Comparative Study between Morphometric Analysis and Fluorescence In-Situ Hybridization (FISH) in Cases of Breast Cancer with Equivocal HER2 Score 2+

Thesis

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Abstract

This study was done on 60 blocks from patients diagnosed with invasive breast cancer with known HER2/neu scores. They were divided according to their scores into 4 groups of 15 blocks each. The blocks were submitted for paraffin sectioning followed by staining with immunohistochemical HER2 stain. All the slides were then scanned by the whole slide scanner. Only the group with HER2 score 2+ was tested by FISH.

The (score 2+) group when scanned and digitally analyzed revealed three slides to be positive, nine slides to be negative and three slides maintained their equivocal results.

The 15 blocks from the equivocal (score 2+) group were further tested by FISH. Two out of the 15 were positive for HER2/neu overexpression, while the remaining 13 turned out to be negative.

The two positive cases detected by the FISH were among the three positive cases detected by the quantitative digital analysis.

Key Words:

Breast Cancer, HER2, Quantitative digital analysis.

Dad

I won't let you down, again.

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List of Abbreviations

AKT	: Protein kinase B
Amp	: Amphiregulin
AOI	: Areas of interest
ASCO	: American Society of Clinical Oncology
CAP	: College of American Pathologists
CEF	: Cyclophosphamide, Epirubicin and Fluorouracil
Cel	: Cellulin
CEP17	: Chromosome enumeration probe
CER genes	: eceriferum
CISH	: Chromogenic in situ hybridization
CMF	: Cyclophosphamide, Methotrexate and Fluorouracil
Cmyc	: Cell myelocytomatosis
EGF	: Epidermal Growth Factor
Epi	: Epinephrine
ER	: Estrogen Receptor
ERBB2	: Erythroblastic leukemia viral oncogene homolog 2
ESHRE	: European Society of Human Reproduction and Embryology
Fc	: Fragment, crystallizable region.
FDA	: Food and Drug Administration
FISH	: Fluorescent in Situ Hybridization
HB-GF	: Heparin-binding Growth Factor
HER2	: Human Epidermal Growth Factor Receptor 2
HMEC	: Human mammary epithelial cell
IgG	: Immunoglobulin G
IHC	: Immunohistochemistry
IQ-FISH	: Interphase Quantitative FISH
ISH	: In situ hybridization

LVEF : Left ventricular ejection fraction

M.I.T. : Massachusetts Institute of Technology

MA.5 : Mammary.5

MAPK : Mtogen-Activated Protein kinase

MEK : MAPK/extracellular signal–related kinase kinase

mTOR : Mammalian Target of Rapamycin.

NCI : National Cancer Institute

NCICCTG : National Cancer Institute of Canada Clinical Trials Group

NRG : Neuregulin

P16 : Protein 16

P95 : Protein 95

PI3K : Phosphatidylinositol 3_ kinase

PR : Progesterone Receptor

pRB : Retinoblastoma protein

qRT-PCR : Quantitative reverse transcriptase polymerase chain reaction

Ras : Rat sarcoma

RNA : Ribonucleic acid

SISH : Silver in situ hybridization

SOS : Son of Sevenless

TGF : Transforming Growth Factor

TNBCs : Triple-Negative Breast Cancers

TOP2A : DNA topoisomerase 2-alpha

USA : United States of America

VEGF : Vascular Endothelial Growth Factor

WSI : Whole Slide Imaging

INTRODUCTION

Breast cancer is the most common malignant tumour among women worldwide. More than half of the incident cases in the world occur in Europe and North America. A Westernized life-style, including older age at giving birth to a first child and fewer children, are among the explanations for the increasing incidence seen worldwide ***(Beiki O et al., 2012).***

Cancer arises from the aberrant growth of cells that have sustained mutations in genes controlling cell proliferation and survival. Breast carcinoma cells also commonly acquire alterations in the Ras-signaling pathway ***(Clark G.J. and Der C.J, 1995)***, which may occur by several mechanisms, most notably amplification or overexpression of the human epidermal growth factor receptor 2 *(HER2/*neu) gene ***(Slamon DJ et al., 1989).***

Human epidermal growth factor receptor 2 is a transmembrane tyrosine kinase receptor and a member of the *ErbB* protein family, more commonly known as the epidermal growth factor receptor *(EGFR)* family. Activation of this class of cellular receptors is known to result in increased activity of a variety of molecular pathways associated with tumor growth and progression ***(Markman M et al., 2013).***

Human epidermal growth factor receptor 2 is overexpressed/ amplified in approximately 15% – 25% of human breast cancers ***(Scaltriti M et al., 2007; Staaf J et al., 2010)*** and its positivity is associated with

worse prognosis (higher rate of recurrence and mortality) in patients with newly diagnosed breast cancer who do not receive any adjuvant systemic therapy ***(Wolff AC et al., 2007).***

Human epidermal growth factor receptor 2 proto-oncogene amplification is usually accompanied by protein over-expression. The gene amplification is generally determined by the fluorescent in situ hybridization (FISH) method or in situ hybridisation (ISH) techniques using DNA probes, analysed by either chromogenic in situ hybridisation (CISH) or silver enhanced in situ hybridization (SISH) detection methods ***(Meijer SL et al., 2011).***

The protein expression is determined by immunohistochemistry (IHC) ***(Yosepovich A et al., 2010)*** using monoclonal or polyclonal antibodies and results are generally scored as 0, 1+, 2+ or 3+. Tumours with *HER2* staining scores of 0 and 1+ are categorised as *HER2*-negative, 2+ as equivocal and 3+ as *HER2*-positive ***(Meijer SL et al., 2011).***

Immunohistochemistry has emerged as a method of choice for regular screening; whereas FISH is used as a confirmatory test particularly in equivocal IHC cases (2+) ***(De P et al., 2010).***

Image morphometry is a quantitative method with two major goals in pathology: to add objective measurement to diagnostic assessment and to improve diagnostic capabilities ***(Marchevsky AM and Bartels PH, 1994).***

Computerized interactive morphometry (CIM) forms a useful technique in providing an objective and a reproducible estimate of the various breast lesions ***(Ruiz A et al., 1999).***

Trastuzumab (Herceptin®), a recombinant humanized monoclonal antibody directed against an extracellular region of *HER2*, was the first *HER2*-targeted therapy approved by the United States Food and Drug Administration (FDA) for the treatment of *HER2*-overexpressing metastatic breast cancer ***(Nahta R and Esteva FJ, 2006).***

In addition, trastuzumab with adjuvant chemotherapy (either in sequence or in combination) significantly improved disease-free and overall survival rates in patients with early stage *HER2*-overexpressing breast cancer ***(Nahta R and Esteva FJ, 2006).***

Because trastuzumab is only effective for patients with *HER2* gene amplified and overexpressing breast carcinomas, reliable determination of the *HER2* status is of great importance ***(Meijer SL et al., 2011).***

Equivocal IHC (2+) results are an important part of the ongoing discussion on optimising methods to assess *HER2* status and comprise about 15% of samples in routine practice ***(Dowsett M et al., 2003; Sawaki M et al., 2006).***

AIM OF THE WORK

This study aims to compare between the findings of quantitative digital analysis and Fluorescence In-Situ Hybridization (FISH) in breast cancer patients with *HER2* Score 2+ (equivocal or borderline) diagnosed by immunohistochemistry.

I. Breast Cancer

Incidence and Risk Factors

Breast carcinoma is the most common malignant tumour and the leading cause of carcinoma death in women, with more than 1,000,000 cases occurring worldwide annually ***(Rosai J et al., 2011).***

Although there has been a steady decrease in breast cancer mortality since the early 90s, due largely to improvements in the early detection and treatment of breast tumours, in the United States of America (U.S.A) approximately 40,000 women will die of breast cancer this year ***(Siegel R et al., 2013).***

However, there is large geographical variation in its incidence; with the exception for Japan, the incidence ranks highest in high-income countries ***(GLOBOCAN, 2008).*** More than half of the incident cases in the world occur in Europe and North America. The incidence of breast cancer has been increasing since the 1970s even in countries with a reported low rate, such as Japan, Korea, India and even Africa which lacks accurate population data ***(Sloan FA and Gelband H, 2007).***

In Egypt, breast cancer is the most common cancer among women, representing 18.9% of total cancer cases (35.1% in women and 2.2% in men) among the Egypt National Cancer Institute (NCI) series of 10 556 patients during the year 2001, with an age-adjusted rate of 49.6 per 100, 000 population ***(Omar S et al., 2003).***

A westernized life-style, including older age at giving birth to a first child and fewer children, are among the explanations for the increasing incidence seen worldwide. Despite the substantial improvement in breast cancer prognosis and survival, it is still the leading cause of cancer mortality in low- and middle-income countries and more than half of the breast cancer mortality is reported from low- and middle-income countries ***(Beiki O et al., 2012).***

Breast cancer should be largely viewed as a disease predominantly influenced by risk factors related to lifestyle, as only approximately 15% of all breast cancer cases can be attributed to familial and genetic influences. Most known risk factors for breast cancer can be linked to hazardous effects of hormonal exposures ***(ESHRE, 2004*)**, although other risk factors such as exposure to ionizing radiation are also relevant in some populations ***(Ronckers CM et al., 2005).***

Major risk factors for breast cancer include: Early menarche, late menopause, nulliparity, late age at first birth ***(Albrektsen G et al., 2005)***, little or no breastfeeding, family history, long-term use of hormone replacement therapy [but apparently not long-term use of oral contraceptives] ***(Beral V, 2003),*** fibrocystic disease and epithelial hyperplasia and smoking ***(Reynolds P et al., 2004)***.

Pathogenesis

Cancer arises from the aberrant growth of cells that have sustained mutations in genes controlling cell proliferation and survival. The great majority of human breast cancers arise from epithelial cells, and genetic analysis of tumour cells obtained from patients has revealed several commonly mutated genes. Mutations in the *p53* tumour suppressor gene occur in over half of all tumours examined ***(Ozbun MA and Butel JS, 1995).***

Breast cancers also frequently carry mutations that deregulate the retinoblastoma protein *(pRB)* pathway including loss of expression of *pRB or Protein 16 (p16) INK4a* ***(Varley JM et al., 1989; Brenner AJ et al., 1996)*** or amplification or overexpression of cyclin D1 ***(Gillett C et al., 1994).*** Breast carcinoma cells also commonly acquire alterations in the Ras-signaling pathway ***(Clark GJ and Der CJ, 1995)***, which may occur by several mechanisms; most notably amplification or overexpression of the Human Epidermal Growth Factor Receptor 2 (*HER2/neu)* gene ***(Slamon DJ et al., 1989)***. Cell myelocytomatosis gene *(C myc*) is also frequently amplified or overexpressed ***(Escot C et al., 1986)***.

Whereas these individual genetic mutations have been cataloged in numerous breast cancers, none is involved universally in all human breast cancers, and the number of mutant genes that coexist in the genome of a naturally arising breast cancer cell is unknown. As a consequence, it has been impossible to know how many mutant genes are required to convert a normal human mammary epithelial cell *(HMEC)* into a tumour cell ***(Elenbaas B et al., 2001).***

The analysis of gene expression patterns by ***Perou CM et al., 2000*** led to the discovery and identification of four distinct molecular subtypes of breast cancer with Ribonucleic acid (RNA) expression profiles dividing the tumours into at least four subgroups.

These subgroups are characterized by variations in overexpression, with the luminal subgroup highly expressing genes normally associated with breast luminal cells, the second subgroup expressing genes typically active in breast basal epithelial cells (basal-like subgroup), and the third subgroup overexpressing human epidermal growth factor receptor 2 (*HER2* subgroup), which is associated with a unique set of genes. The fourth tumour subgroup consists of tumours that cluster with normal breast samples, and are classified as normal-like breast tumours ***(den Hollander P et al., 2013).***

In 2007, ***Perou CM et al.*** extended their initial findings by identifying yet another molecular subtype, the claudin-low tumours, which underexpress genes involved in tight-junctions and cell–cell adhesion, including several Claudin genes and E-cadherin and high expression of endothelial marker. From a clinical perspective, these claudin-low tumours are associated with a poor prognosis. The basal and claudin-low molecular subtypes significantly overlap the clinical triple-negative breast cancers (TNBCs), which have low levels of estrogen receptor (ER), progesterone receptor (PR), and *HER2* proteins, exhibit a high level of molecular heterogeneity and are highly aggressive.

II. Human Epidermal Growth Factor Receptor 2

Discovery and Nomenclature

Epidermal growth factor receptor *(EGFR, ErbB-1, HER1),* the first tyrosine kinase receptor, was discovered by Stanley Cohen and co-workers at Vanderbilt University, USA in 1978 ***(Carpenter G et al., 1978).***

The neu oncogene was discovered in 1982 by a group of scientists (Robert Allan Weinberg Group) at Massachusetts Institute of Technology (M.I.T).

The *ErbB/HER* family of receptor tyrosine kinases consists of four different proteins called *EGFR/ErbB1/HER1, ErbB2/Neu/HER2, ErbB3/ HER3, and ErbB4/HER4.* Under normal physiological conditions, the *ErbB* receptors play crucial roles in propagating signals regulating cell proliferation, differentiation, motility and apoptosis ***(Holbro T et al., 2004).***

The *ErbB-2* was named for its similarity to avian erythroblastosis viral oncogene B *(ERBB)* ***(Ullrich A et al., 1984).*** Neu is so named because it was derived from a rat neuroglioblastoma cell line ***(Shih C et al., 1981).***

Subsequent sequence analysis and chromosomal mapping studies revealed all three genes *(neu, c-erbB-2, and HER-2)* to be the same ***(Coussens L et al., 1985).***

The human epidermal growth factor receptor 2 gene (*ERBB2)* (commonly referred to as *HER2*) is an oncogene located on the long arm of chromosome 17. It encodes *p185* (ErbB-2 or neu) oncoprotein which is a transmembrane tyrosine kinase receptor that can be associated with multiple signal transduction pathways ***(Goud Ket al., 2012).***