonográfica, pelo estatística Kappa. A comparação entre os desempenhos da Ultra-sonografia e da Mamografia será realizada pela estatística do %2.

IV - COMENTÁRIOS DOS RELATORES

O projeto apresenta-se bem redigido, com metodologia adequada. Os critérios de inclusão, exclusão e descontinuação dos sujeitos estão bem definidos; cálculo do tamanho amostrai e análise estatística muito bem embasados por cálculos estatísticos. Os aspectos éticos estão bem discutidos no corpo do projeto e o Termo de Consentimento Livre e Esclarecido é claro e adequado.

Recomendamos apenas que os prefixos de telefone seja corrigido no Termo de Consentimento Livre e Esclarecido.

(f) www.fcm.unicamp.br/pesquisa/etica/index.httnl

[iMP

V - PARECER DO CEP

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP, após acatar os pareceres dos membros-relatores previamente designados para o presente caso e atendendo todos os dispositivos das Resoluções 196/96 e complementares, resolve aprovar sem restrições o Protocolo de Pesquisa, bem como ter aprovado o Termo do Consentimento Livre e Esclarecido, assim como todos os anexos incluídos na Pesquisa supracitada.

O conteúdo e as conclusões aqui apresentados são de responsabilidade exclusiva do CEP/FCM/UNICAMP e não representam a opinião da Universidade Estadual de Campinas nem a comprometem.

VI - INFORMAÇÕES COMPLEMENTARES

O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado (Rés. CNS 196/96 - Item IV.l.f) e deve receber uma cópia do Termo de Consentimento Livre e Esclarecido, na íntegra, por ele assinado (Item IV.2.d).

Pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou (Rés. CNS Item III. 1.z), exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade do regime oferecido a um dos grupos de pesquisa (Item V.3.).

O CEP deve ser informado de todos os efeitos adversos ou fatos relevantes que alterem o curso normal do estudo (Rés. CNS Item V.4.). É papel do pesquisador assegurar medidas imediatas adequadas frente a evento adverso grave ocorrido (mesmo que tenha sido em outro centro) e enviar notificação ao CEP e à Agência Nacional de Vigilância Sanitária - ANVISA -junto com seu posicionamento.

Eventuais modificações ou emendas ao protocolo devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas. Em caso de projeto'do Grupo I ou II apresentados anteriormente à ANVISA, o pesquisador ou patrocinador deve enviá-las também à mesma junto com o parecer aprovatório do CEP, para serem juntadas ao protocolo inicial (Rés. 251/97, Item III.2.e)

Relatórios parciais e final devem ser apresentados ao CEP, de acordo com os prazos estabelecidos na Resolução CNS-MS 196/96.

VII - DATA DA REUNIÃO

Homologado na II Reunião Ordinária do CEP/FCM, em 17 de fevereiro de 2009.

Profa. Dm. Catmen Sílvia Bertuzzo
PRESIDENTE DO COMITÊ DE ÉTICA EM PESQUISA
FCM / UNICAMP

7.4. Anexo 4 – Poster referente ao artigo 2, apresentado no 8th European Breast Cancer Conference (EBCC-8). Vienna, Austria, março de 2012

Poster 85 - Detection, Diagnosis and Imaging

Sonographic Features of BI-RADS®- US Breast Masses in Luminal, HER-2 Overexpression and Triple Negative Phenotypes

Rodrigo Menezes Jales, Luís Otavio Sarian, Renato Torresan, Emílio Francisco Marussi, Beatriz Regina Alvares, Sophie Derchain
E-mail: rodrigoj@hotmail.com

State University of Campinas – Unicamp - Campinas – São Paulo - Brazil

Background: Molecular dissimilarities between breast cancer types often produce different clinical presentations, prognoses and response to various treatments. It has been demonstrated that breast ultrasound (US) has a high degree of accuracy in the differentiation between benign and malignant lesions. Moreover, a growing body of evidence emerged suggesting that the predictive molecular profiling of breast malignancies also correlates with some sonographic findings. However, how well the BI-RADS®-US categorization of breast lesions correlates with the molecular profile of breast cancer remains largely unknown.

Purpose: To assess sonographic characteristics of BI-RADS®-US category 4 breast masses in luminal, HER2 overexpressing and triple-negative phenotypes.

Methods: In this cross-sectional study, we selected 327 sonographic breast masses classified as BI-RADS®-US subcategories 4a, 4b and 4c. Histologic examination of all masses confirmed 132 (40%) malignant or 195 (60%) benign tumors. Malignant lesions were grouped into three phenotypic subtypes: luminal (ER or PR positive, n=89), HER2 overexpressing (ER and PR negative and HER2 positive n=16) and triple-negative (ER, PR and HER2 negative n=27) phenotype categories using previously published Immunohistochemical methods. We compared sonographic features of the malignant lesions to tumor phenotype status.

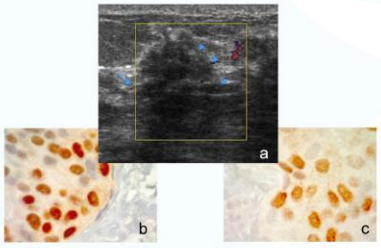

Figure 1: 45-year-old woman with a palpable breast mass. Ultrasound image shows the predominant indistinct (arrow head), also presenting spiculated margins (arrow), with discrete echogenic halo and shadowing mass, classified as BI-RADS®-US 4c (Figure 1a). Pathologic diagnosis: Invasive ductal carcinoma associated with multiple foci of ductal carcinoma *in situ* of solid and cribriform types (luminal phenotype). Positive reaction for ER (Figure 1b) and PR (Figure 1c) was observed as strong brown-black, fine, intranuclear granules (original magnification [100X]).

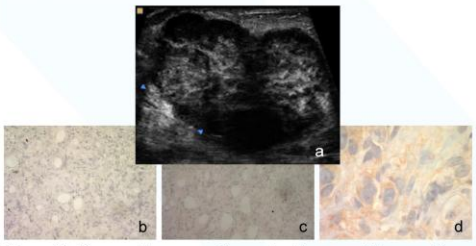

Figure 2: 62-year-old woman with a palpable mass. Ultrasound image shows the circumscribed, abrupt interface, with enhancement immediately deep to the lesion (arrow head) mass, classified as BI-RADS®-US 4a (Figure 2a). Pathologic diagnosis: invasive ductal carcinoma (triple-negative phenotype). Negative reaction for ER (Figure 2b) and PR (Figure 2c) was observed as no brown-black, fine, intranuclear granules (original magnification [40x]). Negative reaction for HER2 (Figure 2d) was observed as a faint/barely perceptible membranous reactivity (score 1). Cytoplasmic staining should be ignored (original magnification [100X]).

Results: BI-RADS®-US subcategories 4a, 4b e 4c were not clearly related to luminal, triple-negative and HER2 overexpressing phenotypes **(table 1)**.

Table 1: distribution of BI-RADS®-US 4a, 4b and 4c malignant masses by molecular profiling

BI-RADS® US	Luminal (ER+ or PR+)		HER-2 overexpression (ER-/PR-/HER2+)		Triple negative (PR-/ER-/HER2-)	
	N(%)	OR(95% CI)	N(%)	OR(95% CI)	N(%)	OR(95% CI)
4 a	16(76)	ref	0	ref	3(15)	ref
4 b	29(48)	0.29(0.09-0.9)	12(21)	11(0.6-195)	17(29)	2.35(0.61-9.1)
4 c	44(74)	0.92(0.3-2.9)	4(7)	3.6(0.2-71)	7(13)	0.84(0.2-3.6)
Total	**89**		**16**		**27**	

OR= odds ratios, CI=confidence interval, ref=reference variable

The luminal phenotype was positively associated with the following sonographic features: spiculated margin (OR=6.4; 95%CI=1.8 to 23.6), indistinct margin (OR=17.2; 95%CI=1.8 to 23.6), echogenic halo (OR=3.8; 95%CI=1.05 to 13.6). The luminal phenotype was negatively associated with enhancement (OR=0.3; 95%CI=0.15 to 0.76). Triple-negative phenotype was negatively associated with spiculated margin (OR=0.13; 95%CI=0.02 to 0.8) and shadowing (OR=0.02; 95%CI=0.01 to 0.47). The HER2 phenotype was not associated with any of the sonographic features **(table 2)**.

Table 2: distribution of sonographic findings by molecular profiling in BI-RADS®-US 4 malignant mass

US findings	Luminal (ER+ or PR+)		HER-2 overexpression (ER-/PR-/HER2+)		T)riple negative (PR-/ER-/HER2-)	
	N(%)	OR(95% CI)	N(%)	OR(95% CI)	N(%)	OR(95% CI)
Mass shape						
Oval	55(62)	Ref	11(13)	Ref	15(18)	Ref
Round	3(60)	0.9(0.14-5.6)	1(20)	1.6(0.16-15.8)	1(20)	1.1(0.1-10.7)
Irregular	31(66)	1.2(0.55-2.4)	4(9)	0.6(0.19-2.11)	11(24)	1.4(0.6-3.5)
Mass margin						
Circumscribed	13(45)	Ref	5(18)	Ref	9(33)	Ref
Microlobulated	33(57)	1.6(0.66-3.9)	8(14)	0.7(0.22-2.5)	14(25)	0.67(0.2-1.8)
Angular	8(61)	1.97(0.5-7.4)	0	0.18(0.01-3.5)	3(27)	0.75(0.1-3.5)
Indistinct	14(93)	17(1.9-149)	1(7)	0.3(0.03-2.98)	0	0.06(0-1.17)
Spiculated	21(84)	6.4(1.7-23)	2(9)	0.4(0.07-2.4)	1(4)	0.13(0.02-0.8)
Mass orientation						
Parallel	72(64)	Ref	14(13)	Ref	21(20)	Ref
Taller	17(63)	0.97(0.4-2.3)	2(8)	0.6(0.12-2.72)	6(24)	1.29(0.4-3.6)
Echo						
Hypoechoic	81(66)	Ref	13(11)	Ref	21(18)	Ref
Complex	8(44)	0.4(0.15-1.1)	3(18)	1.7(0.43-6.6)	6(35)	2.44(0.8-7.3)
Lesion boundary						
Abrupt interface	72(60)	Ref	15(13)	Ref	26(23)	Ref
Echogenic halo	17(85)	3.8(1.0-13)	1(6)	0.4(0.05-3.4)	1(6)	0.2(0.03-1.7)
Posterior acoustic features						
None	57(70)	Ref	9(12)	Ref	9(60)	Ref
Shadowing	13(87)	2.7(0.57-13)	1(7)	0.56(0.07-4.8)	0	0.02(0.01-0.4)
Enhancement	16(44)	0.3(0.15-0.7)	6(17)	1.52(0.49-4.6)	13(37)	0.39(0.1-1.3)
Combined	3(37)	0.25(0.06-1)	0	0.4(0.02-7.7)	5(62)	1.1(0.19-6.4)
Vascularity						
Absent	33(67)	Ref	3(7)	Ref	7(16)	Ref
Present in lesion	45(58)	0.66(0.3-1.4)	13(17)	3(0.8-11.1)	18(24)	1.74(0.6-4.5)
Present adjacent	39(66)	0.95(0.4-2.1)	6(10)	1.6(0.4-6.8)	14(24)	1.73(0.6-4.7)

OR= odds ratios, CI=confidence interval, ref=reference variable

Conclusion: Although some sonographic features were related to luminal and triple-negative phenotypes, BI-RADS®-US subcategories 4a, 4b and 4c were not clearly related to these molecular markers.

Partially supported by Fapesp 2009/17097-1