UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE – UFCSPA
PROGRAMA DE PÓS-GRADUAÇÃO EM PATOLOGIA

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Avaliação da Expressão do Ki67 em Carcinomas Ductais Invasivos Mamários de Subtipos Luminal A e Luminal B (HER2-negativo) nas Áreas de Maior e Menor Proliferação Celular

Porto Alegre
2014

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Dissertação submetida ao Programa de Pós-Graduação em Patologia da Fundação Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Mestre

Orientador: Prof. Dr. Claudio Galleano Zettler

Porto Alegre
2014

Catalogação na Publicação

Luzzatto, Felipe
Avaliação da expressão do Ki67 em carcinomas ductais invasivos mamários de subtipos luminal A e luminal B (HER2-negativo) nas áreas de maior e menor proliferação celular / Felipe Luzzatto. -- 2014.
75 p. : il., graf., tab. ; 30 cm.

Dissertação (mestrado) -- Universidade Federal de Ciências da Saúde de Porto Alegre, Programa de Pós-Graduação em Patologia, 2014.

Orientador(a): Claudio Galleano Zettler.

1. Mama. 2. Carcinoma ductal invasivo. 3. Ki67. 4. Metástases axilares. I. Título.

Sistema de Geração de Ficha Catalográfica da UFCSPA com os dados fornecidos pelo(a) autor(a).

Agradecimentos

Ao meu orientador, Professor Dr. Claudio Galleano Zettler, pelo apoio e sugestões para a elaboração do projeto e desta dissertação.

À minha esposa Darliza Sansone Calliari, pela paciência, compreensão e estímulo nas horas difíceis, sem os quais não teria alcançado os meus objetivos na presente dissertação.

Aos meus pais Rui Luzzatto e Tania Luzzatto por todos os ensinamentos, que nortearam a minha vida pessoal, profissional e o meu modo de ser.

À minha irmã e colega patologista, Laura Luzzatto, pelo auxílio na coleta e análise de dados da dissertação.

Aos funcionários do Serviço de Patologia da Irmandade da Santa Casa de Misericórdia de Porto Alegre (ISCMPA) que auxiliaram na separação de blocos, lâminas e requisições dos arquivos do Laboratório de Patologia.

À secretária do Programa de Pós-Graduação em Patologia da Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Maristela Pasin, pelas dicas e orientações oportunas com relação às disciplinas e dissertação de Mestrado.

Sumário

Lista de Abreviaturas Utilizadas

AJCC: American Joint Committe on Cancer

CDI: carcinoma ductal invasivo mamário

CDIs: carcinomas ductais invasivos mamários

HE: hematoxilina-eosina

HER2/neu: Cerb-B2

INCA: Instituto Nacional do Câncer

Ki67: índice de proliferação celular

RE: receptores de estrógeno

RP: receptores de progesterona

SOE: sem outras especificações

TNM: tumor, lymph node, metastasis

UTDL: unidade terminal ducto-lobular

Resumo da Dissertação

Introdução

A classificação imuno-histoquímica do câncer de mama em subgrupos moleculares, pode substituir o perfil molecular a partir da análise dos anticorpos RE, RP, HER2 e do índice de proliferação celular (Ki67). Entretanto a diferenciação dos subtipos luminal A e luminal B (HER2-negativo) baseada no Ki67 possui limitações.

Objetivos

Comparar a expressão do Ki67 nas áreas de maior proliferação celular (“hot spots”) com as de menor proliferação em CDIs SOE, de subtipos luminal A e luminal B (HER2-negativo), verificando possíveis diferenças entre essas áreas que possam influenciar a classificação dos subgrupos moleculares destas neoplasias. Analisar a relação dos RE e RP nestas áreas e de outros fatores prognósticos (dimensão da neoplasia, grau histológico, invasão vascular) com as metástases axilares.

Material e Métodos

Analisadas retrospectivamente, lâminas de HE e de RE, RP, HER2 e Ki67 de 75 pacientes com CDIs SOE, unifocais, de subtipos luminal A e luminal B (HER2-negativo), avaliando-se o Ki67 nos “hot spots” e nas áreas de menor proliferação celular, comparando estas áreas entre si, e com RE, RP e demais

fatores prognósticos (dimensão, grau histológico da neoplasia, invasão vascular e metástases axilares).

Resultados

Observada uma diferença de 12 pontos percentuais a mais no Ki67 nas áreas de “hot spots” considerando-se o “cutoff” de 14%, o que levou a reclassificar 13 pacientes (17,3%) como subtipo luminal B (HER2-negativo) nestas áreas. A diferença entre os RE e RP nos “hot spots” e demais áreas não atingiu significância e a comparação com demais fatores prognósticos confirma a maior parte dos achados da literatura.

Conclusão

É fundamental a estandardização da análise do Ki67 visto que de acordo com nosso estudo 13 pacientes (17,3%) com CDIs seriam reclassificados como luminal B (HER2-negativo) e o tratamento adjuvante poderia sofrer alterações considerando-se a análise do mesmo em diferentes áreas da neoplasia.

Palavras-chave

Mama; carcinoma ductal invasivo; Ki67; hot spot; metástases axilares

1. Introdução

1.1. Epidemiologia do Carcinoma Mamário

O carcinoma de mama é a segunda neoplasia maligna mais comum no mundo, excetuando-se as neoplasias malignas cutâneas não melanocíticas, sendo, sem dúvidas, o tipo de carcinoma mais comum entre as mulheres, com uma estimativa de cerca de 1,67 milhões de novos casos diagnosticados no ano de 2012 (25% do total dos cânceres). É o tipo mais comum de câncer feminino, tanto nas regiões mais, assim como nas menos desenvolvidas e a quinta causa mais comum de morte dentre as neoplasias malignas em geral (522000 mortes), sendo a mais frequente dentre as mulheres em regiões menos desenvolvidas (324000 mortes, 14,3% do total) e a segunda causa de morte em regiões mais desenvolvidas (198000 mortes, 15,4% do total), após o câncer de pulmão (Ferlay e cols., 2012).

Em 2014, segundo dados do INCA estima-se que ocorram cerca de 57120 casos novos de câncer da mama, no Brasil, com um risco estimado de 56,09 casos a cada 100 mil mulheres. Sem considerar os tumores de pele não melanoma, esse tipo de câncer é o mais frequente nas mulheres das regiões Sudeste (71,18/ 100 mil), Sul (70,98/ 100 mil), Centro-Oeste (51,30/ 100 mil) e Nordeste (36,74/ 100 mil). Na região Norte, é o segundo tumor mais incidente (21,29/ 100 mil) (Instituto Nacional do Câncer, 2014).

A incidência de câncer de mama, assim como em todas as neoplasias epiteliais, aumenta rapidamente de acordo com a idade, elevando-se gradativamente até a menopausa e diminuindo após a mesma. A etiologia é multifatorial, relacionada, por exemplo, à dieta, fatores reprodutivos e

desequilíbrios hormonais e o prognóstico desta doença é melhor se detectado em estágios iniciais (Caplan, 2014; Aragón e cols., 2014).

1.2. Tipos de Carcinomas Mamários

Os carcinomas mamários são divididos em carcinomas “in situ” e carcinomas invasores.

Os carcinomas in situ são classificados em carcinomas dos tipos ductal e lobular “in situ”. O carcinoma ductal “in situ” é considerado uma proliferação de células epiteliais confinadas ao sistema ducto-lobular, caracterizadas por atipia discreta ou acentuada e uma tendência inerente, mas não obrigatória de progressão para o carcinoma invasivo. O carcinoma lobular “in situ” é uma proliferação celular originada na UTDL, geralmente caracterizada por células pequenas e descoesas, com ou sem envolvimento pagetoide de ductos mamários (Page e cols., 1985; Page e cols., 1991; Page e cols., 2003; Lakhani e cols., 2012).

A partir do momento em que ocorre a ruptura da membrana basal de carcinomas ductais e lobulares “in situ”, com invasão dos tecidos adjacentes são considerados carcinomas invasores.

Os carcinomas invasores da mama são um grupo de neoplasias epiteliais malignas caracterizadas pela invasão de tecidos adjacentes como já referido, e uma marcada tendência a metastatizar para sítios distantes. A maior parte destes carcinomas são adenocarcinomas. Acredita-se que sejam derivados do epitélio do parênquima mamário, particularmente da UTDL. São classicamente divididos em carcinomas invasivos dos tipos lobular e ductal e exibem uma enorme variedade de padrões fenotípicos e morfológicos, de

comportamentos clínicos e prognósticos diversos, de acordo com os padrões histopatológicos apresentados.

1.3. Fatores Prognósticos no Carcinoma Mamário

Atualmente muito esforço tem sido feito para a identificação de novos e precisos fatores prognósticos e preditivos no câncer de mama. Os fatores prognósticos contribuem para a avaliação do risco de recaídas de pacientes baseados em indicadores tais como a biologia intrínseca tumoral e o estadiamento da doença na ocasião do diagnóstico, sendo tradicionalmente utilizados para a escolha dos pacientes que poderão ser poupados da terapia adjuvante, considerando somente o risco de recaídas.

A presença ou ausência de metástases em linfonodos axilares, a dimensão tumoral, o grau histológico, o status de receptores hormonais, o tipo histológico do tumor, e o índice de proliferação celular da neoplasia obtido pela análise da proteína Ki-67, que é um marcador de proliferação celular, são aceitos como fatores prognósticos bem definidos. Além disso, a invasão vascular neoplásica peritumoral é outro importante fator prognóstico no câncer de mama visto que quando confirmada, histologicamente, está associada a um risco aumentado de doença metastática, ao aumento do risco de recidiva local e à redução da sobrevida (Rakha e Ellis, 2011; Duraker e Caynak, 2014; de Kruijf e cols., 2014; Munzone e cols., 2014).

É importante ressaltar, que diferentes tipos morfológicos de neoplasias mamárias podem ser divididos, em grupos de prognósticos diversos, como pode ser observado abaixo, de acordo com Elston e cols. (1999):

- Excelente prognóstico (acima de 80% dos pacientes com sobrevida de 10 anos): carcinoma tubular, carcinoma cribriforme invasivo, carcinoma mucinoso e carcinoma túbulo-lobular;
- Bom prognóstico (60-80% dos pacientes com sobrevida de 10 anos): carcinoma tubular misto, carcinoma lobular alveolar, carcinoma misto ductal SOE/tipo especial;
- Prognóstico reservado (50-60% dos pacientes com sobrevida de 10 anos): carcinoma medular, carcinoma papilar invasivo e carcinoma lobular clássico;
- Prognóstico ruim (menos de 50% com sobrevida de 10 anos): carcinoma lobular misto, carcinoma lobular sólido, carcinoma ductal SOE, carcinoma misto ductal SOE/lobular e carcinoma inflamatório (Fisher e cols., 1977; Ridolfi e cols., 1977; Haagensen e cols., 1978; Lucas e Perez-Mesa, 1978; Page e cols., 1983; Carstens e cols., 1985; Clayton, 1986; Weigelt e cols., 2008; Dieci e cols., 2014).

O grau histológico da neoplasia invasora é outro importante fator prognóstico para o tratamento do carcinoma mamário. Vários estudos têm demonstrado uma associação significativa entre o grau histológico da neoplasia invasora e a sobrevida relacionada ao mesmo (Henson e cols., 1991; Elston e Ellis, 1998; Thomas e cols., 2009; Daveau e cols., 2014). É obtido a partir de determinadas características morfológicas da neoplasia, tais como a formação tubular, pleomorfismo nuclear e índice mitótico, definidos segundo parâmetros observados na classificação de Elston e Ellis (vide Tabela 1) (Elston e Ellis, 1991).

Tabela 1. Método semi-quantitativo para a avaliação do grau histológico nas neoplasias mamárias (Elston e Ellis).

Característica	Escore
Formação de Glândulas e Túbulos	
Formação tubular predominante (> 75%)	1
Moderada formação tubular (10-75%)	2
Pouca ou nenhuma formação tubular (<10%)	3
Pleomorfismo Nuclear	
Células pequenas e uniformes	1
Moderado aumento no tamanho e variação nuclear	2
Marcada variação nuclear	3
Índice Mitótico	
Dependente da Área do Microscópio	1 – 3
Grau 1 (bem diferenciado): 3 a 5 pontos Grau 2 (moderadamente diferenciado): 6-7 pontos Grau 3 (pouco diferenciado): 8-9 pontos	

O câncer de mama é uma doença heterogênea com múltiplas alterações moleculares, responsáveis pelo crescimento, a sobrevivência e a resposta ao tratamento. Além do grau histológico e dos demais fatores prognósticos brevemente relatados o tratamento é baseado no padrão de expressão imuno-histoquímica dos receptores de estrógeno (RE) e progesterona (RP), na

presença ou ausência de superexpressão da molécula HER2 (também designada c-*erbB2* e *c-neu*) e do índice de proliferação celular (Ki67) (Barnes e cols., 1996; Fisher e cols., 2005; Zaha, 2014).

Nas últimas duas décadas, os avanços no manejo do câncer de mama têm sido influenciados pela crescente introdução de terapias alvo como resultado da identificação destas novas moléculas. O critério convencional para seleção de um agente antineoplásico tem sido a habilidade da droga de induzir regressão tumoral. A terapia endócrina é o primeiro exemplo de uma abordagem alvo-dirigida, já em uso rotineiro há mais de quatro décadas (Anderson e cols., 2002).

O tamoxifeno, que é um antagonista do RE, tem sido o padrão ouro no tratamento do câncer de mama com expressão de receptores hormonais (Clarke, 2008; Gnant e cols., 2011). Nos últimos anos, os inibidores da aromatase, que inibem a síntese de estrógenos nos tecidos periféricos, incluindo a mama, têm-se mostrado superiores ao tamoxifeno em mulheres na pós-menopausa, com câncer de mama inicial ou avançado (Winer e cols., 2005; Schiavon e Smith, 2014).

A superexpressão do HER2 está presente em 18 a 20% dos pacientes com câncer de mama e está associada a um perfil de doença mais agressivo com pior sobrevida, e resistência à terapia hormonal (Wolff e cols., 2007). O trastuzumab, um anticorpo monoclonal humanizado, tem como alvo o domínio extracelular do HER2, melhorando a sobrevida destas pacientes tanto em estádios iniciais assim como em doença avançada.

Além dos receptores hormonais e o status HER2, outros mecanismos de avaliação prognóstica têm sido observados que são o escore, também

denominado assinatura, ou classificação multigênica. Embora estes fatores tenham valor prognóstico intrínseco relacionado ao risco de recorrência para pacientes que não recebem terapia sistêmica, a sua maior utilidade é indicar ou não a utilização de terapia endócrina (antiestrogênica) ou anti-HER2 (tal como o trastuzumab) (Lonning, 2007; Liu e cols., 2012; Jackson e Chester, 2014). A utilização destes fatores como marcadores preditivos, e não como prognósticos é de fundamental importância na avaliação e cuidado de pacientes diagnosticados com tumor de mama, mas o AJCC achou difícil a inclusão dos mesmos em um esquema do sistema de estadiamento do TNM (Tumor, Lymph node, Metastases) (AJCC, 2010).

1.4. O Índice de Proliferação Celular (Ki67)

A taxa de proliferação celular tumoral há muito tempo tem sido relacionada com o curso clínico das doenças. A partir disso, histopatologistas tem buscado meios para a determinação da mesma, como complemento diagnóstico.

O Ki67 é uma proteína nuclear que, nos humanos, é codificada pelo gene *MKI67* (antígeno identificado pelo anticorpo monoclonal Ki67) e está estritamente relacionado com a proliferação celular. Durante a interfase, o antígeno Ki67 pode ser detectado exclusivamente no núcleo celular, enquanto que durante a divisão celular (mitoses) a maior parte desta proteína é realocada na superfície cromossômica. Está presente durante todas as fases ativas do ciclo celular (G_1, S, G_2, e mitoses), mas ausente em células em repouso (G_0) (Scholzen e Gerdes, 2000; de Azambuja e cols., 2007).

O método mais simples e mais utilizado para a determinação da atividade proliferativa celular é a contagem de figuras mitóticas, entretanto, a quantificação do índice de proliferação celular (Ki67) pelo estudo imuno-histoquímico é um outro método confiável, e de fácil avaliação, do crescimento das neoplasias (Yerushalmi e cols., 2010; Sheri e cols., 2012). O índice de proliferação celular (Ki67) é definido a partir do percentual de núcleos celulares de carcinoma invasor positivos para a reação imuno-histoquímica para este anticorpo, em relação ao total de núcleos de células de carcinoma invasor presentes no corte histológico (Urruticoechea e cols., 2005).

Uma metanálise realizada por de Azambuja e cols. (2007), sustenta que a alta expressão de Ki67 confere um pior prognóstico a pacientes com carcinomas mamários precoces, demonstrando uma associação significativa entre a sua superexpressão e o risco de recidiva, morte, sobrevida livre de doença e a sobrevida em geral. Além disso é também considerado como um possível método de avaliação prognóstica para os efeitos da quimioterapia, embora alguns estudos evidenciem resultados conflitantes (Viale e cols., 2008; Colleoni e cols., 2010; Jones e cols., 2010).

Entretanto o Ki67 ainda não é completamente aceito como um biomarcador de rotina visto que ainda não existem estandardizações a respeito do métodos de sua avaliação (Harris e cols., 2007; Polley e cols., 2013). Atualmente, existem questionamentos de como deve ser realizada a interpretação mais adequada do Ki67, se nas áreas de menor ou de maior atividade proliferativa das células neoplásicas, estas últimas áreas, denominadas “hot spots“ que, à interpretação imuno-histoquímica, são identificadas como locais em que a coloração nuclear das células neoplásicas

pelo Ki67 é particularmente predominante, se comparada ao restante dos cortes imuno-histoquímicos da neoplasia.

Em um estudo realizado por Mikami e cols. (2013) foi avaliada a concordância interobservadores na análise do Ki67, sendo demonstrado que esta era significativamente maior com o sistema de contagem celular, no qual cerca de 1000 células eram quantificadas nas áreas de maior proliferação celular (“hot spots“) e o percentual de células positivas era calculado. No segundo método de avaliação, denominado método visual, as áreas de maior densidade celular eram determinadas e após era realizada uma estimativa visual utilizando um escore de 1 (0–9%) a 10 (90–100%).

O número de células ideal a ser contado para a determinação do Ki67 ainda não é bem estabelecido. Na maior parte dos estudos de 1000 à 2000 células tumorais foram contadas (Colleoni e cols., 2004; Jones e cols., 2010; DeCensi e cols., 2011). Entretanto estudos avaliando a associação entre o número de células e a sua reprodutibilidade ainda não foram publicados, sendo necessárias pesquisas adicionais para a determinação da quantidade ideal de células a ser utilizada para a determinação do índice de proliferação celular. O Grupo Internacional de Pesquisa em Câncer de Mama recomenda que pelo menos 1000 células sejam contadas, mas aceita a contagem de 500 células como número mínimo. Além disso também recomenda que os pontos de maior proliferação celular (“hot spots“) sejam inclusos na contagem final mesmo que um escore médio já tenha sido obtido. Dessa maneira a seleção das áreas de “hot spots“ torna-se fundamental (Dowsett e cols., 2011).

1.5. A importância do Ki67 na Classificação Imuno-Histoquímica dos Subtipos Moleculares de Carcinoma Mamário

Na atualidade, como já referido, o perfil genético vem demonstrando um importante papel na caracterização do comportamento biológico das neoplasias mamárias. Alguns estudos tem demonstrado que a análise imuno-histoquímica do Ki67, RE, RP e HER2 pode ter um valor prognóstico muito similar ao escore prognóstico multigênico em pacientes recebendo terapia anti-hormonal e que a estratificação de pacientes de acordo com os índices do Ki67 pode ajudar a antever a significância da resposta terapêutica em pacientes com tumores mamários hormônio-responsíveis. Além disso, alguns pacientes, com tumores de alto índice de proliferação celular e de subtipos menos químio-sensíveis, podem ter um benefício significativo com a qumioterapia (Cuzick e cols., 2009; Sueta e cols., 2014).

Segundo Cheang e cols. (2009) a subdivisão das neoplasias, nestes casos, em subgrupos moleculares dos tipos clínico-patológicos luminal A, luminal B, HER2, basal e triplo-negativo não basal, pode ser definida fundamentalmente pelo perfil imuno-histoquímico para receptores hormonais (RE, RP) e para HER2 e pelo índice de proliferação celular (Ki67) baseado em um “cutoff” de 14% para a diferenciação das neoplasias em luminal A e luminal B segundo a experiência de um único laboratório de referência. Este estudo foi o primeiro a aplicar escores imuno-histoquímicos visuais quantitativos do Ki-67 em subtipos de câncer de mama que foram classificados pelo perfil de expressão genética. Uma vantagem deste método é que o “cutoff” do Ki-67 de 14%, definido através do estudo imuno-histoquímico, foi determinado em relação a uma distinção importante na biologia básica do câncer de mama, e não em relação ao resultado clínico ou ao valor da média ou mediana do índice

do Ki-67 na população em estudo. Através deste método, o “cutoff” será mais provável de ser diretamente aplicável em outras coortes de pacientes com diferentes regimes de tratamentos e distribuições de riscos. Embora, o perfil de expressão genética ainda seja o método mais sensível, Cheang e cols. (2009) demonstraram que o Ki-67 pode ser utilizado associado ao painel de biomarcadores (RE, RP e HER2), como já descrito, para identificar tumores luminais B adicionais que não foram classificados por esses três marcadores.

A partir desta análise poderá ser elaborado o tratamento das pacientes, que pode ou não ser associado à quimioterapia dependendo das características moleculares e/ou imuno-histoquímicas das neoplasias mamárias (Goldhirsch e cols., 2011; Goldhirsch e cols., 2013).

Observa-se assim, que com o advento dos testes genéticos foi enfatizada a importância dos genes responsáveis pela proliferação celular, dentre eles, o Ki67, como marcador prognóstico e preditivo das neoplasias mamárias.

Dessa maneira, torna-se fundamental uma padronização para a definição precisa do Ki67, visto que a inadequada caracterização do mesmo pode determinar tratamentos clínicos diversos para os pacientes com carcinomas mamários, e a sua quantificação determina importantes informações prognósticas, além das variáveis clínico-patológicas utilizadas tradicionalmente (Dowsett e cols., 2011; Pathmanathan e cols., 2014).

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2. Objetivos

2.1. Objetivo Principal

- Estabelecer uma comparação da imunoexpressão do Ki67 nas áreas de maior índice de proliferação celular (“hot spots”) com a imunoexpressão do Ki67 nas áreas de menor índice de proliferação celular em CDIs NOS, de subtipos luminal A e luminal B (HER2-negativo), verificando se existem diferenças entre estas áreas que possam influenciar a classificação destes subtipos, e, consequentemente, o tratamento.

2.2. Objetivo Secundário

- Estabelecer uma comparação em CDIs NOS, de subtipos luminal A e luminal B (HER2-negativo) da imunoexpressão dos RE e RP nas áreas de menor e maior índice de proliferação celular do Ki67 (“hot spots”), assim como de outros fatores prognósticos (dimensão da neoplasia, grau histológico, invasão vascular peritumoral) com as metástases axilares.

3. Artigo Científico Redigido em Inglês

Analysis of the Ki67 Expression in the Areas of High and Low Proliferation Index in Patient´s with Invasive Ductal Carcinomas NOS, of Luminal A and Luminal B (HER2-negative) Subtypes.

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Keywords: breast; invasive ductal carcinoma; Ki67; hot spot; axillary metastasis.

Abstract

Aims: The molecular biology of breast cancer is very important for patient's treatment but it is not accessible to all population because of its high cost. In its absence, classifications based on immunohistochemistry have been recommended, combining Ki67, Estrogen Receptor (ER), Progesterone Receptor (PR) and HER2/neu. The subdivision of breast cancer in luminal A and luminal B (HER2-negative) based on the proliferative index (Ki67) is subjective and could influence the patient's treatment. The aim of this study is to try to show the failures of the present methodology of Ki67 interpretation.

Methods: The slides of 75 patients were analyzed retrospectively comparing the Ki67 in the higher proliferative areas (hot spots) of Invasive Ductal Carcinomas Not Otherwise Specified (IDC NOS) with the lower proliferative areas. The ER and PR were evaluated in these areas to see if there was a correlation of its expression with the proliferative index. All of these variables as well as the tumor size, tumor grade and vascular invasion were compared with the axillary lymph node metastasis.

Results: There was a significant difference of 12 percentage points more, regarding the Ki67 in the hot spots when considered the cutoff of 14%. According to this data 13 patients (17,3%) with IDC NOS would be reclassified as luminal B (HER2-negative) in the hot spots. The difference between ER and

PR in the higher and lower proliferative areas didn't reach significance. The other prognostic factors in general were consistent with the previous reports in literature.

Conclusions

According to our data 13 patients (17.3%) with IDC NOS would be reclassified as luminal B (HER2-negative) and could have a different adjuvant treatment. It's important to emphasize the need of a standardization of its analysis, in conjunction with other parameters to choose the best patient's treatment.

Introduction

Breast cancer is the second most frequent cancer in the world, excluding non-melanoma skin cancer, and, by far, the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers), being the most common cancer in women both in more and less developed regions.[1] It is considered a heterogeneous disease characterized by striking genetic, phenotypic and clinical differences and the best decision of its treatment depends on a panel of clinical and pathological prognostic and predictive factors.[2]

In the last 10 years the treatment of breast cancer has changed substantially, regarding new information in the molecular biology of this neoplasm. In 2000, Perou and colleagues published the first paper classifying breast cancer into intrinsic subtypes based on gene expression profile.[3]

Since then, several articles have been published about this subject and the benefits of combining biological tumor markers into surrogate molecular subtypes to add prognostic information which may be important for recommendation of systemic therapy.[2,4-7]

With the advent of screening, small estrogen receptor-positive tumors are being diagnosed with a generally good prognosis thanks to the widespread application of hormone therapies. However, current markers do not allow accurate prediction of the probability of recurrence, and improvements are necessary to clearly identify the women that could be classified as low risk to safely avoid the use of chemotherapy and its unwanted adverse effects. Hence, the objective of identifying molecular signatures to select the patients who could

be spared from chemotherapy has been one of the aims in the treatment of the breast cancer.[8,9]

Intrinsic subtypes of breast carcinoma have been broadly accepted in the classification of this neoplasm. In the absence of genetic expression data of the breast carcinoma, classifications based in immunohistochemistry have been recommended. Combining the immunohistochemical markers Ki67, Estrogen Receptor (ER), Progesterone Receptor (PR) and HER2/neu, generally used at the routine for breast carcinoma, has shown useful prognostic and predictive information in addition to the classical clinical factors and could have a very similar prognosis to the multigenic prognostic score as defined by some studies.[10]

According to the St. Gallen Conference in 2013, the breast cancer should be divided into five clinic-pathologic surrogate subtypes called luminal A, luminal B (HER2-negative), luminal B (HER2-positive), HER2 and Triple-negative (Basal).[11] The luminal A tumors expresses ER and PR and are negative for HER2, with a low Ki-67, and the luminal B tumors comprises those luminal cases, which lack the characteristics noted for luminal A disease. Thus, either a high Ki-67 value or a low PR expression may be used to distinguish between luminal A and luminal B (HER2-negative). The importance of making this difference is that the luminal A tumors should receive only endocrine therapy and the tumors classified as Luminal B (HER2-negative) should receive endocrine therapy, associated with cytotoxic therapy for most part of patients.[4,10,11]

The cut-point between the high and low values for Ki-67 varies between laboratories and a level of <14% is best correlated with the gene-expression definition of luminal A, based on the results in a single reference laboratory.[4]

However there are uncertainties of how the Ki67 rate should be determined because there is a high degree of inter-laboratory variation in the Ki67 measurement and, consequently, the possibility of undertreatment of patients with luminal disease who might benefit from chemotherapy.[12]

In the present study we analyze in a transversal retrospective study the Ki67 expression comparing it in the whole immunohistochemical sections of invasive ductal carcinoma (WSIDC) with the immunoexpression of Ki67 in the hot spots of invasive ductal carcinoma (HSIDC), trying to determinate if there is a difference in the immunohistochemical classification of the subtypes luminal A and luminal B (HER2-negative) between these two sites, according to the recommendations of the 13th St. Gallen International Expert Consensus on Breast Cancer.[11] The expression of ER and PR in the HSIDC and in the WSIDC as well as the tumor size, the histological grade of the neoplasm and the peritumoral vascular invasion are also compared with the axillary lymph node metastasis (ALNM), another important prognostic factor.[13]

Materials and methods:

Patient Selection

Seventy-five consecutive patients with Invasive Ductal Carcinoma (IDC) treated with breast conserving surgery or radical modified mastectomy were selected retrospectively between the period of January 2011 and January 2014, from the medical files of the Santa Casa de Misericórdia de Porto Alegre. All of the patients included in the research had a unilateral and unifocal tumor, without any history of a previous carcinoma, in situ or invasive, in the same or contralateral breast. Tumor type has been shown to be a prognostic factor in breast cancer.[14] In that way we selected only cases of Invasive Ductal Carcinoma Not Otherwise Specified (IDC NOS), to exclude possible bias related to ALNM.

Patients with multifocal or multicentric tumours, with neoadjuvant treatment, with lobular carcinoma or special types of breast carcinoma (e.g. tubular carcinoma, mucinous carcinoma, medullar carcinoma), were excluded from the research to avoid any bias related do this different variables. All the patients were submitted to the sentinel lymph node (SLN) biopsy and/or to the axillary dissection, depending on the biologic characteristics of the primary tumor, the positivity of the SLN, or the clinical positivity of the axilla.

Only patients with IDC with positivity for ER and/or PR, classified as luminal A and luminal B and with negativity for HER2/neu according to the immunohistochemistry were chosen to be part of the research. Patients with indeterminate score (2+) for HER2/neu were submitted to the Silver-enhanced *in situ* Hybridization (SISH), and were only included in the research when were proved to be negative as well as for the SISH.

The histopathological information used in the analysis was documented based in the data presented in the original pathology reports and in the review of the slides of hematoxilin-eosin and of the routine imunohistochemistry, selected from the archives of the Pathology Laboratory of the Santa Casa de Misericórdia de Porto Alegre. The clinical information was obtained from the original hospital records.

The research was submitted and approved by the Ethics Committee.

Tumor Analysis

Two experienced investigators in breast pathology reviewed together all of the slides of hematoxilin-eosin (HE) (Figures 1A and 1B) and of the routine imunohistochemistry panel for breast carcinoma composed of Ki67, ER, PR, and HER-2/neu. The tumor size of the IDC was determined on the gross section analysis for large neoplasms and on the microscopic measurement for small neoplasms. The tumor grade was settled according to the Elston & Ellis classification.[15]

The peritumoral vascular invasion was reviewed in the HE slides and was considered as focal when the vascular invasion was detected in only 1 High Power Field (HPF, x 40 objective), and extensive if present in more than 1 HPF.

Sentinel and Axillary Lymph nodes (ALN)

The SN were examined during transoperatory lymph node dissection and submitted to a routine protocol of three semiseriated histological sections,

stained with hematoxilin-eosin, with an interval of 50 micrometers between the sections.

The ALN were analyzed in seriated histological sections stained with HE.

The axilla was considered positive when it was detected at least one micrometastatic deposit (greater than 0.2 mm and/or more than 200 cells, but none greater than 2.0 mm) or macrometastatic disease (greater than 2.0 mm) at the SLNs or at the other ALNs.[16]

Immunohistochemistry

The specimens were fixed according to the routine in 10% neutral phosphate buffered formalin. The cases included in the research were evaluated considering the positivity of the results of the routine immunohistochemistry based in internal and external controls.

The prepared tissue sections (2 µm) of the neoplasm were placed in an incubator during 60 minutes at 60ºC before being allocated at the Automated Benchmark XT from Ventana. Sections were stained according to the specific machine's protocols using the anti-Ki67 Rabbit Monoclonal Primary Antibody (30-9; ~2µg/mL; Ventana Medical Systems, Inc., USA), anti-ER Rabbit Monoclonal Primary Antibody (SP1; ~1µg/mL; Ventana Medical Systems, Inc., USA), anti-PR Rabbit Monoclonal Primary Antibody (1E2; ~1µg/mL; Ventana Medical Systems, Inc., USA) and anti-HER-2/neu Rabbit Monoclonal Primary Antibody (4B5; ~6µg/mL; Ventana Medical Systems, Inc., USA).

The tumors were considered to be positive for ER and PR if 1% or more of the cells showed positive staining. The HER2 status was given in the pathology reports as negative (0), 1+, 2+, or 3+ in accordance with the

guidelines published by Sauter et al.[17] Tumors with a score of 0 or 1+ were regarded as HER2-negative and those with a score of 3+ were considered HER2-positive. Tumors with scores of 3+ were excluded from the research. Tumors with a 2+ staining were tested for gene copy numbers of HER2 by SISH. If the results showed an amplification of the HER2, the patients were excluded from the research.

Ki67, ER and PR Scoring

The Ki67 index was determined using a cellular counting approach. Scoring was performed by two of the authors together, blinded to patient's information according to the recommendations from the International Ki67 in Breast Cancer Working Group.[18] The entire slide was scanned for immunostaining evaluation using light microscope at Low Power Field (LPF, x10 objective). The Ki67 index was determined as a percentage of cells with immunoexpression for the antibody (Figure 1C). All tumor cell nuclei with homogenous granular staining were considered to be positive, regardless of intensity, while any cytoplasmic immunoreactivity was considered non-specific and ignored.

The places in which the invasive neoplasm had a higher proliferative rate denominated as hot spots (Figure 1D), and characterized as the invasive neoplastic areas in which the nuclear positive cells predominated when compared to the other sections of the neoplasm, were chosen by the authors, individualized with a marker pen, analyzed and quantified. The Ki67 at the HSIDC was considered as the percentage of positive malignant cells among a number of at least 500 malignant cells, at HPF. Then this result was compared

with the analysis of the Ki67 at the WSIDC according to the recommendations from the International Ki67 in Breast Cancer Working Group.[18] In the WSIDC at least 1000 malignant cells in 4 random HPF where selected to represent the spectrum of staining seen on initial overview of the whole section. The data of the hot spots previously determined were included in the overall score. After this analysis the immunohistochemical panel (ER and PR) was settled in the correspondent marked areas of the HSIDC and the percentage of positive neoplastic cells were compared with the WSIDC, according to the same protocol used to analyze the Ki67.

Statistical Analysis

Statistical analysis was performed using SPSS software version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

The Wilcoxon's nonparametric test was used to reveal any differences between the expression of ER, PR, and Ki67 in the WSIDC and in the HSIDC as paired samples. On the other hand, the Mann-Whitney's nonparametric test was used to compare the expression of ER, PR and Ki67 in the WSIDC and in the HSIDC for independent samples according to tumor size and the presence of ALNM, with a P-value < 0.05 to denote statistical significance.

For the comparison of age and ALNM it was used the Mann-Whitney's test. For the peritumoral vascular invasion, the tumor grade, according to Elston and Ellis,[14] and for the relation of this variables with the ALNM it was used the Fisher's exact test.

Results

Comparison between Ki67 expression in the HSIDC and in WSIDC

The Ki67 showed a significant difference (P<0.001) in the HSIDC when compared with the WSIDC, presenting an expression of 12 percentage points more in the hot spots (Figure 2A).

According to the expression of Ki67, and considering the cutoff of <14% to classify the IDC as luminal A and of >14% as luminal B,[4] it was obtained the immunohistochemical division of the neoplasms according to Table 1. This table demonstrates the occurrence of 13 patients more classified as Luminal B in the HSIDC, when compared to the immunoexpression of Ki67 in the WSIDC.

Table 1: Immunohistochemical Subtype of IDC according to the Ki67 cutoff of 14% in the WSIDC and in the HSIDC

Immunohistochemical Subtype of Invasive Ductal Carcinoma	WSIDC (n / %)	HSIDC (n / %)
Luminal A	20 (26.7%)	7 (9.3%)
Luminal B (HER2 negative)	55 (73.3%)	68 (90.7%)
Total	75 (100%)	75 (100%)

WSIDC= Whole Section of Invasive Ductal Carcinoma; HSIDC= Hot Spot of Invasive Ductal Carcinoma

Comparison between Ki67 expression in the HSIDC and in the WSIDC with ER and PR

The Table 2 lists the relation of expression of Ki67 in the WSIDC and in the HSIDC with ER and PR (Figures 2B and 2C, respectively). The Wilcoxon signer ranked test found ER to have no significant difference in the WSIDC and in the hot spots. When analyzing PR it was observed a significant difference of 10 percentage points less in HSIDC when compared with the WSIDC (Figure 2C).

Table 2. Comparison between the expression of Ki67, ER and PR in the WSIDC and in the HSIDC

Variable	WSIDC	HSIDC	P-value
Ki-67	17 (1 - 48)	29 (3 - 75)	< 0.001
ER	95 (15 - 95)	95 (10 - 95)	0.11
PR	70 (0 - 95)	60 (0 - 95)	0.002

Data presented as medians (minimum and maximum) / n= 75 (WSIDC= Whole Section of Invasive Ductal Carcinoma; HSIDC= Hot Spot of Invasive Ductal Carcinoma; Estrogen Receptor= ER; Progesterone Receptor= PR).

Comparison between Ki67, ER and PR expressions and other Prognostic Variables with the ALNM

The relationship between Ki67, ER and PR in the WSIDC with the ALNM and the same relation in the HSIDC are presented in Table 3. We didn't find significant difference between the expression of ER, PR and Ki67 in the WSIDC as well as ER and PR in the HSIDC when compared with the ALNM. The Ki67 showed limitrophe significance in the hot spot, when compared to the ALNM.

We also have seen, after analyzing the data that could be a relation of patients with higher Ki67 index in the hot spot with the ALNM. Considering this, we went further to explore this association and found 21% of patients with ALNM compared with only 11% of patients without ALNM when the Ki67 labeling index was >40% in the hot spot (Figure 3). However this finding didn’t reach statistical significance (P=0.30) at Fisher‘s exact test.

The mean tumor size was 2.7 cm (range 0.2 - 9.5). In non-metastatic tumors (n= 46) the median size was 1.7 cm (range 0.2 - 5.5) when compared with metastatic tumors (n= 29), which presented a median size of 2.5 cm (range 0.7 - 9.5) demonstrating significance (P = 0.001) according to Mann-Whitney’s test.

The mean patient’s age was 59.5 years (range 33 - 84). There was no significant difference (P= 0.47) in the patient’s age with ALNM (median 61, range 33 - 79) and without ALNM (median 60, range 43 - 84), according to Mann-Whitney’s test.

The Fisher’s exact test demonstrated no significant difference (P= 0.22) between the histopathological tumor grade and the ALNM.

Among those patients with extensive peritumoral vascular invasion 67%

had axillary metastasis compared with 33% without metastatic axillary disease, demonstrating significance (P=0.002) between the extensive peritumoral vascular invasion and the ALNM.

Table 3. Relation between the expression of ER, PR, and Ki67 in the WSIDC and in the HSIDC with the ALNM

Variable	Without Metastasis n = 46	With Metastasis n = 29	P-value
Ki-67 in the Whole Section of the Invasive Ductal Carcinoma	17 (1 - 40)	18 (5 - 48)	0.13
Estrogen Receptor in the Whole Section of Invasive Ductal Carcinoma	95 (15 - 95)	95 (50 - 95)	0.69
Progesterone Receptor in the Whole Section of Invasive Ductal Carcinoma	75 (0 - 95)	60 (0 - 95)	0.17
Ki-67 in the Hot Spot of Invasive Ductal Carcinoma	25 (3 - 60)	30 (10 - 75)	0.080
Estrogen Receptor in the Hot Spot of Invasive Ductal Carcinoma	95 (10 - 95)	95 (50 - 95)	0.74
Progesterone Receptor in the Hot Spot of Invasive Ductal Carcinoma	70 (0 - 95)	50 (0 - 95)	0.17
Data presented as medians (minimum and maximum) / n = 75			

Discussion

The breast cancer is a heterogeneous disease characterized by different prognostic factors. As discussed before in the absence of genetic expression data of the breast carcinoma, classifications based in immunohistochemistry are recommended combining the immunohistochemical markers Ki67, ER, PR and HER2.[19-21] The distinction, particularly, of the subtypes luminal A from the luminal B is very important because of the diverse prognosis related to each of these neoplasms and to avoid unnecessary treatment with chemotherapy.[4,10,11,22].

The luminal A subtype has a more favorable prognosis compared to the luminal B and the systemic therapy advocated for the patients with luminal A tumors is generally restricted to endocrine therapy, differently from the luminal B tumors, with a worse prognosis, who may benefit from adjuvant chemotherapy combined with endocrine treatment.[23-25]

When considering the differentiation between this two tumor subtypes the cutoff of <14% is best correlated with the gene-expression definition of luminal A, according to a single reference laboratory.[4] However the lack of standardization in the analysis of Ki67 between the different pathology laboratories in the world could lead to undertreatment or overtreatment to the patients considering this cutoff.

The Ki67 labelling index is reported in a number of studies to demonstrate prognostic value for breast cancer patients.[26,27] However, it has not been accepted as a routine biomarker, mainly because there is no standardization of the assay system.[28]

In a study by Mikami et al, it was evaluated the interobserver concordance

of the Ki67, demonstrating that the concordance was significantly higher with the counting system than with the scoring system (visual estimate), indicating that counting cells is useful for reproducible assessment and that an identification of the fields for the assessment is pivotal for the standardized assessment of Ki67 in breast cancer.[29]

In our study it was demonstrated a significant difference of 12 percentage points more, regarding the Ki67, in the HSIDC, when compared with the WSIDC, even when adopted the recommendations from the International Breast Cancer Working Group of including the hot spots data in the overall score to define the Ki67 in the WSIDC.[18] It was demonstrated in our research that in the HSIDC there was a difference of 13 patients more (17.3%) classified as luminal B when compared to the WSIDC. According to these results the patient's treatment based on a cutoff of 14% should be different depending of the analysis of the Ki67 in HSIDC or in the WSIDC.

When analyzing the Ki67 in the WSIDC and in the HSIDC and the ER and PR in these sites, we didn't find significant difference between the expressions of these receptors with the occurrence of ALNM. However when trying to find a relation between the Ki67 alone in the hot spots and the ALNM it was seen that with higher Ki67 rates (>40%) there was a difference of 10 percentage points more in the hot spot of patients with ALNM when compared to patients without metastasis. Despite this finding didn't reach statistical significance, it should be better investigated in larger series and other researches.

Usually the higher is the proliferative index the lower is the expression of ER and PR.[30-32] Trying to define the correlation between the Ki67 and the expressions of ER and PR in the areas of hot spots and in WSIDC, we did not

find significant statistical difference regarding the ER but, when considering the PR we have noted a difference of 10 percentage points less in the HSIDC when compared with the WSIDC.

The presence of different clones in a neoplasm has been demonstrated in some studies.[33,34] These findings could be related to different cell populations or different clones of the neoplasm, presented in the hot spots when compared with the WSIDC. We suggest a genetical analysis of these two different sites to prove this hypothesis.

Among the other prognostic factors studied in the research that can influence the therapy we analyzed the histopathological tumor grade, the tumor size and the peritumoral vascular invasion.

The histopathological tumor grade as well as the patient's age are considered important predictors of prognosis and ALNM and this probability is higher if a patient has a high histopathological grade and a younger age according to some studies.[35-37] According to our research the tumor grade as well as the patient's age didn't reach significance when compared to the ALNM. This fact could be related to the similar clinical characteristics of the luminal A and luminal B (HER2 negative) tumors, both, usually expressing ER and PR but differentiated only by the Ki67 according to the immunohistochemistry.

And finally, the presence of peritumoral vascular invasion and the large tumor size according to our study are related to the ALNM, a finding that is consistent with those of previous studies.[38-43]

Conclusions

In summary, some of the findings presented in our research confirm the results previously observed in the literature like the relation of peritumoral vascular invasion and the tumor size with the ALNM. According to our data 13 patients (17.3%) with IDC NOS would be reclassified as luminal B (HER2-negative) and could have a different adjuvant treatment, considering the analysis of the Ki67 in the hot spots, with a cutoff of 14%. These facts should place a question whether the use of cutoffs for the analysis of Ki67 is the most adequate manner to differentiate the luminal A and luminal B subtypes of IDC according to the immunohistochemistry, because these results could influence the patient's treatment.

Although there is no universal agreement on the evaluation parameters of the Ki67, it's important to emphasize the need of a standardization of its analysis, and the participation of laboratories in quality assurance programmes. Meanwhile each individual institution worldwide should have its own treatment guidelines according to the experience based on their pathology laboratory reports and on their own patient's clinical evolution to determinate the best clinical and therapeutical approach for breast cancer.

Acknowledgements The authors would like to thank all the staff that works at the Archives of the Pathology Department of the Santa Casa de Misericórdia de Porto Alegre that helped in the separation of slides and pathology reports.

Contributors FL, LL and CGZ are the only contributors for this paper. FL did the literature search, the data collection and wrote the paper. LL helped with de data collection. CGZ reviewed and edited the paper, and made changes to it.

Competing interests The authors declares not to have competing interests.

Provenance and peer review Not commissioned; internally peer reviewed.

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Figures:

Figure 1:

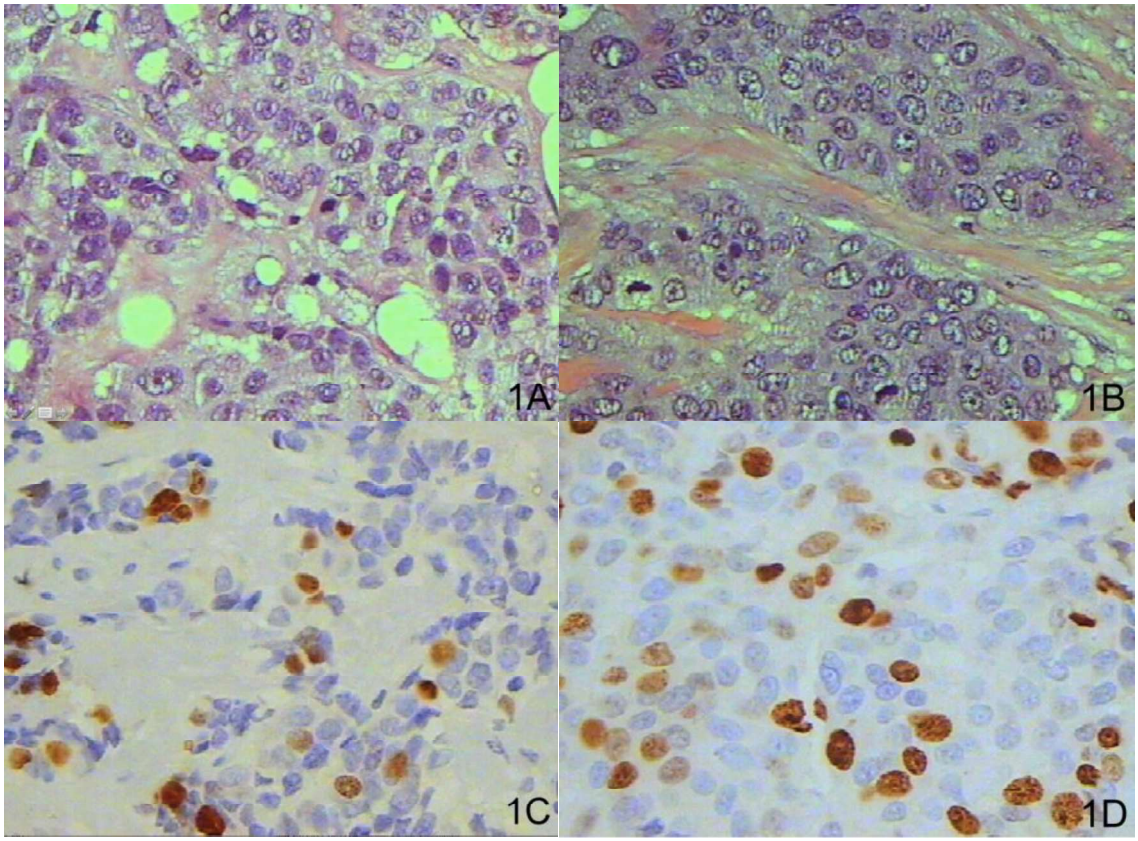

Figure 1 (A,B) High-power fields of hematoxilin-eosin histological sections of an invasive ductal carcinoma; (C) High-power field of the Ki67 in an area of the whole sections of an invasive ductal carcinoma; (D) High-power field of the Ki67 in an area of the hot spot of an invasive ductal carcinoma.

Figure 2:

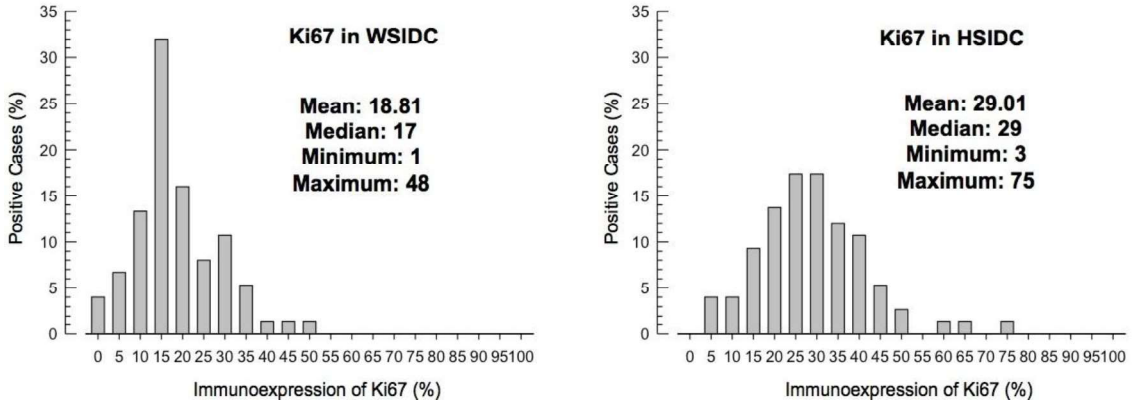

Figure 2A. Comparison between the Ki67 in the WSIDC with Ki67 in the HSIDC; n = 75; P < 0.001.

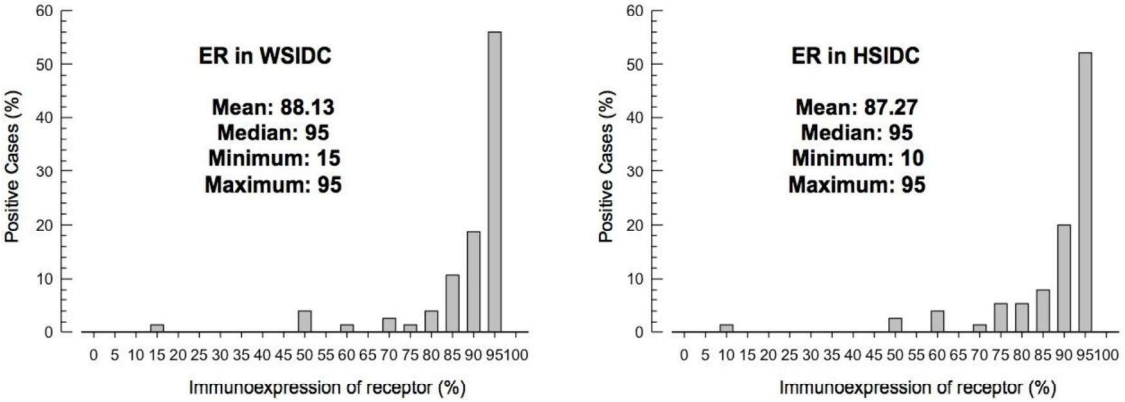

Figure 2B. Comparison between the ER in the WSIDC with ER in the HSIDC; n = 75; P = 0.11.

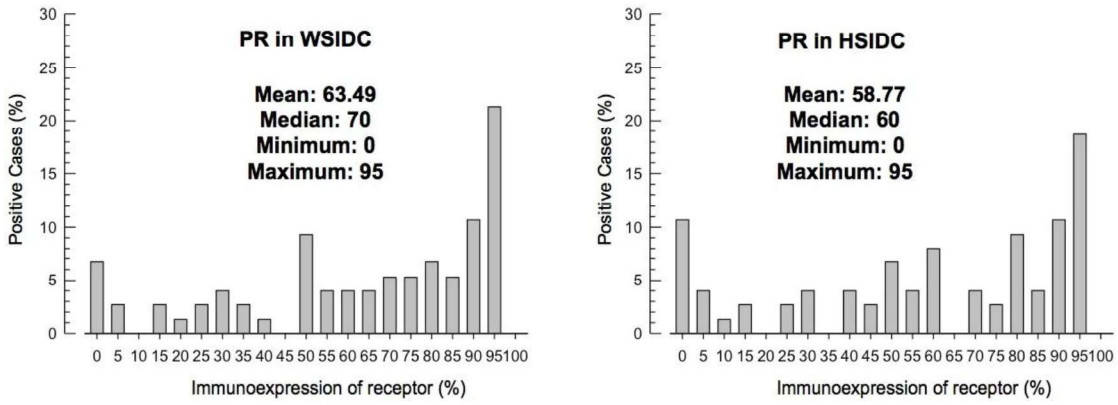

Figure 2C. Comparison between the PR in the WSIDC with PR in the HSIDC; n = 75; P = 0.002.

Figure 2. Comparison between the immunoexpressions of Ki67, Estrogen Receptor (ER), and Progesterone Receptor (PR) in the Hot Spots of Invasive Ductal Carcinoma (HSIDC) and in the Whole Sections of Invasive Ductal Carcinoma (WSIDC).

Figure 3.

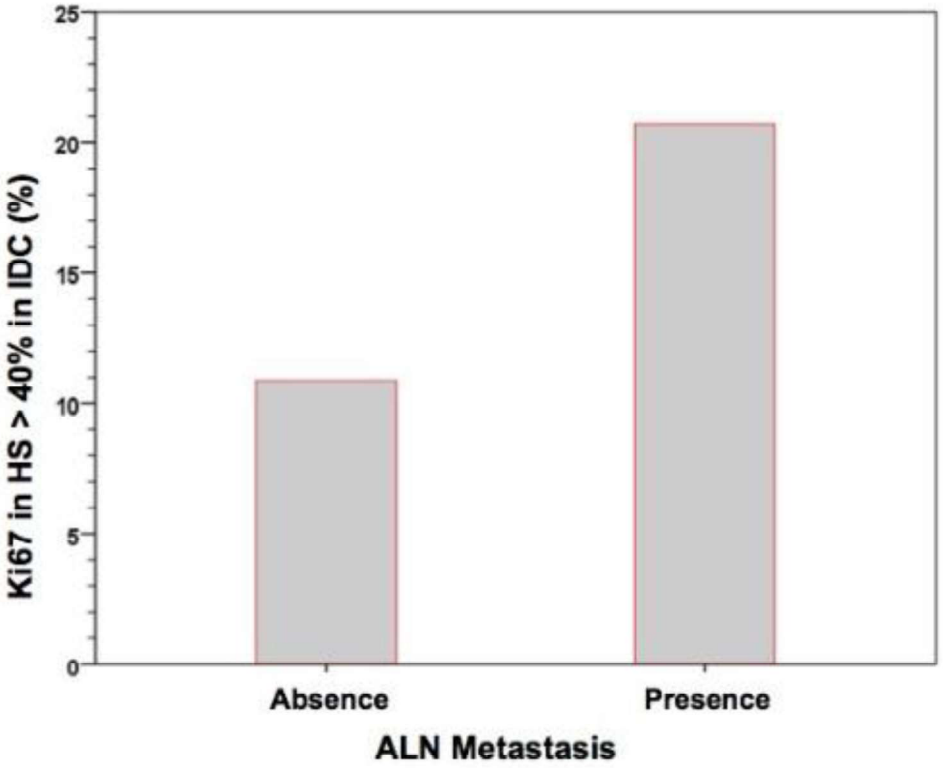

Figure 3. Percentage of patients with Ki67 > 40% in the Hot Spots (HS) of Invasive Ductal Carcinoma (IDC) and the relation with the Axillary Lymph Node (ALN) Metastasis.

A presente dissertação será enviada para publicação na Revista “Journal of Clinical Pathology”

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- Each question must have 5 possible answers, with only *one* answer being correct (the correct answer must be marked with an asterisk)
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4. Considerações finais

A presente dissertação de mestrado objetivou ressaltar as incertezas existentes com relação aos métodos de avaliação do índice de proliferação celular (Ki67) nos carcinomas ductais invasivos mamários SOE, buscando, dessa maneira, obter informações a respeito da sua imunoexpressão nas áreas de maior proliferação celular denominadas “hot spots” e nas áreas de menor índice proliferativo. De acordo com os resultados obtidos nas áreas de “hot spots”, 13 pacientes (17,3%) seriam reclassificados como subtipo luminal B (HER2-negativo) considerando-se o “cutoff” de 14%”, se comparados com a avaliação do Ki67 no restante da neoplasia. É importante ressaltar que as diferenças na classificação imuno-histoquímica dos subtipos luminal A e luminal B de acordo com a expressão do Ki67 nestas duas áreas, poderiam determinar tratamentos clínicos diversos dependendo do local onde o mesmo for analisado.

Além disso, foi realizada uma comparação da imunoexpressão dos RE e RP nas áreas de menor proliferação celular e nos “hot spots” e de outros fatores prognósticos, tais como o grau histológico e a dimensão tumoral, bem como a invasão vascular peritumoral, com as metástases axilares. A maior parte destes dados obtidos foi consistente com os achados previamente referidos na literatura.

Dessa forma, a partir dos dados expostos, pode-se concluir que, embora existam algumas determinações para uma melhor interpretação do Ki67, estas não são muito bem estabelecidas. É importante salientar, dessa forma, que apesar da inexistência de um consenso mundial para a avaliação do Ki67, a necessidade da estandardização da análise do mesmo, com o intuito de

individualizar o tratamento dos pacientes com câncer de mama, torna-se fundamental. Enquanto isso não ocorre os resultados anatomopatológicos devem ser correlacionados com a evolução clínica dos pacientes com carcinoma mamário a partir dos protocolos de tratamento preconizados por cada instituição, buscando assim, a melhor terapia a partir de seus próprios resultados, e não da experiência e informações clínico-patológicas de outras instituições.

5. Anexos

5.1. Parecer do Comitê de Ética da Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA)

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação do índice de proliferação celular (KI67) em neoplasias mamárias nas áreas de maior e menor proliferação e sua relação com as metástases axilares e demais fatores prognósticos.

Pesquisador: Claudio Galleano Zettler

Área Temática:

Versão: 3

CAAE: 16773813.1.0000.5335

Instituição Proponente: Irmandade da Santa Casa de Misericordia de Porto Alegre - ISCMPA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 431.574

Data da Relatoria: 17/10/2013

Apresentação do Projeto:

O carcinoma mamário é o tipo de carcinoma mais comum, perfazendo cerca de 22% dos casos de neoplasias mundiais. Dentre alguns dos fatores prognósticos relacionados com a sobrevida e utilizados para o tratamento clínico destas neoplasias podem ser citados o grau histológico, invasão vascular neoplásica peritumoral, presença ou ausência de metástases axilares, status para os receptores hormonais de estrógeno (RE), progesterona (RP), para a molécula de HER2 e o índice de proliferação celular (Ki67). O Ki67 é considerado um fator importante para o tratamento clínico, entretanto não possui uma padronização para a sua avaliação entre os diferentes Serviços de Patologia.será realizada de forma retrospectiva, no período entre janeiro de 2012 e abril de 2013, uma revisão de lâminas de hematoxilina-eosina e de anticorpos do painel imunoistoquímico de rotina (RE, RP, HER2, citoqueratina 5/6 e Ki67) de espécimes de carcinomas mamários unifocais, sem terapia neoadjuvante, do Serviço de Patologia do Complexo Hospitalar Santa Casa de Porto Alegre.

Objetivo da Pesquisa:

Estabelecer uma comparação entre o perfil imunoistoquímico (RE, RP, HER2 e citoqueratina 5/6) e o grau histológico das neoplasias primárias mamárias com o índice de proliferação celular na neoplasia como um todo e nas áreas de maior proliferação celular ("hot spots") e sua relação com

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as metástases axilares e outros fatores prognósticos em pacientes com carcinomas mamários, unifocais, sem tratamento clínico neoadjuvante.

Avaliação dos Riscos e Benefícios:

A pesquisa não determinará riscos aos pacientes do estudo por se tratar de estudo retrospectivo, com a análise de material já presente nos arquivos do Serviço de Patologia da Santa Casa de Misericórdia de Porto Alegre, sem a necessidade de coleta de novas amostras ou realização de novos procedimentos diagnósticos.

Ainda não existe um consenso a respeito do melhor método para a análise do índice de proliferação celular (Ki67) nas neoplasias mamárias. A definição da maneira mais adequada para a realização desta análise pode determinar um tratamento clínico mais adequado para os pacientes com carcinoma mamário.

Comentários e Considerações sobre a Pesquisa:

Nada a declarar.

Considerações sobre os Termos de apresentação obrigatória:

Ver em conclusões.

Recomendações:

Considerando que a pesquisa utilizará lâminas, recomendo aos pesquisadores atenção especial à legislação citada no parecer anterior.

Conclusões ou Pendências e Lista de Inadequações:

Relatório parcial enviado.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

De acordo com o parecer do relator.

PORTO ALEGRE, 22 de Outubro de 2013

Assinador por:
José Geraldo Vernet Taborda
(Coordenador)

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5.2. Parecer do Comitê de Ética da Irmandade da Santa Casa de Misericórdia de Porto Alegre (ISCMPA)

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação do índice de proliferação celular (KI67) em neoplasias mamárias nas áreas de maior e menor proliferação e sua relação com as metástases axilares e demais fatores prognósticos.

Pesquisador: Claudio Galleano Zettler

Área Temática:

Versão: 3

CAAE: 16773813.1.0000.5335

Instituição Proponente: Irmandade da Santa Casa de Misericordia de Porto Alegre - ISCMPA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 382.042

Data da Relatoria: 03/09/2013

Apresentação do Projeto:

Aprovada.

Objetivo da Pesquisa:

Aprovado.

Avaliação dos Riscos e Benefícios:

De acordo.

Comentários e Considerações sobre a Pesquisa:

De acordo.

Considerações sobre os Termos de apresentação obrigatória:

De acordo.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

Aprovada a emenda de solicitação de envio de rel. semestral para UFCSPA.

Situação do Parecer:

Aprovado

Endereço: R. Profº Annes Dias,285 Hosp.Dom Vicente Scherer
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Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Após a avaliação do relatório parcial de andamento solicitado pela Instituição Co-participante apresentado pelo Pesquisador, o presente Comitê não encontrou óbices quanto a continuação do desenvolvimento do estudo em nossa Instituição.

PORTO ALEGRE, 03 de Setembro de 2013

Assinador por:
Claudio Teloken
(Coordenador)

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