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CARCINOMAS MAMÁRIOS IN SITU DE PADRÃO MISTO: CARACTERIZAÇÃO IMUNOFENOTÍPICA E VARIAÇÃO DIAGNÓSTICA ENTRE OBSERVADORES

Belo Horizonte
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Tese apresentada ao Programa de Pós-graduação em Patologia da Universidade Federal de Minas Gerais, como requisito parcial à obtenção de título de Doutor em Patologia - área de concentração em Patologia Médica.

Orientadora: Helenice Gobbi

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FOLHA DE APROVAÇÃO

"CARCINOMAS MAMÁRIOS IN SITU DE PADRÃO MISTO: CARACTERIZAÇÃO IMUNOFENOTÍPICA E VARIABILIDADE DIAGNÓSTICA ENTRE OBSERVADORES"

DOUGLAS SOLTAU GOMES

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em PATOLOGIA, como requisito para obtenção do grau de Doutor em PATOLOGIA, área de concentração PATOLOGIA MÉDICA.

Aprovada em 21 de fevereiro de 2014, pela banca constituída pelos membros:

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RESUMO

A distinção entre neoplasia lobular (NL) e carcinoma ductal in situ (CDIS) tem importantes implicações terapêuticas. Em alguns casos é difícil determinar se a morfologia da lesão é ductal ou lobular. Este grupo tem sido denominado de carcinomas in situ de padrão misto (CISM). É frequente também a associação de lesões de células colunares (LCC) com NL e CDIS de baixo grau. Os objetivos deste estudo foram avaliar a variação interobservador dos diagnósticos de NL, CDIS e LCC do laudo original e após revisão por patologista especializada em patologia mamária em biopsias enviadas para consultoria e/ou segunda opinião, e avaliar a expressão de E-caderina e β-catenina na caracterização imunofenotípica em CISM. Trata-se de estudo retrospectivo, observacional, do tipo transversal. Para a análise da variação interobservador foram analisados 610 casos de espécimes mamários enviados formalmente para consultoria e/ou segunda opinião ao Laboratório de Patologia Mamária da Faculdade de Medicina da Universidade Federal de Minas Gerais, no período de 2005 a 2010. Foi comparada a variação interobservador entre o laudo original e o laudo de revisão no diagnóstico de LCC, NL e CDIS empregando-se o índice de Kappa. Fez-se ainda estudo imuno-histoquímico para E-caderina e β-catenina de vinte e cinco casos com diagnóstico de CISM diagnosticados entre 1999 e 2011 baseando-se na citologia/arquitetura mista (ductal e lobular), pleomorfismo nuclear e presença de comedonecrose. A expressão imuno-histoquímica dos marcadores foi considerada positiva ou negativa. Observamos concordância fraca no diagnóstico das alterações de células colunares (ACC) (Kappa= 0,38), hiperplasia de células colunares (HCC) (Kappa= 0,32) e ACC com atipias (Kappa= 0,29). A concordância entre os diagnósticos de HCC com atipias foi considerada moderada (Kappa= 0,43). Quanto as NL, a concordância foi considerada boa para hiperplasia lobular atípica (HLA) (Kappa= 0,62) e carcinoma lobular in situ (CLIS) (Kappa= 0,66). Entretanto, a concordância entre os diagnósticos de CLIS pleomórfico foi considerado baixa (Kappa = 0,22). Hiperplasia ductal atípica teve concordância moderada (Kappa= 0,44), assim como CDIS de baixo grau (Kappa= 0,47), CDIS de grau intermediário (Kappa= 0,45) e CDIS com microinvasão (Kappa= 0,56). Houve boa concordância diagnóstica nos casos de CDIS de alto grau (Kappa = 0,68). Dos 25 casos de CISM analisados, dezenove (76%) apresentavam somente citologia e/ou padrão arquitetural misto (ductal e lobular), dois casos (8%) apresentaram somente pleomorfismo nuclear, dois casos (8%) apresentavam citologia mista e pleomorfismo nuclear, e dois casos (8%) tinham comedonecrose e pleomorfismo nuclear. Positividade completa para E-caderina e β-catenina foi observada em 11 casos (44%). Em um caso, com pleomorfismo nuclear e comedonecrose, a lesão foi negativa para ambos os marcadores. Em 13 lesões os marcadores foram negativos em áreas de morfologia lobular e positivos em áreas de morfologia ductal. Nossos dados demonstraram maior concordância diagnóstica no CDIS de alto grau, HLA e CLIS. As LCC sem atipias e o CLIS pleomórfico tiveram os menores índices de concordância diagnóstica. As colorações para E-caderina e β-catenina, associadas com a análise citológica e arquitetural, permitiram destacar diferentes imunofenótipos e auxiliar na classificação dos CISM.

Palavras-chave: câncer de mama; neoplasia lobular; carcinoma ductal in situ; E-caderina; concordância diagnóstica.

ABSTRACT

The distinction between lobular neoplasia (LN) and ductal carcinoma in situ (DCIS) has important therapeutic implications. In some cases, it is very difficult to determine whether the morphology of the lesion is ductal or lobular. This group has been called carcinomas in situ with a mixed pattern (CISM). LN and low grade DCIS are frequently associated to columnar cell lesions (CCL). The aims of this study were to evaluate the inter-observer variability in the diagnosis of LN, CDIS and CCL of the breast comparing the original reports and the review of an expert in breast pathology of cases sent in consultation. We also evaluated the immunoexpression of E-cadherin and β-catenin in a series of CISM. This is a retrospective, observational, cross-sectional study. For the analysis of interobserver variability we analyzed 610 cases of breast specimens formally received in consultation for a second opinion in the Breast Pathology Laboratory, School of Medicine of Federal University of Minas Gerais, from 2005 to 2010. Interobserver variation between the diagnosis of LN, DCIS and CCL of original report and the reviewer report was compared. Statistical analysis was performed using the Kappa test. For the immunohistochemical study, a total of 25 cases of breast biopsies diagnosed with CISM, performed between 1999 and 2011, were analyzed considering cytology/mixed architecture (ductal and lobular), nuclear pleomorphism, loss of cell cohesion, and presence of comedonecrosis. The immunophenotype pattern was considered E-cadherin positive and β-catenin positive, or negative. We observed a poor agreement for the diagnosis of columnar cell change (CCC) (Kappa = 0.38), columnar cell hyperplasia (CCH) (Kappa = 0.32) and CCC with atypia (kappa = 0.29). The agreement between the diagnosis of CCH with atypia was considered moderate (kappa = 0.43). However, the agreement between diagnosis of pleomorphic LCIS was considered poor (Kappa = 0.22). Atypical ductal hyperplasia (ADH) (Kappa= 0.44), low-grade DCIS (Kappa= 0.47), intermediate grade DCIS (Kappa= 0.45) and DCIS with microinvasion (Kappa= 0.56) had a moderate agreement in their diagnosis. There was a good agreement in the diagnosis of high grade DCIS (kappa= 0.68). Of the 25 cases analyzed CISM, nineteen (76%) cases presented a mixed cytology and/or architectural pattern, two (8%) presented nuclear pleomorphism, two (8%) presented mixed cytology and nuclear pleomorphism, and two (8%) presented comedonecrosis and nuclear pleomorphism. A complete positivity for E-cadherin and β-catenin was observed in 11 cases (44%). In one case, the lesion was negative for both markers and showed nuclear pleomorphis. Thirteen lesions showed negative staining in areas of lobular cytology and positive staining in cells presenting ductal pattern. Our data demonstrate a greater diagnostic agreement in high-grade DCIS, LCIS and ALH. The CCL without atypia and pleomorphic LCIS had the lowest rates of diagnostic concordance. The expression of E-cadherin and β-catenin, in association to cytological and architectural analysis, may highlight different immunophenotypes and improve classification of CISM.

Keywords: breast cancer; lobular neoplasia; ductal carcinoma in situ; E-cadherin; diagnostic concordance.

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LISTAS DE ABREVIATURAS E SIGLAS

16q	Braço longo do cromossoma 16
1q	Braço longo do cromossoma 1
ACC	Alterações de células colunares
ADH	*Atypical ductal hyperplasia*
AEP	Atipia epitelial plana
ALH	*Atypical lobular hyperplasia*
BPL	*Breast Pathology Laboratory*
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CCC	*Columnar cell change*
CCH	*Columnar cell hyperplasia*
CCL	*Columnar cell lesions*
CDI	Carcinomas ductais invasivos
CDIS	Carcinoma ductal in situ
CISM	Carcinomas in situ de padrão misto
CISM	*Carcinomas in situ with a mixed pattern*
CK	Citoqueratina
CLI	Carcinoma lobular invasivo
CLIS	Carcinoma lobular in situ
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
DCIS	*Ductal carcinoma in situ*
DNA	Ácido desoxirribonucleico
E-cadherin	*Human Epitelial Cadherin*
EUA	Estados Unidos da América
FAPEMIG	Fundação de Amparo à Pesquisa do Estado de Minas Gerais
FEA	*Flat epithelial atypia*
HCC	Hiperplasia de células colunares
HDA	Hiperplasia ductal atípica
HDU	Hiperplasia de tipo usual
HER2	*Human Epidermal growth factor Receptor 2*
HLA	Hiperplasia lobular atípica
IHC	Immunohitochemistry
LCC	Lesões de células colunares
LCIS	*Lobular carcinoma in situ*
LN	*Lobular neoplasia*
NL	Neoplasia lobular
OMS	Organização Mundial da Saúde
PLCIS	*PleomorphicLobular carcinoma in situ*
RE	Receptores de estrógenos
RNA	Ácido Ribonucleico
TDLU	*Terminal-duct lobular unit*
UDLT	Unidade ducto-lobular terminal
UFMG	Universidade Federal de Minas Gerais
US	*Unspecified*
WHO	*World Health Organization*

SUMÁRIO

1. INTRODUÇÃO

1.1. Epidemiologia do câncer de mama

O câncer de mama é o carcinoma mais comum entre mulheres, sendo responsável por 23% dos cânceres em mulheres no mundo, 14% das mortes por câncer e a segunda causa de morte em mulheres em geral (SIEGEL; NAISHADHAM; JEMAL, 2013). O risco de desenvolver câncer de mama aumentou até a década de 80, tanto em países desenvolvidos, quanto nos países menos desenvolvidos. Os índices de incidência nos países menos desenvolvidos ainda têm aumentado, entretanto, nos países desenvolvidos a incidência deixou de aumentar e tem declinado desde então, principalmente como resultado do rastreamento mamográfico. Desde 2005, ocorre uma diminuição na incidência do câncer de mama em muitos países da Europa e Estados Unidos (EUA), que é atribuído, em parte, a diminuição do uso de terapia de reposição hormonal (LAKHANI et al., 2012). Entretanto, no Brasil, a incidência do câncer de mama cresce a cada ano, sendo estimado para o ano de 2014 cerca de 56,09 casos para cada 100.000 mulheres, com um total de 57.120 novos casos (INSTITUTO NACIONAL DE CÂNCER, 2013).

O prognóstico do câncer de mama é muito bom para aquelas pacientes que são detectadas em um estágio precoce da doença. Desde a década de 70, importantes avanços na sobrevida das pacientes com câncer de mama têm sido observados, principalmente a partir da década de 90, resultantes da combinação dos resultados do rastreamento mamográfico populacional e do tratamento adjuvante com hormonioterapia e quimioterapia, especialmente com a introdução de quimioterápicos de segunda e terceira geração e os inibidores da aromatase (LAKHANI et al., 2012).

A etiologia do câncer de mama é multifatorial e envolve fatores de estilo de vida, reprodutivos e hormonais. Os maiores fatores de risco para o seu desenvolvimento são a idade, predisposição genética, exposição a estrógenos, aumento densidade mamária e o desenvolvimento de lesões precursoras na mama (NELSON et al., 2012).

1.2. Lesões precursoras do câncer de mama

As lesões precursoras do câncer de mama são um grupo heterogêneo de lesões proliferativas da mama associadas a um risco aumentado para o desenvolvimento de um carcinoma invasivo subsequente. De acordo com suas características histológicas, tais como padrão arquitetural, atipia nuclear e índice de proliferação, vários sistemas de classificação e evolução para carcinoma invasor foram propostos para estas lesões. Embora esta classificação fosse inicialmente descritiva, o seu uso ainda é aplicado na atualidade para o manejo terapêutico e profilático das pacientes com base em estudos epidemiológicos de risco relativo para o desenvolvimento de carcinoma invasor (DUPONT; PAGE, 1985; LAKHANI et al., 2012; LOPEZ-GARCIA et al., 2010).

A classificação inicial das lesões precursoras ou pré-invasivas baseava-se na similaridade morfológica entre as lesões in situ e invasoras, onde alguns tipos de câncer surgiriam dos ductos e outros dos lóbulos, dando origem as nomenclaturas tradicionais do carcinoma ductal in situ (CDIS) e carcinoma lobular in situ (CLIS). Entretanto, foi demonstrado que a maioria das lesões precursoras surgem da unidade ducto-lobular terminal (UDLT) (WELLINGS; JENSEN, 1973). Posteriormente, foi proposto o modelo em que as células normais do epitélio mamário da UDTL iriam se transformar e dar origem a lesões precursoras morfologicamente identificáveis, ou seja, hiperplasia de tipo usual (HDU) que, então, avançariam para a hiperplasia ductal atípica (HDA) e, posteriormente, dariam origem ao CDIS de baixo grau. Nesse estágio, as células neoplásicas iriam adquirir alterações genéticas e/ou epigenéticas adicionais e iriam transformar-se em carcinoma invasivo ou progredir para CDIS de alto grau e, então, carcinoma invasivo. Um modelo semelhante também foi proposto para carcinoma lobular invasivo (CLI), onde as células da UDTL se transformariam e dariam origem a hiperplasia lobular atípica (HLA), CLIS e, eventualmente, CLI (LOPEZ-GARCIA et al., 2010). Recentemente, estudos analisando o DNA e/ou RNA através de técnicas de biologia molecular em lesões precursoras previamente micro dissecadas tem colocado em dúvida este modelo.

Fundamentado em análises genômicas e transcriptômicas das lesões precursoras, sugere-se que as lesões in situ de alto e baixo grau, bem como suas respectivas contrapartes invasoras, seriam fundamentalmente diferentes. Este conceito é baseado primariamente na observação de que CDIS de baixo grau e carcinomas ductais invasivos (CDI) de baixo grau são na maioria das vezes diploides e apresentam deleções recorrentes do braço longo do cromossoma 16 (16q), estando presentes em mais de 80% dos casos. Por outro lado, os cânceres de mama de alto grau têm cariótipos mais complexos, são geralmente aneuplóides e abrigam múltiplas amplificações. Apesar da maior complexidade dos genomas dos CDIS e CDI de alto grau, deleções de 16q são encontrados em menos que 30% dos casos. (LOPEZ-GARCIA et al., 2010; REIS-FILHO et al., 2005; SIMPSON et al., 2005).

Embora a influência do grau histológico sobre a biologia do câncer de mama tenha sido confirmada, estudos posteriores demonstram que os cânceres de mama dividem-se essencialmente em dois grupos principais com base na expressão de receptores de estrógenos

(RE) e genes regulados por RE, sugerindo que o expressão de RE e ativação da via dos RE também desempenham um papel importante na determinação das vias de desenvolvimento e progressão do câncer de mama. Apesar de cânceres de mama RE-positivo e RE-negativos serem doenças diferentes em termos de seus fatores de risco, padrões de disseminação, comportamento clínico e resposta às terapias, há evidências que sugerem que o nível de instabilidade genética exibido por uma determinada lesão está relacionada com o seu grau histológico (LAKHANI et al., 2012; LOPEZ-GARCIA et al., 2010).

Inúmeras lesões proliferativas da mama têm sido associadas com um aumento do risco de desenvolvimento de câncer de mama na mesma mama (ipsilateral) ou na mama contralateral. Algumas destas lesões têm se mostrado clonais, e possuem características histológicas, imuno-histoquimicas e moleculares idênticas às dos cânceres de mama invasivos associados, sejam eles síncronos ou metacrônicos; lesões que cumpram estes critérios são consideradas precursoras do câncer de mama (LAKHANI et al., 2012; LOPEZ-GARCIA et al., 2010). Estudos observacionais e moleculares demonstraram recentemente que uma família de lesões in situ da mama coexistem em frequências que não poderiam ser justificadas por acaso. Esta lesões precursoras englobam as lesões de células colunares (LCC), atipia epitelial plana (AEP), HDA, HLA, CLIS e CDIS de baixo grau – também chamadas de "Família das neoplasias de baixo grau nuclear da mama". Essas lesões são caracterizadas por baixo grau histológico, expressão de receptores hormonais, ausência de expressão HER2 e amplificação do gene HER2, aberrações genéticas normalmente encontrados em cânceres de mama de baixo grau, ou seja, deleções de 16q e ganhos de 1q. (ABDEL-FATAH et al., 2007).

1.3. Lesões de células colunares

As lesões de células colunares constituem um espectro de alterações que surgem na UTDL caracterizado pela presença de células epiteliais colunares revestindo UTDL variavelmente aumentadas, com ácinos dilatados, revestidos por células colunares altas, com frequente secreção apical (*apical snouts*); secreção luminal e presença frequente de microcalcificações (LAKHANI et al., 2012). Nos últimos anos, há um interesse aumentado por estas lesões, pois estão sendo encontradas com frequência cada vez maior em biópsias mamárias realizadas devido à presença de microcalcificações na mamografia ou como achado incidental. Estas lesões historicamente têm sido referidas por diferentes nomenclaturas, desde "blunt duct adenosis" até "clinging carcinoma" (EUSEBI et al., 1989). A nomenclatura e critérios diagnósticos das LCC mais utilizados e com maior possibilidade de reprodutibilidade diagnóstica (O'MALLEY *et al.*, 2006), têm sido os de Schnitt e Vincent-Salomon (2003) onde:

- Alteração de células colunares (ACC) – caracterizada por uma ou duas camadas de células colunares com núcleos uniformes e alongados, orientadas perpendicularmente a membrana basal. Os nucléolos são ausentes ou não facilmente percebidos. (FIG. 1A)
- Hiperplasia de células colunares (HCC) - caracterizada por mais de duas camadas de células colunares com núcleos uniformes e alongados, orientados perpendicularmente a membrana basal. Os nucléolos são ausentes ou não facilmente percebidos. Padrões arquiteturais complexos não estão presentes. (FIG. 1B)
- Alteração de células colunares com atipias (ACC com atipias) - caracterizada por uma ou duas camadas de células colunares com atipias citológicas presentes (geralmente de baixo grau). As células ficam semelhantes às do carcinoma tubular; mitoses são incomuns. (FIG. 1C)
- Hiperplasia de células colunares com atipias (HCC com atipias) - caracterizada por mais de duas camadas de células colunares com atipias citológicas presentes (geralmente de baixo grau). As células ficam semelhantes às do carcinoma tubular, mitoses são incomuns. (FIG. 1D)

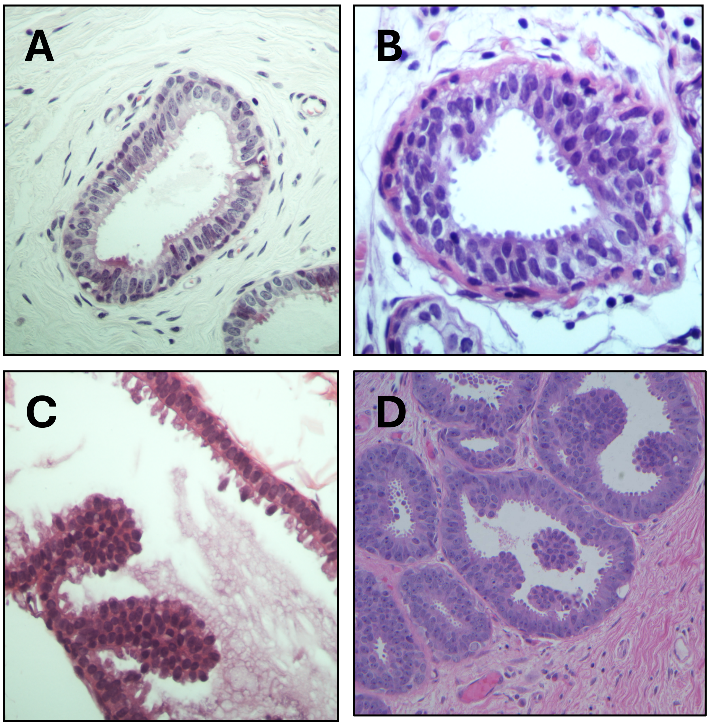

Figura 1 - Espectro das alterações de células colunares. Em A, alteração de células colunares sem atipias. Em B, hiperplasia de células colunares sem atipias. Em C, alteração de células colunares com atipias (formação de micropapilas ou microtufos). Em D, Hiperplasia de células colunares com atipias (GOMES, 2010).

Em 2003, a classificação OMS englobou as LCC com atipias (ACC com atipias e HCC com atipias) sob o termo de "atipia epitelial plana" (AEP) (TAVASSOLI; DEVILEE, 2003), em sua última versão (2012) esta nomenclatura foi mantida sendo realizada a divisão em dois grupos: lesões de células colunares e atipias epiteliais planas (LAKHANI et al., 2012).

Estudos recentes tem demonstrado que as LCC com atipias (ou atipia epitelial plana) apresentam similaridades genéticas e a coexistência com outras neoplasias da família de baixo grau nuclear da mama, bem como CDIS de baixo grau e carcinoma tubular, sugerindo um status de lesão precursora dos carcinomas invasores de baixo grau histológico para estas lesões (ABDEL-FATAH et al., 2007). Entretanto, poucos dados disponíveis, oriundos de séries pequenas, com seguimento clínico muito limitado, tem avaliado o potencial da AEP evoluir para carcinoma invasor. Eusebi et al. (1989) identificou 25 pacientes entre 9.000 biópsias de mama que tinham AEP. Essas pacientes foram submetidas somente a biópsia diagnóstica, sem qualquer tentativa de excisão completa. Apenas uma paciente (4%) tinha AEP recorrente ou persistente, enquanto nenhuma das pacientes desenvolveram câncer de mama em um seguimento médio de 19,2 anos. Entre as pacientes do estudo *EORTC 10853 trial*, que randomizou mulheres com CDIS somente com excisão contra excisão e radioterapia, 59 pacientes foram identificados que estariam atualmente classificadas como tendo AEP. Com um seguimento médio de 5,4 anos, nenhuma dessas pacientes apresentaram recorrência local ou desenvolvimento de um câncer invasivo posterior (BIJKER et al., 2006; DONKER et al., 2013). Boulos et al. (2008) avaliou o risco geral de câncer de 1.261 biópsias com lesões de células colunares em 4.569 mulheres, com seguimento de 17 anos. Foi observado um leve aumento do risco relativo (RR) para o desenvolvimento de câncer de mama quando comparado com o risco de doença em lesões não proliferativas, RR 1,47 (IC 95%, 1,0 - 2,2, $p = 0,05$). Este foi consideravelmente menor do que o risco visto com ADH; RR 3,31 (IC 95% 1,2-9,4, p = 0,02).

O tratamento cirúrgico da AEP pode ser determinada pelo tipo de procedimento de diagnóstico. Quando diagnosticada por uma biópsia excisional, não é necessário terapia adicional ou re-excisão. Quando a AEP é identificada em uma biópsia por agulha, foi encontrada uma taxa de subestimação de CDIS de 1,5% em LCC sem atipias, 9% em LCC com atipias, e de 20% quando LCC estava associado com HDA (VERSCHUUR-MAES et al., 2012). Entretanto, devido às limitações dos estudos e à grande variação nos tempos de seguimento após a biópsia inicial ainda é incerto se há necessidade de se indicar excisão cirúrgica após diagnóstico de AEP. Na ausência de uma anormalidade mamográfica residual vigilância radiológica tem sido defendida por alguns com base em revisões retrospectivas (JARA-LAZARO; TSE; TAN, 2009; LAKHANI et al., 2012).

1.4. Carcinoma ductal *in situ*

Carcinoma ductal *in situ* é uma proliferação neoplásica de células epiteliais confinadas ao sistema ducto-lobular e caracterizadas pela presença de atipias citológicas que vão de sutis a evidentes, e uma tendência inerente, mas não necessariamente obrigatória para progressão para o câncer de mama invasivo (LAKHANI et al., 2012). Fora dos programas de rastreamento mamográfico, o CDIS constitui aproximadamente 5% dos cânceres de mama, mas dentro de programas de rastreamento este índice chega a 20-25% (PINDER, 2010).

A heterogeneidade microscópica do CDIS tem levado ao desenvolvimento de uma série de sistemas de classificação. Historicamente, CDIS foi classificado com base no padrão arquitetural da proliferação, incluindo comedo, cribiforme, micropapilar, sólido ou subtipos mistos. No entanto, a reprodutibilidade do sistema de categorização com base somente no padrão de crescimento é problemática, pois o CDIS mostra na maioria das vezes mais de um padrão arquitetural. Por conseguinte, os sistemas mais recentes tendem a ser baseados no grau nuclear, o que é menos comumente sobreposto (15,7%), alguns incorporando também a presença ou a ausência do necrose luminal (PINDER, 2010; SNEIGE et al., 1999).

Tabela 2 - Classificação do grau histológico do tumor no CDIS

	Baixo grau	Grau intermediário	Alto grau
Grau nuclear	G2 ou G1	G2 ou G1	G3
Necrose	Ausente ou escassa	Presente	Geralmente presente e extensa
Arquitetura	Papilar e micropapilar	Todos os padrões, sobretudo misto e cribriforme	Comedo, misto, sólido, micropapilar, raramente cribriforme

Adaptado de Schmitt e Gobbi (2006)

O grau histológico do CDIS está relacionado com a probabilidade da progressão para carcinoma invasivo e a rapidez com a qual este irá provavelmente a ocorrer. Embora a radioterapia reduza o risco da recorrência local da doença em aproximadamente metade das pacientes, uma série de marcadores de prognóstico do CDIS, em adição ao grau histológico, foram identificados. O padrão de crescimento arquitetural do CDIS, a presença de necrose, idade jovem e detecção sintomática foram relatados como fatores de mau prognóstico para o CDIS e com o aumento na probabilidade de recorrência local da doença; assim como o tamanho da lesão e, em particular, a presença de margens comprometidas.

O tratamento para o CDIS inclui excisão ampla, também chamada de cirurgia conservadora da mama, seguida de radioterapia resulta em taxas de sobrevida comparáveis a mastectomia, embora os índices de recorrência local sejam um pouco maiores com a conservação da mama. O objetivo da cirúrgica conservadora deve ser para extirpar todo o foco de CDIS de alcançar margens negativas. Ampliação de margens ou mastectomia deve ser realizada se necessário para obter margens negativas. Para as mulheres com CDIS tratadas com cirurgia conservadora da mama indica-se o uso de tamoxifen, no entanto, a decisão de administrar tamoxifen em mulheres com CDIS com RE-negativo requer uma discussão individualizada sobre os riscos e benefícios do tratamento (NATIONAL COMPREHENSIVE CANCER NETWORK, 2013).

1.5. Neoplasias lobulares

Desde a sua descrição, há mais de 70 anos, os critérios diagnósticos morfológicos do carcinoma lobular *in situ* sofreram poucas mudanças significativas (FOOTE; STEWART, 1941). Tanto a hiperplasia lobular atípica (HLA) quanto o carcinoma lobular *in situ* (CLIS) são caracterizados pela proliferação de células uniformes, arredondadas ou cuboidais, com núcleos redondos e citoplasma claro. As células ocupam parcialmente ou totalmente o lóbulo, mas a arquitetura de base ainda pode ser reconhecida. A diferenciação entre as duas entidades depende da extensão do envolvimento lobular sendo mais quantitativa do que qualitativa e sujeita a variação na avaliação interobservador (FITZGIBBONS, 2000; LAKHANI et al., 2012).

Page *et al.* (1985; 1991) correlacionando a extensão das lesões lobulares e a evolução das pacientes, definiu HLA quando há preenchimento e expansão de menos de 50% dos ácinos de um ou mais lóbulos por células de citologia lobular proliferadas e CLIS quando há preenchimento e distensão de mais de 50% dos ácinos de uma unidade lobular e perda do lúmem intracelular residual, os riscos de desenvolvimento de carcinoma invasor foram calculados em 4-5 vezes para a HLA e 8-11 vezes para o CLIS (Figuras 2A e 2B). Eusebi et al. (1989) descreveram a variante pleomórfica do CLIS, que incluem o mesmo padrão arquitetural do CLIS, porém com pleomorfismo nuclear acentuado e citoplasma abundante, nucléolos mais evidentes, podendo apresentar áreas de necrose tipo comedo e microcalcificações, com ou sem características apócrinas (Figura 2C) (LAKHANI et al., 2012). Devido a suas características morfológicas, associado ao fato de que o perfil imuno-histoquímico do CLIS pleomórfico ser mais provavelmente negativos para receptores de estrógeno e positivos para HER2 e maior índice proliferativo ao KI-67, estas lesões tem sido associadas a um comportamento biológico mais agressivo que o CLIS clássico, entretanto há carência de estudos epidemiológicos que comprovem esta afirmação (EUSEBI; MAGALHAES; AZZOPARDI, 1992; LAKHANI et al., 2012; O'MALLEY, 2010). A maioria dos carcinomas invasivos, que ocorrem subsequentemente a casos de neoplasia lobular são do tipo ductal. Mas carcinomas lobulares invasivos podem constituir até 45% dos carcinomas subsequentes, número bem acima da incidência esperada (5-14%) de carcinomas lobulares invasivos na população em geral (CHUBA et al., 2005; FISHER, E. R. et al., 2004).

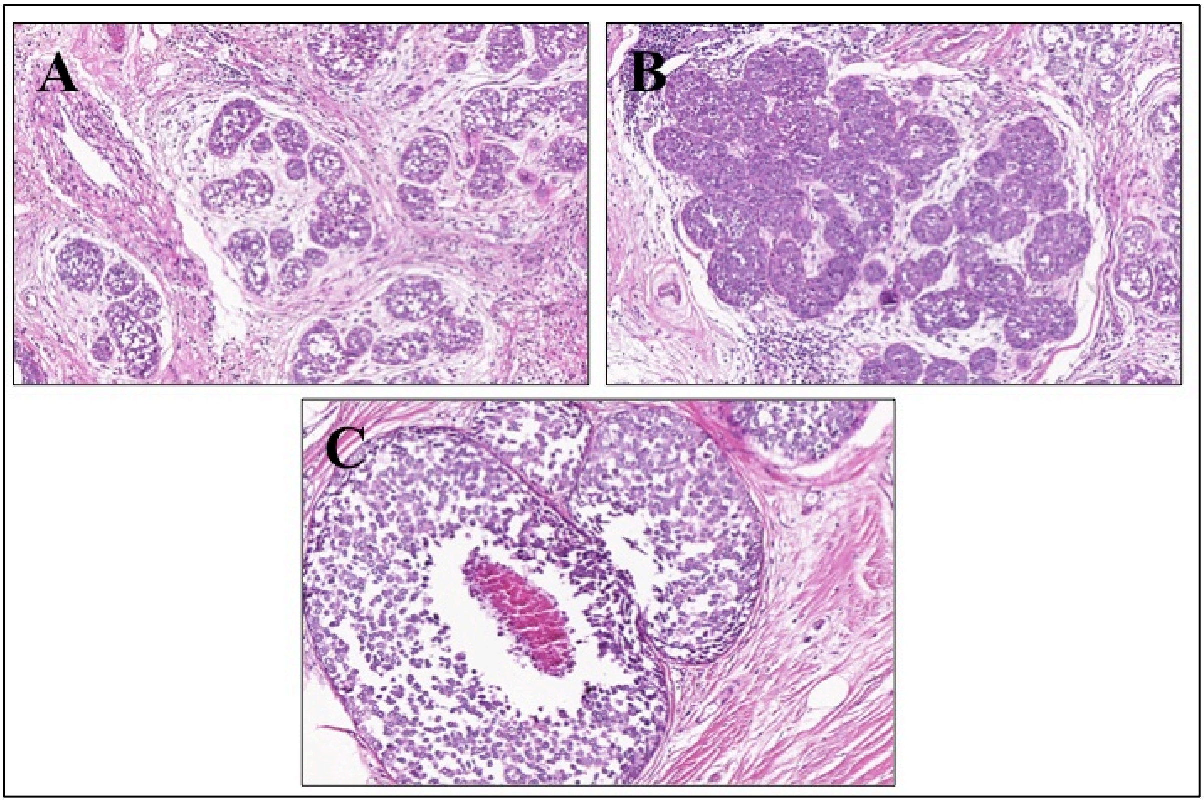

Figura 2 - Espectro das neoplasias lobulares. Em A, hiperplasia lobular atípica (HE – 100x). Em B, carcinoma lobular *in situ* (100x). Em D, CLIS pleomórfico (100x).

As NL são frequentemente multicêntricas e bilaterais (CHUBA et al., 2005). Ocorrem predominantemente em mulheres na pré-menopausa, sendo a maioria dos casos diagnosticados em mulheres entre os 40 e os 50 anos de idade. Dados derivados do *Surveillance, Epidemiology and End Results Program* têm demonstrado que a incidência ajustadas por idade de CLIS entre as mulheres nos EUA aumentou quatro vezes entre a década de 70 (0,9 por 100.000

pessoas/ano) e final da década de 90 (3.2 por 100.000 pessoas/ano). Mulheres com idade entre 50 e 59 anos experimentaram o maior aumento absoluto na incidência entre 1978 -1998. Esta crescente incidência não foi observado em mulheres na pré-menopausa ou em mulheres com mais de 70 anos de idade (LI et al., 2002; O'MALLEY, 2010).

1.6. Carcinomas *in situ* de padrão misto

Em alguns casos os critérios morfológicos para o diagnóstico das NL podem não ser bem identificados, causando confusão com outras lesões proliferativas intraductais. Esses casos, muitas vezes são compostos de células monótonas, pequenas e típicas de CLIS, mas aparecem de forma mais coesa. Alternativamente, estes casos podem assemelhar-se com padrão arquitetural de CDIS com formação de estruturas microacinares, mas apresentam a perda de coesão típica do CLIS (O'MALLEY, 2010). Os principais diagnósticos diferenciais das neoplasias lobulares são: CLIS com carcinoma ductal *in situ* sólido de baixo grau, CLIS pleomórfico e CDIS de alto grau, e carcinomas *in situ* com achados citológicos e/ou arquiteturais que se desviam do padrão não se podendo determinar se a proliferação é lobular ou ductal. Este último grupo tem sido denominado de carcinoma *in situ* de padrão misto ou de padrão indeterminado (GOLDSTEIN et al., 2001; JACOBS et al., 2001).

Existem implicações terapêuticas importantes neste diagnóstico diferencial. Pacientes com CLIS, na maioria das vezes, são acompanhadas clinicamente, podendo ser oferecido tamoxifeno como terapia profilática (FISHER, B. et al., 1998; SCHNITT; MORROW, 1999). Em contraste, pacientes com CDIS devem ser tratadas por excisão da lesão com margens livres e radioterapia, ou até mastectomia (LAKHANI et al., 2006). Quando diagnosticado através de core biopsy, o CDIS deve ser tratado com excisão completa da lesão seguida ou não de radioterapia. Porém ainda existem controvérsias quanto ao significado clínico do achado de CLIS em espécimes de core biopsy (LIBERMAN, 2000; LONDERO et al., 2008; NAGI et al., 2008).

1.7. E-caderina e o complexo caderina-cateninas

Recentemente, foi observado que quase todos os casos de HLA, CLIS e carcinoma lobular invasor (CLI) perdem a expressão imuno-histoquímica na membrana citoplasmática para a E-caderina, em contraste com os carcinomas ductais que mantêm sua expressão (DA SILVA et al., 2008; GOLDSTEIN et al., 2001; JACOBS et al., 2001; MOLL et al., 1993). Entretanto, deve-se ter cuidado ao se basear exclusivamente na expressão positiva da E-caderina para exclusão do diagnóstico de CLIS, pois até 15% dos CLIS podem apresentar expressão aberrante deste marcador (CHOI et al., 2008; DA SILVA et al., 2008).

Uma alternativa para diminuir esta interferência, seria a associação de marcadores imuno-histoquímicos da via das cateninas. Da Silva (2008) analisou através de imuno-histoquímica e técnicas de biologia molecular três casos que apresentavam morfologia e genotipagem característicos de CLI, mas com expressão aberrante de E-caderina. Destes três casos, dois apresentavam expressão negativa de β-catenina, demonstrando falha na formação do complexo caderina-catenina que é requerida para a função normal da célula e manutenção da arquitetura tecidual, inclusive a adesão celular (DA SILVA et al., 2008; DABBS; KAPLAI; et al., 2007). Outra explicação para esta expressão anormal seria a diferença de marcação IHQ entre clones de anticorpos anti-E-caderina. Estudo comparando dois tipos de anticorpos demonstrou discrepância na marcação de lesões lobulares em 6,4% dos casos (CHOI et al., 2008).

As caderinas são proteínas que realizam o contato e comunicação célula-célula em muitos órgãos e tecidos. Elas operam como receptores transmembrana e mediam interações homofílicas cálcio-dependentes entre as células. Esta interação é crucial não somente para a interação com as células vizinhas, mas também para a sinalização celular. As caderinas apresentam características estruturais específicas em sua sequência de aminoácidos que são usadas para a sua classificação em tipo I, tipo II, tipo III e caderinas atípicas. As caderinas do tipo I incluem a caderina epitelial (E), neural (N), placentária (P) e retinal (R). A E-caderina é expressa em todas as células epiteliais da glândula mamária, enquanto que a P-caderina é expressa nas células epiteliais dos ácinos e ductos e também nas células mioepiteliais. N-caderina é expressa nas células mesenquimais do estroma mamário, a R-caderina também é expressa nas células epiteliais da mama (ANDREWS; KIM; HENS, 2012).

Nas células epiteliais normais da mama, a E-caderina é composta por um domínio extracelular, um domínio transmembrana e um domínio intracitoplasmático. Este domínio intracitoplasmático compreende o domínio justamembranar que liga-se a p120 catenina, e o domínio catenina-ligante que interage com a β-catenina; β-catenina liga-se a **α**-catenina, **α**-actina e vinculina, que estabelece uma ligação direta entre o complexo caderina-catenina e o citoesqueleto de actina (Figura 3) (BARANWAL; ALAHARI, 2009; DABBS et al., 2013).

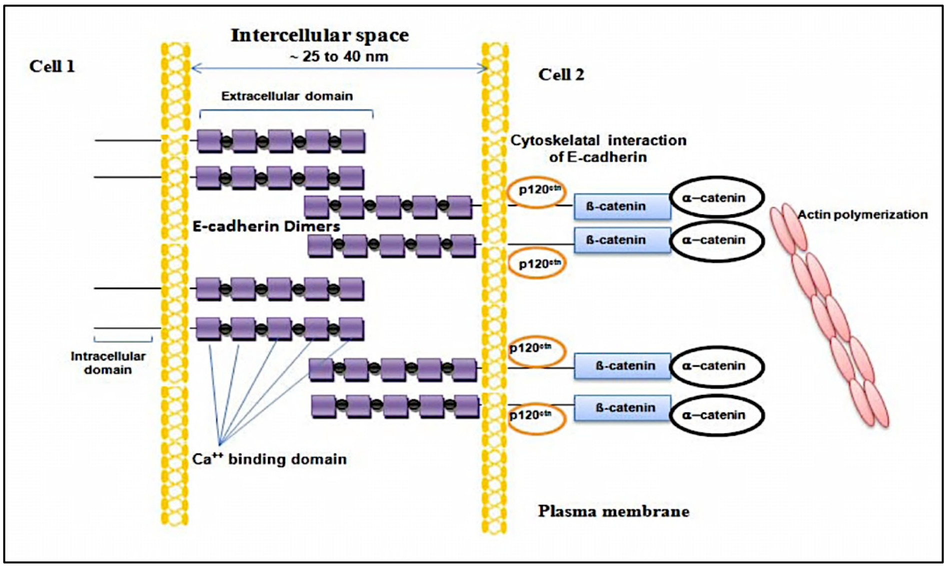

Figura 3 - Ilustração esquemática do complexo caderina-cateninas. (BARANWAL; ALAHARI, 2009)

A E-caderina é o produto proteico do gene *CDH1* (16q22.1), e tem sido identificada como uma proteína supressora tumoral, estando modificada em muitos cânceres, incluindo o câncer de mama e o câncer gástrico difuso. Devido a sua função de promover a adesão e o desenvolvimento de uma arquitetura e fenótipo epitelial normal, sua supressão nas células cancerígenas e é associada aumento na transição epitélio-mesênquima (EMT), motilidade, potencial invasivo e metastático (ANDREWS et al., 2012).

A relação entre E-caderina e β-catenina é bem estudada, e juntas estas moléculas estabilizam a célula epitelial em sua forma polarizada. E-caderina junta-se com a β-catenina para formar o complexo de adesão caderina-catenina, que impede a localização intranuclear da β-catenina, que tem sido demonstrada induzir uma expressão gênica que favorece a invasão tumoral. (ANDREWS et al., 2012; BARANWAL; ALAHARI, 2009).
Caso a E-caderina esteja ausente ou não funcionante, a p120 catenina acumula-se no citoplasma e ativa uma série de eventos intracelulares que resultando no aumento da motilidade celular (SHIBATA et al., 2004). A p120 catenina é uma proteína intracelular que une o complexo das cateninas ao citoesqueleto celular. Quando há perda da expressão da E-caderina, a p120 catenina fica dispersa no citoplasma, o que explica sua expressão citoplasmática CLIS e membranar no CDIS (DABBS; BHARGAVA; CHIVUKULA, 2007; SARRIO et al., 2004)

A inativação ou diminuição da função da E-caderina ocorre através de uma combinação de mecanismos genéticos, epigenéticos e transcricionais. A perda do cromossoma 16q é usualmente acompanhada por mutações, metilação do gene promotor, ou por não codificação de RNAs, levando a uma inativação bialélica do gene e perda da expressão da proteína (BARANWAL; ALAHARI, 2009; DABBS et al., 2013).

Mutações no gene CDH1 têm sido encontradas em 7% das HLA, 100% dos CLIS e em 27%-65% dos CLI, mas são raras no CDI. Estudos de sequenciamento paralelo tem confirmado associação estatisticamente significativa entre carcinomas lobulares e mutações no gene *CDH1*, entretanto nem todos os carcinomas lobulares apresentam mutações neste gene. Mutações idênticas no gene *CDH1* têm sido encontradas no CLIS e nos CLI sincrônicos, suportando o papel precursor dos CLIS para CLI (ANDRADE et al., 2012; DABBS et al., 2013).

1.8. 34betaE12

Outros marcadores imuno-histoquímicos têm sido sugeridos para auxiliar no diagnóstico dos CISM. O 34betaE12, anticorpo monoclonal anti-citoqueratina (CK) de alto peso molecular (CK 1, 5, 10, e 14), tem sido utilizada no diagnóstico diferencial dos carcinomas *in situ* de padrão misto por um grupo de pesquisadores, onde foi observado positividade na marcação imuno-histoquímica nas lesões lobulares e negatividade nas lesões ductais (BRATTHAUER et al., 2002; WHEELER et al., 2004). No entanto, o mesmo grupo ao testar separadamente cada uma das CK 1, 5, 10 e 14 não observou a positividade individual esperada. Após outras análises concluiu-se que a reatividade para 34betaE12 provavelmente deve-se a reação cruzada com outras CK, possivelmente a CK 19. Ou ainda, deve-se a reação com outra proteína semelhante a CK em tecidos parafinados fixados em formalina; ou pela detecção de CK 1, 5, 10 e 14 , mutada ou modificada que não pode ser detectada pelo anticorpo monoclonal individual (BRATTHAUER; MIETTINEN; TAVASSOLI, 2003).

1.9. Justificativa

Estudos demonstraram variabilidade interobservador entre patologistas da comunidade e patologistas especializados no estudo de lesões mamárias no diagnóstico de neoplasias mamárias *in situ* e hiperplasias epiteliais (PEREZ et al., 2013; SALLES; SANCHES; et al., 2008). Estas diferenças foram mais marcantes nas lesões proliferativas intraductais onde foram observadas discordâncias diagnósticas que implicariam mudanças terapêuticas em 26,4% dos casos com repercussão clínica para os pacientes (SALLES; GOUVÊA; et al., 2008). Entretanto, estudos sobre a variação interobservador no diagnóstico de NL e LCC em seu diagnóstico são escassos.

Os carcinomas *in situ* de padrão misto são raros e apresentam frequência indeterminada na rotina de laboratórios de anatomia patológica. Estudos que avaliem o diagnóstico diferencial dessas lesões são escassos e com pequeno número de pacientes nas séries da literatura variando entre 17-27 casos (BRATTHAUER et al., 2002; CHOI et al., 2008). Durante o trabalho de mestrado, foi avaliado uma série de neoplasias lobulares, incluindo casos de carcinomas lobulares *in situ* da mama, todos provenientes do Hospital das Clínicas da UFMG e excluímos casos recebidos em consultoria de outras instituições (GOMES et al., 2011). Identificamos em nossa série alguns casos com padrão misto, em geral de difícil reconhecimento por patologistas sem treinamento específico com estas lesões.

Devido a raridade destas lesões e dos poucos estudos sobre elas na literatura, a importância de conhecer sua biologia e evolução para melhor definição de tratamento das pacientes, interessamos em continuar o estudo durante o doutorado e fazer caracterização imunofenotípica e análise interobservador no diagnóstico destas lesões.

2. DESENVOLVIMENTO

2.1. Objetivos:

Os objetivos deste estudo foram:

1. Avaliar a variabilidade interobservador dos diagnósticos entre o laudo original e após revisão por consultor especialista em patologia mamária de lesões precursoras e lesões proliferativas intraductais, principalmente NL, LCC e CDIS, em biopsias mamárias enviadas para consultoria.

2. Avaliar a expressão de E-caderina e β-catenina na caracterização imunofenotípica dos carcinomas *in situ* de padrão misto.

2.2. Métodos, resultados e discussão

Conforme resolução n° 01/2013, de 14 de janeiro de 2013, que regulamenta a defesa de dissertações e teses no Programa de Pós-Graduação em Patologia. Os métodos, resultados obtidos e sua consequente discussão serão apresentados de forma alternativa, como dois artigos científicos.

O primeiro artigo; denominado: **"Inter-observer variability between general pathologists and a specialist in breast pathology in the diagnosis of lobular neoplasia, columnar cell lesions, and ductal carcinoma in situ of the breast"**; foi publicado no periódico *Diagnostic Pathology* (Fator de Impacto 1,85 – Qualis B1 – Medicina II) em 26/06/2014. O artigo está no formatado original da revista.

O segundo artigo; denominado **"Usefulness and limitations of E-cadherin and β-catenin in the classification of breast carcinomas in situ with mixed pattern"**; foi publicado no periódico *Diagnostic Pathology* (Fator de Impacto 1,85 – Qualis B1 – Medicina II) em 9/7/2013. O artigo está no formatado original da revista.

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DIAGNOSTIC PATHOLOGY

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Inter-observer variability between general pathologists and a specialist in breast pathology in the diagnosis of lobular neoplasia, columnar cell lesions, atypical ductal hyperplasia and ductal carcinoma in situ of the breast

Douglas S Gomes, Simone S Porto, Débora Balabram and Helenice Gobbi*

Abstract

Background: This study aimed to assess inter-observer variability between the original diagnostic reports and later review by a specialist in breast pathology considering lobular neoplasias (LN), columnar cell lesions (CCL), atypical ductal hyperplasia (ADH), and ductal carcinoma in situ (DCIS) of the breast.

Methods: A retrospective, observational, cross-sectional study was conducted. A total of 610 breast specimens that had been formally sent for consultation and/or second opinions to the Breast Pathology Laboratory of Federal University of Minas Gerais were analysed between January 2005 and December 2010. The inter-observer variability between the original report and later review was compared regarding the diagnoses of LN, CCL, ADH, and DCIS. Statistical analyses were conducted using the Kappa index.

Results: Weak correlations were observed for the diagnoses of columnar cell change (CCC; Kappa = 0.38), columnar cell hyperplasia (CCH; Kappa = 0.32), while a moderate agreement (Kappa = 0.47) was observed for the diagnoses of flat epithelial atypia (FEA). Good agreement was observed in the diagnoses of atypical lobular hyperplasia (ALH; Kappa = 0.62) and lobular carcinoma in situ (LCIS; Kappa = 0.66). However, poor agreement was observed for the diagnoses of pleomorphic LCIS (Kappa = 0.22). Moderate agreement was observed for the diagnoses of ADH (Kappa = 0.44), low-grade DCIS (Kappa = 0.47), intermediate-grade DCIS (Kappa = 0.45), and DCIS with microinvasion (Kappa = 0.56). Good agreement was observed between the diagnoses of high-grade DCIS (Kappa = 0.68).

Conclusions: According to our data, the best diagnostic agreements were observed for high-grade DCIS, ALH, and LCIS. CCL without atypia and pleomorphic LCIS had the worst agreement indices.

Virtual Slides: The virtual slide(s) for this article can be found here: http://www.diagnosticpathology.diagnomx.eu/vs/1640072350119725.

Keywords: Breast cancer, Lobular neoplasia, Columnar cell lesions, Atypical ductal hyperplasia, Ductal carcinoma in situ, Inter-observer variability, Agreement

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Abstract

Introdução: O objetivo do estudo foi avaliar a variabilidade interobservador entre os diagnósticos do laudo original e após revisão por especialista em patologia mamária considerando as neoplasias lobulares (NL), lesões células colunares (LCC), hiperplasia ductal atípica (HAD) e carcinoma ductal in situ (CDIS) em biopsias mamárias.

Métodos: Estudo retrospectivo, observacional, do tipo transversal. Um total de 610 casos de espécimes mamários que foram enviados formalmente para consultoria e/ou segunda opinião ao Laboratório de Patologia Mamária da Universidade Federal de Minas Gerais foram analisados entre janeiro de 2005 e dezembro de 2010. A variabilidade interobservador entre o laudo original e o laudo de revisão foi comparada entre os diagnósticos de NL, LCC, HDA e CDIS. A análise estatística foi realizada pelo índice de Kappa.

Resultados: Observamos uma concordância fraca para os diagnósticos de alterações de células colunares (ACC; Kappa = 0,38), e hiperplasia de células colunares (HCC; Kappa = 0,32), enquanto uma concordância moderada (Kappa = 0,47) foi observada para o diagnóstico de atipia epitelial plana (AEP). A concordância foi considerada boa para os diagnósticos de hiperplasia lobular atípica (Kappa = 0,62) e carcinoma lobular *in situ* (CLIS; Kappa = 0,66). Entretanto, a concordância foi considerado baixa para o diagnóstico de CLIS pleomórfico (Kappa = 0,22). Concordância moderada foi observada para os diagnósticos de HLA (Kappa = 0,44), CDIS de baixo grau (Kappa = 0,47), CDIS de grau intermediário (Kappa = 0,45) e CDIS microinvasor (Kappa = 0,56). Boa concordância foi observada para o diagnóstico de CDIS de alto grau (Kappa = 0,68).

Conclusão: De acordo com nossos dados, as melhores concordâncias diagnósticas foram observadas entre CDIS de alto grau, HLA e CLIS. As LCC sem atipias e o CLIS pleomórfico tiveram os piores índices de concordância.

Background

Despite advances in the understanding of the molecular biology of breast cancer progression and new molecular markers, the histopathological analysis remains the most widely used diagnostic method of precursor and intraductal proliferative lesions of the breast [1].

Currently, increasing number of breast lesions are discovered during the pre-clinical phase due to the more widespread use of mammography screening and the incorporation of new imaging technologies for the diagnosis of breast cancer. There has also been an increase in the diagnosis of intraductal proliferative and precursor breast lesions, which exhibit uncertain behaviour. These include lobular neoplasia (LN), columnar cell lesions (CCL), atypical ductal hyperplasia (ADH), and ductal carcinoma in situ (DCIS). The differential histologic diagnosis between some of these lesions can be difficult and presents challenges to pathologists; especially those not specialized in breast pathology [2,3].

Reproducibility studies are useful when evaluating the applicability of histological criteria for the classification of breast lesions and when determining the level of agreement amongst pathologists regarding morphological diagnoses. Studies conducted by our group have revealed significant inter-observer variability between the diagnoses made by general pathologists and those made by breast pathology experts in the diagnosis for DCIS and ADH; this discrepancy could have significant therapeutic implications [4,5]. Although there have been various studies on the diagnostic agreement considering DCIS, few studies have analysed the diagnostic agreement considering LN and CCL [6,7].

Our study aimed to assess the frequency of detection rate of precursor lesions and intraductal proliferative lesions, primarily CCL and LN, in breast biopsies sent for consultation as well as the inter-observer variability in the diagnoses made during the original report and a later review by a specialist consultant in breast pathology.

Methods

A retrospective, observational, cross-sectional study was conducted. Files from the Breast Pathology Laboratory at the School of Medicine of Federal University of Minas Gerais (UFMG), Brazil, were reviewed between January 2005 and December 2010, and 673 cases of breast lesions were identified as having been formally sent for consultation or second opinion. The analysed data were obtained from the original pathologist reports and from the consulting report conducted by a single pathologist (HG) with an expertise on breast pathology. A total of 63 cases were excluded from the analysis; these cases did not have the original reports for comparison or they had insufficient and/or damaged material that prevented the review.

Data were collected through the use of a structured form, and the following items were analysed in both the original report and the review: type of specimen, specialty of the referring physician, and presence of intraductal proliferative lesions (columnar cell lesions [CCL], ADH, and DCIS) and the LN (atypical lobular hyperplasia [ALH], lobular carcinoma in situ [LCIS], and pleomorphic LCIS) associated or not with invasive carcinoma.

The histological classification of LN originally reported by Page *et al.* and adopted by the 2012 World Health Organization (WHO) Classification of Breast Tumours was used [1]. Lobular neoplasia refers to the entire spectrum of atypical epithelial lesions originating in the terminal-duct lobular unit (TDLU) and characterized by a proliferation of generally small, non-cohesive cells. ALH is defined as a filling or expansion of less than 50% of the acini in one or more lobular units by proliferating small, uniform cells. LCIS was defined as a filling and distension of greater than 50% of the acini of a lobular unity and a loss of the residual intracellular lumen [8,9]. The criteria used to diagnose pleomorphic LCIS were those originally described by Eusebi *et al.*, which included the same architectural configuration as LCIS but with increased nuclear pleomorphism, larger nucleoli, with or without comedo necrosis [10].

CCLs are a group of lesions of the terminal ductal-lobular units that are characterized by variably enlarged dilated acini lined by columnar epithelial cells without cytological or architectural atipia [1]. Flat epithelial atypia (FEA), the term adopted by the WHO Classification of Breast Tumours, since 2003, refers to a neoplastic alteration of the TDLUs characterized by replacement of the native epithelial cells by one to several layers of a single epithelial cell type showing low-grade (monomorphic) cytological atypia [1,11]. These lesions differ from those with sufficient architectural and cytological findings for a differential diagnosis of ADH or DCIS. In the present study, we used the diagnostic criteria proposed by Schnitt and Vincent-Salomon, who previously suggested the classification of the FEA group into two groups, CCC and columnar cell hyperplasia (CCH) with atypia according to the number of layers of proliferating epithelial cells (Table 1) [12,13].

ADH was defined as a proliferation of regularly distributed monomorphic cells to form regular, uniform, and circular secondary lumens. These lesions are small, and the cells have 2 partial ducts or "spaces" involved, and are less than 2 mm in size. DCIS is characterised as an epithelial proliferation of atypical cells with 2 complete "spaces" or ducts involved or are more than 2 mm in overall size. The criteria of Elston and Ellis were used to diagnose DCIS with microinvasion [14], which are recognised by predominant DCIS as well as the infiltration of neoplastic cells beyond the basal membrane of the unspecialised or extralobular connective tissue up to 1 mm in size. The histological grades (low, moderate, and high) of DCIS were determined after considering the grade of nuclear atypia as well as the presence and extension of necrosis according to the criteria of Lagios [15]. Currently, the Breast Pathology Laboratory uses the new WHO (2012) Classification for Breast Tumours as a diagnostic reference [1].

To tabulate the data, cases with more than one type of breast lesion were classified according to the lesion with the greatest risk or potential to develop into a carcinoma. For LN, the risk classification was as follows: pleomorphic LCIS > LCIS > ALH; for CCL: CCH atypia > CCC atypia > CCH > CCC; and for DCIS: microinvasive DCIS > high-grade DCIS > intermediate-grade DCIS > low-grade DCIS. Cases of DCIS for which the grade had not been evaluated during the original report or the review were classified as unspecified (US).

The SPSS program (version 17.0; SPSS Inc., Chicago, IL, USA) was used to analyse the inter-observer variability between the original diagnosis and the histopathological review conducted by the consultant pathologist, using the Kappa index. This index was interpreted according to the following values proposed by Landis and Koch [16]: < 0.20 (bad); 0.21–0.40 (poor); 0.41–0.60 (moderate); 0.61–0.80 (good); and 0.81–1.00 (excellent). The significance level *(p)* was defined as 0.05. This study was approved by the Research Ethic's Committee of the UFMG.

Results

A total of 610 cases of breast lesions that had been referred for second opinion and satisfied the inclusion criteria were analysed; of these, 56.9% were breast specimens from segmental mastectomies or quadrantectomies, 29.8% from core-biopsies, 5.9% from mastectomies, and 7.4% were other specimen types. The referring practitioner was specialised in breast surgery in 60% of the cases, in oncology in 5.2% of the cases, and in pathology in 4.1% of the cases. Patients' ages ranged from 13–94 years with a mean age of 53.6 years (±13.3 years).

LN was present in 11.0% (67/610) of the original reports and 11.8% (72/610) of the later reviews. Of 67 cases from the original reports, ALH was present in 25.4%, LCIS in 67.2%, and pleomorphic LCIS in 7.5%. Of the 72 LN cases from the later reviews, ALH was present in 30.6%, LCIS in 63.9%, and pleomorphic LCIS in 5.6% (Table 2; Figure 1). There were good agreements between the original reports and later reviews regarding the diagnoses of ALH (Kappa index = 0.62; [$p < 0,05$]) and LCIS (Kappa index = 0.66; [$p < 0,05$]). However, there was a poor agreement between the diagnoses of pleomorphic LCIS (Kappa index = 0.22; [$p < 0,05$]).

CCL were present in 14.4% (88/610) of the original reports and 25.1% (153/610) of the reviews. Of 88 cases from the original reports, CCC were present in 64.8%, CCH in 12.5%, CCC with atypia in 8.0%, and CCH with atypia in 14.8% of the cases. Of the 153 cases from the reviews, CCC were present in 74.5%, CCH in 9.8%, CCC with atypia in 6.5%, and CCH with atypia in 9.2% of the cases (Table 3; Figure 2). There were weak diagnostic agreements between the original report and later review for CCC (Kappa index = 0.38; [$p < 0,05$]), CCH (Kappa

Table 1 Diagnostic criteria for columnar cell lesions used in the present study

	Columnar cell change	Columnar cell hyperplasia	Columnar cell change with atypia	Columnar cell hyperplasia with atypia
			Flat epithelial atypia	
Topography	Terminal duct-lobular unit with variable dilation	Terminal duct-lobular unit with variable dilation	Terminal duct-lobular unit with variable dilation	Terminal duct-lobular unit with variable dilation
Architecture	1 or 2 cell layers	Cell stratification greater than 2 layers, complex cellular configurations are not present	1 or 2 cell layers	Cellular stratification of more than 2 layers, complex cell configurations are not present
Cytology	Columnar cells with ovoid to elongated nuclei orientated perpendicular to the basal membrane; nucleolus absent or inconspicuous.	Columnar cells with ovoid to elongated nuclei orientated perpendicular to the basal membrane; "hobnail" cells might appear with absent or inconspicuous nuclei.	Cytological atypia present (usually low-grade); the cells resemble tubular carcinoma. Mitoses are uncommon.	Cytological atypia present (usually low-grade); the cells resemble tubular carcinoma. Mitoses are uncommon.
Apical decapitation	Often present, not usually prominent.	Often present, might be exaggerated.	Often present, might be exaggerated.	Often present, might be exaggerated.
Intraluminal secretions	Might be present but are not usually prominent.	Might be present and prominent.	Might be present and prominent.	Might be present and prominent.
Calcifications	Might be present	Usually present, might be psammomatous.	Usually present, might be psammomatous.	Usually present, might be psammomatous.

Adapted from Schnitt and Vincent-Salomon [12], Fraser *et al.* [13], Tavassoli, & Devilee [11].

index = 0.32). The agreement was moderate (Kappa index = 0.47; [$p < 0,05$]) between the diagnoses of FEA (CCC with atypia and CCH with atypia).

ADH was present in 12.1% (74/610) of the original reports and 8.4% (51/610) of the later reviews (Table 4; Figure 2B and Figure 3). There was a moderate agreement between the original reports and later reviews regarding the diagnosis of ADH (Kappa index = 0.44; [$p < 0,05$]). Of the 74 cases of ADH present in the original reports, in 41.9% (31/74) the reviewer confirmed the diagnosis of ADH. In 58.1% (43/74) cases the ADH was over-diagnosed, these, 58.1% (25/43) the reviewer downgrade diagnosis for usual ductal hyperplasia, in 14.0% (6/43) the diagnosis was increased to DCIS, and 27.8% (12/43) could not evaluate this information only by the reports.

The DCIS frequencies in the original reports and later reviews were 37.7% (230/610) and 39.0% (238/610), respectively. Of 230 DCIS cases from the original reports, low-grade DCIS was present in 25.6%, intermediate-grade DCIS in 23.0%, high-grade DCIS in 39.1%, DCIS-US in 10.9%, and microinvasive DCIS in 1.3%. Of the 238 DCIS cases encountered during later review, low-grade DCIS was present in 26.5%, intermediate-grade DCIS in 20.6%, high-grade DCIS in 44.9%, DCIS-US in 5.9%, and microinvasive DCIS in 2.1% (Table 5). Good diagnostic agreement was observed between the original reports and later reviews for high-grade DCIS (Kappa index = 0.68; [$p < 0,05$]). However, moderate diagnostic agreement was observed for low-grade DCIS (Kappa index = 0.47;

Table 2 Diagnostic agreement between the original report and later review of lobular neoplasia

	LN report review				
Original LN report	**Absent**	**ALH**	**LCIS**	**Pleomorphic LCIS**	**Total**
Absent	521	10	11	1	543
ALH	7	8	2	0	17
LCIS	8	4	31	2	45
Pleomorphic LCIS	2	0	2	1	5
Total	538	22	46	4	610

LN: lobular neoplasia; ALH: atypical lobular hyperplasia; LCIS: lobular carcinoma in situ.

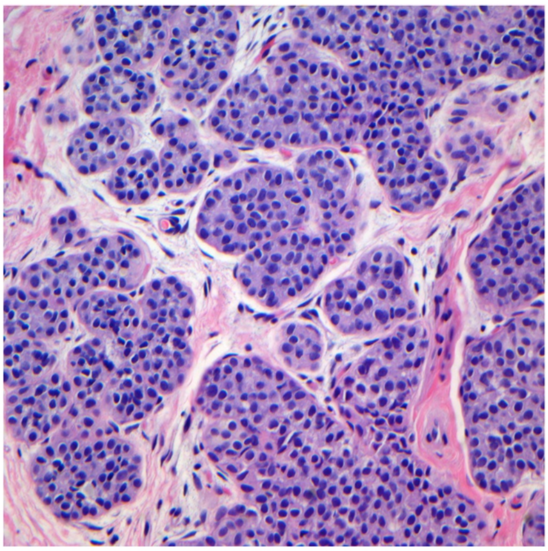

Figure 1 Lobular neoplasia: this case was originally diagnosed as lobular carcinoma in situ and considered atypical lobular hyperplasia after review. Less than 50% of lobular units are involved and expanded by uniform cells. (Hematoxylin and eosin; x200).

Table 3 Diagnostic agreement between the original report and later review of columnar cell lesions

	CCL report review					
Original CCL report	Absent	CCC	CCH	CCC with atypia	CCH with atypia	Total
Absent	437	66	10	2	7	522
CCC	16	36	1	3	1	57
CCH	2	5	3	1	0	11
CCC with atypia	1	3	1	2	0	7
CCH with atypia	1	4	0	2	6	13
Total	457	114	15	10	14	610

CCL: columnar cell lesions; CCH: columnar cell hyperplasia; CCC: columnar cell change.

[$p < 0,05$]), intermediate-grade DCIS (Kappa index = 0.45; [$p < 0,05$]), and microinvasive DCIS (Kappa index = 0.56; [$p < 0,05$]).

Discussion

In this study, we analysed the LN, CCL, ADH and DCIS diagnostic agreements and reproducibility between general pathologists and a specialist pathologist with training and experience in breast pathology in cases received in consultation for a second opinion.

The importance of LN morphological classification is attributed to the Page group and collaborators, who correlated lobular lesion extension with patient evolution. The risks of developing invasive carcinomas were calculated as 4–5% for ALH and 8–11% for LCIS [8,9]. Eusebi *et al.* described the pleomorphic variant of LCIS; this variant features the same architectural arrangement as LCIS but exhibits marked nuclear pleomorphism and abundant cytoplasm, more evident nucleoli, and possible areas of comedo necrosis and microcalcifications either with or without apocrine characteristics [10]. Given the morphological characteristics in association with the fact that the imunohistochemical profile of pleomorphic LCIS is more likely oestrogen receptor-negative and HER-2-positive along with a higher Ki-67 proliferation index that classic LCIS, these lesions have been correlated with a more aggressive biological behavior than that of classic LCIS; however, epidemiological studies to prove this assumption are lacking [1,10]. Recently, molecular analyses of synchronous LCIS and both classic ILC type and the pleomorphic variant of invasive lobular carcinoma demonstrated similarities in the genomic profiles [17]. LCIS is considered both a risk factor and a non-obligate precursor lesion for subsequent invasive carcinomas in either breast, of either ductal or lobular type, but only a minority of women actually develop invasive breast cancer after a long-term follow up [1].

Table 4 Diagnostic agreement between the original diagnosis of atypical ductal hyperplasia and the reviewer´s diagnosis

	Reviewer´s diagnosis of ADH		
Original diagnosis of ADH	Absent	ADH	Total
Absent	516	20	536
ADH	43	31	74
Total	559	51	610

ADH: atypical ductal hyperplasia.

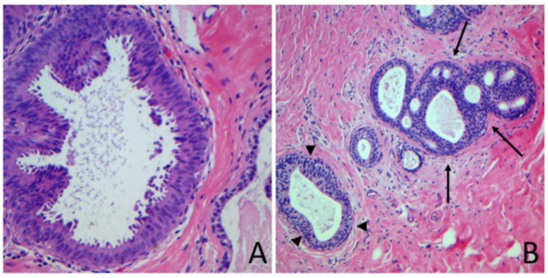

Figure 2 Diagnostic disagreements between the original diagnosis of flat epithelial atypia and a low-grade ductal carcinoma in situ and the reviewer´s diagnosis. A: Case originally diagnosed as flat epithelial atypia and considered a columnar cell change without atypia by the reviewer. (Hematoxylin and eosin; x400). **B**: Case diagnosed by the reviewer as atypical ductal hyperplasia (arrows) adjacent to columnar cell change without atypia (arrowheads) and originally considered by the referral pathologist a low-grade ductal carcinoma in situ. (Hematoxylin and eosin; x100).

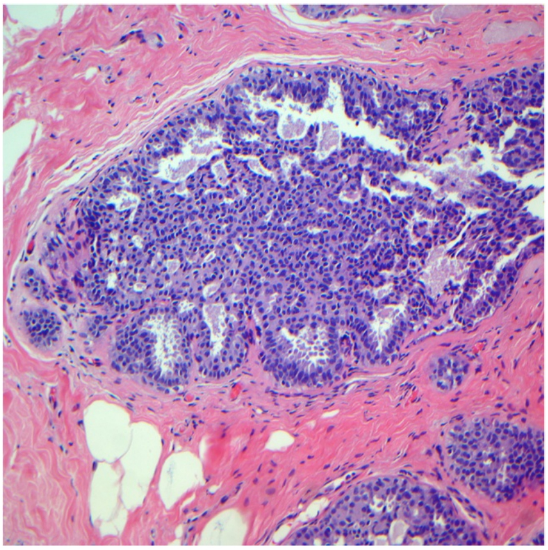

Figure 3 Case originally diagnosed as atypical ductal hyperplasia and as usual ductal hyperplasia after review. Note the epithelial cells displaying a haphazard orientation, and the presence of slit-like secondary lumina peripherally located. (Hematoxylin and eosin; x100).

Table 5 Diagnostic agreement between the original report and later review of DCIS

	DCIS report review						
Original DCIS report	**Absent**	**LG DCIS**	**IG DCIS**	**HG DCIS**	**US DCIS**	**MIC DCIS**	**Total**
Absent	333	16	9	19	3	0	380
LG DCIS	16	32	9	0	2	0	59
IG DCIS	8	7	23	12	1	1	53
HG DCIS	6	3	5	72	2	2	90
US DCIS	8	5	3	3	6	0	25
MIC DCIS	0	0	0	1	0	2	3
Total	372	63	49	107	14	5	610

DCIS: ductal carcinoma in situ; LG DCIS: low-grade carcinoma in situ; IG DCIS: intermediate grade carcinoma in situ; HG DCIS: high-grade carcinoma in situ; US DCIS: unspecified carcinoma in situ; MIC DCIS: microinvasive carcinoma in situ.

In our study, a good diagnostic agreement was observed between the reports from generalist pathologists and a breast pathology specialist regarding the diagnoses of ALH (Kappa index = 0.62) and LCIS (Kappa index = 0.66). Our data are similar to those of Fitzgibbons, who analysed the responses of 2,952 pathologists to clinical cases from The College of American Pathologists Performance Improvement Program in Surgical Pathology [6]. That study analysed the agreement (%) regarding the diagnosis of ALH; 58% of the pathologists correctly diagnosed ALH, whereas 17% diagnosed LCIS. When LN (ALH and LCIS) cases were assessed together, the agreement rate was 74%. However, there were other conflicting diagnoses for these cases, including ADH (14%), DCIS (1.4%), and usual ductal hyperplasia (10%) [6]. In fact, the differential diagnosis of LN and intraductal proliferative lesions can be difficult, especially when concerning classic LN versus low-grade solid DCIS, and pleomorphic LCIS versus high-grade DCIS [2,18]. Despite the low number of pleomorphic LCIS cases in our study, the interobserver agreement was poor (Kappa index = 0.22). The immunophenotypic criteria of E-cadherin, β-catenin, and p120-catenin expression in combination with the careful identification of cytological and architectural alterations are useful tools in the morphological classification of these lesions [18,19].

The correct diagnosis of LN cases will affect the treatment options and counseling. Patients with LN are at risk of developing invasive ipsilateral and contralateral breast carcinomas. For this reason, most patients diagnosed with LN are clinically monitored, and tamoxifen might be administered as a prophylactic therapy against the development of invasive carcinomas [20]. In very specific cases in which there are other associated risk factors, a bilateral prophylactic mastectomy might be offered [21]. The management of LN after core-biopsy diagnoses remains controversial. Complete excision of the lesion is recommended in patients who have been diagnosed with various forms of LCIS; however, the current evidence does not support the routine excision of conventional LCIS diagnosed via core-biopsy in cases with a clinical-radiological correlation and in which suspected area on the image has been properly sampled [1,2].

CCL and FEA are a group of breast lesions whose diagnostic criteria were defined only in recent years [12]. With the widespread use of mammography screening, CCLs have often been identified in breast biopsies and are present in as many as half of the biopsies performed for microcalcifications detected via mammography. Recent studies have shown that flat epithelial atypia shares genetic similarities with ADH, low-grade DCIS, and tubular carcinomas, suggesting that these lesions act as precursors of invasive, low-grade carcinomas [22].

Despite advances in genetic studies of these lesions, few studies have assessed the diagnostic reproducibility of CCL amongst pathologists. In our study, we observed weak diagnostic agreements regarding CCC and CCH between generalist pathologists and a breast pathology specialist. When we assessed FEA, the agreement was moderate with better agreement for lesions with more pronounced atypia. Our data differed from those of O'Malley *et al.* [7], who observed excellent agreement (Kappa index = 0.83) regarding the diagnoses of CCL without atypia and FEA. The agreement was better when detecting the absence of FEA (92.8%) than when confirming its presence (90.4%). However, in contrast to our study, the agreement was assessed in selected cases and amongst pathologists experienced in breast pathology using images of the cases with pre-established diagnostic criteria [7]. Haupt *et al.* analysed the diagnostic agreement regarding previously selected CCL cases between residents and fellows both before and after conducting a tutorial on the diagnostic criteria of CCL. Before conducting the tutorial, the diagnostic agreement of FEA was weak (Kappa index = 0.39); after the training, there was a statistically significant increase in the ability to recognise FEA (Kappa index = 0.60) [23]. A similar study was conducted by Tan *et al.*, who analysed the diagnostic agreement of CCL amongst pathologists from the same department after a tutorial;

the agreement obtained varied from weak to moderate (Kappa index range = 0.44–0.71) between the group of pathologists and the pathologist tutor [24].

The clinical importance of a correct diagnosis of FEA is that these lesions often coexist alongside LN, ADH, low-grade DCIS, and low-grade invasive carcinomas such as tubular carcinomas [22,25]. In a recent meta-analysis, Verschuur-Maes *et al.* analysed 24 studies that reported the presence of carcinoma in situ after diagnosing CCL in needle biopsies. DCIS underestimation rates of 1.5%, 9%, and 20% were observed in cases of CCL without atypia, CCL with atypia, and CCL associated with ADH, respectively [26]. However, given the limitations of the studies and the large variation in the time of follow-up after the initial biopsy, the WHO Classification of Breast Tumours (2012) notes that it remains uncertain whether the indication of surgical excision is necessary after a diagnosis of FEA via core needle biopsy. Radiological and pathological correlations are recommended to determine the subsequent procedure [1]. Moreover, epidemiological studies such as that conducted by Boulos *et al.* have revealed that CCL are associated with only a relative 1.55-fold risk of developing invasive carcinomas in subsequent years; however, the risk associated with these lesions is not entirely independent of the risk associated with other concomitant proliferative lesions [27].

ADH and DCIS comprise 10% and 15–20%, respectively, of all breast lesions detected using mammography screening. The relative risk of developing invasive carcinoma ranges from 4 to 5-fold among patients with a diagnosis of ADH and from 8- to 10-fold among patients with DCIS [1,28]. However, the histopathological diagnoses of these intraductal proliferative lesions may be difficult, and the inter- and intra-observer agreements between the pathologists differ [5,29,30]. This diagnostic inconsistency could be primarily a result of the morphological criteria used to classify and appropriately select the diagnostic fields [30,31]. The correct diagnosis and the differentiation between ADH and DCIS have repercussions for the treatment of these lesions. When diagnosed via core biopsy, ADH lesions must be completely removed to search for DCIS in the excision specimen to avoid a missed detection of an invasive component. In cases in which the diagnosis of ADH was upheld after an extended biopsy, no further treatment is necessary. However, given the greater risk of progressing to an invasive carcinoma, DCIS has been treated by complete excision of the lesion with free margins, with complementary radiotherapy in cases for which breast-conserving surgery has been performed, and the use of tamoxifen as a prophylaxis against local recurrence [32].

In our study, the diagnostic agreements between the original reports and later reviews were moderate for ADH (Kappa index = 0.44), low-grade DCIS (Kappa index = 0.47), intermediate-grade DCIS (Kappa index = 0.45), and microinvasive DCIS (Kappa index = 0.56). Elston *et al.* analysed the level of inter-observer agreement in the diagnoses of ADH and DCIS amongst 23 pathologists who used pre-defined diagnostic criteria. In this study, the Kappa indices were considered poor (0.35) for ADH and good (0.78) for DCIS. However, when DCIS cases were stratified by histological grades, significant variations were observed in the inter-observer diagnoses, as the Kappa indices were 0.51 for low-grade DCIS, 0.19 for intermediate-grade DCIS, and 0.41 for high-grade DCIS [31]. However, in our study, we obtained a better agreement for the diagnosis of high-grade DCIS (Kappa index = 0.68). Similar findings have been described by other authors that conducted studies on the inter-observer variability with regard to the nuclear grade of DCIS. Sneige *et al.* evaluated the inter-observer variability among six pathologists who assessed 125 cases of DCIS and observed a better diagnostic agreement for high-grade DCIS [33,34]. The nuclear grade of DCIS is an important factor when determining the therapeutic approach because high nuclear-grade lesions are associated with a poor prognosis and are often associated with local recurrence and/or progression into invasive lesions, a greater chance of metastasis, and greater required care during local surgical procedures [4,28].

Various strategies have been used in an attempt to improve the diagnoses of ADH and DCIS, including a review of the diagnostic criteria and continuing education programs [33]. Recently, Jain *et al.* revealed that the agreement between nine pathologists regarding the diagnosis of ADH was poor (Kappa index = 0.34) and that an immunohistochemical analysis of cytokeratin (5, 14, 7 and 18) and p63 protein expression significantly improved the level of agreement among pathologists (Kappa index = 0.5) [30]. The influence of automated methods of interpretation [35] and the use of telepathology and virtual slides has been evaluated to improve the accuracy of diagnosis and as a tool for education, quality control, and second opinion in pathology [36,37].

As our study was retrospective, it includes some limitations. Proliferative lesions may or may not be associated with invasive carcinomas, a factor that might cause the generalist pathologist to underreport a case when faced with a more aggressive diagnosis. Although our data were based on pathological reports, upon assessing the aims of this study, we believe that this methodology is similar to that used in clinical practice.

Our study was the first to assess the diagnostic agreement regarding CCL and LN in cases that were sent for consultation according to reports by generalist pathologists. Interestingly, in our series, a total of 60% of cases were sent for consultation by breast surgeons and 5.2% of cases were sent by oncologists. Only 4.1% of cases were sent for consultation by pathologists. We believe that formal requests for second opinions regarding precursor and borderline breast lesions should be encouraged, especially amongst general pathologists, with the aim of reducing errors in diagnosis and thereby assuring appropriate therapeutic conduct and guaranteeing patient safety [4,5].

Conclusions

Our findings show a low degree of inter-observer diagnostic agreement between generalist pathologists and a specialist in breast pathology with regard to CCL without atypia and pleomorphic LCIS, moderate agreement for FEA, ADH, and low-grade, intermediate, and microinvasive DCIS, and good agreement for high-grade DCIS, ALH, and LCIS. We believe that the use of standardised diagnostic criteria and specific training in breast pathology might improve the reproducibility of these diagnoses, thereby improving the reliability of the pathological reports in the definition of the best therapeutic approach for each patient.

Abbreviations

ADH: Atypical ductal hyperplasia; ALH: Atypical lobular hyperplasia; CCC: Columnar cell change; CCH: Columnar cell hyperplasia; CCL: Columnar cell lesions; DCIS: Ductal carcinoma in situ; FEA: Flat epithelial atypia; LCIS: Lobular carcinoma in situ; TDLU: Terminal-duct lobular unit LN: lobular neoplasia; UFMG: Federal University of Minas Gerais; WHO: World Health Organization.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

DSG conceived the study, and drafted the manuscript. SSP participated in the design of the study. DB performed the statistical analysis, and drafted the manuscript. HG participated in design and coordination of the study, participated in the histological review, and drafted and reviewed the manuscript. All authors have read and approved the final manuscript.

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DIAGNOSTIC PATHOLOGY

RESEARCH

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Usefulness and limitations of E-cadherin and β-catenin in the classification of breast carcinomas in situ with mixed pattern

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Abstract

Background: The distinction between lobular neoplasia of the breast and ductal carcinoma *in situ* has important therapeutic implications. In some cases, it is very difficult to determine whether the morphology of the lesion is ductal or lobular. The aim of this study was to evaluate the value of E-cadherin and β-catenin expression through the immunophenotypical characterization of carcinoma *in situ* with mixed pattern (CISM).

Methods: A total of 25 cases of CISM were analyzed considering cytology/mixed architecture (ductal and lobular), nuclear pleomorphism, loss of cell cohesion, and presence of comedonecrosis. The immunophenotype pattern was considered E-cadherin positive and β-catenin positive, or negative.

Results: Nineteen (76%) cases presented a mixed cytology and / or architectural pattern, two (8%) presented nuclear pleomorphism, two (8%) presented mixed cytology and nuclear pleomorphism, and two (8%) presented comedonecrosis and nuclear pleomorphism. A complete positivity for E-cadherin and β-catenin was observed in 11 cases (44%). In one case, the lesion was negative for both markers and showed nuclear pleomorphis. Thirteen lesions showed negative staining in areas of lobular cytology and positive staining in cells presenting the ductal pattern.

Conclusions: The expression of E-cadherin and β-catenin, combined with cytological and architectural analysis, may highlight different immunophenotypes and improve classification of CISM.

Virtual Slides: The virtual slide(s) for this article can be found here: http://www.diagnosticpathology.diagnomx.eu/vs/1693384202970681

Keywords: E-cadherin, β-catenin, Breast cancer, Lobular neoplasia, Ductal carcinoma *in situ*, Immunohistochemistry

Additional non-English language abstract - Portuguese

Introdução: A distinção entre neoplasia lobular e carcinoma ductal *in situ* tem importantes implicações terapêuticas. No entanto, em alguns casos, é muito difícil determinar se a morfologia da lesão é ductal ou lobular. O objetivo deste estudo foi avaliar a expressão de E-caderina e β-catenina na caracterização imunofenotípica dos carcinomas *in situ* de padrão misto (CISM).

Métodos: Um total de vinte e cinco casos de CISM foram analisados considerando a citologia/arquitetura mista (ductal e lobular), pleomorfismo nuclear e presença de comedonecrose. A expressão imuno-histoquímica foi considerada positiva para E-caderina e β-catenina, ou negativa.
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Resultados: Dezenove (76%) casos apresentavam somente citologia e/ou padrão arquitetural misto (ductal e lobular), dois casos (8%) apresentaram somente pleomorfismo nuclear, dois casos (8%) apresentavam citologia mista e pleomorfismo nuclear, e dois casos (8%) tinham comedonecrose e pleomorfismo nuclear. Uma positividade completa para E-caderina e β-catenina foi observada em 11 casos (44%). Em um caso a lesão foi negativa para ambos marcadores e apresentava pleomorfismo nuclear e comedonecrose. Em 13 lesões o imunofenótipo foi negativo em áreas lobulares e positivo em áreas ductais.

Conclusão: A caracterização imunofenotípica com E-caderina e β-catenina, combinada com a análise citológica e arquitetural, pode destacar diferentes imunofenótipos e auxiliar na classificação dos CISM.

Background

In situ breast carcinomas are classified, according to their morphology, as ductal carcinoma *in situ* (DCIS) or lobular neoplasia (LN), which includes lobular carcinoma *in situ* (LCIS) and atypical lobular hyperplasia (ALH). According to the 2012 WHO classification of tumors of the breast, classic LCIS is diagnosed when more than half of the acini of a lobular unit are distended and distorted by a dyshesive proliferation of cells with small, uniform nuclei. Lesser involvement by the characteristic cells is diagnosed as ALH. Lesions that show marked nuclear pleomorphism, with or without apocrine features and comedonecrosis are referred as pleomorphic LCIS (PLCIS) [1].

In some cases, the diagnostic criteria based on the morphology of LN is not clear, leading to mistaken diagnosis of intraductal proliferative lesions. The main differential diagnoses of lobular neoplasia are: LN with solid low-grade DCIS, PLCIS and high-grade DCIS. Some *in situ* carcinomas present unusual cytological and / or architectural features, making it difficult to determine whether the proliferation is lobular or ductal. This group has been called carcinomas *in situ* with a mixed or indeterminate pattern (CISM) [2,3].

The differential diagnosis of the CISM carries some important implications. Patients with LN are usually clinically monitored and can be offered tamoxifen as a prophylactic therapy to prevent the development of invasive carcinoma [4,5]. On other hand, patients with DCIS should be treated by surgical removal of the lesion, with clear margins followed by radiotherapy, or mastectomy [6]. When diagnosed by core biopsy, DCIS should be treated with complete excision of the lesion. However, the clinical significance and therapeutic implications of finding LN in core biopsy specimens are still controversial [7,8].

The diagnosis of CISM is extremely rare and studies assessing the differential diagnosis of these lesions are scarce and include only a few patients. The largest series reported between 12 and 28 cases [9,10]. Previous studies by our group identified 0.08% of CISM among breast biopsies performed in our general hospital [11]. Although rare, when analyzed under light microscope, the CISM lesions are difficult to diagnose and there is lack of epidemiological data linked to their biological behavior.

A great progress in the diagnosis of these lesions came with the observation that almost all cases of LN and invasive lobular carcinoma (ILC) lose the immunohistochemistry (IHC) signal for E-cadherin and β-catenin expression in the cytoplasm membrane, whereas the expression of these proteins is maintained in both *in situ* and invasive ductal carcinomas [3,12,13]. The cadherins comprise a large number of adhesion molecules localized in the intercellular junctions, keeping cells connected through homophilic protein-protein interactions. The observation that cadherins play an important role in the establishment of the epithelial phenotype, cell migration, cell differentiation, and tumor dissemination has stimulated great interest in this family of adhesion molecules. Among them is the Human Epithelial Cadherin (E-cadherin), a calcium-dependent transmembrane glycoprotein directly involved in the process of cell adhesion [14]. The α, β, p, and γ catenins play important roles in intercellular signal transduction. The β-catenin, specifically, binds to the cytoplasmatic portion of the E-cadherin and to the structure of actin microfilaments of the cytoskeleton as well, being involved in cell adhesion [15,16]. The E-cadherin gene mutation is the major mechanism responsible for its inactivation in cancer cells and is associated with other carcinomas, such as hepatocellular carcinoma, diffuse-type gastric cancer, thyroid and colorectal cancer [16,17]. Another route resulting in inactivation of E-cadherin is attributed to dysfunctional promoter activity or DNA methylation at the promoter region [17,18].

The aim of this study was to evaluate the expression of E-cadherin and β-catenin for the immunophenotypical characterization of carcinomas *in situ* with mixed pattern, and identify potential morphological patterns that could assist in the diagnosis of the different types of CISM lesions.

Methods

This is a retrospective, descriptive study that analyzed 25 cases of breast biopsies (wide local excision or mastectomy) performed between 1999 and 2011. The study analyzed the histopathological reports and slides available at the Laboratory of Breast Pathology at the School of Medicine at the Federal University of Minas Gerais (UFMG), Brazil. We selected one or more slides stained with hematoxylin and eosin (HE) and which were representative of each diagnosis of *in situ* carcinoma with mixed pattern. The slides were analyzed simultaneously by two of the authors (HG and DSG) using a dual-view microscope and classified according to the standard morphological patterns and immunohistochemical expression of E-cadherin and β-catenin. Cases without available slides and / or paraffin blocks were excluded from the study. The study was approved by the UFMG research ethics committee.

Morphological evaluation

In situ lesions matching the LN morphological pattern were characterized according to the proliferation of generally small, dyshesive cells, with uniform round nuclei and clear cytoplasm. The acini are partially or completely expanded, but the lobular architecture is maintained [1]. Lesions classified as DCIS presented proliferation of monomorphic cells with regular distribution, and hyperchromatic nuclei forming regular secondary, rounded, and uniform lumens. The *in situ* lesions characterized as mixed pattern presented cytological or architectural features common to the ductal and lobular lesions and were classified into three main patterns according to criteria previously described by Jacobs *et al.* [3]: Group 1 – those presenting architectural and cytological findings of LN but with areas of comedo-type necrosis; Group 2 – those with CISM lesions characterized by small and uniform cells, either growing in a solid pattern with focal microacinar-like structures but with cellular dyshesion, or growing in a mosaic pattern with occasional intracytoplasmic vacuoles; Group 3 – those with marked cellular pleomorphism and nuclear atypia, however, the LN discohesive pattern remains.

Immunohistochemical evaluation

Sequential 5 μm thick histological sections were obtained from the paraffin blocks from the breast specimens and mounted on silanized slides. Sections were deparaffinized in two consecutive baths of xylene, for 20 minutes each, and rehydrated in ethanol series with decreasing concentrations and finally in distilled water. For antigen retrieval, a buffer solution of 10 mM citrate pH 6.0 was used in an electrical pressure-cooker. Immunohistochemistry was performed automatically using Ventana Benchmark XT equipment (Ventana Medical Systems Inc., Tucson, AZ, USA). E-cadherin (clone 36) and β-catenin (clone 18) antibodies were used, at titers of 1:600 and 1:800, respectively. Antibodies were purchased from BD Transduction (cat# C19220), San Jose, CA, USA.

The non-biotinylated polymer system (Novolink®, Leica Microsystens) technique was used for reaction amplification. A diaminobenzidine (DAB) solution was used as chromogen and the slides were counter-stained using Harris hematoxylin. External positive and negative controls were used. Normal ducts and lobules, adjacent

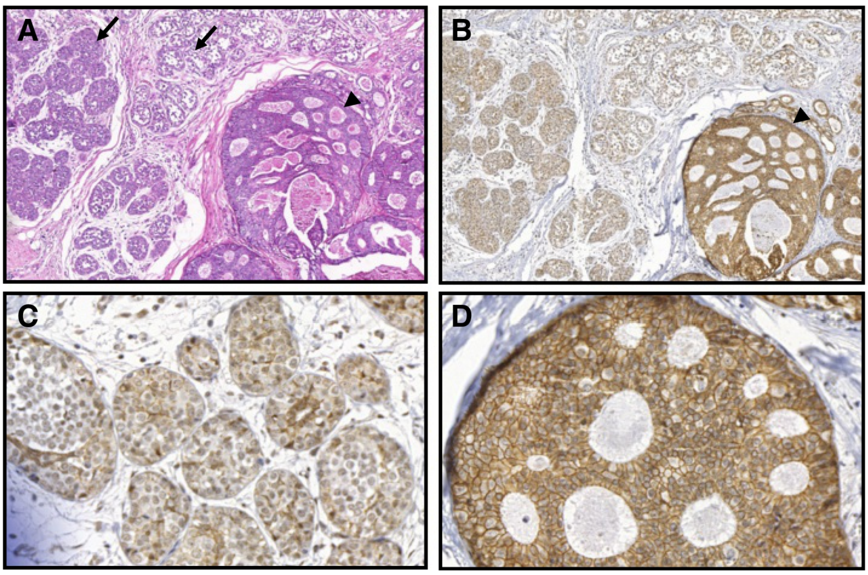

Figure 1 Case # 1: Lobular neoplasia (arrows) and ductal carcinoma in situ of cribriform type (arrowheads) are present in the same breast field (hematoxylin and eosin; A – 100×). Cells of ductal carcinoma stain positive (**B**,100× and **D**, 400×) and cells of lobular neoplasia are negative for E-cadherin (**B**, 100× and **C**, 400×).

to the lesions and expressing E-cadherin and β-catenin in the epithelium, were used as internal controls.

Staining for E-cadherin and β-catenin was considered positive when the staining intensity around the entire circumference of the membrane was similar to that seen in the normal luminal epithelial cells. No staining was considered as negative (Figure 1).

Results

Twenty-five cases of CISM were identified from the Breast Pathology Laboratory during the study period. The average patient age was 52.7 (± 11.5) years. Nineteen cases (76%) presented morphological pattern showing cytology and / or architectural mixed pattern (ductal and lobular), two cases (8%) showed lobular architectural pattern with nuclear pleomorphism, two cases (8%) showed mixed cytology and nuclear pleomorphism, and two cases (8%) showed comedonecrosis and nuclear pleomorphism (Table 1).

Immunohistochemistry for E-cadherin was performed in all 25 cases. Eleven cases (44%) were positive for E-cadherin (Figure 2). Thirteen cases (52%) showed mixed immunophenotype with positive E-cadherin staining the ductal cells and negative in the lobular areas. In one case, the cells were completely negative for E-cadherin.

In all cases in which both markers were analyzed (20 cases) the immunohistochemical results agreed with both E-cadherin and β-catenin. Immunohistochemistry for β-catenin was not performed in five cases due to sample processing artifacts and insufficient material for the preparation of new slides.

Nineteen cases were composed by small, uniform cells varying from low to intermediate nuclear grade, growing in solid pattern, with some microacinar-like structures admi-

Table 1 Morphology and immunophenotype of 25 breast carcinomas *in situ* with mixed pattern analyzed from breast biopsies

Case #	Morphological pattern of *in situ* lesion			Morphology of *in situ* lesion with mixed pattern			E-cadherin immunophenotype		β-catenin immunophenotype	
	Lobular	Ductal	Mixed	Nuclear pleomorphism	Comedonecrosis	Mixed cytology or architecture	Lobular areas	Ductal areas	Lobular areas	Ductal areas
1	Yes	Yes	Yes	No	No	Yes	-	+	-	+
2	No	No	Yes	No	No	Yes	-	+	-	+
3	No	No	Yes	No	No	Yes	+	+	+	+
4	No	No	Yes	No	No	Yes	+	+	+	+
5	No	No	Yes	Yes	No	No	-	+	-	+
6	No	No	Yes	No	No	Yes	+	+	+	+
7	Yes	No	Yes	No	No	Yes	+	+	+	+
8	No	No	Yes	No	No	Yes	+	+	+	+
9	No	No	Yes	Yes	Yes	No	-	+	-	+
10	No	No	Yes	No	No	Yes	-	+	-	+
11	No	Yes	Yes	No	No	Yes	+	+	+	+
12	No	No	Yes	No	No	Yes	+	+	NA	NA
13	No	No	Yes	Yes	Yes	No	-	-	-	-
14	Yes	Yes	Yes	No	No	Yes	-	+	-	+
15	No	Yes	Yes	No	No	Yes	+	+	+	+
16	No	No	Yes	Yes	No	Yes	-	+	NA	NA
17	Yes	No	No	Yes	No	Yes	-	+	-	+
18	Yes	Yes	Yes	No	No	Yes	-	+	NA	NA
19	No	Yes	Yes	No	No	Yes	+	+	+	+
20	No	No	Yes	No	No	Yes	+	+	+	+
21	No	No	Yes	No	No	Yes	-	+	-	+
22	Yes	Yes	Yes	No	No	Yes	-	+	NA	NA
23	No	No	Yes	No	No	Yes	-	+	-	+
24	No	No	Yes	Yes	No	No	-	+	NA	NA
25	No	Yes	Yes	No	No	Yes	+	+	+	+

NA Not applied.

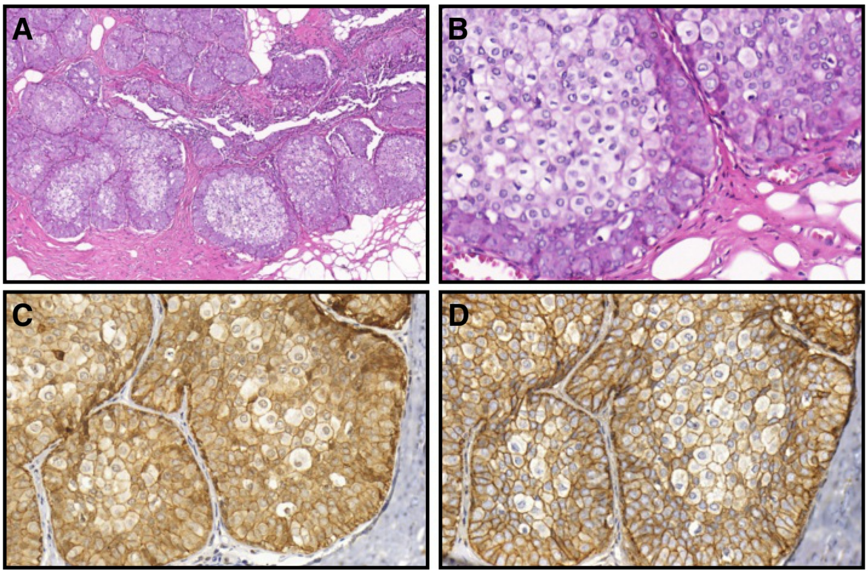

Figure 2 Case # 8: Carcinoma in situ with mixed pattern (CISM) showing dual cell population (Group 2) stained for hematoxylin and eosin (A – 100×, B – 400×). A stronger positive membrane staining for E-cadherin (**C** – 400×) and β-catenin (**D** – 400×) can be appreciated in the outer layer of cells of ductal pattern. A weaker staining is seen in cells of lobular pattern present in the center of the units.

xed with groups of low nuclear grade dyshesive cells, in a mosaic pattern. Of these, 11 (57.9%) presented positive immunohistochemistry for E-cadherin and β-catenin. In these cases, solid architecture with low-grade cytology was the most common morphological pattern. Eight of these cases (42.1%) presented the mixed immunophenotype and in four of them, the mixed pattern resulted from a "collision" of the lesions showing areas positive and areas negative for both markers in the same duct-lobular unit (Figure 3).

Two cases presented the lobular architectural pattern with nuclear pleomorphism and two cases presented mixed cytology and nuclear pleomorphism. The last two cases were considered as mixed immunophenotype. Of the two cases that presented comedonecrosis and nuclear pleomorphism, one was completely negative for both markers (Figure 4) and the other presented cells positive and cells negative for both markers (Table 2).

Discussion

In this study, we sought to evaluate the expression of E-cadherin and β-catenin for the immunophenotypical characterization of CISM. We also searched for potential morphological patterns that could help in the diagnosis of different types of CISM lesions. We adopted the morphological classification described by Jacobs *et al.* that defines CISM as "carcinomas with indeterminate features"[3]. According to this classification CISM lesions are divided in three main groups, namely: (I) presence of necrosis, (II) cytology and / or mixed architecture, and (III) nuclear polymorphism. This classification is highly reproducible and addresses the main morphological groups described in our study.

A total of 25 CISM cases were evaluated in this study. The most common morphological pattern of lesions identified belonged to group (II) with 19 out of 25 cases (76%), followed by group (III) (2/25 cases - 8%), and overlapping patterns between groups (I) and (II) (2/25 cases - 8%) and groups (II) and (III) (2/25 cases - 8%). Our findings are in agreement with those reported by Jacobs *et al.* who observed, in 28 cases of CISM, 60% of the lesions in group (II) (17/28 cases), 21% in group (I) (5/28 cases) , and 18% in group (III) (5/28 cases).

However, it is noteworthy that the terminology and morphologic criteria used for the diagnosis of CISM are heterogeneous. Fisher *et al.* termed it as "ductolobular carcinoma *in situ*" lesions with monomorphic cells with foci of necrosis or cribriform pattern [4]. Acs *et al.* described 14 cases of CISM referring to the lesions as "with ductal carcinoma *in situ* and lobular features" and adopted, as a diagnostic criteria, LN *in situ* lesions with cytological and architectural patterns, with central areas of comedonecrosis or lobules, or large duct units populated by non-cohesive cells with marked nuclear pleomorphism [19]. Maluf *et al.* analyzed 12 cases of "solid low grade carcinoma *in situ* of the breast" and included "low-grade solid DCIS, LCIS and DCIS and LCIS associated with invasive carcinomas of any type. Cases showing only unequivocal areas of LCIS or DCIS of nonsolid type were excluded" [20].

Even among experts in the pathology of breast tumors, the descriptions of these lesions are divergent. Page and

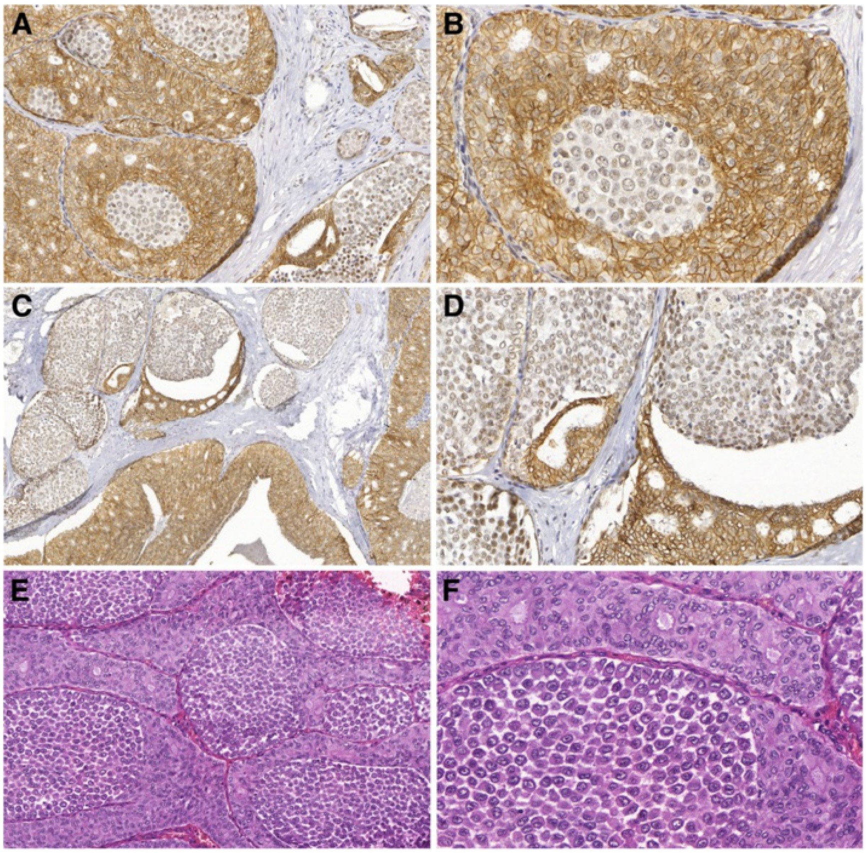

Figure 3 Case # 16: Carcinoma in situ with mixed pattern (CISM) showing dual cell population stained for hematoxylin and eosin, involving a papilloma and adjacent ducts. Areas of pleomorphic lobular pattern show discohesive atypical cells (arrows). Areas of "ductal" pattern show proliferation of cohesive cells with hyperchromatic nuclei forming secondary, rounded, and uniform lumens (arrowheads), (**A**, 200× and **B**, 400×). A mixed pattern resulted from a "collision" of both cell types in the same duct-lobular unit. "Ductal" cells are positive for E-cadherin (arrowheads) and lobular cells are negative (**C** and **E**– 200×, **D** and **F** – 400×).

Anderson state that in most cases an attempt should be made to classify the lesions as LN or DCIS [21]. However, on rare occasions this might not be possible and the diagnosis of "*in situ* carcinoma of ductal or lobular type" needs to be made. These authors recommend that if more than one focus of necrosis is found, the lesion should not be classified as LN. Rosen describes two main types of CISM: "concurrent intraductal and lobular carcinoma *in situ*" for lesions that present a cytology of lobular pattern and distended ducts and central necrosis or calcifications, and "coexistent intraductal and lobular carcinoma *in situ* in a single duct-lobular unit." The author uses this description to refer to the more unusual intraductal lesions characterized by the presence of two distinct architectural and cytological patterns [22].

Recently, *in situ* lesions with lobular cytological features of classic LCIS but with marked nuclear pleomorphism, comedonecrosis, and with or without apocrine cytology have been described as pleomorphic LCIS [1]. Some reports suggest that these variants are more aggressive than classic LN and a surgical treatment similar to that applied to DCIS is recommended. However , there are no prospective epidemiological studies showing that these variants have different clinical significance and appropriate management of pleomorphic LCIS is currently uncertain [1].

In our series, we observed a frequent association between immunophenotype and morphology (cytoarchitectural features). Lesions in group (II), with solid architecture and low-grade cytology, were more often associated with expression of E-cadherin. Our data differ from those reported by Acs *et al.*, in which no expression of E-cadherin was observed in the 14 CISM cases analyzed. The most frequent morphological pattern observed in that study was presence of lobular cytology with comedonecrosis (n = 9) [19]. Maluf *et al.* analyzed 12 CISM cases and detected E-cadherin expression in five, while another five cases showed no expression of the protein and two presented a mixed population of cells in this

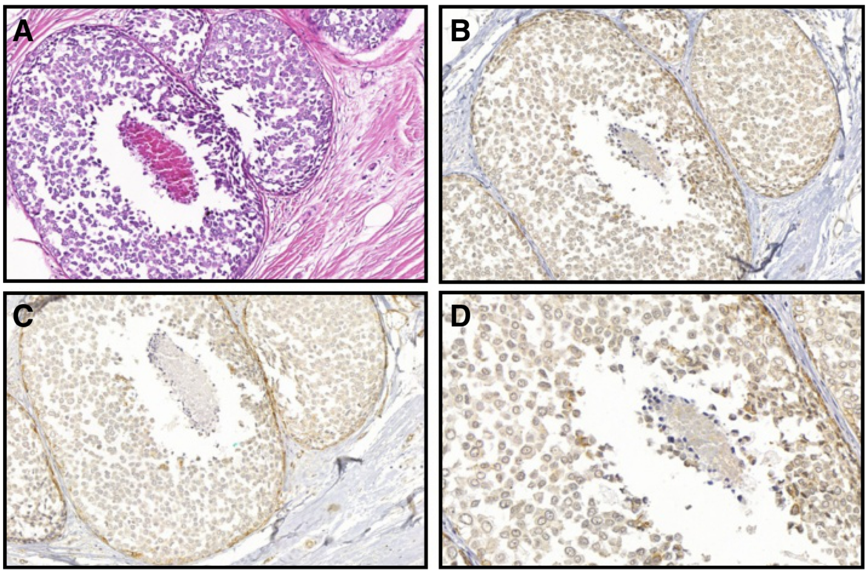

Figure 4 Case # 13: Pleomorphic lobular carcinoma in situ showing discohesive atypical cells, with nuclear pleomorphism and comedonecrosis. Hematoxylin and eosin (**A** – 100×). Lobular cells are negative for E-cadherin (**B** – 100×; **D** – 400×) and for β-catenin (**C** – 100×).

regard. These authors did not observe the prevalence of a specific morphological pattern over others [20]. Similarly to the study by Jacobs *et al.*, lesions in group (II) were the most frequent lesion associated with expression of E-cadherin in our study, however we observed them at a higher frequency. A dual cell population in the same terminal duct-lobular unit was observed in four cases. This is likely due to the coexistence of LN and DCIS in the same terminal duct-lobular unit.

Since first reported, the immunohistochemical reaction of E-cadherin has been proposed as an aiding tool in the differential diagnosis between ductal and lobular lesions, either invasive or *in situ*. However, it should be noted that up to 15% of lobular lesions may exhibit aberrant expression of E-cadherin and thus, the lack of E-cadherin expression should not be used as the sole criterion for LN diagnosis [12]. Choi *et al.* observed variability in the immunohistochemical staining of E-cadherin, and detected abnormal staining patterns, both in ductal and lobular lesions, making the differential diagnosis between *in situ* lobular and invasive lesions very difficult through immunohistochemistry [10]. An alternative to reduce this interference and improve diagnosis is the combined use of immunohistochemical markers of the catenin pathway. Using IHC and molecular biology techniques Da Silva *et al.* analyzed three cases presenting morphological characteristics and genotyping that agreed with invasive lobular carcinoma, with nonetheless aberrant expression of E-cadherin. Of these three cases, two did not express β-catenin, indicating that the formation of the cadherin-catenin complex, which is required for the normal function of the cell and maintenance of tissue architecture, including cell adhesion, failed [12,23]. In our study, we observed that expression of E-cadherin agreed with expression of β-catenin in all cases here observed.

Table 2 Category morphology and immunophenotype for E-cadherin of carcinoma *in situ* with mixed pattern

Morphology	Cases	E-cadherin		
		Positive	Negative	Mixed
Group 1	0	0	0	0
Group 2	19	11	0	8
Group 3	2	0	0	2
Group 2 and 3	2	0	0	2
Group 1 and 3	2	0	1	0
Total	25	11	1	13

Other explanations for the abnormal expression of E-cadherin found in other studies may be related to technical difficulties and pitfalls that may occur during the different stages in the immunohistochemical reaction. In our study, we had some difficulties in the pre-analytical reaction such as material loss and weak staining in some cases. This may reflect the fact that we used specimens coming from the routine diagnosis laboratory of a general hospital; and other cases were sent to us for a second opinion. In many cases, there was no control of the pre-analytical phase or standardization of time of formalin fixation and unbuffered formalin was used. Goldstein *et al.* showed that the reactivity level varies with the number of blocks and thickness of the sample sections in the pre-analytical process [2]. Different clones of antibodies against E-cadherin and different antigens may also have an effect on the quality of the immunohistochemical staining. A comparison between two types of antibodies revealed discrepancy in the staining of lobular lesions in 6.4% of the cases [7]. Finally, there is a lack of consensus regarding the interpretation of the positivity of immunohistochemical staining of E-cadherin. The established cutoff of a positive signal varies between basal membrane expression and presence of any positivity to 20% of expressing cells [12,23]. Semiquantitative evaluations of the intensity of staining and association of different criteria forming scores of staining intensity have also been proposed [19].

Other immunohistochemical markers have been suggested to aid in the diagnosis of CISM. The p120 catenin is an intracellular protein that promotes the binding between the complex of catenins and cell cytoskeleton. When E-cadherin expression is absent, p120 catenin is dispersed in the cytoplasm, which explains its expression in the cytoplasm in LN, and in the membrane in DCIS [23,24].

Conclusions

The immunophenotypic characterization of carcinomas *in situ* using E-cadherin and β-catenin, combined with the analysis of cytological and architectural patterns, is a useful tool for the morphological and immunophenotypical classification of CISM. However, a negative staining for these markers should not be used as the sole criterion of lobular phenotype because aberrant expression in lobular neoplasia and loss of expression in ductal cancers can both occur.

Abbreviations

ALH: Atypical lobular hyperplasia; CISM: Carcinoma *In Situ* with mixed pattern; DCIS: Ductal carcinoma *In Situ*; LN: Lobular neoplasia; LCIS: Lobular carcinoma *In Situ*; IHC: Immunohistochemistry; E-cadherin: Human epithelial cadherin; PLCIS: Pleomorphic lobular carcinoma *In Situ*; UFMG: Federal University of Minas Gerais.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

DSG conceived the study, participated in the histological review, and drafted the manuscript. *SSP* participated in the design of the study. RMG: Performed the immunohistochemistry reactions. *HG* participated in design and coordination of the study, participated in the histological review, and drafted and reviewed the manuscript. All authors have read and approved the final manuscript.

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2.3. Considerações finais

Atualmente, vivemos em um momento singular no estudo das vias moleculares e genéticas do desenvolvimento do câncer de mama, assim como de seu diagnóstico e tratamento. O número de artigos publicados sobre este tema aumenta a cada ano. Uma simples pesquisa com o descritor "*breast cancer*" na base de dados do PUBMED demonstra que o número de artigos publicados com este tema aumentou de 5.441 em 2003, para 13.777 em 2013. Embora estes avanços sejam importantes, a melhora da sobrevida e da qualidade de vida das pacientes dependem do correto diagnóstico do carcinoma invasor e/ou de suas lesões precursoras. A equipe multidisciplinar envolvida no cuidado da paciente com lesões mamárias, especialmente mastologistas e oncologistas, definem sua abordagem terapêutica, entre outros parâmetros, com base no laudo histopatológico emitido pelos patologistas. Por este motivo, é importante que este modo de comunicação seja claro, reprodutível e preciso, mesmo que seja necessário a utilização de ferramentas complementares como a imuno-histoquímica ou discussão do caso com patologistas mais experientes.

Durante o trabalho do mestrado, analisamos uma série histórica de casos de NL, e sua associação com as lesões benignas, pré-malignas e malignas. Concluímos que, NL e LCC estavam frequentemente associadas. No entanto, diferente de outras séries, classificamos a maioria dos casos como LCC sem atipias. Observamos também maior frequência de carcinoma invasor, mais comumente CLI, associados às formas mais desenvolvidas de NL (CLIS) (GOMES, 2010; GOMES et al., 2011). Estes dados nos fizeram questionar se estes diagnósticos eram reprodutíveis entre os patologistas em geral, motivando a pesquisa de doutorado.

No presente estudo, procuramos analisar a reprodutibilidade diagnóstica de lesões precursoras do câncer de mama, em especial LCC e NL entre patologistas generalistas e um patologista com treinamento e experiência em patologia mamária em espécimes enviados para consultoria e/ou segunda opinião, assim como lesões mamárias mais bem estudadas em relação a reprodutibilidade como o CDIS. Em 56,9% dos casos os espécimes mamários eram provenientes de setorectomias ou quadrantectomias, 29,8% de core biopsy, 5,9% de mastectomias e 7,4% de outros tipos de espécimes. As solicitações de consultoria e/ou segunda opinião foram solicitadas em 60% dos casos pelos cirurgiões mamários, em 5,2% por oncologistas e em somente 4,1% dos casos por patologistas.

Analisamos, também, um subgrupo de carcinomas in situ da mama de difícil diagnóstico diferencial entre lesão ductal ou lobular, mesmo para patologistas experientes, denominados de carcinomas in situ de padrão misto. Utilizamos a ferramenta da reação imuno-histoquímica para E-caderina e β-catenina comparando com padrões morfológicos previamente descritos na tentativa de melhor definir estas lesões.

Observamos que dos 67 casos de NL nos laudos originais, HLA estavam presentes em 25,4%, CLIS em 67,2% e CLIS pleomórfico em 7,5 %. Dos 72 casos de NL após a revisão, HLA estavam presentes em 30,6%, CLIS em 63,9% e CLIS pleomórfico em 5,6%. Notamos uma concordância boa para o diagnóstico entre o laudo do original e após a revisão da HLA e CLIS. Entretanto, a concordância entre os diagnósticos de CLIS pleomórfico foi considerada baixa. Acreditamos que a boa concordância observada para os diagnóstico de HLA e CLIS se deva aos critérios diagnósticos e potencial precursor já bem estabelecidos, entretanto ainda restam algumas dúvidas ao mastologista e/ou oncologista quanto ao seu melhor manejo em determinadas situações.

O CLIS pleomórfico é uma lesão de difícil diagnóstico diferencial com o CDIS de alto grau, pois compartilham características morfológicas semelhantes como o pleomorfismo nuclear e em alguns casos a presença de necrose tipo comedo, embora mantenha a arquitetura lobular e a perda de coesão celular típicas das NL. Apesar de não avaliado em nosso estudo, estas lesões podem, em sua maioria, ser diagnosticadas como CDIS de alto grau podendo induzir a um tratamento mais agressivo das pacientes. Outros diagnósticos diferenciais, que apresentam as mesmas implicações terapêuticas do CLIS pleomórfico, são o de NL com carcinoma ductal *in situ* sólido de baixo grau, e carcinomas *in situ* com achados citológicos e/ou arquiteturais que se desviam do padrão não se podendo determinar se a proliferação é lobular ou ductal, também chamados de CISM.

O uso da imuno-histoquímica para E-caderina e β-catenina foi avaliado em nosso estudo para o diagnóstico diferencial destas lesões. Um total de vinte e cinco casos de CISM foram selecionados e analisados considerando a citologia/arquitetura mista (ductal e lobular), pleomorfismo nuclear e presença de necrose tipo comedo. Consideramos a expressão imuno-histoquímica positiva para E-caderina e β-catenina, ou negativa. Dezenove (76%) casos apresentavam somente citologia e/ou padrão arquitetural misto (ductal e lobular), dois casos (8%) apresentaram somente pleomorfismo nuclear, dois casos (8%) apresentavam citologia mista e pleomorfismo nuclear, e dois casos (8%) tinham comedonecrose e pleomorfismo nuclear. Uma positividade completa para E-caderina e β-catenina foi observada em 11 casos (44%), lesões com arquitetura sólida e citologia de baixo grau, foram mais frequentemente associada com a expressão de E-caderina. Em um caso a lesão foi negativa para ambos marcadores e apresentava pleomorfismo nuclear e comedonecrose. Em 13 lesões o imunofenótipo foi negativo em áreas lobulares e positivo em áreas ductais. Acreditamos que expressão imuno-histoquímica para E-caderina associada com a β-catenina e combinada com a análise citológica e arquitetural da lesão, podem destacar diferentes imunofenótipos e auxiliar na classificação de lesões de difícil diagnóstico como os CISM. Entretanto, esta técnica não deve ser utilizada de rotina para o diagnóstico de NL com padrão histológico bem característico.

As LCC as estavam presentes em 14,4% os laudos originais e em 25,1% dos laudos de revisão, percebemos uma fraca concordância diagnóstica para ACC, HCC e ACC com atipias, entre patologista generalistas e um especialista em patologia mamária. Quando avaliamos a atipia epitelial plana (LCC com atipias e ACC com atipias) a concordância foi moderada, demonstrando uma maior concordância em lesões com atipias mais pronunciadas. Acreditamos que esta discrepância possa ser devida a dificuldade, por muito tempo, de critérios diagnósticos reprodutíveis para estas lesões, e a falta de dados epidemiológicos que confirmem o seu potencial como lesão precursora que têm sido bem evidenciado por estudos genéticos. Estes dilemas podem induzir o patologista a uma subnotificação deste diagnóstico, principalmente quando acompanhadas de outras lesões com potencial biológico mais bem estudado, ou ter a tendência de classificá-las como HDA ou CDIS de baixo grau. Até termos maiores informações sobre o comportamento e potencial precursor destas lesões, o diagnóstico de LCC, principalmente AEP, deve servir de alerta ao patologista para o diagnóstico de outras lesões proliferativas e/ou invasoras comumente associadas como HDA, CDIS de baixo grau, CLI e carcinoma tubular.

Diversos estudos, incluindo uma linha de pesquisa de nosso grupo (PEREZ et al., 2013; SALLES; GOUVÊA; et al., 2008; SALLES; SANCHES; et al., 2008), têm avaliado a reprodutibilidade diagnóstica do CDIS entre patologistas. As maiores dificuldades encontradas por nós e outros autores reside no diagnóstico diferencial entre HDA e CDIS de baixo grau, CDIS microinvasor e a correta graduação histológica do CDIS. Estes diagnósticos diferencias são acompanhados de importantes implicações terapêuticas; como, por exemplo, conduta a expectante na HDA diagnosticada por biópsia excisional até ressecção com margens amplas e radioterapia como ocorre no CDIS. Em nossa série, a frequência de CDIS presentes laudos originais foi de 38,4% e de 39,7% dos laudos de revisão. Observamos uma boa concordância para o diagnóstico entre o laudo do original e após a revisão do CDIS de alto grau. Entretanto, a concordância entre os diagnósticos de HDA, CDIS de baixo grau, CDIS de grau intermediário e CDIS microinvasor foi considerado moderada. Acreditamos, assim como outros autores, que a constante revisão de critérios diagnósticos, programas de educação continuada e controle de qualidade possam ser utilizadas para tentar melhorar o diagnóstico das lesões proliferativas intraductais.

Por se tratar de um estudo retrospectivo, algumas limitações metodológicas podem ser encontradas. Devido à baixa frequência de algumas lesões estudadas, optamos por incluir todos os casos conclusivos que apresentavam laudos originais e de revisão para comparação. Por este motivo, as lesões precursoras estudadas poderiam estar associadas ou não a carcinoma invasivo, podendo gerar subnotificações no laudo original. Outra limitação encontra-se na fase pré-analítica da imuno-histoquímica realizada no grupo de CISM. Nosso material era proveniente de blocos de parafina arquivados em alguns casos há vários anos. Em algumas situações o material era escasso ou inadequado para novos cortes. Além disso, não tivemos controle de algumas variáveis importantes como o tempo de fixação e o uso de formol tamponado que podem comprometer a técnica da reação imuno-histoquímica. Entretanto, devido ao fato dos CISM serem lesões pouco frequentes, não haveria tempo suficiente para se realizar um estudo prospectivo.

Como principais aplicações práticas de nosso estudo podem-se destacar, que fomos os primeiros a avaliar a concordância diagnóstica de LCC e NL a partir de casos enviados para consultoria a partir de laudos de patologistas generalistas. Estes dados podem servir de referência para práticas de educação, treinamento, e controle de qualidade, bem como estimular os patologistas a solicitarem revisão e/ou segunda opinião de lesões precursoras com fraca a moderada concordância diagnóstica com patologistas mais experientes no estudo das lesões mamárias. Apesar de outras séries terem realizado imuno-histoquímica para E-caderina em CISM, nossa contribuição nesta área foi associar o uso de um segundo marcador do complexo das cateninas, a β-catenina, que pode minimizar a falha do método da E-caderina no diagnóstico das NL devido a sua expressão aberrante. Contudo não observamos esta expressão em nossa série, pois concordância na expressão de E-caderina e β-catenina em todos os casos estudados.

Acreditamos que como perspectivas futuras de pesquisa na área das lesões precursoras mamárias, são necessários estudos que avaliem a clonalidade destas lesões em carcinomas mamários invasores sincrônicos e metacrônicos. A associação a estes dados de informações sobre a evolução clínica e resposta terapêutica, poderão fornecer um maior conhecimento a respeito do potencial evolutivo para o desenvolvimento de carcinoma invasor, assim como identificar marcadores tumorais preditivos e prognósticos que auxiliem no manejo destas pacientes.

3. CONCLUSÕES

1. Nossos achados demostram uma concordância interobservador entre os patologistas generalistas e um patologista especialista patologia mamária considerada fraca para as LCC sem atipias e CLIS pleomórfico; moderada para LCC com atipias (ou atipia epitelial plana), HDA e CDIS de graus baixo, intermediário e microinvasor. O CDIS de alto grau, HLA e CLIS tiveram concordância diagnóstica considerada boa em nossa série.
2. A caracterização imunofenotípica com E-caderina e β-catenina, combinada com a análise citológica e arquitetural, pode destacar diferentes imunofenótipos e auxiliar na classificação dos CISM.

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ANEXOS

ANEXO A - Produção científica durante o período do doutorado

Artigos completos publicados em periódicos

**GOMES, D. S.; BALABRAM, D; PORTO, SS ; GOBBI, H. . Lobular Neoplasia: Frequency and Association with Other Breast Lesions. Diagnostic pathology, v. 6, p. 74, 2011. (Anexo 2)
***GOMES, D. S.**; PORTO, SS ; ROCHA, RM ; GOBBI, H. . Usefulness and limitations of E-cadherin and beta-catenin in the classification of breast carcinomas in situ with mixed pattern. Diagnostic Pathology, v. 8, p. 114, 2013.
GOMES, D. S.; PORTO, SS ; BALABRAM, D ; GOBBI, H. . Inter-observer variability between general pathologists and a specialist in breast pathology in the diagnosis of lobular neoplasia, columnar cell lesions, atypical ductal hyperplasia and ductal carcinoma in situ of the breast Diagnostic Pathology, v. 9, p. 121, 2014.

Resumos publicados em anais de congressos

*GOMES, D. S. ; PORTO, SS ; BALABRAM, D ; GOBBI, H. . Diagnósticos histopatológicos de lesões mamárias feitos por patologistas gerais e revisão especializada: implicações terapêuticas. In: XVI CONGRESSO BRASILEIRO DE MASTOLOGIA, 2011, GOIÂNIA - GO. REVISTA BRASILEIRA DE MASTOLOGIA, 2011. v. 21. p. 62.
** GOBBI, H. ; GOMES, D. S. ; BALABRAM, D ; PORTO, SS . Lobular neoplasia: frequency and association with others breast lesions. In: XXVII th International Congress of the International Academy of Pathology, 2010, São Paulo. Histopathology, 2010. v. 57. p. 33-33.

Apresentações de Trabalho

PORTO, SS ; GOMES, D. S. ; BALABRAM, D ; GOBBI, H. . Carcinomas in situ da mama com características indeterminadas (CIS-I): frequência e associação com outras lesões mamárias. 2011. (Apresentação de Trabalho/Comunicação).
GOMES, D. S. ; PORTO, SS ; BALABRAM, D ; GOBBI, H. . Diagnósticos histopatológicos de lesões mamárias feitos por patologistas gerais e revisão especializada: implicações terapêuticas. 2011. (Apresentação de Trabalho/Comunicação).
**GOMES, D. S. ; BALABRAM, D ; PORTO, SS ; GOBBI, H. . Fatores clínicos e anatomopatológicos que influenciam a sobrevida livre de doença em pacientes com carcinoma lobular invasor. 2010. (Apresentação de Trabalho/Congresso).
**GOBBI, H. ; GOMES, D. S. ; BALABRAM, D ; PORTO, SS . Lobular Neoplasia: Frequency and Association With Other Breast Lesions. 2010. (Apresentação de Trabalho/Congresso).
Relacionados com o trabalho do doutorado.
** Relacionados com o trabalho do mestrado.

ANEXO B - Artigo referente ao trabalho de mestrado

Gomes *et al. Diagnostic Pathology* 2011, **6**:74
http://www.diagnosticpathology.org/content/6/1/74

DIAGNOSTIC PATHOLOGY

RESEARCH Open Access

Lobular neoplasia: frequency and association with other breast lesions

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Abstract

Background: Using new molecular biology techniques, recent studies have implicated a common evolutionary pathway between lobular neoplasia, lobular carcinomas, and columnar cell lesions. Our aims were to assess the frequency of lobular neoplasia in a series of breast biopsies that were performed and examined in the same institution and to analyze the association between subtypes of lobular neoplasia and benign and malignant breast lesions.

Methods: Cases were selected after reviewing archived pathological reports in the Breast Pathology Laboratory, School of Medicine of Federal University of Minas Gerais (1999-2008). Cases of lobular neoplasia were reviewed and classified as atypical lobular hyperplasia, ductal involvement by cells of atypical lobular hyperplasia, lobular carcinoma *in situ*, and pleomorphic lobular carcinoma *in situ*. Coexistence of lobular neoplasia with other breast lesions, including columnar cell lesions, invasive ductal carcinoma and invasive lobular carcinoma, was evaluated. The association between lobular neoplasia and breast lesions was analyzed by Fisher's exact test and chi-square test for linear trend.

Results: We analyzed 5650 breast specimens, selecting 135 breast specimens (2.4%) that had a diagnosis of lobular neoplasia, corresponding to 106 patients. Hematoxylin and eosin-stained slides were available for 84 cases, 5 of which were excluded because they contained only "indeterminate" *in situ* lesions. Of the 79 remaining cases, columnar cell lesions were present in 78.5%, primarily with columnar cell changes without atypia (67.7%). Invasive carcinoma was present in 45.6% of cases of lobular neoplasia–a similar frequency (47.2%) as invasive ductal carcinoma and invasive lobular carcinoma. We noted a significant linear trend ($p < 0.03$) of a higher frequency of invasive carcinomas that were concomitant with lobular carcinoma *in situ* compared with atypical lobular hyperplasia. Invasive lobular carcinomas were associated with lobular carcinoma *in situ* in 33% of cases, compared with 2.8% of atypical lobular hyperplasia cases.

Conclusions: Our findings confirm a frequent association between lobular neoplasia and columnar cell lesions, the majority of which lacked atypia. We also observed a greater frequency of invasive carcinoma, more commonly invasive lobular carcinoma, associated with more developed forms of lobular neoplasia (lobular carcinoma *in situ*).

Keywords: breast cancer, lobular neoplasia, ductal carcinoma *in situ*, columnar cell lesions

Background

Lobular carcinoma *in situ* (LCIS) was first described by Foote and Stewart in 1941, designated as such due to its cytological similarities with invasive lobular carcinoma (ILC): cuboidal and regular and harboring discohesive cells, often containing cytoplasmic vacuoles. LCIS was originally considered a precursor of invasive carcinoma due to its frequent association with invasive lobular carcinoma [1]. Subsequent epidemiological studies demonstrated that the risk of developing invasive lesions was not as high as expected, progressing slowly and forming in the ipsilateral and contralateral breast [2].

Other studies confirmed the indolent nature of LCIS; clinically, LCIS was considered a risk marker for invasive breast cancer. The consequent risk was proportional to the extent of disease and was evaluated, based on

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distention of the lobular units in the ducts that were affected by neoplastic cells [3,4]. Due to its indolent behavior, Haagensen *et al.* proposed replacing the term "lobular carcinoma" with "lobular neoplasia" to decrease the impact of the malignancy and the link to mortality that is associated with the term "carcinoma" [2].

Page *et al.* correlated the extension of lobular involvement and the risk of breast cancer, proposing a semiquantitative stratification method–designating lobular lesions in atypical lobular hyperplasia (ALH) for less extensive lesions and LCIS for more extensive lesions. A 4- to 5-fold relative risk of developing invasive carcinoma was observed for ALH lesions, whereas for CLIS, the relative risk was 8 to 11 times greater than the general population [4]. The ductal involvement by cells of atypical lobular hyperplasia (DIALH), also called pagetoid spread, carried an intermediate risk of developing carcinoma of 6.8-fold [5].

Although Page's classification has been used widely over the past 20 years, the latest World Health Organization (WHO) classification of tumors groups these lesions under lobular neoplasia (LN), without considering their development [6].

Recent molecular biology studies have revealed more about lobular neoplasia. Genetic similarities, such as the loss of chromosomal material on 16q and gains on 1q, have been observed in LN and other low-nuclear grade breast lesions. Similar genetic alterations were detected in columnar cell lesions (CCLs), low-grade ductal carcinoma *in situ* (DCIS), tubular carcinoma (TC), and ILC. These similarities suggest a common evolutionary pathway, in which low-grade precursor lesions progress to low-grade invasive and *in situ* carcinomas [7-9].

Columnar cell lesions coexist frequently with DCIS, and low-grade invasive carcinomas, particularly TC and ILC [10-13]. However, few studies have evaluated this association, based on the diagnosis of LN, in routinely removed breast specimens [14,15].

The aims of this study were to assess the frequency of LN in a series of breast biopsies that were performed and examined in the same institution and to analyze the association between subtypes of LN with benign and malignant breast lesions.

Methods

We accessed the archives of the Breast Pathology Laboratory (BPL) of the School of Medicine of Federal University of Minas Gerais from August 1999 to December 2008, selecting all breast specimens with diagnoses of ALH, DIAL, LCIS, and pleomorphic LCIS. Cases of LN with original hematoxylin and eosin (H&E)-stained slides were reviewed by DSG and HG using a double-headed optical light microscope and included in the study. Breast biopsy specimens from the same patient were considered one case. Cases with only core needle biopsy specimens were excluded.

We used the histological criteria per Page *et al.* to classify ALH, DIALH, and LCIS [3-5]. LCIS was defined as complete involvement of the lobules by neoplastic cells, with greater than 50% of a lobule completely replaced and distended by neoplastic and monomorphic cells. ALH was defined as lobules that were partially distended by neoplastic cells, failing to meet the criteria for LCIS. DIALH was diagnosed when the ALH cells extended between the epithelial layer and the basement membrane of the terminal duct.

The criteria that we used to diagnose pleomorphic LCIS was described by Eusebi *et al.*–the same architectural pattern as LCIS but with larger nucleoli and nuclear pleomorphism [16]. Cases that harbored more than one subtype of LN were classified by the lesion with the greatest risk of developing carcinoma: pleomorphic LCIS > Classic LCIS > DIALH > ALH. The term "indeterminate *in situ* lesions" (IILs) or "mixed type lesions" was used to describe certain breast carcinomas *in situ*, in which the cytological or architectural properties and distribution deviated from the typical patterns, rendering it difficult, if not impossible, to determine whether the proliferation was lobular or ductal, based only on morphological criteria [17]. These cases were not included in our analysis.

The frequency of the association of LN was analyzed for the following diagnoses: CCL, per Schnitt and Vincent-Salomon [18], who divided the lesions into columnar cell change without atypia (CCC); columnar cell change with atypia (CCC with atypia); columnar cell hyperplasia without atypia (CCH); and columnar cell hyperplasia with atypia (CCH with atypia). The presence of *in situ* and invasive carcinoma was noted, as were their type and histological tumor grade. The tumors were classified per Page *et al.* and the American College of Pathology [19,20]. The Nottingham grading system was used for histological grading [21].

Statistical analysis was performed using SPSS (version 17.0, SPSS Inc, Chicago, IL, USA). Differences in mean age between LN groups was calculated by ANOVA, and the association between LN and breast lesions was analyzed using Fisher's exact test, χ^2 test, and χ^2 test for trend. The study was approved by the ethical committee of the UFMG.

Results

During the study period, 5650 breast specimens from the same institution were analyzed. From the original reports, 135 breast specimens (2.4%) were diagnosed with a subtype of lobular neoplasia, corresponding to 106 patients, 21 of whom had 2 or more consecutive biopsies. H&E-stained slides were available for 84

Table 1 Frequency of subtypes of lobular neoplasia (LN) and mean age of patients

LN	n	%	Mean age (years) ± SD	
ALH	22	26.2	50.2	± 9.0
DIALH	25	29.8	50.2	± 9.7
LCIS	29	34.5	51.3	± 10.6
LCIS pleo	3	3.6	49.3	± 8.1
IIL	5	6.0	58.2	± 8.1
Total	84	100.0	52.0	± 9.7

ALH = atypical lobular hyperplasia; DIALH = ductal involvement by cells of atypical lobular hyperplasia; LCIS = lobular carcinoma *in situ*; LCIS pleo = pleomorphic LCIS; IIL = indeterminate *in situ* lesions. There was no difference in mean age among patients with different lesions (p = 0.425); n = number of cases; SD = standard deviation.

patients, slides for 5 of whom were excluded because they contained only indeterminate *in situ* lesions. The frequencies of LN subtypes and the average patient ages are shown in Table 1. There was no significant difference in patient age between subgroups of patients with LN (p = 0.425).

We observed a frequent association of LN with CCL (62/79 cases, 78.5%) and with most cases of CCC without atypia (42/62 cases, 67.7%). We observed a significant linear association (p = 0.03), wherein the frequency of LN tended to correlate negatively with the degree of atypical columnar lesions (Table 2). Twenty-three cases (29.1%) presented with coexisting LN, CCL, and invasive carcinoma (Table 2). Twenty cases (87%) comprised CCC or CCH without atypia, and 3 cases (13%) had CCC or CCH with atypia. There were no significant differences in the association of columnar lesions with or without atypia with regard to histological type and tumor grade of the invasive carcinomas. The coexistence of TC, LN, and CCL, reported by some groups as "Rosen's triad" [11], was observed in 1 case.

Moderate or usual ductal hyperplasia without atypia and atypical hyperplasia were present in 40% and 10.1% of 79 LN cases, respectively, but no significant difference in the association with LN subtypes was observed.

Table 2 Frequency of association between subtypes of lobular neoplasia and columnar cell lesions (CCL)

CCL	ALH		DIALH		LCIS		LCIS pleo		Total	
	n	%	n	%	n	%	n	%	n	%
CCC	14	22.6	13	21.0	15	24.2	0	0	42	67.7
CCH	4	6.5	3	4.8	2	3.2	0	0	9	14.5
CCC with atypia	1	1.6	3	4.8	4	6.5	1	1.6	9	14.5
CCH with atypia	0	0	0	0.0	1	1.6	1	1.6	2	3.2
Total	19	30.6	19	30.6	22	35.5	2	3.2	62	100.0

ALH = atypical lobular hyperplasia; DIALH = ductal involvement by cells of atypical lobular hyperplasia; LCIS = lobular carcinoma *in situ*; LCIS pleo = LCIS pleomorphic; CCC = columnar cell change; CCH = columnar cell hyperplasia; CCC with atypia = columnar cell change with atypia; CCH with atypia = columnar cell hyperplasia with atypia; n = number of cases. χ^2 test for trend: p = 0.03.

Table 3 Association between subtypes of lobular neoplasia and histological grade of ductal carcinoma *in situ*

Histological grades of DCIS	ALH		DIALH		LCIS		Total	
	n	%	n	%	N	%	n	%
Low	0	0.0	2	11.8	2	11.8	4	23.5
Intermediate	1	5.9	0	0.0	1	5.9	2	11.8
High	2	11.8	4	23.5	5	29.4	11	64.7
Total	3	17.6	6	35.3	8	47.1	17	100.0

ALH = atypical lobular hyperplasia; DIALH = ductal involvement by cells of atypical lobular hyperplasia; LCIS = lobular carcinoma *in situ*; DCIS = ductal carcinoma *in situ*. There was no difference between groups.

LN was associated with DCIS in 21.5% of cases, and high-grade DCIS correlated more often with LN (64.7% of cases). There were no cases that of concomitant DCIS and pleomorphic LCIS. Although there was no significant difference between LN subtypes, LCIS was most often associated with DCIS (47.1%) (Table 3). We noted 7 cases (8.9%) of LN and DCIS without concurrent invasive carcinoma–5 high-grade, 1 moderate, and 1 low-grade. Invasive carcinomas were present with LN in 45.6% of cases, with similar rates of association with invasive ductal carcinomas (IDCs) and ILC (47.2%).

With regard to cases of ILC, however, we observed a higher frequency of ILC that was associated with LCIS (33.3%) compared with DIALH (11.1%) and ALH (2.8%) (Table 4). No significant difference was noted in the link between histological grade of the invasive carcinoma and LN subtype in any group (Table 5).

Discussion

The frequency of diagnosis of lobular neoplasia in our study was 2.4% in a consecutive series of routinely removed breast specimens in a general hospital. The rate of LCIS ranges from 0.5% to 3.6% of breast specimens [2,3]. Because there are no obvious clinical or radiological features, the true incidence of LN in the general population is unknown [22,23].

Table 4 Association subtypes of lobular neoplasia and histological type of invasive carcinomas

Histological types	ALH		DIALH		LCIS		Total	
	n	%	n	%	N	%	n	%
IDC	4	11.1	4	11.1	9	25.0	17	47.2
ILC	1	2.8	4	11.1	12	33.3	17	47.2
Tubular carcinoma	0	0.0	1	2.8	0	0.0	1	2.8
Micropapillary	0	0.0	0	0.0	1	2.8	1	2.8
Total	5	13.9	9	25.0	22	61.1	36	100.0

ALH = atypical lobular hyperplasia; DIALH = ductal involvement by cells of atypical lobular hyperplasia; LCIS = lobular carcinoma *in situ*; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma;. χ^2 test for trend: p = 0.03. There was no difference between groups.

Table 5 Association between subtypes of lobular neoplasia and histological tumor grade of invasive carcinomas

Tumor grade	ALH		DIALH		LCIS		Total	
	n	%	n	%	n	%	n	%
Low	1	2.8	3	8.3	12	33.3	16	44.4
Intermediate	2	56	4	11.1	9	25.0	15	41.7
High	2	5.6	2	5.6	1	2.8	5	13.9
Total	5	13.9	9	25.0	22	61.1	36	100.0

ALH = atypical lobular hyperplasia; DIALH = ductal involvement by cells of atypical lobular hyperplasia; LCIS = lobular carcinoma *in situ*; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma. There was no difference between groups.

The diagnosis of LN is typically related to an incidental finding on breast biopsies that are performed for other indications. With the increasing use of mammography, lobular neoplasia has been observed in association with microcalcifications in up to 40% of cases that are diagnosed by core needle biopsy [24]. Microcalcifications rarely form within LNs and they usually correlate with other benign or malignant breast lesions–the diagnosis of LN is most often incidental [23].

Columnar cell lesions (CCLs) comprise a spectrum of morphological alterations of the duct epithelial lining, acquiring a columnar cell appearance and involving variably dilated acini of the terminal duct lobular unit (TDLU) [25]. There has been recent, increasing interest in these lesions, because they are detected in up to 42% of the breast biopsies that are performed due to the presence of microcalcifications by mammography [26].

For instance, many terms have been used to describe CCLs, from "blunt duct adenosis" to "clinging carcinoma" [25,27]. Nevertheless, Schnitt and Vincent-Salomon's nomenclature and diagnostic criteria of CCL have been the most widely used [18], whereas in the most recent WHO guidelines, CCL was included under the term "flat epithelial atypia" (FEA) [6]. After the release of the WHO classification, Schnitt began referring to CCC and CCH with atypia as "flat atypia" [28].

CCLs have been linked to lobular neoplasia, low-grade DCIS, and invasive carcinoma. Further, similar genetic abnormalities have been found in CCC and CCH with atypia or FEA and the associated low-grade DCIS and invasive carcinoma. These findings have led to the reasonable conclusion that CCC and CCH with atypia are the earliest morphologically identifiable precursor lesions of low-grade DCIS and invasive carcinoma [8,15,25].

Yet, there are no prospective randomized trials, and few epidemiological studies with patients with only CCC and CCH with atypia have evaluated the prognosis of these lesions. Several studies, comprising a limited number of cases, demonstrated little or no risk for progression to invasive carcinoma [29-32]. Thus, there remains no consensus on the ideal treatment for these atypical lesions.

In our series, LN and CCL coexisted in 78.5% of cases, most often as mild forms of the spectrum of CCL–eg, CCC without atypia (67.7%). Our data are consistent with a recent study that examined 68 core needle biopsy specimens with a diagnosis of LN due to the excision of microcalcifications. The authors demonstrated an association between LN and CCL in 54% of cases, none of which presented with CCC or CCH with atypia after wide excision biopsy [15]. However, after analyzing 111 breast biopsies with LN but no other *in situ* or invasive carcinomas, Leibl *et al.* noted that LN was associated with FEA–ie, CCC and CCH with atypia in 86.5% of cases [33]. Our studies and other reports have observed a frequent association of CCL with LN, but they differ regarding the presence or absence of atypia.

There are many terms for LCC. Moreover, the WHO morphological definition of FEA is imprecise and does not describe the cytological and architectural features that are necessary for its diagnosis. In our study, all cases were reviewed by 2 observers, including a well-trained breast pathologist (HG). We used well-defined diagnostic criteria per Schnitt and Vincent-Salomon and noted fewer cases of CCC and CCH with atypia than what has been reported [10,11,33]. We believe that in many series and cases in our Breast Consulting Laboratory, FEA is being overdiagnosed, which could lead to the implementation of more aggressive treatments [34].

The frequency of invasive carcinomas that were associated with LN in our series was 45.6%, and we observed a similar frequency of ILC (47.2%) and invasive ductal carcinoma (IDC). However, when LN subtypes were analyzed separately, we observed a 4-fold higher frequency of IDC that was associated with ALH versus ILC and a greater link between ILC (33.3%) and LCIS compared with IDC. We also noted a 12-fold increase in the correlation between ILC and LCIS (33.3%) compared with ALH (2.8%).

Our data are consistent with a series of 775 cases of LN [14]. Bratthauer and Tavassoli stratified the LNs as "lobular intraepithelial neoplasias" (LINs) and evaluated the frequency of association between LIN subtypes (1, 2, and 3) and invasive carcinoma. The percentage of LIN 1 (equivalent to ALH) that was associated with invasive carcinoma was 14%, and 89% of these tumors were IDCs. In the patients with LIN 3 (equivalent to LCIS), the frequency of association with IDC and ILC was 23% and 86%, respectively. The authors concluded that the advance from LIN 1 to LIN 3 was linked to a 64% increase in the frequency of invasive carcinoma and a greater than 700% rise in the likelihood of ILC [14].

Our results corroborate other studies and suggest that lobular neoplasia is not only a risk indicator but also a nonobligate precursor of invasive breast carcinoma [23]. Invasive carcinomas that develop after a diagnosis of ALH are 3 times more likely to arise in the ipsilateral rather than contralateral breast [35].

Lobular neoplasia and ILC are detected together frequently in the same specimen and location of the tumor–in up to 90% of cases of ILC [10]. These lesions have similar immunohistochemical profiles, including the loss of expression of E-cadherin and β-catenin and the cytoplasmic localization of p120-catenin [36]. Invasive and *in situ* lobular carcinomas confer similar genetic gains and losses, often bearing the same mutations in the gene that encodes E-cadherin (*CDH1*) [7,37,38].

However, it is unknown why LCIS carries a greater risk of progression to invasive disease and is associated more frequently with invasive lobular carcinoma compared with ALH. Mastracci *et al.* demonstrated that somatic alterations in *CDH1* are a hallmark of LCIS but not ALH [39]. This disparity suggests that mutations that inactivate *CDH1* can distinguish LNs that are able to progress to invasive disease, explaining our morphological data [39].

Conclusions

Our findings confirm a frequent association between lobular neoplasia and CCL without atypia, thereby differing from other studies in which the majority of CCL is classified as CCL with atypia or FEA. We also noted a higher frequency of invasive carcinoma, more commonly ILC, that was associated with more developed forms of LN (LCIS).

List of abbreviations used

ALH: Atypical lobular hyperplasia; BPL: Breast Pathology Laboratory; CCC: Cell change without atypia; CCH: Columnar cell hyperplasia; CCL: Columnar cell lesions; *CDH1:* Gene that encodes E-cadherin; DCIS: Ductal carcinoma *in situ*; DIALH: Ductal involvement by cells of atypical lobular hyperplasia; FEA: Flat epithelial atypia; IDC: Invasive ductal carcinoma; IIL: "indeterminate *in situ* lesions"; ILC: Invasive lobular carcinoma; LCIS: Lobular carcinoma *in situ*; LIN: Lobular intraepithelial neoplasia; LN: Lobular neoplasia; TC: Tubular carcinoma; UFMG: Federal University of Minas Gerais; WHO: World Health Organization.

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Authors' contributions

DSG conceived the study, participated in the histological review, and drafted the manuscript. *DB* participated in the study design, performed the statistical analysis, and drafted the manuscript. *SSP* participated in the design of the study. *HG* participated in design and coordination of the study, participated in the histological review, and drafted and reviewed the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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ANEXO C - Protocolo de coleta de dados para o artigo Usefulness and limitations of E-cadherin and β-catenin in the classification of breast carcinomas in situ with mixed pattern

Protocolo	
Nº questionário: ___ ___ ___	
Nº Registro: ___ ___ ___ ___ ___ ___ ___	
Exame nº: ___ ___ ___ ___ ___ ___ ___ ___	
Diagnóstico da lesão no HE	**0-Não 1 - Sim**
Lobular	HELOB___
Ductal	HEDUCTAL ___
Mista	HEMISTA___
Lesão Mista	**0-Não 1 - Sim**
Pleomorfismo nuclear	LMPLEOM ___
Presença de necrose	LMNECROSE ___
Presença de citologia mista	LMCITO___
E-CADERINA : BLOCO: ___	**0-NEGATIVA 1 - POSITIVA**
Coloração Lobular	COLORLOB___
Coloração Ductal	COLORDUCTO ___
B-CATENINA : BLOCO: ___	**0-NEGATIVA 1 - POSITIVA**
Coloração Lobular	COLORLOB___
Coloração Ductal	COLORDUCTO ___
P120-CATENINA : BLOCO: ___	**0-NEGATIVA 1 - POSITIVA**
Coloração Lobular	COLORLOB___
Coloração Ductal	COLORDUCTO ___
MUDANÇA DE DIAGNÓSTICO 0-Não 1 - Sim	___
Observações:	

ANEXO D - Protocolo de coleta de dados para o artigo "Inter-observer variability between general pathologists and a specialist in breast pathology in the diagnosis of lobular neoplasia, columnar cell lesions, and ductal carcinoma in situ of the breast

Protocolo de Revisão AP

Nome:_______________________________ Idade: __ __ Registro HC: ______

Nº do Laudo original: ______________ Lab. de Origem: _________________

Procedência: ___ 1 - BH 2-Interior 3- Outro estado:___________________

Médico Solicitante: ________________________________

Especialidade: 1-Mastologista 2-Oncologista 3-Patologista 4-Paciente 5-Outro:____

Tipo de espécime: ___ ____

1-core bx 2- bx incisional 4-Setorectomia 8- Mastectomia 16- EA 32- BLS 99-SI

Nº Biopsia revisão: ___ ___ ___ ___ ___ ___ ___ Data: ___/___/___.

LAUDO ORIGINAL:___ ___ **0-Benigno 1- Maligno**

LESÃO LOBULAR:	**0-Não 1 - Sim**
HLA	HLA ___
CLIS	CLIS ___
CLIS pleomórfico	CLISPLEOM ___
Lesão Mista	LOBMISTA ___

HIPERPLASIA EPITELIAL	**0-Não 1 - Sim**
Ductal leve	HDLEVE ___
Ductal moderada/florida	HDMF ___
Ductal atípica	HDA___

CARCINOMA DUCTAL IN SITU	**0-Não 1 - Sim**
CDIS baixo grau	CDISBG ___
CDIS Grau Intermediário	CDISINT___
CDIS alto grau	CDISAG ___
CDIS Microinvasivo	CDISMICRO___

NEOPLASIA INVASORA **NEOINVASIV: __ __ __**

1- Ductal (soe) 2 - Lobular 4-Tubular 8-Colóide 16-Papilar 32-Micropapilar
64-Medular 128-Apócrino 256-Outro 888-NA 999-SI

GRAU TUMORAL: **GRAUTUMOR: __**

LAUDO PÓS REVISÃO:___ ___ **0-Benigno 1- Maligno**

LESÃO LOBULAR:	**0-Não 1 - Sim**
HLA	HLA ___
CLIS	CLIS ___
CLIS pleomórfico	CLISPLEOM ___
Lesão Mista	LOBMISTA ___

HIPERPLASIA EPITELIAL	**0-Não 1 - Sim**
Ductal leve	HDLEVE ___
Ductal moderada/florida	HDMF ___
Ductal atípica	HDA___

CARCINOMA DUCTAL IN SITU	**0-Não 1 - Sim**
CDIS baixo grau	CDISBG ___
CDIS Grau Intermediário	CDISINT___
CDIS alto grau	CDISAG ___
Paget	PAGET___

NEOPLASIA INVASORA **NEOINVASIV: __ __ __**

1- Ductal (soe) 2 - Lobular 4-Tubular 8-Colóide 16-Papilar 32-Micropapilar
64-Medular 128-Apócrino 256-Outro 888-NA 999-SI

GRAU TUMORAL: **GRAUTUMOR: __**

CONCORDÂNCIA GERAL: ___ **0-Não 1 - Sim**

CONCORDÂNCIA ESPECÍFICA: ___ **0-Não 1 - Sim**

ANEXO E - Parecer do Comitê de Ética da UFMG.

UFMG

Universidade Federal de Minas Gerais
Comitê de Ética em Pesquisa da UFMG - COEP

Parecer nº. ETIC 597/07

Interessado(a): Profa. Helenice Gobbi
Departamento de Anatomia Patológica
Faculdade de Medicina-UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 12 de dezembro de 2007, o projeto de pesquisa intitulado **"Lesões lobulares mamárias: freqüência, detecção e associação com outras lesões"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

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