Paula Carvalho de Abreu e Lima Brito

Características histopatológicas e fenótipo molecular do câncer de mama associado à gravidez

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Dissertação apresentada ao Programa de Pós-Graduação em Patologia do Centro de Ciências da Saúde da Universidade Federal de Pernambuco, para obtenção do título de Mestre em Patologia

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“It is much more important to know what sort of a patient has a disease than what sort of a disease a patient has”

William Osler

Resumo

Introdução: O câncer de mama associado à gravidez (CMAG), definido como aquele diagnosticado durante a gravidez ou até um ano após o parto, é uma doença distintamente agressiva. Embora estudos mostrem que a gravidez por si só não é um preditor prognóstico independente, um parto recente (até 2 anos do diagnóstico) parece ser um marcador independente de mau prognóstico. Ainda não é claro se o CMAG possui características histopatológicas distintas. Carcinomas com diferenciação basal constituem um subtipo agressivo, comum em mulheres jovens. Nenhum estudo até hoje avaliou a ocorrência de diferenciação basal no CMAG. **Objetivo:** Definir a frequência de diferenciação basal e a distribuição dos tipos moleculares no CMAG e avaliar se esse possui características histopatológicas distintas. **Métodos:** Foram selecionados os casos de câncer de mama diagnosticados em mulheres com idade ≤35 anos, no Hospital de Câncer de Pernambuco, de 1995 a 2011. Desses, 39 foram diagnosticados durante a gravidez ou até dois anos do último parto e 32 em mulheres nulíparas, sendo as últimas selecionadas como grupo controle. Foram comparadas as características clinicopatológicas, a frequência de diferenciação basal e a distribuição molecular nos dois grupos. **Resultados:** Não houve diferenças quanto ao tipo histológico, *status* dos receptores hormonais e Her-2, subtipos moleculares ou diferenciação basal entre os grupos. Os carcinomas de grau histológico 3 foram mais frequentes no grupo controle. Observou-se diferenciação basal em 18.4% e 21.9% dos tumores no CMAG e grupo controle, respectivamente. Foram identificados dois casos de carcinoma micropapilar invasivo, que ainda não haviam sido relatados no CMAG. **Conclusão:** O CAMG não parece ter características histopatológicas distintas daquelas dos tumores diagnosticados em nulíparas.

palavras-chave: neoplasias da mama, gravidez, imunoistoquímica

Abstract

Background: Pregnancy-associated breast cancer (PABC), defined as cancer diagnosed during or within one year of the last pregnancy, is a distinctively aggressive disease. Although studies have shown that pregnancy is not an independent predictor of mortality, a recent labor (up to 2 years prior to diagnosis) appears to be an independent prognostic marker. Data are conflicting regarding whether PABC has distinct pathologic features. Carcinomas with basal differentiation are an aggressive subtype of breast carcinoma, being common in young women. No study to date has addressed the occurrence of basal differentiation in PABC. **Objective:** To determine the frequency of basal differentiation and molecular type distribution in PABC, and determine if it has distinct pathologic features. **Method:** All cases of invasive breast carcinoma diagnosed in women ≤35 years at Hospital de Câncer de Pernambuco, Brazil, from 1995 to 2011, were selected. Of all cases, 39 were diagnosed during or within 2 years of the last pregnancy and 32 in nulliparous women, which were selected as controls. The clinicopathologic characteristics, basal differentiation and molecular distribution were compared between the groups. **Results:** No significant differences were seen with regard to histologic type, hormone receptor and Her-2 positivity, basal differentiation and molecular subtypes. Nulliparous women were more likely to harbor grade 3 carcinomas. Basal differentiation was seen in 18.4% e 21.9% of tumors in PABC and non-PABC, respectively. Two PABC cases were invasive micropapillary carcinomas, which had not been reported in PABC before. **Conclusion:** PABC shares similar clinicopathologic characteristics with cancer diagnosed in nulliparous women.

Keywords: breast neoplasms, pregnancy, immunohistochemistry.

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Lista de abreviaturas e siglas

BRCA-1: gene breast cancer 1, early onset

CK: citoqueratina

CMAG: câncer de mama associado à gravidez

EGFR: epidermal growth factor receptor

FISH: hibridização in situ fluorescente

HCP: Hospital de Câncer de Pernambuco

HER-2: human epidermal growth factor receptor-2

IDC: invasive ductal carcinoma

IGF-1: insulin growth factor 1

IMPC: invasive micropapillary carcinoma

PABC: pregnancy-associated breast carcinoma

p53: proteína p53

pT: pathologic T stage

pT: pathologic N stage

RE ou ER: receptor de estrógeno

RP ou PR: receptor de progesterona

TN: triple-negative

Sumário

1. APRESENTAÇÃO

O câncer de mama é o câncer mais comum entre as mulheres e a causa mais comum de morte por câncer em todo o mundo (RAKHA *et al*, 2010). Embora infrequente em jovens – apenas cerca de 5% das mulheres com câncer de mama tem menos de 40 anos (SARIEGO, 2010) – nessa população os tumores costumam ser mais agressivos e de prognóstico desfavorável (XIONG *et al*, 2001; CHUNG *et al*, 1996). Aproximadamente 10% das pacientes com câncer de mama e menos de 40 anos desenvolvem a doença durante a gravidez (NUGENT e O´CONNELL, 1985).

A incidência de câncer de mama na gravidez e no período pós-parto varia de 2,3 a 40 casos por 100.000 mulheres (BURÉ *et al*, 2011). Contudo, considerando que a idade materna à primeira gravidez continua a aumentar, espera-se que a incidência de câncer de mama em grávidas aumente (BEADLE *et al*, 2009).

Estudo epidemiológico realizado na década de setenta (MACMAHON, COLE e BROWN, 1973), depois confirmado em grande estudo prospectivo (ROSNER, COLDITZ e WILLETT, 1994), demonstrou que a gravidez está associada à redução do risco de câncer de mama. Contudo, já no início dos anos 80 reconheceu-se que o efeito protetor da gravidez a termo não era imediato nem constante e que, na verdade, haveria um efeito promotor antes que seu efeito protetor fosse obtido (JANERICH e HOFF, 1982). O aumento transitório do risco de câncer de mama após a gravidez mostrou atingir o seu pico no 6º ano após o parto e persistir por aproximadamente dez anos; esse risco revelou-se mais marcante em mães que tiveram gravidez mais tarde, em comparação com primíparas jovens (ALBREKTSEN *et al*, 2005).

Além disso, estudos mostraram que o efeito protetor da gravidez depende fundamentalmente da idade ao primeiro parto. Pesquisa envolvendo 16000 mulheres identificou a idade de 35 anos como ponto crucial, ou seja, gravidez antes dos 35 anos conferiria algum grau de proteção, e após os 35 anos levaria a um permanente aumento de risco (TRICHOPOULOS *et al*, 1983). Alguns estudos subsequentes mostraram que idade materna avançada na primeira gravidez não eliminaria totalmente o efeito protetor da gravidez (ALBREKTSEN *et al*, 2005); outros constataram que mulheres cuja primeira gravidez ocorreu após os 35 anos tinham um aumento de risco persistente, comparável ao das nulíparas (COLDITZ e ROSNER, 2000).

O câncer de mama é o tumor maligno mais frequentemente diagnosticado durante a gravidez (SMITH *et al*, 2003). Para a maioria dos autores, o termo “câncer de mama

associado à gravidez" (CMAG) se refere ao câncer de mama diagnosticado durante a gravidez ou até 1 ano após o parto (HALASKA *et al*, 2009; BEADLE *et al*, 2009; RODRIGUEZ *et al*, 2008; MATHELIN *et al*, 2008), contudo, há variações nessa definição, com alguns incluindo um intervalo de até 5 anos após parto (MCDANIEL *et al*, 2006). Estudos mostram que mulheres com CMAG, independentemente da idade, tem prognóstico menos favorável (RODRIGUEZ *et al*, 2008; WHITEMAN *et al*, 2004; BLADSTROM, ANDERSON e OLSSON, 2003; DALING *et al*, 2002). Principalmente quando diagnosticado até dois anos do último parto, o CMAG é de prognóstico sombrio, com alta mortalidade por metástases (DALING *et al*, 2002).

Os fatores responsáveis pelo mau prognóstico do câncer de mama na gravidez e puerpério são pouco conhecidos. Especula-se que a maior agressividade da doença deva-se ao retardo do diagnóstico, à idade jovem, às influências hormonais e imunológicas inerentes à gravidez, às alterações involutivas sofridas pela mama no puerpério (SCHEDIN, 2006) e ao aumento da vascularização induzido pela gravidez (STENSHEIM *et al*, 2009). A gravidez e o puerpério aumentam a densidade do parênquima mamário, tornando o exame clínico e mamográfico mais difícil de interpretar (SCHEDIN, 2006). A gravidez expõe a mama a níveis elevados de estrógeno, progesterona, IGF1 (insulin growth fator 1), os quais podem exercer efeito pró-tumorigênico (SCHEDIN, 2006). Os níveis elevados de hormônios na gravidez poderiam acelerar o crescimento tumoral ou o desenvolvimento de metástases, através de um efeito intrínseco na biologia do tumor ou do aumento da vascularização (RODRIGUEZ *et al*, 2008). Estudos em culturas de células e em modelos animais de câncer de mama mostraram que o aumento dos hormônios na gravidez pode aumentar o tamanho e a proliferação do tumor (THORNE e LEE, 2003; GOTTARDIS *et al*, 1989). Um dado interessante é que gestantes com pré-eclâmpsia, doença caracterizada por níveis sistêmicos baixos de estrógeno e IGF1 (GIUDICE e IRWIN, 1999), possuem risco duas vezes menor de desenvolver câncer de mama associado à gravidez (INNES e BYERS, 2004). Contudo, ainda não se sabe a real influencia dos hormônios na gravidez na progressão tumoral do CMAG, já que há uma baixa frequência de tumores positivos para receptores hormonais nessa população, os quais teoricamente não responderiam aos estímulos hormonais.

A mudança no padrão reprodutivo das mulheres nas últimas décadas, com retardo da primeira gravidez e diminuição da paridade, possivelmente contribuirá para o aumento da incidência do câncer de mama. A idade avançada à primeira gestação está aumentando o número de casos de câncer que coincidem com a gravidez, podendo representar um problema importante para um futuro próximo.

Os estudos etiopatogênicos e clinicopatológicos do câncer de mama nesse período são escassos. É fundamental que se conheçam as características histopatológicas e os fatores de implicação prognóstico-preditiva (expressão dos receptores hormonais e Her-2) dos tumores da mama no ciclo gravídico-puerperal, para que se compreenda a sua associação com maior agressividade clínica e para que se contribua com o conhecimento de uma condição de grande impacto para a sociedade e para família, pelo óbito de mulheres jovens e mães de lactentes que se tornam precocemente órfãos.

Este trabalho teve como objetivo geral comparar o CMAG com o câncer de mama de mulheres nulíparas, em relação às características clínicopatológicas, aos marcadores imunoistoquímicos prognósticos e aos marcadores basais. Os objetivos específicos foram: observar se existem diferenças quanto às características clínicopatológicas, aos marcadores imunoistoquímicos prognósticos, à prevalência dos carcinomas com diferenciação basal, e à distribuição molecular, entre o CMAG e o câncer de mama de mulheres nulíparas.

2. REVISÃO DA LITERATURA

Para a maioria dos autores o termo “câncer de mama associado à gravidez” se refere ao câncer de mama diagnosticado durante a gravidez ou até 1 ano após o parto (BEADLE *et al*, 2009; HALASKA *et al*, 2009; MATHELIN *et al*, 2008; RODRIGUEZ *et al*, 2008), contudo, há variações nessa definição, com alguns incluindo um intervalo de até 5 anos após parto (MCDANIEL *et al*, 2006).

A maioria dos estudos sobre o câncer de mama durante a gravidez mostrou que essas pacientes têm pior prognóstico (BEADLE *et al*, 2009; RODRIGUEZ *et al*, 2008; MATHELIN *et al*, 2008). Estudos sugerem que o mau prognóstico do câncer de mama em gestantes resulte do fato de que: essas pacientes são jovens e o câncer de mama em jovens é sabidamente mais agressivo (BEADLE *et al*, 2009; MIDDLETON *et al*, 2003) e tem estadiamento mais avançado, por retardo do diagnóstico (WOO, YU e HURD, 2003). Contudo, estudos não mostraram pior prognóstico das gestantes, quando se ajustou para tamanho tumoral e estadiamento (KROMAN e MOURIDSEN, 2003; LETHABY *et al*, 1996), não parecendo ser, a gravidez na ocasião do diagnóstico, um marcador prognóstico negativo independente (SCHEDIN, 2006). Porém, um parto recente parece ser um preditor independente de mau prognóstico (STENSHEIM *et al*, 2009; WHITEMAN *et al*, 2004; KROMAN e MOURIDSEN, 2003; BLADSTROM *et al* 2003; DALING *et al*, 2002; LETHABY *et al*, 1996). A doença é especialmente mais agressiva nas pacientes diagnosticadas dentro de um ou dois anos do último parto (WHITEMAN *et al*, 2004; KROMAN e MOURIDSEN, 2003; DALING *et al*, 2002; OLSON *et al*, 1998). Hipóteses levantadas para justificar esse achado seriam o efeito pro-tumorigênico da involução do parênquima mamário após o parto e lactação (O´BRIEN *et al*, 2010; MCDANIEL *et al*, 2006), a baixa proporção de tumores positivos para receptores hormonais nessa população (RODRIGUEZ *et al*, 2008; BLADSTROM *et al,* 2003) e a seleção de tumores com características associadas a mau prognóstico, induzida pelos hormônios da gravidez (SCHEDIN, 2006). O número de gestações anteriores ao diagnóstico parece não ter influencia sobre a sobrevida dessas pacientes (STENSHEIM *et al*, 2009).

Características associadas a mau prognóstico, além do estadiamento, incluem: grau histológico, tipo histológico, negatividade para os receptores hormonais, expressão do Her-2, dentre outros. Os tumores que mostram ausência de imunoexpressão para receptores hormonais (receptor de estrógeno e progesterona), imunopositividade para Her-2, ou

negatividade para os três marcadores (i.e., triplo-negativos) estão associados a maior agressividade, sendo, em geral, tumores de alto grau histológico.

Na realidade, estudos sobre os marcadores prognósticos do câncer de mama associado à gravidez são escassos (SCHEDIN, 2006). Além disso, os dados são conflitantes, principalmente pela variabilidade dos critérios usados na seleção do grupo controle. Daling *et al* (2002) relataram que os tumores de pacientes cujo parto ocorreu em até dois anos do diagnóstico tinham maior probabilidade de serem negativos para o receptor de progesterona, de expressar p53 e de ser de maior grau histológico, quando comparado a nulíparas. Outros grupos relataram menor expressão de receptor de estrógeno (HALASKA *et al*, 2009; MATHELIN *et al*, 2008; RODRIGUEZ *et al*, 2008; AZIZ *et al*, 2003; BONNIER *et al*, 1997) e progesterona (MATHELIN *et al*, 2008; RODRIGUEZ *et al*, 2008; AZIZ *et al*, 2003; BONNIER *et al*, 1997) no CMAG. Outros estudos não detectaram diferenças significativas na expressão de receptor de estrógeno ou grau histológico (BEADLE *et al*, 2009; MIDDLETON *et al*, 2003).

Uma nova taxonomia para o câncer de mama foi recentemente proposta com base em estudos de expressão gênica em *microarrays*, em que foram caracterizados cinco subtipos moleculares: luminal A, luminal B, mama normal, Her-2 e basal-*like* (PEROU *et al*, 2000). Estudos subsequentes mostraram o valor prognóstico dessa classificação molecular (SORLIE *et al*, 2001). Por exemplo, o grupo denominado basal-*like*, que corresponde a cerca de 15% dos casos de câncer de mama (CAREY *et al*, 2006; NIELSEN *et al*, 2004), tem sido objeto de intenso estudo na literatura recente, está associado a mau prognóstico e corresponde a maior parte dos tumores triplo-negativos (negativos para receptores hormonais e Her-2). Recebe essa denominação por expressar gens comuns às células basais/mioepiteliais da mama, tais como as citoqueratinas de alto peso molecular e o EGFR. Esses tumores costumam ter alto grau histológico, bordas não infiltrativas, alto índice mitótico, áreas de necrose central, infiltrado linfocítico conspícuo, características medulares e áreas metaplásicas (RAKHA e REIS-FILHO, 2009). Possuem padrão metastático distinto, disseminando-se principalmente para vísceras como cérebro e pulmões (RAKHA e REIS-FILHO, 2009), e menos para linfonodos e ossos que os demais tipos (FULFORD *et al*, 2007; BANERJEE *et al*, 2006; HICKS *et al*, 2006; TSUDA *et al*, 2000). Ao contrário dos tumores positivos para os receptores hormonais e para Her-2, que recebem tratamento-alvo específico com antiestrógenos e trastuzumab, respectivamente, os carcinomas basal-*like* carecem de terapia especifica, embora uma parcela desses seja muito sensível à quimioterapia neoadjuvante (LIEDTKE *et al*, 2008). Contudo, outros trabalhos mostram que os carcinomas triplo-

negativos que expressam citoqueratinas basais (carcinomas de tipo basal) responderiam menos à quimioterapia com antraciclina que os triplo-negativos que não expressam esses marcadores (CONFORTI *et al*, 2007). Esse subtipo é relatado como mais prevalente nas mulheres de descendência africana e hispânica (CAREY *et al*, 2006), costuma acometer mulheres jovens (CAREY *et al*, 2006; CALZA *et al*, 2006), e compartilha muitas características com os tumores associados à mutação do BRCA-1 (gene breast cancer 1, early onset) (FOULKES *et al*, 2003). Um dado interessante é o de que fatores classicamente associados à diminuição do risco de câncer de mama, como alta paridade e idade jovem à primeira gravidez, estão relacionados ao aumento de risco para o câncer de tipo basal; já o aumento da gordura abdominal parece elevar o risco tanto para carcinomas de tipo basal, quanto para os carcinomas positivos para os receptores hormonais (MILLIKAN *et al*, 2008). A amamentação associa-se à diminuição de risco para o tipo basal (MILLIKAN *et al*, 2008).

Infelizmente, a maior parte dos gens avaliados nos estudos de perfil de expressão gênica (considerado padrão-ouro, porém, inviável pelo custo e necessidade de tecido fresco ou congelado) não possuem anticorpos imunoistoquímicos correspondentes que pudessem ser utilizados na prática clínica, em blocos de parafina. Contudo, embora a correlação entre o carcinoma de tipo basal definido pelo perfil de expressão gênica e o determinado por exame imunoistoquímico não seja exata, muitos estudos têm proposto painéis imunoistoquímicos com boa sensibilidade e especificidade (RAKHA *et al*, 2007; LAAKSO *et al*, 2005; NIELSEN *et al*, 2004). Porém, ainda não há consenso internacional sobre qual o painel imunoistoquímico preferencial para identificação do subtipo basal na rotina clínica. Alguns autores recomendam um painel que incluiria RE, RP, Her-2, CK (citoqueratinas) 5/6, CK14, CK17 e EGFR (RAKHA e REIS-FILHO, 2009). Mais recentemente, sugeriu-se um painel triplo com CK14, EGFR e 34βE12 (citoqueratina de alto peso molecular) como o de melhor combinação de sensibilidade e especificidade (78% e 100%, respectivamente), frente ao padrão-ouro (perfil de expressão gênica) (THIKE *et al*, 2010). Há estudos que defendem a avaliação rotineira dos marcadores basais nos carcinomas triplo-negativos, uma vez que a distinção entre tipo basal e não-basal (sendo o primeiro de maior agressividade clínica) útil da decisão sobre a conduta terapêutica a ser adotada (SASA *et al*, 2008; CHEANG *et al*, 2008). Outros estudos mostraram que a simples expressão das citoqueratinas basais conferiria pior prognóstico e sobrevida mais curta (RAKHA *et al*, 2007; POTEMSKI *et al*, 2005; VAN DE RIJN *et al*, 2002;), independentemente de serem os tumores triplo-negativos ou não (RAKHA *et al*, 2007).

Não há, no nosso conhecimento, estudos relacionados à prevalência dos carcinomas de tipo basal no CMAG. Também não há estudos que tenham avaliado a expressão de marcadores imunoistoquímicos basais, excetuando-se o EGFR (AZIZ *et al* 2003), no CMAG. Considerando que o CMAG ocorre tipicamente em mulheres jovens e que a maioria mostra negatividade para os receptores hormonais (BEADLE et al, 2009; MATHELIN *et al*, 2009; HALASKA *et al*, 2008; RODRIGUEZ *et al*, 2008; MIDDLETON *et al*, 2003; DALING *et al*, 2002; BONNIER *et al*, 1997) e Her-2 (HALASKA *et al*, 2008; MIDDLETON *et al*, 2003; DALING *et al*, 2002), é possível que grande parte corresponda a tumores basal-*like*. Além disso, o CMAG mostra maior frequência de positividade para EGFR (AZIZ *et al*, 2003), sendo o último um marcador tipicamente expresso pelos tumores basal-*like*. Um estudo de grande casuística, que incluiu 797 casos de CMAG, mostrou maior prevalência do CMAG em mulheres hispânicas (RODRIGUEZ *et al*, 2008). Além disso, observou que mulheres de descendência africana tinham um risco de morte 68% maior que mulheres brancas (RODRIGUEZ *et al*, 2008). Sabendo-se, portanto, que é mais frequente a ocorrência do carcinoma basal-*like* na população hispânica e afrodescendente, é possível que prevalência de carcinoma basal-*like* no CMAG seja alta, o que poderia justificar o prognóstico sombrio dessa condição.

2.1. Artigo de revisão

Do pregnancy-associated breast cancers have distinct pathological features? A review.

Introduction

Although pregnancy has been classically associated with a protective effect against breast cancer, epidemiologic studies have demonstrated a still under-recognized transient increase in risk shortly after pregnancy (1-8), which peaks around the 6th year of postpartum (9). Pregnancy-associated breast cancer (PABC) is defined by most authors as breast cancer diagnosed during pregnancy or within one year after delivery. However, some include patients diagnosed up to 5 years of postpartum (10). Albeit uncommon, PABC is not a rare condition. Seven to 15% of premenopausal breast cancers occur during pregnancy (11). Breast cancer is the most common malignancy diagnosed during pregnancy and the postpartum period, occurring in 1 in 3000 pregnant women (12). Considering that the maternal age at first pregnancy continues to rise around the world, the incidence of PABC is expected to increase (13). Most studies have shown that it is associated with a poor outcome, likely due to delayed diagnosis, young age of the patients, and lack of standardized treatment. However, the majority of studies have concluded that it is not an independent prognostic factor when adjusted for stage at presentation. In addition, pregnancy subsequent to a breast cancer diagnosis does not seem to adversely affect the prognosis (14), and termination of pregnancy does not appear to improve outcome (11, 15-18). In contrast, breast cancer diagnosed in the postpartum period, especially within 5 years of delivery, has been shown to be an independent predictor of mortality (19-22), and associated with adverse prognostic profiles (20, 23-26). The prognosis is even worse for mothers diagnosed within 2 years of postpartum (19, 25). The reasons for that are uncertain, but it could be that pregnancy and/or lactation select for a specific cancer phenotype of increased aggressiveness. Data regarding intrinsic tumor characteristics such as histologic features and hormone receptor status are scarce, with many studies being based at specialized institutions with small sample sizes. Furthermore, only very few studies have evaluated Her-2 and p53 in this population. These histopathologic characteristics are reviewed and discussed in this paper.

Methodology

Data for this review were identified by searches of PubMed using the keywords "pregnancy", "immunohistochemistry", and the free text words “breast carcinoma” and “pregnancy-associated breast cancer”. Searches were restricted to papers written in the English language. No time limit was used. Relevant references were also included. All papers that evaluated the hormone receptor status of tumors by immunohistochemistry were selected. Studies that evaluated non-pregnant patients diagnosed within 2 years of postpartum were also included. Reviews and case reports were excluded.

Studies

The search retrieved 25 studies from 1993 through 2011.
Most studies were carried out in the past decade, with only three being published in the 1990s. This could be explained by the more widespread availability and routine use of immunohistochemistry in the management of breast cancer patients, and/or an increased interest in the theme.

Samples

Samples varied from 5 to 797 PABC cases, and 10 to 4,177 controls. Many studies were hampered by small sample sizes, with many containing 40 or fewer cases (30-34). Thirteen of 24 articles defined PABC as cancer diagnosed during pregnancy or within 1 year of postpartum. Nevertheless, definitions also included cancer diagnosed exclusively during pregnancy, during pregnancy or lactation or within 2 months of stopping lactation, during pregnancy or within 12 days of postpartum, during pregnancy or within 6 months of postpartum, during pregnancy or within 2 years of postpartum, or exclusively within the second year of postpartum. Consequently, there was a lot of variation in the definition of case and control groups. Most defined controls as patients that did not meet the definition criteria for PABC. Therefore, control groups could have been quite heterogeneous as far as including nulliparous women, multiparous women, and women diagnosed during the second year of postpartum (the latter being included in the very definition of PABC in other studies) (19, 25, 33). Siegelmann-Danieli et al [31], for instance, reported that only 11% of their controls were nulliparous patients, and the interval from last pregnancy to diagnosis ranged from 1.1 to 30 years in parous patients.

Two problems with study designs were raised by some authors (35). First, since postpartum cases have been shown to be of worse prognosis than cases diagnosed during pregnancy (19, 22, 32, 36-39), it may be more appropriate to separate these groups when analyzing data (35). In addition, while the increased risk of dying associated with postpartum carcinomas is more marked within 5 years of delivery, it could extend up to 15 years after pregnancy (40). Therefore, in order to avoid any influence of pregnancy on prognosis, the ideal control group would probably be composed of nulliparous patients only. Only three of 17 studies selected nulliparous women exclusively as the control group.

Histologic type

The most common histologic type of PABC was invasive ductal carcinoma (IDC). This is in keeping with the known highest frequency of ductal carcinoma in the general population as well as in young women. However, some studies purposely excluded histologic types other than IDC (19), breast sarcoma and in situ disease (41, 42). Lobular carcinomas were infrequent and varied from 0% to 12.5%, and mucinous carcinomas comprised 4.1% to 14% of the cases, except for one very small case series (which included only 5 PABC cases), in which two were IDC (40%), two were lobular carcinomas (40%) and one (20%) was mucinous carcinoma (43). Mixed ductal and lobular carcinomas corresponded to 1.9% to 3% of the cases (13, 28). In situ carcinomas were reported in 0% to 7% of the cases. Medullary carcinomas were mentioned by three groups and corresponded to 1.6%, 2.7% and 5% of the cases. Invasive cribriform carcinoma was described in only one case by one group (44). Focal spindle cell differentiation and squamous differentiation were reported in two cases (45). No studies have reported the occurrence of tubular carcinoma in PABC.

None of the studies has identified statistically significant differences in histologic type between PABC and controls. Reed et al [14] found similar rates of extensive intraductal component in pregnancy, lactation and control groups (10%, 24% and 23%, respectively), and no difference was seen in regard to whether ductal carcinoma in situ (DCIS) was localized within or in the periphery of the tumor. Shousha [29] found that the presence of cancerization of lobules, which is more commonly encountered in young women (46), was more likely present in PABC cases, and that foci of mucinous carcinoma was almost exclusively seen in patients with a current or recent history of lactation. This has not been confirmed nor evaluated in other studies. Shousha speculated that the more actively growing breast lobules of pregnant women could be more susceptible to invasion, and that the foci of mucinous

carcinoma associated with lactation could be explained by the increased insulin sensitivity of the breast during late pregnancy and lactation (47).

Histologic grade

In one study (48), only 25.3% of tumors were grade 3, but for a large percentage of cases (51.7%) grade was unknown. All other studies found a high percentage of grade 3 tumors in PABC, ranging from 40% to 84%. This could be attributed to the young age of the patients. However, 6 of 13 studies concluded that PABC was more likely to be of high histologic grade. One study that grouped grade 2 and 3 tumors together found that 86.7% of pregnant and 100% of postpartum patients had grade 2/3 tumors, respectively (44). The percentage of grade 1 tumors varied from 0 to 17.5%.

Immunohistochemical markers

The percentage of estrogen receptor (ER) positive PABC cases varied from to 19% to 55%. All studies except for one (33), obtained ER positivity in half or less of the cases. Importantly, PABC was shown to be significantly more likely to be ER negative in 7 of 13 studies.
Progesterone receptor (PR) positivity was seen in 24% to 83.3% of PABC cases. Looking solely at postpartum patients, Reed et al [14] reported PR positivity in only 23% of cases. The reason for such broad variation is unknown, but may be related to small sample sizes. The study that reported the highest percentage of positivity had PR tested on only 12 cases. Five of 13 studies concluded that PABC was significantly more likely to be PR negative. One showed only a borderline significant difference in PR expression ($p=0.05$), attributed to the large number of patients for whom PR status was unknown (13).
Six studies did not report ER and PR status separately, but rather “ER and/or PR expression” or “hormone receptor” positivity, making comparison of data difficult. Two of them reported PABC to be more likely ER/PR negative and ER and/or PR negative, respectively (30, 31). Importantly, in one of the studies control patients were significantly older and more likely to be Jewish, which could have interfered with the results (31).
None of the studies have given a more detailed quantitative description for ER and PR positivity, such as percentage of positive cells and intensity of staining, and most did not specify the criteria used for determining ER and/or PR positivity (11, 24, 29, 31, 33, 34, 37, 43 48, 49, 50-52). The most commonly utilized criterion was nuclear staining in 10% of cells

or more (14, 28, 44, 45), but some utilized the "H-score" (30, 32) or other specific scores (27). Some studies had a large percentage of cases for which ER (13, 37, 48, 49), and PR status was unknown (13, 37, 48).

Her-2 is amplified in 25% to 30% of all breast cancers and is associated with aggressive disease (53). Fourteen papers evaluated Her-2 status in PABC. Her-2 positive cases ranged from 6% to 60%. This wide variation may be attributed to small sample sizes, large percentage of cases for which Her-2 was unknown (52), different techniques utilized for evaluating Her-2 status, and variation in the criteria used for determining positivity. Most papers evaluated the Her-2 status by immunohistochemistry. Porta et al [54] tested Her-2 on only 7 cases, all determined by fluorescent in situ hybridization (FISH). Only one study from 1993 which included only 15 PABC cases, reported PABC to be more likely Her-2 positive. Importantly, in this study the Her-2 status was divided into negative, low positive and positive, according to a specific score (27), which differed from the criteria used by other groups (14, 44). Middleton et al grouped cases showing 2+ and 3+ positive staining together as "positive cases". Another group evaluated 2+ cases by FISH (28). Most studies have shown similar frequencies of Her-2 positive tumors in PABC and non-PABC populations.

Triple-negative (TN) carcinomas tend to occur in younger women and are associated with poor clinicopathologic features such as large tumor size, high-grade tumors, increased risk of distant recurrence, and death (55-57). Only one study evaluated the frequency of TN tumors in PABC, and concluded that it was more likely to be triple-negative. In contrast to the frequency of TN carcinomas in the general population, which ranges from 12% to 17% (55-57), TN carcinomas corresponded to 34.2% of PABC cases (33), which could explain the poor prognosis of PABC. Interestingly, the association with triple-negativity and hormone receptor negativity was particularly strong in women who were pregnant or lactating, or within 6 months of pregnancy at diagnosis. The authors suggested that the poor biologic features of PABC form a decrescent continuum through pregnancy, lactation and the early postpartum period. However, this contrasts with the worse outcome reported for postpartum cases (19, 22, 32, 36-39), a reason why some discourage postponing treatment to the postpartum period (35). Others have reported no difference in overall survival between pregnant and postpartum patients (13, 48).

Positivity for p53 was evaluated by six studies, and ranged from 11% to 58%. Only one group concluded that women diagnosed within 2 years of postpartum were more likely to have p53 positive tumors (19). The authors suggested that early tumors or preneoplastic cells harboring p53 mutations may be selected for enhanced growth via exposure to pregnancy-related

hormones. Criteria for determining positivity were based on a specific score (30), finding nuclear staining in more than 5% (45) or 10% of tumor cells (19), and were not clearly defined by three groups (29, 33, 43).

EGFR (epidermal growth factor receptor) positivity was evaluated by only one group, and was present in 33% of the patients (30). EGFR has been reported to be positive in 45% of premenopausal patients with breast cancer (58) and more likely positive in younger patients (59, 60). EGFR is also considered a marker of basal-like carcinomas (61), which are usually associated with young age and triple-negativity (62). Thus, the high EGFR positivity seen in PABC may just reflect the young age of the patients and high incidence of hormone-negative tumors. However, EGFR has been demonstrated to be significantly more likely positive in PABC cases (30).

Discussion

PABC are typically high-grade, hormone-negative invasive ductal carcinomas. Thus, the pathologic features of PABC are similar to those of nonpregnant young women. However, when controlled for age, PABC seems to have even more aggressive tumor biology, with a higher frequency of grade 3, ER, PR and triple-negative tumors. Importantly, the 3 studies that included women diagnosed within 2 years of postpartum demonstrated an increased likelihood for being ER negative (25, 33), PR negative (19, 33), grade 3 (19, 33), and p53 positive (19) tumors. Thus, it seems that specifically the postpartum population is more likely to harbor tumors with poorer histopathologic features. This only partly explains the unfavorable outcome of postpartum cases, since its higher mortality has been shown to be only minimally accounted for by tumor grade and PR status, and not affected at all by the ER (25) or p53 status (19). However, another group has confirmed that ER and PR-negativity confer a worse prognosis on multivariable analysis (37).

Women submitted to fertility therapy (FT), which has been associated with a transient increase in breast cancer risk (63), also tend to develop ER negative, high-grade tumors. Interestingly, the reported adverse prognostic features in patients exposed FT, such as locally advanced disease, metastatic disease and poor clinical outcome, are mostly notable when they are diagnosed within 2 years of an FT cycle (31). However, unlike a recent labor, FT itself has not been proven to be an independent negative marker (31).

The high frequency of triple-negative phenotype in PABC raises the possibility that many are basal-like carcinomas, known to be of aggressive behavior. The high percentage (33%) and

increased likelihood for EGFR positivity further supports this idea (30). These findings suggest that PABC may in fact be an inherently more aggressive disease.

A recent study has reported a lower expression of hormone signaling genes, such as *ERα*, *PGR*, and *ERBB2* in normal breast tissue from parous women, particularly within 2 years of the last pregnancy, compared with nulliparous women (64). This may partly explain the significantly lower hormone positivity in tumors of postpartum women, and the protective effect of pregnancy on the risk of developing hormone-positive tumors. In other words, if the *ERα*-positive cell population is reduced in parous women, the potential tumor development and progression driven by estrogen should also be reduced (64). Alternatively, having an overrepresented *ERα*-negative cell population might also increase the likelihood of developing ER-negative tumors. What causes the downregulation of hormone-related genes after a pregnancy is unknown. Bonnier et al [24] suggested that the high rate of hormone negativity in PABC could be due to the processing and downregulation of re-induction of receptors induced by the high serum levels of estrogen and progesterone.

A recent study has shown that miR-21, a microRNA frequently overexpressed in solid tumors (65, 66) that down-regulates tumor suppressor-genes such as PTEN and BCL2 (67-69), is overexpressed in the tumor and normal breast tissue of PABC patients (34). In addition, a significantly higher expression of miR-21 was found in normal breast tissue of PABC cases, when compared to normal breast from non-PABC controls. The authors suggested that differential miRNA expression may occur in the physiological reorganization of the breast during pregnancy, lactation or involution and that may modify gene expression and contribute to tumor development and progression.

A worrisome observation raised by Mathelin et al is that PABC hormone-dependence may be underestimated by using ER and PR immunohistochemistry. When testing the immunohistochemical expression of pS2-TTF1, an estrogen-regulated protein which had been routinely used in the early 90s and shown to be predictive of hormone therapy response (70), they obtained a much larger proportion of positive cases (57.5%) when compared to ER (47.5%) and PR (32.5%) immunostains. Considering that hormone therapy is largely avoided or delayed in PABC patients and only applied to hormone positive tumors, negative immunohistochemical results may prevent patients from receiving a potentially beneficial therapy. Testing for pS2-TTF1 may thus be particularly useful in PABC cases.

Conclusion

As a noncoded and uncommon disease, PABC remains a poorly understood entity, typically described in retrospective studies, and suboptimally defined due to the potential biases inherent to such studies. Another problem is the large variation in study designs. The solution to this problem is the development of prospective studies, using more uniform case and control groups, and pathologic parameters.

Table 1 Case series of PABC

Study and country	Sample	Histologic type	Histologic grade	Immunohistochemical results
Berry et al, 1999, USA	PABC: 22 (pregnant patients only)	Invasive ductal (%): 100	II (%): 24 III (%): 76	ER positive cases (%): 10 PR positive cases (%): 10 ER and PR positive (%): 15 ER and PR negative (%): 65
Middleton et al, 2003, USA	PABC: 39 (diagnosed during pregnancy or within 12 days of postpartum)	Invasive ductal (%): 100	I or II (%): 16 III (%): 84	ER positive cases (%): 28 PR positive cases (%): 24 ER and PR positive (%): 16 Her-2 positive cases (%): 28 p53 positive cases (%): 48
Ives et al, 2004, Australia	PABC: 148 (during pregancy or within one year of delivery)	Invasive ductal (%): 85.1 Invasive lobular (%): 4.7 In situ (%): 2.1 Other (%): 7.4 Unknown (%): 0.7	I (%): 2.8 II (%): 15.9 III (%): 44.8 Unknown (%): 36.6	ER positive cases (%): 35.1 Unknown (%): 31.1
Porta et al, 2004, Italy	PABC: 7 (during pregnancy or within 1 year of postpartum)	Ductal (%): 100	Not reported	ER/PR positive cases (%): 28.5 Her-2 positive cases (%): 60 [a]
Gentilini et al, 2005, Italy	PABC: 21 (during pregnancy) and 17 (within one year of delivery while lactating)	pregnant: Invasive ductal (%): 95 Invasive cribriform (%): 5 postpartum: Invasive ductal (%): 100	I (%): 13.3 II + III (%): 86.7 I (%): 0 II + III (%): 100	ER and PR positive cases (%): 8 ER or PR positive cases (%): 7 Her-2 positive cases (%): 23.8 ER and PR positive cases (%): 6 ER or PR positive cases (%): 2 Her-2 positive cases (%): 14.3

Bodner-Adler et al, 2007, Austria	PABC: 5 (during pregnancy)	Invasive ductal (%): 40 Invasive lobular (%): 40 Mucinous (%): 20	I (%): 0 II (%): 20 III (%): 80	ER positive cases (%): 40 PR positive cases (%): 40 Her-2 positive cases (%): 20 p53 positive cases (%): 40
Cardonick et al, 2010, USA	PABC: 130 (during pregnancy or within 6 weeks of delivery)	Invasive ductal (%): 75.8 Invasive lobular (%): 5.6 Medullary (%): 1.6 unknown (%): 12.1	not reported	ER positive cases (%): 42 PR positive cases (%): 35 Her-2 positive cases (%): 25
Rouzier et al, 2011, France	PABC: 48 (during pregnancy or within one year of delivery)	Invasive ductal (%): 96 Invasive lobular (%): 2 Other (%): 2	I (%): 2 II (%): 31 III (%): 67	Hormonal receptor positive cases (%): 46 Her-2 positive cases (%): 19 Unknown Her-2 status: 31

PABC: pregnancy-associated breast carcinoma; ER: estrogen receptor; PR: progesterone receptor

[a] determined by fluorescent in situ hybridization only

Table 2 Studies that compared clinicopathologic characteristics of PABC with non-PABC

Study and country	Sample	Histologic type	Histologic grade	Immunohistochemical results	Statistically significant differences
Elledge et al, 1993, USA	PABC: 15 (pregnant patients only)	Not reported	Not reported	ER positive cases (%): 50 PR positive cases (%): 83.3 Her-2 positive cases (%): 58	PABC more likely to be Her-2 positive
	Control: 411 (non-pregnant)	Not reported	Not reported	ER not performed PR not performed Her-2 positive cases (%): 16	
Bonnier et al, 1997, France	PABC: 154 (pregnant or within 6 months postpartum)	Ductal (%): 88.2 Lobular (%): 8.2 Medullary (%): 2.7	I (%): 12 II (%): 48 III (%): 40	ER positive cases (%): 46.7 [a] PR positive cases (%): 34.2 [a]	None
	Control: 308	Ductal (%): 87.6 Lobular (%): 6 Medullary (%): 4.2	I (%): 17.9 II (%): 46 III (%): 36.1	ER positive cases (%): 53.9 PR positive cases (%): 65.8	
Ibrahim et al, 2000, Saudi Arabia	PABC: 72 (pregnant patients only)	Invasive ductal (%): 95.8 Mucinous (%): 4.1	I (%): 0 II (%): 40 III (%): 60	ER positive cases (%): 33 PR positive cases (%): 69	None
	Control: 216 (non-pregnant)	Invasive ductal (%): 97.2 Medullary (%): 2.7	I (%): 3 II (%): 37 III (%): 60	ER positive cases (%): 43 PR positive cases (%): 46	
Shousha, 2000, England	PABC: 14 (during pregnancy, or within 4 months of delivery, or during lactation or within 2 months of stopping lactation)	Invasive ductal (%): 71 Mucinous (%): 14 Invasive lobular (%): 7 DCIS (%): 7 Cancerization of lobules(%): 79	I (%): 0 II (%): 20 III (%): 80	ER positive cases (%): 50 PR positive cases (%): 30 Her-2 positive cases (%): 44 p53 positive cases (%): 11	PABC less likely to be ER positive and more likely to be grade III, and to have cancerization of lobules
	Control: 13	Invasive ductal (%): 69	I (%): 11	ER positive cases (%): 91	

		Mucinous (%): 0 Invasive lobular (%): 15 DCIS (%): 15 Cancerization of lobules (%): 15	II (%): 56 III (%): 33	PR positive cases (%): 64 Her-2 positive cases (%): 18 p53 positive cases (%): 18	
Daling et al, 2002, USA	PABC: 60 (diagnosed within 2 years of postpartum)	Invasive ductal (%): 100	I (%): 4.6 II (%): 31.8 III (%): 63.6	ER positive cases (%): 50 PR positive cases (%): 45 Her-2 positive cases (%): 50 p53 positive cases (%): 56.7	Tumors diagnosed within 2 years of postpartum were more likely to be PR negative, p53 positive, and of high histological grade
	Control: 214 (nulliparous women only)	Invasive ductal (%): 100	I (%): 19.1 II (%): 40.3 III (%): 40.7	ER positive cases (%): 61 PR positive cases (%): 64.8 Her-2 positive cases (%): 45.5 p53 positive cases (%): 32.7	
Aziz et al, 2003, Pakistan	PABC: 24 (diagnosed during pregnancy or within 1 year after pregnancy)	Invasive ductal (%): 100	Not reported	ER/PR positive cases (%): 29 Her-2 positive cases (%): 42 p53 positive cases (%): 58 EGFR positive cases (%): 33	PABC more likely to be ER/PR negative and EGFR positive
	Control: 48	Not reported	Not reported	ER/PR positive cases (%): 59 Her-2 positive cases (%): 44 p53 positive cases (%): 65 EGFR positive cases (%): 19	
Siegelmann-Danieli et al, 2003, Israel	PABC: 23 tumors from 22 patients(during pregnancy or within 1 year of delivery)	not reported	III (%): 68	ER and/or PR negative cases (%): 53 [b]	PABC more likely to be ER and/or PR negative and grade III
	Control: 192 patients with 201 tumors	not reported	III (%): 32	ER and/or PR negative cases (%): 25	
Reed et al, 2003, Norway	Pregnancy group: 20 (birth up to 9 months after diagnosis)	Ductal (%): 80 In situ (%): 0 Lobular (%): 5 Other (%): 15	I (%): 10 II (%): 40 III (%): 50	ER positive cases (%): 22 PR positive cases (%): 33 Her-2 positive cases (%): 44	None

	Lactation group: 102 (birth 1-12 months before diagnosis)	Ductal (%): 92 In situ (%): 5 Lobular (%): 2 Other (%): 1	I (%): 3 II (%): 42 III (%): 55	ER positive cases (%): 37 PR positive cases (%): 23 Her-2 positive cases (%): 44	
	Control: 51 (birth more than 9 months after diagnosis)	Ductal (%): 86 In situ (%): 5 Lobular (%): 0 Other (%): 9	I (%): 13 II (%): 40 III (%): 48	ER positive cases (%): 44 PR positive cases (%): 42 Her-2 positive cases (%): 28	
Phillips et al, 2004, Australia	PABC: 80 (diagnosed within 2 years of postpartum)	Not reported	I (%): 6 II (%): 29 III (%): 56 Unknown (%): 9	ER positive cases (%): 41 Unknown (%): 1 PR positive cases (%): 56 Unknown (%): 1	PABC more likely to be ER negative
	Control: 202 (nulliparous women only)	Not reported	I (%): 11 II (%): 34 III (%): 47 Unknown (%): 8	ER positive cases (%): 57 Unknown (%): 6 PR positive cases (%): 61 Unknown (%): 6	
Mathelin et al, 2008, France	PABC: 40 (during pregnancy or within one year postpartum)	Ductal (%): 82.5 Lobular (%): 12.5 Medullary (%): 5 Tubular (%): 0	I (%): 17.5 II (%): 22.5 III (%): 55 Not determined (%): 5	ER positive cases (%): 47.5 PR positive cases (%): 32.5	PABC more likely to be ER and PR negative
	Control: 61	Ductal (%): 92 Lobular (%): 7 Medullary (%): 0 Tubular (%): 1	I (%): 21 II (%): 36 III (%): 41 Not determined (%): 2	ER positive cases (%): 69 PR positive cases (%): 61	
Rodriguez et al, 2008, USA	PABC: 797 (during pregnancy or within one year postpartum)	Comedocarcinoma (%): 4 Invasive ductal (%): 78.2 Lobular (%): 1.1	not reported	ER positive cases (%): 29.7 Unknown (%): 31 PR positive cases (%): 27.5	PABC more likely to be ER and PR negative

		Other (%): 16.7		Unknown (%): 32.8	
	Control: 4,177	Comedocarcinoma (%): 3.7 Invasive ductal (%): 75.4 Lobular (%): 3.2 Other (%): 17.7	not reported	ER positive cases (%): 37.5 Unknown (%): 34.5 PR positive cases (%): 35.2 Unknown (%): 36.4	
Halaska et al, 2009, Czech Republic	PABC: 32 (during pregnancy or within one year after delivery)	Ductal (%): 97 Lobular (%): 0 Ductolobular (%): 3	I (%): 6.2 II (%): 46.9 III (%): 46.9	ER positive cases (%): 36.7 PR positive cases (%): 36.7 Her-2 positive cases (%): 33.3	PABC more likely to be ER negative
	Control: 32	Ductal (%): 97 Lobular (%): 3 Ductolobular (%): 0	I (%): 9.3 II (%): 43.7 III (%): 46.9	ER positive cases (%): 64.5 PR positive cases (%): 54.8 Her-2 positive cases (%): 35.5	
Beadle et al, 2009, USA	PABC: 104 (during pregnancy or within one year after pregnancy)	Invasive ductal (%): 93.3 Invasive lobular (%): 2.9 Invasive mixed (%): 1.9 Unknown/other (%): 1.9	Not reported	ER positive cases (%): 34.6 Unknown (%): 16.3 PR positive cases (%): 28.8 Unknown (%): 18.3	PABC more likely to be PR negative (borderline significance)
	Control: 564	Invasive ductal (%): 93.1 Invasive lobular (%): 2 Invasive mixed (%): 2.1 Unknown/other (%): 2.8	Not reported	ER positive cases (%): 41.1 Unknown (%): 19.3 PR positive cases (%): 33 Unknown (%): 26.6	
Moreira et al, 2010, Brazil	PABC: 87 (during pregnancy or up to 12 months after parturition or abortion)	Ductal/lobular (%): 90.8 Other (%): 9.2	I or II (%): 23 III (%): 25.3 unknown (%): 51.7	ER or PR positive cases (%): 44.8 Not determined (%): 41.4	None
	Control: 252	Ductal/lobular (%): 92.1 Other (%): 7.9	I or II (%): 24.2 III (%): 32.1 unknown (%): 43.7	ER or PR positive cases (%): 34.5 Not determined (%): 50	
Pilewskie et al, 2011, USA	PABC: 38 (during pregnancy or within 0-2 years of the last	Not reported	I or II (%): 24	ER positive cases (%): 55	PABC more likely to be grade III, ER and PR negative and triple-negative

	pregnancy)		III (%): 76	PR positive cases (%): 47 Her-2 positive cases (%): 25 p53 positive cases (%): 31 Triple-negative cases (%): 34	
	Control: 114 (nulliparous only)	Not reported	I or II (%): 65 III (%): 35	ER positive cases (%): 85 PR positive cases (%): 75 Her-2 positive cases (%): 12.5 p53 positive cases (%): 22 Triple-negative cases (%): 11.5	
Walter et al, 2011, USA	PABC: 25 (during pregnancy or within one year of delivery or during lactation)	Invasive ductal (%): 100	II (%): 32 III (%): 68	ER positive cases (%): 19 PR positive cases (%): 38 Her-2 positive cases (%): 6	None
	Control: 10	Invasive ductal (%): 100	II (%): 28 III (%): 72	ER positive cases (%): 30 PR positive cases (%): 30 Her-2 positive cases (%): 30	
Murphy et al, 2011, USA	PABC: 99 (during pregnancy or within one year of delivery)	Not reported	0 (%): 3 I (%): 2 II (%): 11 III (%): 84	ER positive cases (%): 39 PR positive cases (%): 26 Her-2 positive cases (%): 20	PABC more likely to be grade III, ER negative, and PR negative
	Control: 186	Not reported	0 (%): 6 I (%): 4 II (%): 25 III (%): 65	ER positive cases (%): 65 PR positive cases (%): 55 Her-2 positive cases (%): 19	

[a] evaluated by enzyme immunoassay
[b] evaluated by immunohistochemistry or dextran-coated charcoal methods
DCIS: ductal carcinoma in situ
ER: estrogen receptor
PR: progesterone receptor

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3. MÉTODOS

3.1 Área

O estudo foi realizado no Hospital de Câncer de Pernambuco (HCP), o qual é um hospital referência para pacientes portadores de câncer do Norte e Nordeste do Brasil. Está localizado na Av. Cruz Cabugá, número 1597, Santo Amaro, Recife, Pernambuco. É dedicado à prevenção, diagnóstico e tratamento do câncer e realiza cerca de 470 cirurgias mensais. O Departamento de Patologia do HCP avalia cerca de 5000 espécimes por mês, incluindo biópsias, peças cirúrgicas, citologia ginecológica e citologia por punção aspirativa com agulha fina.

3.2 População

3.2.1 Seleção da amostra

O universo de casos (518) de câncer de mama diagnosticados em mulheres com idade menor ou igual a 35 anos no período de 1995 a 2011 foram selecionados.

3.2.2 Critérios de inclusão e exclusão

Dessa população (518 casos), foram selecionados todos os casos diagnosticados durante a gravidez ou até dois anos do último parto (39 casos), e os casos ocorridos em nulíparas à ocasião do diagnóstico (32 casos). Foram excluídos os casos ocorridos em mulheres com mais de 35 anos e aqueles sem informação sobre paridade. Dentre o grupo com idade menor ou igual a 35 anos, foram excluídos os casos ocorridos em mulheres não grávidas cujo diagnóstico foi estabelecido após 2 anos do último parto.

3.3. Período de Referência

O estudo foi realizado no período de agosto de 2011 a dezembro de 2011.

3.4. Desenho e tipo de estudo

O estudo é analítico observacional (caso-controle).

Trata-se de um estudo analítico, pois envolve a avaliação aprofundada de informações disponíveis na tentativa de explicar o contexto de um fenômeno e é subordinado a uma ou mais questões científicas, as "hipóteses", que relacionam eventos: uma suposta "causa" e um dado "efeito", ou "exposição" e "doença", respectivamente. Procura esclarecer uma dada associação entre uma exposição, em particular, e um efeito específico.

Trata-se de estudo observacional, pois os indivíduos da amostra não foram designados aos grupos por processo aleatório, mas já estavam classificados nos respectivos grupos, no início da pesquisa. Ou seja, a inclusão de indivíduos em um grupo caso ou grupo controle está fora do controle do pesquisador.

3.5. Método de coleta

3.5.1 Avaliação clinicopatológica

A partir do banco de dados do Departamento de Patologia do HCP, foram levantados todos os casos de câncer de mama diagnosticados em mulheres até os 35 anos de idade, ao longo de um período de 17 anos (1995-2011). Foram recolhidos, em ficha-padrão elaborada especificamente para essa pesquisa (APÊNDICE), os dados disponíveis no prontuário clínico sobre a ocorrência do diagnóstico durante a gravidez ou até dois anos do último parto ou a nuliparidade ao diagnóstico. Na ausência dessas informações no prontuário, foi realizada entrevista telefônica, através do número telefônico cadastrado no sistema do hospital. Nos casos que foram diagnosticados durante a gravidez ou até dois anos do último parto, foram obtidos dados sobre: a idade que tinha quando engravidou do primeiro filho, a idade do último filho quando recebeu o diagnóstico ou o tempo decorrido de gravidez ao diagnóstico, a paridade, a amamentação, o tipo de tratamento recebido e a sobrevida (os dois últimos também foram obtidos das pacientes nulíparas ao diagnóstico). Foram então consultados os

laudos anatomopatológicos e imunoistoquímicos do Departamento de Patologia para obtenção de dados como: tipo de cirurgia, lateralidade da mama, tipo e grau histológico do tumor, tamanho do tumor, *status* das margens cirúrgicas, *status* dos linfonodos, ocorrência de metástases à distância, estadiamento e resultados do painel prognóstico. Posteriormente, foram re-examinadas todas as preparações histológicas coradas pela hematoxilina e eosina para reavaliação e confirmação do tipo histológico, graduação histológica, *status* das margens cirúrgicas, *status* dos linfonodos, estadiamento e para determinação de eventuais dados não mencionados no relatório inicial. Foi então selecionado um bloco representativo do tumor de cada paciente, priorizando os blocos contendo tecido com melhor preservação e, quando possível, incluindo controle interno (parênquima mamário não-tumoral) para repetição do painel imunoistoquímico prognóstico (RE, RP, Her-2). Nos casos que revelaram negatividade para os três marcadores, foi realizado estudo imunoistoquímico com marcadores basais. Finalmente, os dados levantados foram digitados e organizados sistematicamente em banco de dados para avaliação e correlação das diversas variáveis estudadas e posterior análise estatística.

3.5.2 Avaliação imunoistoquímica

Para o exame imunoistoquímico, cortes histológicos de 4µm de espessura foram colocados em lâminas histológicas silanizadas e postos em estufa a 58°C por 12 horas. Após essa etapa, foram desparafinadas com xileno, hidratadas em soluções decrescentes de álcool e lavadas em água destilada. Depois foram submetidas à recuperação antigênica sendo incubadas em tampão citrato buffer (DBS) pH 6,0 (para RP e Her-2) e pH 9,0 (para RE) sendo então colocados na câmara pascal a 120°C por 40 minutos (RE e RP) e no steamer por 20 minutos (Her-2). A recuperação antigênica para CK5/6 foi automatizada (PT link) e para EGFR utilizou-se pronase a 0,1%. Após inibição da peroxidase endógena (lavagem com água destilada e tampão citrato) receberam os reagentes (anticorpos) por 20 minutos. Os anticorpos utilizados foram RE (clone SP1, Spring), RP (clone SP4.2, Spring), Her-2 (c-erbB-2, DAKO), CK5/6 (clone D5/16 B4, DAKO) e EGFR (clone 31G7, Zymed). Procedeu-se à lavagem em água destilada, contracoloração com hematoxilina, desidratação, e montagem para posterior leitura.

3.6. Definição das variáveis

3.6.1 Variável Dependente

Agressividade do câncer de mama associado à gravidez: potencial metastático dos tumores diagnosticados durante a gravidez ou até dois anos do último parto.

3.6.2 Variável Independente

- Grau histológico: Conjunto de características arquiteturais (formação tubular), nucleares (diâmetro nuclear) e índice mitótico da neoplasia, de acordo com o Sistema Scarf-Bloom-Richardson modificado por Ellston-Ellis.
- Tipo histológico: categorização dos tumores baseada na histologia (arquitetura e citologia), descrita na Classificação da Organização Mundial da Saúde dos Tumores da Mama e Órgãos Genitais Femininos, 2003.
- Tamanho do tumor: mensuração em centímetro da maior dimensão da neoplasia, após obtenção de três medidas: altura, largura e profundidade.
- Comprometimento axilar: presença de tumor metastático nos linfonodos axilares.
- Estadiamento: Estratificação prognóstico-terapêutica baseada no tamanho tumoral e presença de metástases linfonodais e à distância de acordo com o Manual de Estadiamento do Cancer do American Joint Committte on Cancer, 7ª edição, 2010.
- Status das margens cirúrgicas: a presença de carcinoma invasivo ou *in situ* na margem de ressecção cirúrgica pintada será classificada como “margem positiva” e a ausência como “margem negativa”.
- Marcadores prognósticos (RE, RP, Her-2): Anticorpos avaliados nas células tumorais pelo exame imunoistoquímico, cuja presença ou ausência de expressão tem valor prognóstico-preditivo comprovado. RE e RP serão considerados positivos se expressos por pelo menos 1% das células neoplásicas; Para o Her-2 escore 0 e 1 serão considerados negativos, 2 indeterminado e 3 positivo; os casos indeterminados serão enviados para FISH (hibridização *in situ* fluorescente) para determinação da presença ou ausência de amplificação do gen Her-2.
- Marcadores basais (EGFR, CK5/6): Anticorpos tipicamente expressos em células basais/mioepiteliais normais do parênquima mamário, avaliados através de exame

imunoistoquímico. Ambos foram considerados positivos quando se observou coloração de membrana e/ou citoplasma, fraca ou forte, em qualquer porcentagem de células tumorais (NIELSEN ET AL, 2004).

- Idade: Tempo de vida em anos no momento do diagnóstico.
- Idade ao primeiro filho: Tempo de vida em anos quando engravidou do primeiro filho.
- Tempo de gravidez ao diagnóstico: tempo de gravidez em semanas decorrido até o dia em que recebeu o diagnóstico de câncer de mama.
- Idade do último filho ao diagnóstico: tempo de vida, em anos, do filho mais novo na ocasião do diagnóstico.
- Paridade: número de gestações que progrediram por mais de 20 semanas.
- Amamentação (exclusiva ou não e por quanto tempo): aleitamento materno realizado de maneira exclusiva ou não, por quanto tempo em meses em todas as gestações.
- Tipo de tratamento recebido: modalidade terapêutica recebida (radioterapia, quimioterapia neoadjuvante ou adjuvante, tipo de cirurgia - se segmentectomia, mastectomia, adenomastectomia subcutânea, com ou sem dissecção axilar, e com ou sem biópsia de linfonodo sentinela - hormonioterapia, trastuzumab).

3.7. Método de análise

Os dados foram analisados pelo pesquisador e seu orientador, anotados e computados em formulário específico (APÊNDICE). Os dados categóricos foram resumidos em frequências absolutas e percentual. As comparações entre os grupos de nulíparas e PABC foram realizadas através do teste exato de Fisher ou teste qui-quadrado. A idade foi resumida através da média e desvio padrão. A comparação da idade média entre os dois grupos foi realizada utilizando-se o teste t Student para amostras independentes. A análise estatística foi realizada com o Software Stata 12.1 SE.

3.8. Considerações éticas

Toda a apreciação metodológica do presente estudo atendeu às normas éticas, de acordo com a Resolução nº 196 de 10 de Outubro de 1996 CNS (Conselho Nacional de Saúde) sobre pesquisas científicas desenvolvidas com seres humanos. Para tal, o projeto foi

submetido à avaliação pelo Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal de Pernambuco, para análise e consentimento para a realização da pesquisa. Foi solicitada autorização, ao chefe do departamento de patologia do HCP, para uso do material biológico arquivado. Também foi encaminhado ao Comitê de Ética em Pesquisa do Hospital de Câncer de Pernambuco para apreciação e parecer. Não ocorreu qualquer agressão à integridade física dos pacientes, pois todas as amostras biológicas (blocos de parafina e lâminas) a serem estudadas foram provenientes do arquivo já existente no Departamento de Patologia do HCP.

Resultados (artigo original)

Pathologic features and molecular phenotype of pregnancy-associated breast carcinoma

Abstract

Background. Pregnancy-associated breast cancer (PABC), defined as cancer diagnosed during pregnancy or within one year of delivery, is a distinctively aggressive disease with high mortality. However, studies have shown that pregnancy is not an independent predictor of mortality, and its poor prognosis is likely due to delayed diagnosis and high frequency of hormone receptor-negative tumors. In contrast, a recent labor (up to 2 years prior to diagnosis) has been demonstrated to be an independent prognostic marker. Data are conflicting regarding whether PABC has distinct pathologic features, and many studies show a similar prevalence of hormone receptor-negative and Her-2 positive tumors, when compared to non-PABC. Basal-like carcinomas are an aggressive subtype of breast carcinoma, being more common in young women. Surprisingly no study to date has evaluated the prevalence of basal-like carcinomas or the distribution of molecular phenotypes in PABC**. Methods.** We examined the pathologic features, prevalence of carcinomas with basal differentiation and distribution of molecular phenotypes in PABC, using routine immunohistochemical markers and grade as surrogates. **Results.** We found a greater proportion of PABC cases had either luminal A or triple-negative tumors (35.9% each). Overall, tumors with basal differentiation comprised 18.4% of PABC. Her-2 and Luminal B types corresponded to 12.3% and 17.9% of tumors, respectively. Compared to carcinomas from nulliparous women, no significant differences were seen with regard to histologic type, hormone receptor positivity, Her-2 status, or molecular phenotype. However, in contrast to previous studies, nulliparous women were more likely to harbor grade 3 tumors. **Conclusions.** Both PABC and non-PABC are enriched for tumors with poor prognostic features.

Keywords: breast cancer, pregnancy, immunohistochemistry, molecular phenotypes

Although pregnancy has been classically associated with a protective effect against breast cancer, epidemiologic studies have demonstrated a still under-recognized transient increase in risk shortly after pregnancy,[1-7] which peaks around the 6th year of postpartum.[8] Pregnancy-associated breast cancer (PABC) is defined by most

authors as breast cancer diagnosed during pregnancy or within one year after delivery. However, some include patients diagnosed up to 5 years of postpartum.[9]

Albeit uncommon, PABC is not a rare condition. Seven to 15% of premenopausal breast cancers occur during pregnancy.[10] Furthermore, breast cancer is the most common malignancy diagnosed during pregnancy and the postpartum period, occurring in 1 in 3000 pregnant women.[11] Because maternal age at first pregnancy continues to rise around the world, the incidence of PABC is expected to increase.[12-14]

PABC is associated with a poor outcome, likely due to delayed diagnosis, young age of the patients, and lack of standardized treatment. However, the majority of studies have concluded that pregnancy is not an independent prognostic factor when adjusted for stage. In addition, pregnancy subsequent to a breast cancer diagnosis does not seem to adversely affect the prognosis,[14] and termination of pregnancy does not appear to improve outcome.[10,15-18] In contrast, breast cancer diagnosed in the postpartum period, especially within 5 years of delivery, has been shown to be an independent predictor of mortality,[19-22] and associated with adverse prognostic profiles.[20,23-27] The prognosis is even worse when diagnosed within 2 years of postpartum.[19,25] The reasons for that are uncertain, but it could be that pregnancy and/or lactation select for a specific cancer phenotype of increased aggressiveness.

Data regarding differences in histologic features and hormone receptor status between PABC and non-PABC are conflicting. Yet, it is well-known that women with PABC have a high prevalence of hormone receptor-negative tumors, as do young women in general. Whether PABC has distinct pathologic features is still unknown.

Gene expression microarray-based studies have proposed five molecular breast cancer subtypes: luminal A, luminal B, normal breast-like, Her-2 and basal-like.[28,29] The latter group has characteristic pathologic features, including high tumor grade, hormone receptor and Her-2 negativity,[30] and expression of CK5/6 and/or EGFR.[31,32] Basal-like carcinomas are more prevalent in young, African-American, and Latin women,[33] and are associated with a worse outcome.[29] Because PABC are typically high-grade, hormone receptor-negative, aggressive carcinomas that affect young women, a high prevalence of tumors with basal differentiation is expected. To the best of our knowledge there are no data regarding basal cell differentiation using established basal markers in PABC. The aim of this study was to define the molecular type distribution and occurrence of basal differentiation in PABC using routine pathologic parameters (grade, hormone receptor, and Her-2 status) and basal markers, and examine whether it has distinct pathologic features.

Patients and methods

After approval from the Ethical Committee of Hospital de Cancer de Pernambuco, we used a retrospectively collected database to identify women with breast cancer diagnosed between January 1, 1995, and December 31, 2011. Women were included if they were diagnosed with invasive breast carcinoma at age 35 years or less, and information on parity and date of last labor were available. PABC cases were defined as women diagnosed during pregnancy or within 2 years of delivery. Nulliparous women 35 years or younger were used as the reference group. We recorded age at diagnosis, histologic type, histologic grade, tumor size, number of positive lymph nodes, and tumor marker status (hormone receptors and Her-2). Histologic type was reviewed according to the most recent WHO Classification.[34] Histologic grade was determined according to standard guidelines.[35] All histologic slides were retrieved and reviewed, and representative areas were selected for repeat immunohistochemical stains. Immunostains were performed on core biopsies, lumpectomy and mastectomy specimens.

Four µm sections were re-stained with ER (SP1, DAKO), PR (SP2.4, DAKO) and Her-2 (c-erbB-2, DAKO). Triple-negative (TN) cases were stained with CK5/6 (D5/16 B4, DAKO) and EGFR (31G7, Zymed). Detection was by Envision FLEX (DAKO) with diaminobenzidine chromogen as per routine protocol.

Staining results were assessed by two pathologists. For ER (estrogen receptor) and PR (progesterone receptor), a result was considered positive when at least 1% of tumor cells showed nuclear staining.[36] Her-2 scores were determined according to standard guidelines.[37] Tumors showing undetermined (2+) Her-2 scores were sent for FISH analysis. Cytokeratin 5/6 and EGFR were scored positive if any (weak or strong) cytoplasmic and/or membranous invasive carcinoma cell staining was observed. Using tumor grade, hormone receptor status and Her-2 overexpression as surrogates,[38] we defined molecular subtypes as follows: luminal A (ER and/or PR +, Her-2 -, grade 1-2 tumors), luminal B (ER and/or PR +, Her-2 -, grade 3 or ER and/or PR + and Her-2 + tumors), Her-2 type (ER/PR -, Her-2 + tumors), triple-negative (ER/PR/Her-2 – tumors), and tumors with basal differentiation (ER/PR/Her-2 -, CK5/6 and/or EGFR +).

Results were analyzed using statistical software STATA 12.1 SE. The relationship between clinicopathologic characteristics, immunohistochemical results, and molecular phenotypes was assessed using chi-square and Fisher´s exact tests. A p-value <0.05 defined statistical significance.

RESULTS

Clinicopathologic features

Of 518 women aged ≤ 35 years diagnosed with invasive breast carcinoma between 1995 and 2011 at Hospital de Cancer de Pernambuco, and known date of last labor, 39 (7.5%) were diagnosed during pregnancy or within two years of delivery, and 32 (6.2%) were nulliparous at diagnosis. Ten (1.9%) patients were pregnant at diagnosis. Clinical characteristics are summarized in Table 1. The mean age at diagnosis was 30.2 years (range 21–35 years) for the PABC group, and 30.9 years (range 25–35 years) for the nulliparous group (p= 0.334). The most commonly evaluated specimen in both groups was mastectomy with lymph node dissection (74.4% and 65.6% in PABC and non-PABC, respectively). Lumpectomy specimens were significantly more common in the nulliparous group (21.9% versus 5.1%; p=0.042).

Invasive ductal carcinoma (IDC) was the commonest type in both groups (94.9%, and 93.8%, of PABC and non-PABC cases, respectively). Two tumors in each group were classified as pure invasive micropapillary carcinomas (IMPC), all of which were diagnosed on either lumpectomy or mastectomy specimens. The two IMPC in the PABC group were diagnosed in pregnant patients (Fig. 1). After the identification of IMPC cases, we searched for foci of micropapillary growth pattern in cases otherwise diagnosed as IDC. We found that 4 additional PABC and 5 non-PABC cases had a minor micropapillary component (i.e., comprising less than 50% of the tumor volume). IDC with medullary features was identified in one PABC case and two nulliparous patients. No invasive lobular carcinomas were seen in either group. IDC with apocrine differentiation and IDC with focal squamous differentiation were each diagnosed in one nulliparous patient.

Only 7.7% of PABC cases had grade 1 tumors. None of the nulliparous patients harbored grade 1 tumors. Nulliparous women were more likely to have grade 3 carcinomas (81.2% versus 53.8%; p=0.011).

Most patients harbored either T2 or T3 tumors (83.7% and 78.1% in PABC and non-PABC, respectively). Only 8.1% and 15.3% of tumors were classified as T1 in PABC and reference groups, respectively. Positive lymph nodes were present in 69.5% and 63.3% of PABC and non-PABC cases, respectively. Half of PABC patients had stage III disease (50%), while most nulliparous women had stage II disease (54.8%). Metastases to ovaries and to bone were seen in one and two PABC cases, respectively. Four nulliparous patients had bone metastases at diagnosis. One nulliparous patient had bone, lung and brain metastases, and one had bone and brain metastases. Although distant metastases were more common in nulliparous women (18.8% versus 8.1%) this difference did not reach statistical significance.

No significant differences in histologic type, tumor size, lymph node status, distant metastases or stage were evident between the groups. To further examine the PABC group, we performed additional analyses by pregnancy or postpartum status at diagnosis, and found that there remained no difference between the groups.

Immunohistochemical results and molecular phenotypes

Of PABC cases, 21 (53.8%) tumors were ER positive, 12 (30.8%) PR positive, 7 (17.9 %) Her-2 positive, and 14 (35.9%) TN. For nulliparous patients, 16 (50%) tumors were ER positive, 14 (43.7%) PR positive, 11 (34.4%) Her-2 positive, and 11 (34.4%) TN. Of TN PABC and non-PABC tumors, 23.1% (3/13) and 54.5% (6/11) showed CK5/6 positivity, respectively, and 53.8% (7/13) and 54.5% (6/11) stained for EGFR, respectively.

We found a greater proportion of PABC cases had either luminal A or TN tumors (35.9% each) (Table 2). Nulliparous women had a greater proportion of Luminal B cancers (37.5%). The distribution of Her-2-positive and TN types was similar between the groups (12.3% vs. 15.6%, and 35.9% vs. 34.4%, in PABC and non-PABC, respectively). TN carcinomas in the PABC group were mostly grade 3 (12 of 14, 85.7%), T2 tumors (8 of 13, 61.5%), with positive lymph nodes (9 of 13, 69.2%). Similarly, TN cancers in nulliparous women were mostly grade 3 (10 of 11, 90.9%), T2 tumors (7 of 11, 63.6%) with positive lymph nodes (6 of 11, 54.5%). Basal differentiation was seen in 53.8 % and 63.6% of TN tumors in PABC and nulliparous patients, respectively (Fig. 2). Overall, tumors with basal differentiation comprised 18.4% and 21.9% of all carcinomas in each group, respectively. No significant difference in the prevalence of any molecular type was seen between the groups. After additional analysis by pregnancy or postpartum status at diagnosis, there remained no difference in molecular type between the groups.

DISCUSSION

Despite its known aggressive behavior, it is still unclear whether PABC represents a distinct disease, which may be related to the marked variation in study designs. Most studies have defined PABC as cancer diagnosed during pregnancy or within one year after delivery.[13,39-41] However, definitions vary from cancer diagnosed "exclusively during pregnancy",[42] "during pregnancy or within 12 days of postpartum",[43] "during pregnancy or within 6 months of postpartum",[24] to "during pregnancy or within 2 years of the last pregnancy"[44] etc. In addition, most

defined controls as patients that did not meet definition criteria for PABC.[13,39-41] Therefore, control groups were likely heterogeneous as far as including nulliparous women, parous women, and even women diagnosed during the second year of postpartum - the latter being included in the very definition of PABC in other studies.[19,25,44] Because receiving a diagnosis within 2 years of postpartum has been shown to be an independent predictor of mortality,[19,25,44] this interval was included in our PABC definition. Considering that the increased risk of dying associated with postpartum carcinomas could extend up to 15 years after pregnancy,[45] we sought to avoid any influence of pregnancy/postpartum status on tumor biology in the control group by including only nulliparous women. Interestingly, while previous studies that had only nulliparous controls had at least twice as many controls as cases,[19,25,44] the number of cases in our study exceeded that of controls. This could be related to the fact that 91.6% of women in our state have children before 35 years old.[46] In addition, only 13% of Brazilian women remain nulliparous upon completion of their reproductive period,[47] which makes young nulliparous women with breast cancer a rare kind of patient in Brazil.

Similarly to prior studies, no significant difference in histologic type between PABC and controls was noted. The most common histologic type was IDC, which corresponded to 94.9% of PABC cases. This is in keeping with the reported frequency in other studies, which varied from 71 to 100%.[40,48,49]

Lobular carcinomas are infrequent corresponding to 0% to 12.5% of cases.[50,51] In our study, no lobular carcinomas were identified in either group. Although mucinous,[42,48,52] invasive cribriform,[53] and spindle cell carcinomas[43] have been reported in PABC, none of these types were seen by our group. Medullary carcinomas occur in 2.7% to 5% of PABC cases.[24, 50] IDC with medullary features was identified in one case (2.6%) and two (6.2%) controls, respectively.

Pure IMPC is a rare special type that accounts for only 0.7-3% of all breast cancers.[54] However, up to 7.4% of all breast carcinomas harbor foci of micropapillary growth pattern usually admixed with IDC of no special type.[55] IMPC has been associated with lymph node metastases, lymphovascular invasion, and loco-regional recurrence.[56-58] Interestingly, two patients in each group had IMPC (5.1% and 6.2% of PABC and non-PABC tumors, respectively). Importantly, no study to date has described the occurrence of IMPC in PABC. Studies suggest that the presence of even small foci of micropapillary morphology are associated with a poor prognosis,[59,60] and mixed IMPC (i.e., IDC with a micropapillary component) are more closely related to pure

IMPC than to IDC of no special type at the genomic level,[61] making the recognition of minor micropapillary components clinically important. The intriguing finding of IMPC cases led us to review the slides in search for minor micropapillary components in all cases. Four additional tumors in the PABC and 5 in the nulliparous group had focal micropapillary morphology. Grouping pure and mixed IMPC together, we found a prevalence of 15.4% and 21.9% in PABC and non-PABC cases, respectively, which are at least twice as large as that reported for women in general.[55] This finding suggests that IMPC may be more prevalent in young Brazilian women, although our sample is small. Alternatively, the presence of micropapillary foci in IDC may be underreported in the literature, as previously demonstrated.[55,56]

The frequency of grade 3 tumors in PABC ranges from 40% to 84%.[24,39] In our study, a high percentage (53.8%) of PABC corresponded to grade 3 tumors. Although some studies concluded that PABC was more likely to be high-grade,[19,39,44] we found nulliparous women more likely to harbor grade 3 tumors ($p=0.011$). The reason for that is uncertain but may be related to small sample sizes. The high prevalence of grade 3 tumors in both groups could be attributed to the young age of the patients.

The prevalence of ER and PR positivity in PABC varies from 19% to 55% and 24% to 83.3%, respectively.[43,44,49,62] This broad variation may be explained by small samples and differences in criteria used for determining positivity, which were not defined in many studies.[24,39,41,44,48,49,52,63-66] The most commonly used criterion was nuclear staining in 10% of cells or more,[14,43,51,53] but some utilized the "H-score"[40,50] or other specific scores.[62] In addition, in some studies a large percentage of cases had unknown ER and/or PR status.[13,41,63,65] Some groups have shown that PABC is significantly more likely to be ER and PR negative,[39-41,44,50] while others found no differences.[14,42,49] In our study, 53.8% and 30.8% of PABC stained for ER and PR, respectively, and no difference in positivity was seen when compared to the control group.

Her-2 is amplified in 25% to 30% of all breast cancers and is associated with aggressive disease.[67] Her-2-positive PABC cases range from 6% to 60%.[49,68] This wide variation may be attributed to small sample sizes, different techniques used for evaluating Her-2 status, and variation in positivity criteria. Only one study from 1993 which included 15 PABC cases reported PABC to be more likely Her-2 positive.[62] Importantly, in this study Her-2 status was divided into negative, low-positive and positive according to a specific score, which

differed from that used by other groups.[14,53] In our study, 17.9% of PABC were Her-2 positive. In keeping with most studies, we found no difference in Her-2 positivity between the groups.

TN carcinomas tend to occur in younger women and are associated with poor clinicopathologic features such as large tumor size, high-grade tumors, increased risk of distant recurrence, and death.[69-71] TN tumors corresponded to 35.9% of PABC cases. This frequency is slightly larger than that reported for young women in general (Table 3).[72] Only one prior study had evaluated the frequency of TN tumors in PABC, which comprised 34.2% of cases, and concluded that PABC was more likely to be triple-negative.[44] In our study, the prevalence of TN tumors did not differ between the groups.

Only one unpublished study had evaluated the distribution of molecular phenotypes in PABC, and showed that nulliparous women were more likely to harbor luminal A tumors.[73] In contrast, no differences were seen by our group with regard to any molecular phenotype. In fact, we found a larger percentage of luminal A tumors in the PABC group (35.9% versus 12.5%, respectively), and luminal B tumors in nulliparous controls (37.5% versus 17.9%, respectively), although these differences were non-significant (p=0.078). The reason for this disparity may be related to small sample sizes and ethnic differences. The prevalence of Her-2 phenotype was similar to that previously reported (12.3% vs. 11%).[73]

To the best of our knowledge no prior study has evaluated basal differentiation in PABC. Basal-like carcinomas correspond to 12% of all breast cancers.[74] CK5/6 and EGFR are considered markers of basal differentiation.[75] CK5/6 and EGFR stained 23.1% and 53.8% of TN PABC cases, respectively. Carcinomas with basal differentiation corresponded to 53.8% of TN and 18.4% of overall tumors in the PABC group. Only one prior group had tested EGFR in PABC.[40] EGFR staining was seen in 33% of all cases (which also included hormone receptor-positive carcinomas), and significantly more likely to be present in PABC. In our study, basal marker positivity was similar between the groups. Thus, the high frequency of basal differentiation in PABC may just reflect the young age of the patients and high prevalence of TN tumors. Grouping PABC and non-PABC together, TN carcinomas and tumors with basal differentiation comprised 35.2% and 20% of all tumors, respectively. These findings mirrored those published by another Brazilian group that evaluated non-pregnant patients under 35 years old, which showed a prevalence of 34.3% and 25.8%, respectively.[76] Although our study groups are largely composed of Hispanic women and women of African descent, the percentage of tumors with

basal differentiation were not as high as that previously reported (39%) for premenopausal African American women.[33] On the other hand, the overall prevalence of TN tumors (in PABC and non-PABC) slightly exceeded that reported for premenopausal Mexican patients (35.2% vs. 25.5%, respectively).[77]

In conclusion, there appears to be an overrepresentation of carcinomas associated with a poor outcome in PABC, such as TN tumors and carcinomas with basal differentiation, compared to women in general. However, PABC shares similar histopathologic characteristics and molecular type distribution with carcinomas from young nulliparous women. The development of prospective studies with more uniform designs and detailed histopathologic evaluation are imperative to further clarify the biological characteristics of these aggressive neoplasms.

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Conflict of interest

There are no financial disclosures to report for any of the contributing authors.

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Table 1 Clinicopathologic characteristics of PABC and non-PABC

Characteristics	PABC N (%)	Nulliparous N (%)	P-value [a]
Number of patients	39	32	
Mean age	30.2	30.9	
Histologic type			NS
IDC	37 (94.9)	30 (93.8)	
IMPC	2 (5.1)	2 (6.2)	
Grade			0.025
I	3 (7.7)	0 (0.0)	
II	15 (38.5)	6 (18.8)	
III	21 (53.8)	26 (81.2)	
T classification*			NS
T1	3 (8.1)	5 (15.3)	
T2	17 (45.9)	18 (56.2)	
T3	14 (37.8)	7 (21.9)	
T4	3 (8.1)	2 (6.2)	
Unknown	2	0	
N classification*			NS
N1	9 (25.0)	10 (33.3)	
N2	6 (16.7)	6 (20.0)	
N3	10 (27.8)	3 (10.0)	
N0	11 (30.6)	11 (36.7)	
Unknown	3	2	
Distant metastasis*			NS
Present	3 (8.1)	6 (18.8)	
Absent	34 (91.9)	26 (81.2)	
Unknown	2	0	
Stage*			NS
I	3 (8.8)	1 (3.2)	
II	11 (32.3)	17 (54.8)	
III	17 (50.0)	7 (22.6)	
IV	3 (8.8)	6 (19.3)	
Unknown	5	1	
Specimens			0.033
Core biopsy	3 (7.7)	0 (0.0)	
Lumpectomy	2 (5.1)	7 (21.9)	
Lumpectomy and LN dissection	2 (5.1)	4 (12.5)	
Mastectomy	3 (7.7)	0 (0.0)	
Mastectomy and LN dissection	29 (74.4)	21 (65.6)	

PABC, pregnancy-associated breast carcinoma; NS, non-significant; IDC, invasive ductal carcinoma; IMPC,invasive micropapillary carcinoma; LN, lymph node

[a] Fisher's exact test

* percents omit unknown data

Table 2 Molecular subtypes in PABC and non-PABC

Subtype	PABC N (%)	Nulliparous N (%)	P-value [a]
Luminal A	14 (35.9)	4 (12.5)	NS
Luminal B	7 (17.9)	12 (37.5)	
Her-2 +	4 (12.3)	5 (15.6)	
TN [b]	14 (35.9)	11 (34.4)	
Basal-like	7 (18.4)*	7 (21.9)	
Total	39	32	

PABC, pregnancy-associated breast carcinoma; NS, non-significant; TN,triple-negative
[a] Fisher's exact test
[b] Includes basal-like and non-basal-like carcinomas
*percent omits unknown data

Table 3 Distribution of molecular subtypes in PABC, young women and general population

Study	Sample	Luminal A (%)	Luminal B (%)	Her-2 + (%)	TN (%)	Basal-like (%)
Abreu-e-Lima et al	PABC	35.9	17.9	12.3	35.9	18.4
Collins et al [72]	women ≤ 40 years	33	35	11	21	-
Yang et al [74]	Population-based series	69	6	8	-	12

PABC, pregnancy-associated breast carcinoma; TN, triple-negative

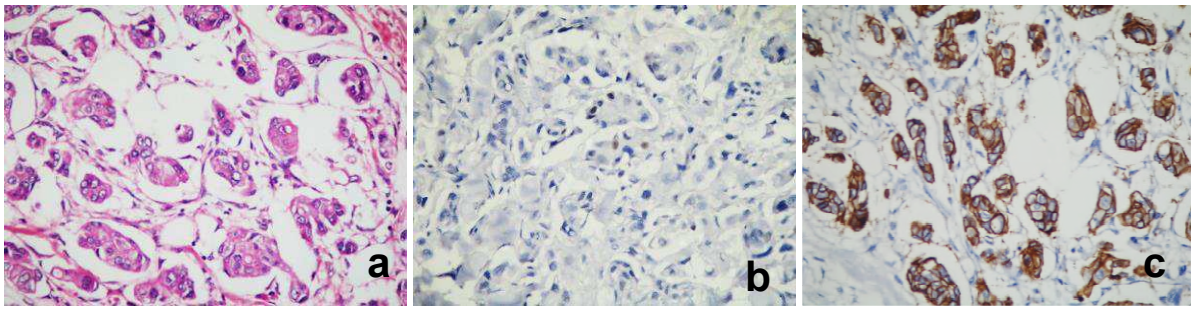

Fig. 1 Invasive micropapillary carcinoma in a pregnant patient. **a** micropapillary growth (H&E x 400). **b** ER positivity in approximately 3% of tumor cells (x 400). **c** tumor cells were positive for Her-2 (3+) (x 400). This tumor was classified as Luminal B

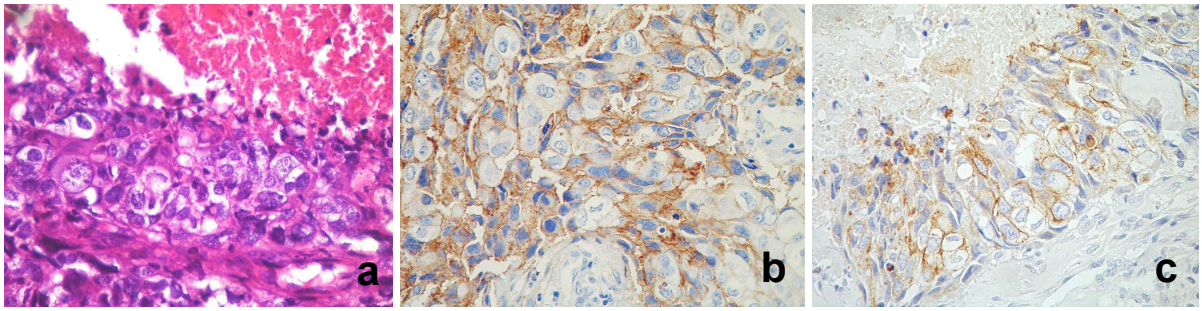

Fig. 2 Pregnancy-associated breast carcinoma showing basal differentiation. **a** Grade 3 invasive ductal carcinoma with necrosis (H&E x 400). **b** EGFR positivity (x 400). **c** CK5/6 positivity (x 400)

Conclusões

Usando um grupo controle ideal para o estudo do câncer de mama associado à gravidez (CMAG), isto é, mulheres de mesma faixa etária, sem interferência da gravidez ou estado pós-parto sobre o prognóstico, pode-se concluir que:

1. Não houve diferenças significativas entre o câncer de mama associado à gravidez e aquele que ocorre em jovens nulíparas, no que tocam os aspectos clinicopatológicos e imunoistoquímicos estudados.
2. Os resultados são semelhantes aos da literatura no que tange a maior prevalência de carcinoma ductal invasivo de alto grau e alta frequência de casos triplo-negativos no CMAG, e contribuem com a literatura pela demonstração de casos com diferenciação basal.
3. Os carcinomas grau 3 foram significativamente mais frequentes no grupo de nulíparas, diferentemente dos resultados obtidos em estudos anteriores. Essa diferença pode ser atribuível ao pequeno número de casos estudados.
4. Embora sem diferença significativa entre os grupos, foram relatados os primeiros casos da literatura de carcinoma micropapilar invasivo no CMAG, podendo ser esse um tipo histológico mais frequente nas mulheres jovens do Nordeste do Brasil.
5. Por fim, acredita-se que o estudo de um maior número de casos poderá estabelecer diferenças entre os grupos, principalmente em relação à prevalência de tumores luminais A e triplo-negativos no CMAG.

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Apêndice

FICHA-PADRÃO DE PACIENTES DO HCP COM DIAGNÓSTICO DE CÂNCER DE MAMA COM ≤ 35 ANOS DE 1995 A 2011

INFORMAÇÕES GERAIS:

Número da ficha:

Ano:

Idade:

Registro hospitalar:

Nome:

Telefone:

INFORMAÇÕES CLÍNICAS

Gestante ao diagnóstico: ( ) sim ( ) não

Diagnóstico estabelecido até 2 anos do último parto: ( ) sim ( ) não

Nulípara ao diagnóstico: ( ) sim ( ) não

Gesta: Para: Aborto:

Idade dos filhos:

Amamentação: ( ) sim ( ) não

Se sim, exclusiva? ( ) sim ( ) não

Por quantos meses amamentou:_____

Tipo de tratamento não cirúrgico:

Radioterapia ( ) Quimioterapia neoadjuvante ( ) Quimioterapia adjuvante ( )

Hormonioterapia ( ) Trastuzumab ( )

Tipo de espécime avaliado:

Core biopsy ( ) Segmentectomia ( ) Mastectomia simples ( ) Mastectomia ( ) adenomastectomia subcutânea ( ) dissecção axilar ( ) linfonodo sentinela ( )

Lateralidade do tumor: Direita ( ) Esquerda ( )

Estado atual: óbito ( ), data do óbito (/ /);

viva ( ) follow-up até data (/ /)

Número(s) do(s) laudo(s) anatomopatológico(s):

Grau histológico:

Tipo histológico:

Tamanho do tumor:

Número de linfonodos positivos:

Margens cirúrgicas: Positivas ( ); Negativa ( )

Estadiamento: T N M

Marcadores:

RE: Positivo ( ); Negativo ( )

RP: Positivo ( ); Negativo ( )

Her-2: Positivo ( ); Negativo ( ); Indeterminado ( )

FISH para Her-2: Positivo ( ); Negativo ( );

CK5/6: Positivo ( ); Intensidade ( ) Porcentagem ( ); Negativo ( )

EGFR: Positivo ( ); Intensidade ( ) Porcentagem ( ); Negativo ( )

Anexos

Anexo A

MINISTÉRIO DA SAÚDE
Conselho Nacional de Saúde
Comissão Nacional de Ética em Pesquisa - CONEP

PROJETO RECEBIDO NO CEP		CAAE - 0318.0.172.447-11
Projeto de Pesquisa Características clinicopatológicas e imunoistoquímicas do câncer de mama associado à gravidez.		
Área(s) Temática(s) Especial(s) Não se aplica	**Grupo** III	**Fase** Não se aplica
Pesquisador Responsável		
CPF 29500087472	**Pesquisador Responsável** Maria do Carmo Carvalho de Abreu e Lima	**Assinatura**
Comitê de Ética		
Data de Entrega 30/06/2011	**Recebimento:** _____ **Assinatura**	

Anexo B

HCP
HOSPITAL
DE CÂNCER
DE PERNAMBUCO

DECLARAÇÃO

Declaramos que o projeto de Pesquisa **nº 34/2011 (CAAE: 0318.0.172.447-11)** intitulado: **"CARACTERÍSTICAS CLINICOPATOLÓGICAS E IMUNOISTOQUÍMICAS DO CÂNCER DE MAMA ASSOCIADO À GRAVIDEZ"**, apresentado pela pesquisadora responsável **Maria do Carmo Carvalho de Abreu e Lima,** foi aprovado nesta data, pelo Comitê de Ética e Pesquisa em Seres Humanos da Sociedade Pernambucana de Combate ao Câncer - SPCC / Hospital de Câncer de Pernambuco - HCP.

Os autores deverão remeter cópia do artigo publicado para arquivo na Biblioteca da SPCC / HCP e terão que mencionar nas publicações a Instituição onde o trabalho foi realizado.

Recife, 09 de agosto de 2011.

Dr. Glauber Leitão
Coordenador
Comitê de Ética em Pesquisa
Sociedade Pernambucana de Combate ao Câncer
Hospital de Câncer de Pernambuco

Anexo C

SERVIÇO PÚBLICO FEDERAL
UNIVERSIDADE FEDERAL DE PERNAMBUCO
Comitê de Ética em Pesquisa

Of. Nº. 336/2011 - CEP/CCS

Recife, 08 de julho de 2011

Registro do SISNEP FR – 422588
CAAE – 0207.0.172.000-11
Registro CEP/CCS/UFPE Nº 237/11
Titulo: Estudo clinico-patológico e imunoistoquimíco do câncer de mama associado á gravidez.
Pesquisador Responsável: Maria do Carmo Carvalho de Abreu e Lima

Senhor (a) Pesquisador (a):

Informamos que o Comitê de Ética em Pesquisa Envolvendo Seres Humanos do Centro de Ciências da Saúde da Universidade Federal de Pernambuco (CEP/CCS/UFPE) registrou e analisou de acordo com a Resolução N.º 196/96 do Conselho Nacional de Saúde, o protocolo de pesquisa em epígrafe, liberando-o para início da coleta de dados em 06 de julho de 2011.

Ressaltamos que a aprovação definitiva do projeto será dada após a entrega do relatório final, conforme as seguintes orientações:

a) Projetos com, no máximo, 06 (seis) meses para conclusão: o pesquisador deverá enviar apenas um relatório final;
b) Projetos com períodos maiores de 06 (seis) meses: o pesquisador deverá enviar relatórios semestrais.

Dessa forma, o ofício de aprovação somente será entregue após a análise do relatório final.

Atenciosamente

Prof. Geraldo Bosco Lindoso Couto
Coordenador do CEP/ CCS / UFPE

A
Profa. Maria do Carmo Carvalho de Abreu e Lima
Departamento de Patologia

Anexo D

HCP
HOSPITAL
DE CÂNCER
DE PERNAMBUCO

DEPARTAMENTO DE PATOLOGIA

Recife, 09 de junho de 2011.

Declaração

Autorizamos a Drª Paula Abreu e Lima a utilizar os arquivos de blocos, lâminas e fichário do Departamento de Patologia do HCP, com fins de Tese de mestrado do programa de Pós-graduação em Patologia, UFPE sobre câncer de mama no ciclo grávido-puerperal. Conforme contacto prévio com a pesquisadora deverá ser mencionado o local do estudo (HCP) e garantindo o sigilo quanto a identificação das pacientes.

Atenciosamente,

Dr. Adonis R.L. de Carvalho
Chefe do Departamento de Patologia

Av Cruz Cabugá 1597
Santo Amaro-Recife/PE
CEP: 50040-000
PABX - (81) 3423.2088
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Anexo E

Annals of
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Instructions to Contributors

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Sample References for Style Reference

Journal

1. Badgwell B, Cormier JN, Xing Y, et. al. Attempted Salvage Resection for Recurrent Gastric
or Gastroesophageal Cancer. Ann Surg Oncol 2009; 16:42–50.

Journal article with DOI reference

2. Xing M: BRAF Mutation in Papillary Thyroid Microcarcinoma: The Promise of Better Risk Management. Ann Surg Oncol DOI: 10.1245/s10434-008-0298-z [Online January 22, 2009].

Book

3. Bland KI, Sarr MG, Büchler, MW, et. al. General Surgery Principles and International Practice, Second Edition. New York: Springer-Verlag, 2009.

Book Chapter

4. Jones SM, Roh MS. Results of Surgical Resection for Hepatocellular Carcinoma.. In: Talamonti MS, Pappas SG, eds. Liver-Directed Therapy for Primary and Metastatic Liver Tumors. Massachusetts:: Kluwer Academic Publishers, 2001:59-76.

Web-Based Resource

5. Society of Surgical Oncology (2007). Position Statement on Prophylactic Mastectomy. Available: http://www.surgonc.org/default.aspx?id=47 [accessed January 28, 2009]

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Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ 2010;340:c332.

Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, Elbourne D, Egger M, Altman DG, for the CONSORT Group. CONSORT 2010 Explanation and Elaboration: updated guidelines for reporting parallel group randomised trial. BMJ 2010;340:c869.

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