ELIZA DEL FIOL MANNA

ASSOCIAÇÃO ENTRE A EXPRESSÃO IMUNOISTOQUÍMICA DA TOPOISOMERASE IIα, HER2 E RECEPTORES HORMONAIS E A RESPOSTA À QUIMIOTERAPIA PRIMÁRIA EM PACIENTES COM CÂNCER DE MAMA

Dissertação de Mestrado

ORIENTADOR: Prof. Dr. LUIZ CARLOS TEIXEIRA
CO-ORIENTADOR: Prof. Dr. MARCELO ALVARENGA

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Curso de Pós-Graduação em Tocoginecologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas

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Dedico este trabalho...

Ao meu marido e amigo, Anderson,
Aos meus filhos, Vinícius e Pedro,
Pelo amor, apoio e compreensão
Nos momentos em que estive ausente.

À minha querida e especial madrinha Maria Cecília
Pelo incentivo, amizade e disponibilidade.

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"Não basta saber,

é preferível saber aplicar.

Não é bastante querer,

é preciso saber querer".

Goethe

Estrutura da Tese

Esta tese está sendo apresentada no formato alternativo de Dissertações de Mestrado da Universidade Estadual de Campinas (Unicamp) e de acordo com o disposto em ***Normas, Procedimentos e Orientações para Publicações de Dissertações e Teses da Faculdade de Ciências Médicas*** (2005).

Inclui uma introdução ao tema, os objetivos do projeto de pesquisa, e um artigo original submetido no ***The Breast***. Os métodos e os resultados obtidos estão apresentados no artigo. Em seguida, a tese apresenta as conclusões e as referências bibliográficas. No anexo foram incluídos os instrumentos utilizados para coleta de dados e a metodologia.

Sumário

Símbolos, Siglas e Abreviaturas

AC	Doxorrubicina, Ciclofosfamida
AJCC	*American Joint Committee on Câncer*
CAISM	Centro de Atenção Integral à Saúde da Mulher
CEF	Ciclofosfamida, Epidoxorrubicina, Fluorouracil
CMF	Ciclofosfamida, Metotrexate, Fluorouracil
CISH	*Chromogenic In Situ Hybridization*
DAB	3,3´- diaminobenzidine
DE	Doença Estável
DMSO	*Dimethylsulphoxide*
DP	Doença Progressiva
DTG	Departamento de Tocoginecologia
EC	Epidoxorrubicina, Ciclofosfamida
EDTA	*Ethylenediaminetetraacetic acid*
ER	*Estrogen receptor*
EUA	Estados Unidos da América
FAC	5-Fluorouracil, Doxorrubicina, Ciclofosfamida
FEC	5-Fluorouracil, Epidoxorrubicina, Ciclofosfamida
FISH	Hibridização por Fluorescência *In Situ*
FCM	Faculdade de Ciências Médicas
HER2	HER-2/neu

IHQ	Imunoistoquímica
LN	Linfonodo (s)
m^2	Metro (s) Quadrado (s)
mg	Miligrama (s)
mm^2	Milímetro (s) Quadrado (s)
n	Número de Casos
OMS	Organização Mundial da Saúde
p	Significância Estatística
PBS	*Phosphate Buffered Saline*
PgR	*Progesterone Receptor*
QT	Quimioterapia
R0	Ausência de Tumor Residual
R1	Tumor Residual Microscópico
R2	Tumor Residual Macroscópico
RC	Resposta Completa
RCp	Resposta Completa Patológica
RE	Receptor de Estrógeno
RP	Resposta Parcial
RPg	Receptor de Progesterona
RECIST	*Response Evaluation Criteria in Solid Tumors*
SBR	*Scarff, Bloom and Richardson*
UICC	União Internacional Contra o Câncer
Unicamp	Universidade Estadual de Campinas
Topo II	Topoisomerase II
Topo IIα	Topoisomerase Iiα
Topo IIβ	Topoisomerase Iiβ
Yo	*Years*

Resumo

Objetivo: O objetivo deste estudo foi avaliar a associação entre a expressão imunoistoquímica da topoisomerase IIα, HER2 e receptores hormonais e a resposta à quimioterapia primária baseada em antraciclina em carcinoma invasivo de mama. **Materiais e Métodos:** Analisamos 109 prontuários de pacientes tratadas com quimioterapia primária baseada em antraciclina no Centro Atenção Integral à Saúde da Mulher da Universidade Estadual de Campinas, no período de 1996 a 2004. As respostas clínica e patológica à quimioterapia primária foram associadas com a superexpressão da topoisomerase IIα e do HER2 e com a negatividade dos receptores hormonais. A análise estatística foi realizada através do teste qui-quadrado ou teste exato de Fisher. **Resultados:** A freqüência da superexpressão da topoisomerase IIα foi de 41%. Não houve associação estatística entre a resposta clínica e a superexpressão da topoisomerase IIα, do HER2 e negatividade dos receptores hormonais. Entretanto, houve associação entre a resposta completa patológica e a negatividade dos receptores hormonais (p=0,0289). **Conclusões:** O presente estudo sugere que esses marcadores não deveriam ser considerados fatores preditivos de resposta à quimioterapia primária com antraciclina, sendo necessários estudos prospectivos desenhados para esse propósito.

Summary

Background: The aim of this study was to evaluate the association between immunohistochemical expression of topoisomerase IIα, HER2 and hormonal receptors and response to primary anthracyclin-based chemotherapy in invasive breast carcinoma. **Materials and Methods:** We analyzed 109 medical charts of patients treated with primary anthracyclin-based chemotherapy in Women's Integral Health Care Center of State University of Campinas from 1996 to 2004. The clinical and pathological response to primary chemotherapy was associated with overexpression of topoisomerase IIα and HER2 and hormonal receptor negativity. Statistical analysis was performed using Chi-square or Fisher's Exact Test. **Results:** The frequency of topoisomerase IIα overexpression was 41%. No statistical association between clinical response and overexpression of topoisomerase IIα, HER2 and hormonal receptor negativity was found. However, there was an association between complete pathological response and hormonal receptor negativity (p=0.0289). **Conclusions:** The present study suggested that these markers should not be considered predictors of response to primary anthracyclin-based chemotherapy, and prospective studies must be designed for this purpose.

1. Introdução

O câncer de mama localmente avançado compreende 30% a 60% dos casos diagnosticados nos países em desenvolvimento e 10% a 20% dos casos nos EUA (Valero et al., 1996; Kaufmann et al., 2003). A quimioterapia primária constitui parte integral do tratamento do câncer de mama nesse estádio, pois permite o tratamento sistêmico precoce da doença, aumenta a taxa de cirurgias conservadoras e testa individualmente a quimiossensibilidade do tumor *in vivo*, sem comprometer a sobrevida (Scholl et al., 1994; Bonadonna et al., 1998; Van Der Hage et al., 2001; Chollet et al., 2002; Mano e Awada, 2004).

Uma resposta favorável à quimioterapia primária está associada à maior sobrevida (Bonadonna et al., 1998; Fisher et al., 1998; Pierga et al., 2000). A resposta completa patológica está relacionada à maior sobrevida livre de doença e à maior sobrevida global (Kuerer et al., 1999; Chollet et al., 2002).

As antraciclinas são as drogas mais utilizadas em quimioterapia primária. A doxorrubicina é um dos quimioterápicos mais ativos contra o câncer de mama, tendo como alvo a topoisomerase II (Topo II). Existem duas isoformas de Topo

II nas células humanas: a topoisomerase IIα (Topo IIα) e a topoisomerase IIβ (Topo IIβ) (Wang, 1996). A Topo IIα é a enzima-chave na replicação do DNA e tem como ação reduzir a torção e condensação das hélices de DNA, criando uma quebra na dupla-hélice, permitindo a passagem de uma segunda dupla-hélice e conseqüente religação com as hélices quebradas (MACGrogan et al., 2003). As antraciclinas induzem à formação de complexos topoisomerase-DNA covalentes, que provocam estabilização das quebras da dupla-hélice, impedindo a religação do DNA e induzindo à apoptose (Kellner et al., 2002). A função da Topo IIβ ainda é pouco conhecida (Wang, 1996).

Estudos *in vitro* demonstraram que a sensibilidade aos inibidores da Topo II é dependente do nível de expressão da Topo IIα nas células-alvo malignas (Gudkov et al., 1993; Nitiss e Beck, 1996; Withwoff et al., 1996; Zhou et al., 1999; Järvinen et al., 2000). Células com menor concentração da proteína da Topo IIα são menos sensíveis aos inibidores da Topo II do que as que apresentam concentração elevada (Järvinen e Liu, 2003).

Em câncer de mama, a superexpressão da Topo IIα esteve associada à superexpressão do HER2, à negatividade dos receptores hormonais, ao alto grau histológico, a elevados níveis do antígeno Ki-67, à mutação do p53 e à aneuploidia (Järvinen et al., 1996; Rudolph et al., 1999; Depowski et al., 2000; Järvinen e Liu, 2003; Koren et al., 2004). A expressão da Topo IIα é dependente do ciclo celular e está relacionada a altas taxas de proliferação celular (Järvinen e Liu, 2003). A superexpressão da Topo IIα também esteve associada a um pior

prognóstico, com maior probabilidade de recorrência da doença e pior sobrevida global (Rudolph et al., 1999; Depowski et al., 2000; Koren et al., 2004).

Acredita-se que a amplificação do gene da Topo IIα possa levar à superexpressão da proteína da Topo IIα e, conseqüentemente, à maior sensibilidade aos inibidores da Topo II (Smith et al., 1993; Järvinen et al., 2000). A superexpressão imunoistoquímica da Topo IIα mostrou correlação satisfatória com a amplificação do gene da Topo IIα detectada pela hibridização por fluorescência *in situ* (FISH) (Järvinen et al., 2000). A pesquisa da superexpressão da Topo IIα através da imunoistoquímica é mais prática e mais barata do que a pesquisa da amplificação de seu gene pela FISH (Martin-Richard et al., 2003).

Entretanto, outros autores não encontraram correlação entre a amplificação e a superexpressão da Topo IIα, sugerindo que a amplificação do gene da Topo IIα nem sempre leva à superexpressão da proteína, pelo menos quando esta é avaliada pela imunoistoquímica (Coon et al., 2002; Mueller et al., 2004; Durbecq et al., 2004; Petit et al., 2004). A regulação da expressão da Topo IIα em tumores sólidos ainda não é bem compreendida (Durbecq et al., 2004).

Alguns trabalhos sugerem que a correlação entre a amplificação do gene da Topo IIα e a superexpressão da proteína avaliada pela imunoistoquímica ocorra em somente 60% dos casos (Coon et al., 2002; Cardoso et a., 2004; Durbecq et al., 2004). Acredita-se que esse mecanismo seja mais complexo, podendo envolver outros processos celulares, incluindo apoptose e proliferação (Durbecq et al., 2003). Como a Topo IIα é regulada pelo ciclo celular, sugere-se

que seus níveis mais elevados detectados pela imunoistoquímica estejam mais relacionados à taxa de proliferação celular do que a sua real expressão no núcleo (Järvinen et al., 1996; Järvinen et al., 1998).

O gene da Topo IIα está localizado na banda cromossômica 17q 12-q21, próximo ao gene do HER2 (Järvinen et al., 2000). O HER2 é um gene que codifica uma proteína transmembrânica de 185-Kd com atividade tirosina-quinase intracelular que leva à indução e ao crescimento tumoral (Yamauchi et al., 2001). A amplificação do HER2 pode ocorrer concomitante a aberrações (amplificação ou deleção) de vários outros genes do mesmo *locus*, entre eles o gene da Topo IIα (Järvinen e Liu, 2003).

Aberrações no gene da Topo IIα (amplificação ou deleção) podem ser demonstradas em quase 90% das amostras de câncer de mama com HER2 amplificados, enquanto que dificilmente são encontradas aberrações na ausência de amplificação do HER2 (Järvinen et al., 1999; Järvinen e Liu, 2003). A amplificação concomitante da Topo IIα ocorre em cerca de 44% dos casos que apresentam amplificação do HER2 (Järvinen e Liu, 2003). Como a prevalência da amplificação do HER2 é de 20% a 30%, estima-se que a amplificação da Topo IIα ocorra em 5% a 15% de todos os casos de câncer de mama (Järvinen et al., 2000).

O HER2 é considerado um fator prognóstico, estando sua superexpressão associada à menor sobrevida (Paik et al., 1990; Toikkanen et al., 1992). O HER2 também já foi estudado como um possível fator preditivo de resposta a diferentes formas de quimioterapia e hormonioterapia (Yamauchi et al., 2001).

Em quimioterapia adjuvante, as pacientes com superexpressão do HER2 apresentaram maior benefício com esquema de quimioterapia baseado em antraciclina (Muss et al., 1994; Paik et al., 1998; Thor et al., 1998). Ainda não são bem conhecidos os mecanismos biológicos que explicam a associação entre a amplificação e/ou superexpressão do HER2 e a sensibilidade aos inibidores da Topo II (Järvinen et al., 2000).

Em quimioterapia primária, a amplificação e a superexpressão do HER2 já estiveram associadas à maior taxa de resposta à quimioterapia com antraciclina (Coon et al., 2002; Campiglio et al., 2003; Park et al., 2003; Penault-Llorca et al., 2003). Entretanto, grande parte dos trabalhos não encontrou essa associação, existindo grande controvérsia na literatura (Rozan et al., 1998; Vargas-Roig et al., 1999; zhang et al., 2002).

Em pacientes com câncer de mama localmente avançado tratadas com quimioterapia primária baseada em antraciclina, foi observada resposta local favorável nos casos em que havia amplificação concomitante do HER2 e da Topo IIα (Coon et al., 2002; Park et al., 2003). Sugere-se que a associação da amplificação/superexpressão do HER2 à quimioterapia com antraciclina possa depender de alterações genéticas associadas, sendo a Topo IIα um bom exemplo (Järvinen e Liu, 2003).

Em linhagens celulares de câncer de mama, a amplificação e a deleção da Topo IIα estão associadas à maior ou menor sensibilidade aos inibidores da Topo II, respectivamente (Järvinen et al., 1999; 2000; Järvinen e Liu, 2003).

Estudos *in vitro* sugerem que a deleção da Topo IIα pode ser considerada um mecanismo de resistência aos inibidores da Topo II, sendo necessários estudos clínicos para confirmação (Järvinen et al., 2000).

A interação entre o HER2 e a Topo IIα pode não estar presente somente a nível de DNA, pois já foi demonstrado em linhagens celulares que a expressão do HER2 ativa a expressão da Topo IIα diretamente, aumentando a quimiossensibilidade aos inibidores da Topo II *in vitro* (Di Leo et al., 2002; Järvinen e Liu, 2003).

A superexpressão da Topo IIα esteve associada a maiores taxas de resposta em quimioterapia primária com antraciclina em câncer de mama localmente avançado (Coon et al., 2002; MACGrogan et al., 2003; Martin-Richard et al., 2003). Da mesma maneira, a perda da superexpressão da Topo IIα após a quimioterapia primária também esteve correlacionada com maior resposta ao tratamento com antraciclina, sugerindo ação seletiva nas células que apresentam superexpressão (Martin-Richard et al., 2003).

Cerca de 15% dos tumores com amplificação do HER2 podem apresentar populações de células tumorais adjacentes com deleção ou amplificação no gene da Topo IIα (Järvinen e Liu, 2003). A heterogeneidade intratumoral no *locus* da Topo IIα foi demonstrada em tumores de pacientes sem tratamento prévio, sugerindo ocorrência espontânea (Järvinen e Liu, 2003). Esse fato também pode explicar a perda gradual da eficácia dos inibidores da Topo II através da seleção de clones resistentes que podem apresentar deleção da Topo IIα (Järvinen e Liu,

2003). Também foi encontrada discordância de 19% entre a superexpressão da Topo IIα no tumor primário e nos linfonodos axilares ipsilaterais comprometidos, podendo explicar a diferença de resposta à quimioterapia entre esses locais (Cardoso et al., 2001).

A expressão dos receptores hormonais é considerada um fator preditivo de resposta à hormonioterapia, mas ainda não está bem estabelecido seu papel como fator preditivo de resposta à quimioterapia primária. Grande parte dos trabalhos encontrou associação entre a negatividade do receptor de estrógeno ou de ambos os receptores e a maior taxa de resposta à quimioterapia primária (Colleoni et al., 2003; 2004; Petit et al., 2004). A positividade do receptor de estrógeno esteve associada à pior resposta patológica (Colleoni et al., 2000; Stearns et al., 2003; Ogston et al., 2004).

Também já foram avaliados como fatores preditivos de resposta à quimioterapia primária o grau histológico, p53, MDR1/gp170, índice de proliferação, fase S, ciclina D, ploidia e índice apoptótico (Masters et al., 1987; Chang et al., 2000; Colleoni et al., 2000; Aas et al., 2003; Anelli et al., 2003; Bonnefoi et al., 2003; Mano e Awada, 2004). Evidências recentes apontam o perfil de expressão gênica dos tumores de mama como o fator preditivo de resposta patológica completa mais promissor na quimioterapia primária (Ayers et al., 2004).

Na prática clínica, existe grande diferença de resposta à quimioterapia primária entre as pacientes. A indicação da quimioterapia é geralmente empírica e baseada em conclusões de estudos que são extraploladas para casos

individuais. Dessa maneira, muitas pacientes são tratadas sem se poder prever qual a real chance de resposta ao esquema de quimioterapia indicado.

A identificação de fatores preditivos de resposta à quimioterapia poderia permitir a individualização do tratamento. Assim, muitas pacientes poderiam ser poupadas de tratamentos com efeitos adversos indesejáveis quando a chance de resposta for baixa. Existem outras modalidades terapêuticas promissoras sendo estudadas, como a hormonioterapia primária com inibidores de aromatase em pacientes pós-menopausadas que apresentam expressão dos receptores hormonais (Kaufmann et al., 2003). Caso fosse possível prever qual tratamento teria maior chance de resultar em uma resposta completa patológica, a seleção do melhor esquema terapêutico poderia ter impacto na sobrevida.

O presente estudo avaliou a expressão imunoistoquímica da Topo IIα, do HER2 e dos receptores hormonais e sua associação com as respostas clínica e patológica à quimioterapia primária com antraciclina em câncer de mama localmente avançado, procurando contribuir para a literatura na identificação de fatores preditivos de resposta para a melhor individualização do tratamento.

2. Objetivos

2.1. Objetivo geral

Avaliar a associação entre a expressão imunoistoquímica da Topo IIα, HER2 e receptores hormonais e a resposta à quimioterapia primária com antraciclina em pacientes com câncer de mama nos estádios IIIA e IIIB.

2.2. Objetivos específicos

- Avaliar a freqüência da superexpressão imunoistoquímica da Topo IIα;
- Avaliar a associação entre a superexpressão imunoistoquímica da Topo IIα e do HER2, tanto concomitante como isoladamente, e a resposta clínica e patológica à QT primária;
- Avaliar a associação entre a expressão imunoistoquímica dos receptores hormonais e a resposta clínica e patológica à QT primária.

3. Publicação

Manna EDF, Teixeira LC, Alvarenga M. Association between immunohistochemical expression of topoisomerase IIα, HER2, and hormonal receptors and response to primary chemotherapy in patients with breast cancer. **Breast** (submetido)

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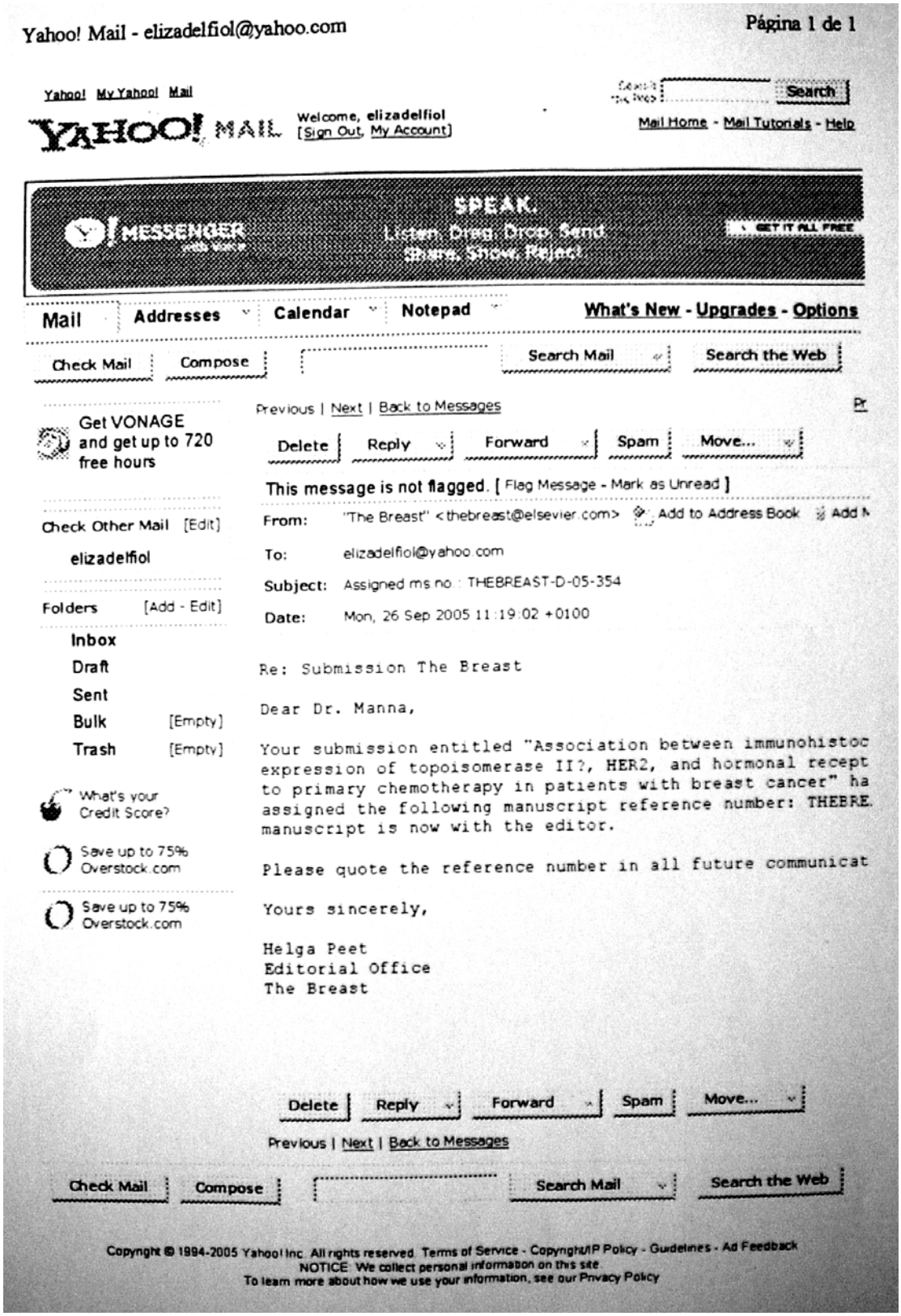

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Association between immunohistochemical expression of topoisomerase IIα, HER2, and hormonal receptors and response to primary chemotherapy in patients with breast cancer

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Summary

The aim of this study was to evaluate the association between immunohistochemical expression of Topoisomerase IIα, HER2 and hormonal receptors and response to primary anthracyclin-based chemotherapy in invasive breast carcinoma. We analyzed 109 medical charts of patients treated with primary anthracyclin-based chemotherapy in Women's Integral Health Care Center from 1996 to 2004. The clinical and pathological response to primary chemotherapy was associated with overexpression of topoisomerase IIα and HER2 and hormonal receptor negativity. Statistical analysis was performed using chi-square and Fisher's Exact Test. The frequency of topoisomerase IIα overexpression was 41%. No statistical association between clinical response and overexpression of Topoisomerase IIα, HER2 and hormonal receptor negativity was found. However, there was an association between complete pathological response and hormonal receptor negativity (p=0.0289). The present study suggested that these markers should not be considered predictors of response to primary anthracyclin-based chemotherapy, and prospective studies must be designed for this purpose.

Key words: breast cancer, HER2, primary chemotherapy, Topoisomerase IIα

Introduction

Locally advanced breast cancer accounts for 30% to 60% of the newly diagnosed breast cancer cases in developing countries and constitutes between 10% and 20% of all new breast cancers in the USA [1, 2]. Primary chemotherapy is an integral part of breast cancer treatment in this stage, allowing early systemic treatment of the disease, increasing rates of breast-conserving surgeries and individualizing patient treatment with chemosensitivity testing for tumors *in vivo*, without compromising survival [3, 4, 5, 6, 7].

Anthracyclin-based chemotherapy is a widely used treatment for breast cancer patients. Doxorubicin is one of the most active chemotherapeutic against breast cancer, targeting topoisomerase IIα (Topo IIα), which is the key enzyme in DNA replication [8]. Topo IIα reduces torsion and condensation of the DNA helix, inducing breaks in the double helix, passing a second double helix through the gap and consequently rejoining the broken strands. Anthracyclin creates covalent topoisomerase-DNA complexes, stabilizing double-strand breaks, preventing rejoining of DNA and inducing apoptosis [9].

In vitro studies have indicated that sensitivity to topoisomerase II inhibitors (Topo IIα) is dependent on the level of Topo IIα expression on tumor target cells [10, 11, 12, 13, 14]. Topo IIα gene amplification may lead to Topo IIα protein overexpression and ultimately to increased sensitivity to Topo II inhibitors [10, 15].

In breast cancer, Topo IIα overexpression has been linked to cell proliferation and HER2 protein overexpression [16]. The Topo IIα gene is located next to the HER2 gene on chromosome band 17q 12-q21 [10]. HER2 is a gene that encodes a 185-Kd transmembrane glycoprotein with intracellular tyrosine-kinase activity leading to tumor

induction and growth [17]. Aberrations of the Topo IIα gene (amplification or deletion) may be demonstrated in 50%-90% of breast cancer samples with amplified HER2, while aberrations in the absence of HER2 amplification are seldom found [18, 19]. Topo IIα has been shown to be coamplified in 44% of HER2 amplified breast cancer cases [10]. Since the prevalence of HER2 amplification is 20% to 30%, it is estimated that Topo IIα amplification occurs in 5% to 15% of all breast cancer cases [10].

Studies in the adjuvant setting have suggested that anthracycline-based chemotherapy is particulary effective in treating women with HER2-positive breast cancers [20, 21]. The biological mechanism for this finding has not yet been fully explained, but Topo IIα is believed to be involved in the altered chemosensivity to Topo II inhibitors [22].

Recent data has suggests that amplification and deletion of Topo IIα may determine both relative chemosensitivity and resistance to anthracycline-based treatment, depending on the specific genetic defect at the Topo IIα locus [18, 19]. Topo IIα amplification and overexpression have been associated with higher response rates to primary anthracycline-based chemotherapy [23, 34].

Hormone receptors are predictors of response to endocrine therapy. In the neoadjuvant setting, studies have suggested that tumors that do not express both estrogen and progesterone receptors have a significantly higher response to chemotherapy [25, 26, 27].

The antitumor activity of chemotherapy in breast cancer varies among patients. A favorable response to primary chemotherapy predicts an increase in disease-free survival [6, 28, 29, 30]. It is essential to identify the patients who are most likely to benefit from these drugs and those most unlikely to receive any benefit from this treatment. Patients who gain no benefit should thus be spared from the risks of side effects. In view of such

evidence, it became necessary to establish the predictors of tumor response to primary chemotherapy. Many other markers have already been studied, including histologic grade, p53, MDR1/gp170, proliferation index, S phase, cyclin D, ploidy and apoptotic index [3, 31, 32, 33, 34, 35, 36].

In a multigenic disease as breast cancer, it is unlikely that a single marker can be used to predict the response to any type of treatment [24]. There are recent evidences that tumor gene expression profile is the most promising predictor of complete pathological response to primary chemotherapy in breast cancer [37].

The present study retrospectively evaluated the association between immunohistochemical expression of Topo IIα, HER2, hormonal receptors and the clinical and pathological response to primary anthracyclin-based chemotherapy, in an attempt to contribute to the most appropriate treatment of choice for each patient.

Patients, Materials, and Methods

Medical records were retrospectively selected from patients with ductal breast carcinoma stages IIIA and IIIB, according to AJCC [38], who had undergone anthracyclin-based primary chemotherapy, from 1996 to 2004, at CAISM –Women's Integral Health Care Center/ State University of Campinas - Brazil.

Sufficient paraffin-embedded tissue from pretreatment biopsy specimen was required for immunohistochemical investigation of Topo IIα, HER2 and hormonal receptor expression. The records included were of patients undergoing primary anthracyclin-based chemotherapy, consisting of AC: doxorubicin (50-60mg/m^2) and cyclophosphamide (500-600mg/m^2); FAC: doxorubicin (50mg/m^2), cyclophosphamide (500mg/m^2) and

fluorouracil (500mg/m^2) and FEC: epidoxorubicin (50mg/m^2), cyclophosphamide (500mg/m^2) and fluorouracil (500mg/m^2). The cases excluded were those of bilateral carcinoma; metastatic sites, except *in situ* cervical carcinoma and epidermoid or basal cell skin carcinomas; excisional biopsy or lumpectomy; biopsies performed in other services; more than one line of primary chemotherapy and finally those who had been previously treated with hormone therapy or other chemotherapeutic agents.

To assess tumor response to chemotherapy, we used the criteria recommended by RECIST ("Response Evaluation Criteria in Solid Tumors"), defining a complete response as disappearance of all target lesions at clinical evaluation and partial response as at least a 30% decrease in the sum of the largest diameter of target lesions. Stable disease is defined when neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum of the largest diameters since treatment started. Progressive disease is defined as at least a 20% increase in the sum of the largest diameter recorded since treatment started or the appearance of one or more new lesions [39].

R0 (complete pathological response) was defined as tumor disappearance at histopathological evaluation, R1 as the presence of only microscopic tumor and R2 as the presence of macroscopic tumor (TNM, 2002) [40].

An objective response was classified as a complete response or partial response. On the other hand, stable or progressive disease were defined as a lack of an objective response.

The principles established in the Declaration of Helsinki (Association Medica Mundial, 2000) [41] and in Resolution 196/96 of the Ethical Research Commitee [42] were followed, and the patient identity was preserved.

Immunohistochemistry Technique. Immunohistochemical assessment of Topo IIα, HER2 and estrogen receptor (ER) and progesterone receptor (PR) protein expression was performed on formalin-fixed and paraffin-embedded tissue. Tissue slides (4 μm-thick sections) were deparaffinized in xylene at 110° C and rehydrated with graded ethanol baths, water and PBS. Endogenous peroxide block was performed in 10% hydrogen peroxide. Antigens were unmasked in a heated T-fall® pressure cooker using EDTA (RE, RP and Topo II) or citrate (HER2) buffer for 30 min. The slides were then placed in vertical humid chambers with PBS solution until incubation with specific primary antibodies. Monoclonal antibodies used were specific for: ER (clone 1D5, 1/300; clone 6F11, 1/80, Novocastra); PR (clone 1A6, 1/100, Novocastra) and Topo IIα (clone M 3532-1, 1/50, DAKOCYTO). A polyclonal antibody specific for HER2 was also used (clone A 0485-129, 1/300, DAKOCITO). Subsequently the slides were refrigerated overnight at 4 °C. Following incubation with the primary antibodies, the slides were rinsed 3 times in PBS and dried with an absorbent wipe. Envision plus® (anti-mouse) was used for ER, PR and Topo IIα and Envision (code K1491, DAKO - anti-mouse/anti-rabbit) was used for HER2. The slides were sterilized at 37°C for 1 hour and preheated to 37°C in PBS. Staining was developed with DAB chromogen substrate (3.3´-diaminobenzidine sigma, code 5637) in 60 mg DAB to 100 ml PBS, 10% 1.5 ml H_2O_2 and 1 ml dimethylsulphoxide (DMSO) a 37°C for 5 minutes. Then the slides were couterstained with Mayer hematoxylin for 30 to 60 seconds, dehydrated and mounted with DPX solution.

To test for immunohistochemical positivity, external controls previously positive for the antigen of interest were used. Normal breast tissue was used for ER and PR; Positive (3+) ductal carcinoma of the breast was used for HER2 and normal tonsil was used for Topo IIα. Slides were viewed under light microscope at 40x objective. To

assess hormone receptor positivity, the percentage of cells with receptor expression was considered. Cases were considered positive when at least 10% of malignant cells stained for ER and PR [43], and more than 15% for Topo IIα [44]. Figure1 shows Topo IIα expression in about 50% of tumor cells (positive). Regarding HER2, membrane staining was graded according to intensity (0-3+) using the HercepTest criteria, and only 3+ was considered positive (complete and strong membrane staining in at least 10% of tumor cells) [45]. Staining results were reproducible and assessed by a pathologist. The percentage of tumor cell staining for each marker was averaged for final classification. All immunohistochemical assessments were performed blindly as to clinical data.

Statistical methods. The frequency of Topo IIα overexpression was analyzed. The clinical and pathological response to primary anthracyclin-based chemotherapy was associated with overexpression of Topo IIα, HER2 and lack of hormone receptor expression. The chi-square or Fisher's Exact Test [46] were used to determine the association between variables. *P* value < 0.05 was considered statistically significant.

Results

Patient, disease and tumor characteristics

One hundred and nine medical charts of breast cancer patients were selected. Patient and disease characteristics may be observed in Table 1; tumor characteristics are described in Table 2. In 30 patients, diagnosis was made by incisional biopsy and in 79 by core needle biopsy. An increased frequency of high histological and nuclear grade

was found, according to the Scarff, Bloom and Richardson (SBR) grading system. The largest diameter of breast cancers was a mean of 72.93 mm, median of 70 mm, and values ranged from 20 mm to 150 mm. Most tumors were classified as T3, T4b and T4d.

The percentage of cells stained by the anti-topoisomerase IIα antibody in tissues ranged from 0 to 90%, with a median of 15% and mean of 23%. The frequency of Topo IIα overexpression was 41.3% (Fig 2). Of the 109 patients, 23 (21.1%) presented HER2 3+ overexpression. Regarding hormone receptors, 58 (53.2%) patients had both negative receptors and 51 (46.8%) patients had at least one or both positive receptors. There was a statistical association between Topo IIα and HER2 overexpression (Table 3). No association between Topo IIα overexpression and negativity of hormonal receptors was found (Table 4).

Treatment performed and chemotherapy response

Regarding the primary chemotherapy regimen performed, 91 patients were treated with AC, 12 with FAC and 6 with FEC. The mean number of primary chemotherapy cycles was 3.34, with a median of 3, ranging from 2 to 6. Anthracyclin dose intensity ranged from 10 mg/m^2/week to 33 mg/m^2/week, with a median of 22 mg/m^2/week.

Modified radical mastectomy was performed in 87 patients; Halstead radical mastectomy in 14 patients; simple mastectomy in 2 patients and modified radical mastectomy with immediate reconstruction in 5 patients.

There was a 63.3% rate of objective clinical response, with 36.7% of the patients presenting stable or progressive disease. Complete pathological response (R0) occurred in

only 2 patients, and 4 patients presented microscopic pathological response (R1). Affected lymph nodes after surgery were a median of 5, ranging from 0 to 30 lymph nodes.

Association between response to chemotherapy and markers

There was no association between Topo IIα and HER2 overexpression, alone or combined, and clinical and pathological response (table 5). Hormonal receptor negativity was not associated with clinical response, but with pathological response (Table 6).

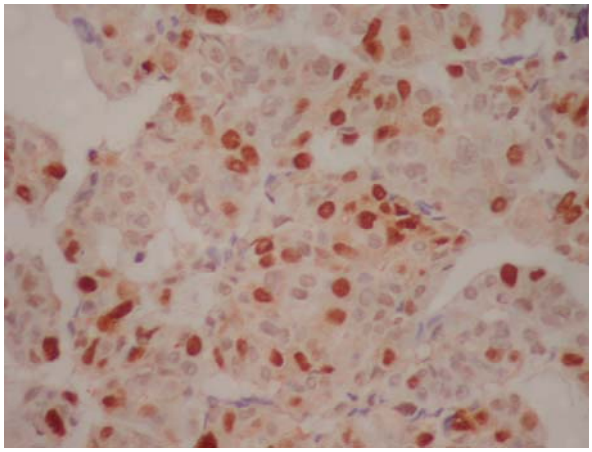

Figure 1. Invasive ductal carcinoma with Topo IIα expression in about 50% of tumor cells (positive). Photomicroscopy Nikon, 250x

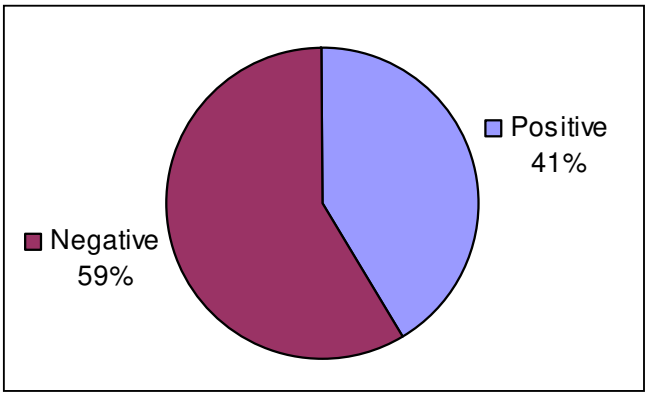

Figure 2: Graph showing sectors of frequency of Topo IIα expression

Table 1: Patients and disease characteristics

		n-109	(%)
Age	≤ 35 yo	5	4,6
	> 35 yo	104	95,4
Menopausal status	premenopausal	62	56,9
	postmenopausal	47	43,1
Clinical stage	IIIA	41	37,7
	IIIB	68	62,3
T	T2	8	7,4
	T3	32	29,3
	T4a	3	2,8
	T4b	45	41,3
	T4c	2	1,8
	T4d	19	17,4
N	N0	10	9,1
	N1	38	34,9
	N2	61	56,0

Table 2: Tumor characteristics

		n-109	(%)
Histological grade	II	9	8,3
	III	94	86,2
	Indeterminated	6	5,5
Nuclear grade	II	44	40,3
	III	60	55,1
	Indeterminated	5	4,6
Hormone receptors	ER+ and/or PR+	51	46,8
	ER- and PR -	58	53,2
HER2	Positive	23	21,1
	Negative	86	78,9
Topo IIα	Positive	45	41,3
	Negative	64	58,7

Table 3: Association between Topo IIα and HER2 overexpression

HER2	Positive		Negative		*p* value
	n	%	n	%	
Topo IIα					
Positive	14	60.90	31	36.00	**0.0312***
Negative	9	39.10	55	64.00	
Total	23	21.10	86	78.90	

*chi-square test.

Table 4: Association between Topo IIα overexpression and hormone receptor negativity

Hormone receptors	ER- and PgR-		ER+ and/or PgR+		*p* value
	n	%	n	%	
Topo IIα					
Positive	24	41.4	21	41.2	**0.9829**
Negative	34	58.6	39	58.8	
Total	58	53.2	51	46.8	

Table 5: Association between clinical response and marker expression

		Response (%)	*p* value
Topo IIα overexpression			
Present	(n = 45)	30 (43.5%)	0.5412
Absent	(n = 64)	39 (56.5%)	
HER2 overexpression			
Present	(n = 23)	15 (21.7%)	0.8302
Absent	(n = 86)	54 (78.3%)	
Overexpression of Topo IIα and HER2			
Present	(n = 14)	8 (11.6%)	0.6085
Absent	(n = 95)	61 (88.4%)	
Expression of hormone receptors			
ER+ e/ou PgR+	(n = 51)	32 (46.3%)	
ER-and PgR-	(n = 58)	37 (53.7%)	0.9098

Table 6: Association between pathological response and marker expression

		R0 + R1	R2	*p* value
Topo IIα overexpression				
Present	(n = 45)	3	42	0.6894
Absent	(n = 64)	3	61	
HER2 overexpression				
Present	(n = 23)	0	23	0.3391
Absent	(n = 86)	6	80	
Topo IIα and HER2 overexpression				
Present	(n = 14)	0	14	1
Absent	(n = 95)	6	89	
Expression of hormone receptors				
ER+and/or PgR+	(n = 51)	0	51	**0.0289***
ER- and PgR-	(n = 58)	6	52	

* Fisher's Exact Test

Discussion

In clinical practice, there is a little recorded information in the literature about the predictive value of Topo IIα overexpression and/or amplification for tumor response to primary anthracyclin-based chemotherapy in locally advanced breast cancer.

In this study, the rate of Topo IIα overexpression was 41%. The prevalence of Topo IIα overexpression ranged from 31% to 49% in the literature [23, 47, 48, 49]. In the majority of cases, Topo IIα overexpression was determined by the percentage of stained cells, predominant in 10% [23, 24, 26, 50]. In the current study, the cut-off was 15%, corresponded to the median value found.

Immunohistochemical examination of Topo IIα overexpression showed satisfactory correlation with fluorescence *in situ* hybridzation (FISH) [10]. The possibility of applying immunohistochemical studies of Topo IIα status could simplify and reduce costs. However, other authors found no correlation between amplification and overexpression of Topo IIα, suggesting that Topo IIα gene amplification does not always result in protein overexpression, particularly when this protein is evaluated by immunohistochemistry [26, 51, 52, 53]. The regulation of Topo IIα expression in solid tumors remains incompletely understood [53].

Several studies have suggested that the Topo IIα gene amplification and protein overexpression are correlated in only 60% of the cases evaluated by immunohistochemistry [24, 51, 53]. It may be more complex, involving other cell processes, e.g. apoptosis and proliferation [53]. Since Topo IIα is regulated by the cell cycle, it is suggested that the

higher levels detected by immunohistochemistry are more likely related to cell proliferation rate than the actual level of nuclear overexpression [16, 22].

An important prognostic and potentially therapeutic finding is the association of Topo IIα and HER oncoprotein overexpression. This study found an association between HER2 and Topo IIα overexpression, similar to what was described in other reports [16, 49, 51]. This suggests that Topo IIα is preferentially expressed in a more aggressive subset of breast tumors (overexpressing HER2) [16, 49]. However, another report found no statistical association between overexpression of both markers [23].

Topo IIα overexpression has been associated with hormonal receptor negativity, high histologic grade, p53, high Ki-67 antigen levels and aneuploidy [16, 49, 54, 55]. In this study, there was no association between Topo IIα overexpression and hormone receptor negativity. Similarly, no association was found in other studies [47, 49]. Topo IIα overexpression was related to a worse prognosis, with higher frequency of disease recurrence and worse overall survival [48, 49, 55].

The present study showed no association between clinical response to primary anthracyclin-based chemotherapy and overexpression of HER2 and Topo IIα (concomitant or alone). In cell lines, it has already been demonstrated that HER2 expression directly activates Topo II expression, increasing chemosensitivity to Topo II inhibitors *in vitro* [19, 56].

Reports have described that amplification and overexpression of HER2 are correlated with tumor response to primary anthracyclin-based chemotherapy [51, 57]. The predictive value of HER2 amplification/overexpression for doxorubicin response is still controversial [17, 24, 50, 56, 58]. The majority of studies failed to find an association between amplification and/or HER overexpression and response to primary

anthracyclin–based chemotherapy [59, 60, 61]. However, a higher rate of tumor response to doxorubicin-based chemotherapy was found in HER2-positive cases by CISH (chromogenic *in situ* hybridization) [51, 57].

Patients with locally advanced breast cancer treated with primary anthracyclin-based chemotherapy showed a favorable local response when HER2 and Topo IIα were concurrently amplified [51, 57]. Studies suggest that the combination of HER2 amplification/overexpression and anthracyclin-based chemotherapy may depend on genetic alterations associated, and Topo IIα is a good example [19, 56].

Two studies have investigated the potential predictive role of both the Topo IIα gene and its protein, assessed by FISH and immunohistochemistry, respectively. More than 430 archival primary tumor samples were obtained from patients entered into a multicenter phase III trial, to compare classical CMF (cyclophosphamide, methotrexate, 5-fluorouracil) with EC (epirubicin and cyclophosphamide), as adjuvant therapy for node-positive breast cancer patients [50, 56]. Both studies suggested that Topo IIα amplification or overexpression was probably associated with as anthracycline-based adjuvant chemotherapy [50, 56].

Several studies have found an association between Topo IIα overexpression and response to primary chemotherapy with anthracycline, despite the heterogeneity of these studies [23, 44, 51]. In the same manner, loss of Topo IIα overexpression after primary chemotherapy was also correlated with a higher response rate to anthracycline-based therapy, suggesting selective action on cells that overexpress Topo IIα [23]. Amplification of Topo IIα was also associated with response to anthracyclin-based chemotherapy as detected by FISH and CISH [51, 57].

Topo IIα gene status seems to be a more specific marker, concerning anthracyclines, since it is not influenced by other factors [53]. Nevertheless, the predictive value of topo IIα amplification when it does not translate into protein overexpression remains to be determined, and both Topo IIα amplification and overexpression might be useful to define the tumor profile with regard to sensitivity to anthracyclines [24, 53]. Since the target fot anthracyclines is the Topo IIα protein and not the gene, biologically it makes sense that the protein status can help predict those patients who are likely to respond [24]. Topo IIα protein status might be particularly interesting in Topo IIα non-amplified tumors (85%-95% of breast cancer cases).

Hormone receptor negativity was not associated with clinical response to chemotherapy, although it was associated with pathological response. This may be explained by the few cases that achieved a complete response. Lack of hormone receptor expression was predictive of complete clinical response in some studies [25, 26, 27, 33, 62]. Other studies have suggested that patients with an estrogen-receptor positive tumor achieve a worse pathological response [33, 63].

Because this was a retrospective study, tissue fixation was not standardized at biopsy, and significant differences in fixation time may have ocurred. Tissue sampling may not have represented the patient's entire tumor burden, thus interfering with immunohistochemical results. There is no standard cut-off for Topo IIα protein positivity evaluated by immunohistochemistry. In several studies, the cut-off varied from 10% to 35% of positive cells [23, 24, 26, 44, 47, 48, 50, 51]. Considerable variation was found among immunohistochemical techniques.

The decreased clinical pathological response to primary chemotherapy found in this study may be explained by the larger mean tumor size, since response rate is higher

in smaller tumors [6, 64]. The median number of primary chemotherapy cycles before evaluating tumor response was 3. Part of the studies indicated that response was assessed after 6 cycles of primary chemotherapy [26, 44, 51]. Evidence is accumulating that taxanes and sequential treatments can increase the rates of complete clinical and pathological response [65].

There seems to be no uniformity among the studies regarding study design, subjects included, inclusion or exclusion criteria of inoperable cases and inflammatory carcinoma, in addition to chemotherapy regimens and doses.

In the current study, these markers were not considered predictors of tumor response to primary anthracycline-based chemotherapy, because no statistically significant association was found. Prospective studies with a larger number of patients and radiological methods to assess a more accurate response are required. Furthermore, the correlation between immunohistochemistry and FISH should be assessed.

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4. Conclusões

- A freqüência da superexpressão da Topo IIα foi de 41%;

- Não houve associação entre a superexpressão da Topo IIα e do HER2, tanto concomitante como isoladamente, e a resposta clínica e patológica à QT primária com antraciclina;

- Não houve associação entre a negatividade dos receptores hormonais e a resposta clínica; porém houve associação com a resposta patológica.

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7. Anexos

7.1. Anexo 1 – Metodologia

7.1.1. Desenho do estudo

Estudo de coorte retrospectivo.

7.1.2. Tamanho amostral

Baseado na diferença de proporções (Pocock, 1987) para a resposta positiva (RC, RCp e RP) e resposta negativa (DE e DP) para a Topo IIα (MacGrogan et al., 2003), o tamanho amostral mínimo calculado para análise estatística foi de 142, considerando-se um $\alpha = 5\%$ e um $\beta = 20\%$.

7.1.3. Variáveis independentes

- Receptor de estrógeno
- Receptor de progesterona
- HER2
- Topo IIα

7.1.4. Variável dependente

Resposta clínica do tumor à quimioterapia: avaliada pelos critérios preconizados pelo RECIST ("Response Evaluation Criteria in Solid Tumors"), em que se comparam as somas dos maiores diâmetros iniciais e finais do tumor (Therasse et al., 2000):

- Resposta completa: desaparecimento do tumor na avaliação clínica;
- Resposta parcial: redução de, no mínimo, 30% na soma dos maiores diâmetros das lesões selecionadas;
- Doença estável: quando nem a redução é suficiente para caracterizar resposta parcial e nem o aumento é suficiente para caracterizar doença progressiva, tendo como referência o menor valor da soma dos maiores diâmetros desde o início do tratamento;
- Doença progressiva: aumento de, no mínimo, 20% na soma dos maiores diâmetros, tendo como referência o menor valor alcançado desde o início do tratamento ou aparecimento de uma ou mais lesões novas.

Resposta patológica do tumor: avaliada pelo TNM (2002):

- R0: ausência de tumor residual
- R1: tumor residual microscópico
- R2: tumor residual macroscópico

7.1.5. Critérios de inclusão

- Prontuários de pacientes portadoras de carcinoma ductal de mama atendidas de 1996 a 2004 no Ambulatório de Oncologia Clínica (CAISM);
- Estádios clínicos IIIA e IIIB;

- QT primária com antraciclina (AC, FAC, FEC);
- Avaliação precisa de resposta clínica no prontuário;
- Tecido suficiente em bloco de parafina da biópsia pré-tratamento para a realização de IHQ para a pesquisa da expressão da Topo IIα, HER2 e receptores hormonais.

7.1.6. Critérios de exclusão

- Carcinoma bilateral;
- Neoplasia de outras localizações, exceto carcinoma *in situ* do colo uterino e carcinomas espinocelulares e basocelulares da pele;
- Biópsia excisional ou nodulectomia;
- Biópsias realizadas em outros serviços;
- Realização de mais de uma linha de QT primária;
- Tratamento prévio com hormonioterapia ou outros agentes quimioterápicos.

7.1.7. Técnica imunoistoquímica

A pesquisa da expressão imunoistoquímica da Topo IIα, HER2 e receptores hormonais foi realizada em tecido da biópsia inicial previamente fixado em formol a 10% e incluído em parafina. Os cortes nas lâminas (de 4µm) foram desparafinados em banho de xilol a 110^{o}C e *re*-hidratados em concentrações decrescentes de álcool etílico, água e PBS. O bloqueio da peroxidase endógena foi feito com banhos em peróxido de hidrogênio a 10%. Para o desmascaramento dos antígenos foi utilizada panela a vapor T-fall® contendo tampão citrato de sódio pH 6.0 para o HER2 e EDTA pH 8,9 para os receptores hormonais e Topo IIα. Foram então

colocadas em solução de PBS até a incubação com os anticorpos primários específicos. Foram utilizados os anticorpos monoclonais específicos para: receptor de estrógeno (clone 1D5, 1/300; clone 6F11, 1/80, Novocastra); receptor de progesterona (clone 1A6, 1/100, Novocastra) e Topo IIα (clone M 3532-1, 1/50, DAKOCYTO). Foi utilizado o anticorpo policlonal específico para o HER2 (clone A 0485-129, 1/300, DAKOCITO). Posteriormente foram colocadas em geladeira a 4°C "overnight". Terminado o período de incubação com o anticorpo primário, as lâminas foram submetidas a 3 lavagens em PBS e secadas com papel de filtro para posteriormente ser colocado o Sistema Envision Peroxidase. Para os receptores hormonais e topoisomerase IIα foi utilizado o Envision plus® (anti-mouse) e para o c-erbB-2 o Envision (código K1491, DAKO - anti-mouse/anti-rabbit). As lâminas foram levadas à estufa a 37°C por 1 hora para posteriormente serem colocadas em PBS pré-aquecido a 37°C. A revelação foi feita com substrato cromógeno DAB (3,3´-diaminobenzidine sigma, código 5637) na proporção de 60mg de DAB para 100ml de PBS, 1,5ml de H_2O_2 a 10% e 1 ml de dimetilsulfóxido (DMSO) a 37°C, durante 5 minutos. Em seguida, as lâminas foram contra-coradas com hematoxilina de Mayer por 30 a 60 segundos, de acordo com sua concentração. Foram, então, lavadas em água corrente, passadas por alguns segundos em água amoniacal, água corrente e água destilada. Os cortes foram posteriormente desidratados em 3 banhos de álcool absoluto e 3 banhos de xilol absoluto e, em seguida, montadas com lamínulas e resina Entellan.

Para atestar a positividade da reação imunoistoquímica foram utilizados controles externos sabidamente positivos. Para o controle positivo de receptores de

estrógeno e de progesterona foi usado tecido mamário normal; do HER2, carcinoma ductal da mama sabidamente positivo (3+) e da Topo IIα, amígdala normal. A leitura das lâminas foi realizada em microscópio óptico comum, no aumento de 40 vezes, de modo semi-objetivo. Para avaliação da positividade dos receptores hormonais foi considerada a percentagem de células com expressão de receptores, sendo considerados positivos os casos com 10% ou mais de células marcadas e negativos os casos com menos de 10% (Goldhirsch et al., 2001). Em relação ao HER2 foram utilizados os critérios do Hercep Test, sendo considerados positivos somente os casos com expressão 3+ (expressão forte da membrana em 100% da circunferência citoplasmática em pelo menos 10% da população de células neoplásicas) (Jacobs et al., 1999). Em relação à Topo IIα, foram considerados positivos os casos com >15% de células marcadas e negativos os casos com ≤15% (MacGrogan et al., 2003).

7.1.8. Análise estatística

Foi analisada a freqüência da superexpressão da Topo IIα. A análise foi realizada através de testes de associação entre variáveis, como o qui-quadrado e teste exato de Fisher (Fisher et al., 1991). Foi considerado estatisticamente significativo o valor de $p < 0,05$.

7.1.9. Instrumentos para coleta de dados

Foi preparada uma lista de verificação (Anexo 1) para a pré-seleção dos prontuários. Os dados foram coletados através de uma ficha codificada e pré-testada (Anexo 2). Para cada prontuário foi atribuído um número de identificação.

7.2. Anexo 2 – Lista de Verificação

Pesquisa: Associação entre a expressão imunoistoquímica da topoisomerase IIα, HER2 e receptores hormonais e a resposta à quimioterapia primária em pacientes com câncer de mama

1. Critérios de inclusão:	**Aceita**	**Rejeita**
1.1 Paciente tem diagnóstico cito-histológico de carcinoma ductal invasivo no estádio IIIA ou IIIB?	_____	_____
1.2 Paciente tem tumor mensurável ao exame clínico?	_____	_____
1.3 Paciente tem estadiamento completo não evidenciando doença a distância?	_____	_____
1.4 Paciente tem avaliação clínica objetiva da resposta à quimioterapia primária?	_____	_____
1.5 Paciente realizou tratamento antiblástico primário com antraciclina?	_____	_____
2. Critérios de exclusão:		
2.1 Paciente é portadora de carcinoma bilateral?	_____	_____
2.2 Paciente é portadora de neoplasia de outras localizações, excetuando-se carcinoma *in situ* do colo uterino e carcinomas epidermóides ou basocelulares de pele?	_____	_____
2.3 Paciente realizou outro tratamento prévio à QT?	_____	_____
2.4 Paciente foi submetida à biópsia excisional do tumor ou nodulectomia?	_____	_____
2.5 Tecido suficiente em bloco de parafina?	_____	_____

- Prontuário deverá conter todos dados necessários para avaliação
- Paciente aceita para ser ficha nº __ __ __

7.3 Anexo 3 – Ficha de Coleta de Dados

Pesquisa: Associação entre a expressão imunoistoquímica da topoisomerase IIα, HER2 e receptores hormonais e a resposta à quimioterapia primária em pacientes com câncer de mama

1. N^{O} da ficha __ __ __ 2. Idade: __ __ anos

3. *Status* menstrual: 1. Pré __ 2. Pós __ 3. Desc __

4. Diagnóstico: 1. PAAF __ 2. TRU-CUT __ 3. Biópsia incisional __

4.1 Número do Exame: _____ 4.2 Data: __/__/__

5. Medidas do tumor pré-QT: 5.1 mama _____ mm

5.2 axila _____ mm

5.3 soma _____ mm

6. Grau histológico: 1. GI __ 2. GII __ 3. GIII __ 4. Desc. __

7. Grau nuclear: 1. GI __ 2. GII __ 3. GIII __ 4. Desc. __

8. Tumor: 1. T1 __ 2. T2__ 3. T3__ 4. T4a __ 5. T4b __ 6. T4c __ 7. T4d __

9. Linfonodo: 1. N0 __ 2. N1 __ 3. N2 __

10. Estádio clínico: 1. IIIA __ 2. IIIB __

11. Receptores hormonais: 11.1 N Exame: _____ 11.2 Data: __/__/__

11.3 Receptor de estrógeno 1. Positivo __ 2. Negativo __ 3. Desc. __

11.4 Receptor de progesterona 1. Positivo __ 2. Negativo __ 3. Desc. __

12. Expressão HER2:

1. Positiva __ 2. Negativa __ 3. Desc. __

13. Expressão topo IIα

1. Positiva __ 2. Negativa __ 3. Desc. __

14. Esquema de QT primária:

1. FAC __ 2. AC __ 3. EC __ 4. FEC __

15. Intensidade de dose de antraciclina: _____mg/m^2/sem

16. Medidas do tumor pós-QT: 16.1 mama ____ mm

16.2 axila ____ mm

16.3 soma _____ mm

17. Resposta clínica: 1. RC __ 2. RP __ 3. DE __ 4. DP __ 5. Não avaliável __

18. Cirurgia: 1. Sim __ 2. Não __ 3. Desconhecido __ 18.1 Data: __/__/__

19. Número do Anatomopatológico: _____ 19.1 Data: __/__/__

20. Medidas patológicas: 20.1 mama ____ mm

20.2 axila ____ mm

20.3 soma _____ mm

21. Resposta patológica:

1. R0 __ 2. R1 __ 3. R2 __ 4. RX __ 5. S/ cirurgia __