CLASSIFICATION OF INVASIVE BREAST CARCINOMA ACCORDING TO ST GALLEN CLASSIFICATION 2011 WITH EMPHASIS ON Ki67 INDEX AMONG SABAHAN POPULATION

By

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Table of Contents

List of abbreviations

ER: Estrogen Receptor

H&E: Hematoxylin and Eosin

HER2: Human Epidermal Receptor 2

HO: HER2 Overexpression

HQE: Hospital Queen Elizabeth

IBC: Invasive Breast Carcinoma

IHC: Immunohistochemistry

LA: Luminal A

LB: Luminal B

LIS: Laboratory Information System

LN: Lymph Node

NMRR: National Medical Research Registry

NST: No Specific Type

PR: Progesterone Receptor

TBNC: Triple Negative Breast Cancer

List of tables

List of figures

Abstrak

Latarbelakang: Kanser Payudara Tanpa Jenis Spesifik (IBC NST) terbahagi kepada empat subtaip menggunakan ujian immunokimia tisu iaitu Estrogen Receptor (ER), Progesterone Receptor (PR) dan Human Epidermal Receptor 2 (HER2). Ia diklasifikasikan kepada Luminal A (LA), Luminal B (LB), HER2 overexpressed (HO) dan triple negative (TN). Pengkelasan St. Gallen 2011 menyatakan penggunaan indeks proliferatif, Ki67 antibodi untuk menentukan subtaip LB daripada LA. LB mempunyai prognosis yang lebih teruk dan rawatan yang berbeza.

Objektif: Kajian ini bertujuan untuk menentukan HER2 negatif subtaip Luminal B menurut Pengkelasan St Gallen 2011 menggunakan antigen Ki67 dan membandingkan ciri-ciri klinikopatologi bagi setiap subtaip luminal.

Kaedah: Kes-kes IBC NST yang mempunyai spesimen biopsi tisu yang disusuli mastektomi diambil daripada arkib Hospital Queen Elizabeth di Kota Kinabalu, Sabah. Biopsi tisu LA diuji menggunakan antibody terhadap Ki67. Kes-kes LA dengan Ki67 ≥14% diklasifikasikan semula sebagai subtaip LB manakala Ki 67< 14% kekal sebagai LA. Perbandingan setiap subtaip luminal dengan tahap barah, saiz barah dan kewujudan metastasis ke noda limfa seterusnya dibuat. Analisis univariasi menggunakan regresi logistik mudah diadakan untuk menentukan peratusan ekspresi Ki67 dalam kes-kes IBC NST. Ujian McNemar's digunakan untuk analisis kategori berkembar. Semua

pengiraan dibuat menggunakan SPSS versi 22 dan nilai-p <0.05 diset sebagai signifikan.

Keputusan: LA adalah subtaip yang biasa (43%; 68/158), diikuti LB yang lebih agresif (33%; 52/158). Hanya 37 daripada 68 kes luminal A diuji dengan Ki67 disebabkan sampel yang terhad dan 43% (16/37) menunjukkan Ki67 ≥14% (dikelaskan semula sebagai subtaip LB). Penggunaan Ki67 memberikan perbezaan keputusan yang signifikan secara statistik (P<0.001; P<0.05). Walau bagaimanapun, subtaip LB menunjukkan keputusan statistik yang tidak signifikan apabila dibandingkan antara tahap, gred dan status noda limfa.

Kesimpulan: Klasifikasi barah payudara menurut Pengkelasan St Gallen 2011 menggunakan Ki67 antigen selain ER, PR and HER2 dalam mengenali kes subtaip LB, yang mempunyai prognosis yang lebih teruk.

Abstract

Background: Invasive Breast Carcinoma of No Specific Type (IBC NST) is divided into four subtypes using Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Receptor 2 (HER2) immunohistochemistry markers. They are classified into Luminal A (LA), Luminal B (LB), HER2 Overexpressed (HO) and Triple Negative (TN) subtype. The St. Gallen 2011 Classification recognizes the use of Ki67 proliferative index to identify LB subtype from LA group. LB has a worse prognosis and different approach of treatment.

Objective: This study aimed to identify HER2 negative LB subtype according to St Gallen Classification 2011 using Ki67 and to compare the clinicopathological features of different subtypes.

Methods: Tissue biopsies of LA subtype were stained with antibody towards Ki67. LA cases with Ki67 ≥14% were reclassified as LB subtype. Luminal subtypes with corresponding stage (tumour size), histological grade and lymph node metastases were compared. Univariate analysis using simple logistic regression was performed to determine the percentage of Ki67 expression among all IBC NST cases. McNemar's test was used for paired categorical analysis. All calculations performed using SPSS version 22 and a p-value of <0.05 was set to denote statistical significance.

Results: LA is the most common subtype (43%; 68/158), followed by LB (33%; 52/158). Only 37 out of 68 cases were stained with Ki67 due to sample limitations. From these LA cases, 43% (16/37) showed Ki67 ≥14% (reclassified

as LB subtype). There was significant result when using Ki67 ($P<0.001$; $P<0.05$). However, LB subtype showed statistically insignificant result when compared with between stage, grade and lymph node status.

Conclusion: The classification according to the St Gallen Classification 2011 utilized Ki67 marker in addition to ER, PR and HER2 in identifying luminal B subtype cases, which have a worse prognosis.

CHAPTER 1: INTRODUCTION

1.1 Literature review

Breast cancer remains as the major cause of death for women in the 21st century and the most common female cancer worldwide (Peter Boyle, 2008). The aetiology and pathogenesis are diverse and there are still not well understood (Abdulkareem, 2013). It is the number one cause of death due to cancer in Malaysian women (Leong, 2007; Cheng Har Yip, 2006). The incidence varies among ethnicities in Malaysia, mostly affecting Chinese (Pathy *et al.*, 2011). Malaysian women have a 1 in 20 chance of developing breast cancer during their lifetime whereas in Sabah, where the population is 3.39 million with more than 30 ethnic groups, most cases presented with advanced disease (Agarwal *et al.*, 2007; Benjamin Dak Keung Leong, 2007; Leong, 2007; Ibrahim *et al.*, 2012; de Deus Moura *et al.*, 2015). The percentage of breast cancer detected at stage I and II was 58% (Goldhirsch *et al.*, 2011). To date, there was no specific study focusing on Invasive Breast Carcinoma of No Specific Type (IBC NST) in Sabah (CH Yip, 2014).

The most significant classification of tumours of the breast was that produced by the World Health Organization (Hoda *et al.*, 2009; Tavassoéli, 2012; Sinn and Kreipe, 2013). IBC NST are tumours devoid of special features thus the designation NST (Edwin R. Fisher, 1975). The special types of invasive breast carcinoma includes tubular, cribriform, mucinous, medullary, lobular, metaplastic, adenoid cystic and invasive secretory

carcinoma (Page, 2003; Alessandra Fabbri, 2008; Sinn and Kreipe, 2013). IBC NST is further subclassified into molecular subtypes (Goldhirsch *et al.*, 2011; Malhotra *et al.*, 2014). Different subtypes carry different prognosis and different treatments (Ferguson *et al.*, 2013). Most typing is possible by way of studying the morphology on H&E staining whereby molecular study is the more accurate way of subtyping albeit costly (Eroles *et al.*, 2012).

Several studies have shown the heterogeneity of IBC NST and new classifications were produced that can alter management and outcome of patients (Goldhirsch *et al.*, 2011; Hortobagyi, 2012a; Molloy *et al.*, 2012). The most popular method of subtyping is by surrogating using immunohistochemistry staining (Cheang *et al.*, 2009; Maisonneuve *et al.*, 2014). The importance is because of different response to therapy and the availability of specific targeted treatment using hormonal and chemotherapeutic agents (Lim and Winer, 2011; Boyle *et al.*, 2013a). Different subtypes also carry different prognosis in term of metastasis and relapse (Guarneri *et al.*, 2009; Kennecke *et al.*, 2010; Voduc *et al.*, 2010; Nuria Ribelles, 2013).

There are 4 subtypes of IBC NST according to the St Gallen classification 2011 (Goldhirsch *et al.*, 2011) *(Table 4).* In previous classification each subtypes are characterized by the expression of hormonal receptors namely Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epithelial Receptor (HER2) on the tumour cells *(Figure 1)* (Onitilo *et*

al., 2009). In addition to ER, PR and HER2, the classification in 2011 incorporates Ki67, a proliferative marker. Determining subtypes is important because of different prognosis and treatment modalities (Chen *et al.*, 2014; Criscitiello *et al.*, 2014). This study concentrates on the use of Ki-67 labelling index, as the means of identifying Luminal B (LB) subtype of IBC NST. Cases of Luminal A (LA) with Ki67 of 14% and more are classified as HER2 negative LB.

Determining luminal subtypes is a part of prognostic factor aimed to foresee the outcome of patients irrespective of treatment. On the other hand, predictive factors intend to assess the outcome of patients associated with sensitivity or resistance to therapy. Thus, a predictive factor is also a therapeutic target such as in the case of ER and HER2. Evaluation by immunohistochemistry (IHC) of ER, PR, and HER2 status represent consolidated and standardized prognostic factors. ER, PR and HER2 status are also validated predictive markers. Other accepted prognostic markers are represented by morphological findings such as tumour size (stage), grade, lymphovascular invasion, lymph node status and Ki67 (Rampaul *et al.*, 2001; Faratian and Bartlett, 2008; Hoda *et al.*, 2009; Song *et al.*, 2011; Haroon *et al.*, 2013; Inic *et al.*, 2014; Francisco Acevedo, 2015; Liu *et al.*, 2015). There is potential for Ki67 as a predictive factor as well, in the case where patients with high Ki67 benefit from adjuvant chemotherapy (Criscitiello *et al.*, 2014). However there were contradicting study regarding this (Ferguson *et al.*, 2013).

1.1.1 Stage

Pathological staging of breast cancer takes into account the size of the tumour, denoted as a capital T, and can be staged as T1 to T4. Tumours may be measured clinically or pathologically; the pathologic size of the lesion may be measured either macroscopically or microscopically. The clinical size of a breast lesion as measured by mammograms or physical examination may differ from the macroscopic pathologic size. It is possible that the presence of a macroscopically measurable lesion has a poorer outcome (Singletary, 2002; Edge, 2010).

1.1.2 Histologic grade

Besides staging, histologic grading system also plays an important prognostic factor. The most commonly used grading system, the Nottingham Histologic Score (also referred to as Elston-Ellis modification of Scarff-Bloom-Richardson grading system), combines nuclear grade, tubule formation, and mitotic rate to classify invasive breast carcinomas into three groups that are highly correlated with survival *(Table 1) (Meyer, 2005; Hoda et al., 2009)*. Survival for patients with well-differentiated grade 1 carcinomas gradually declines to 70% at 24 years. In contrast, most deaths for poorly differentiated grade 3 carcinomas occur in the first 10 years, and 45% of patients survive long-term. Patients with moderately differentiated grade 2 carcinomas have better survival initially, but their long-term survival is only

slightly better than grade 3 carcinomas (Ellis., 1991; Leslie W. Dalton, 1994; S. E. Pinder, 1998; Howayda Abd El All, 2001). A grade of low, intermediate or high is obtained through a composite sum by assigning a 'score' based on the nuclear assessment, mitotic index assessment, and tubular assessment (*Table 1*). The nuclear assessment is based on the nuclear pleomorphism within the invasive cells. The tubular assessment refers to an approximate, quantitative account of the amount of cell groupings that remain in their normal 'tubular' shape. The less the percentage of tubular structures in comparison to other shapes, the higher the score. The mitotic index refers to patterns of cell division through assessing the numbers of dividing daughter cells, measured per square millimetre. Mitoses are only counted in the invasive area of the tumour (V. Le Doussal, 1989; Meyer, 2005).

1.1.3 Lymph node involvement

Axillary lymph node status at the time of diagnosis is the most significant and durable prognostic factor in breast cancer patients. To date, only nodal status has been consistently associated with survival outcomes in occult primary breast cancer (Ivkovic-Kapicl *et al.*, 2006; Hoda *et al.*, 2009).

1.1.4 The St Gallen Classification

Clinicians, pathologists and researchers convene in St Gallen, Switzerland to give a consensus regarding breast cancer treatment including what is theoretically feasible in patient risk stratification, treatment and daily practice management (Giuseppe Curigliano, 2013). In the earlier consensus given in 1998, patients with breast cancer were classified into different risk groups according to lymph node status, tumour size, grade, receptor status and age *(Table 2)*. Such classifications were made to guide oncologists in giving adjuvant chemotherapy as studies have shown that most patients benefit from chemotherapy regardless of lymph node status (Salisbur, 2002) *(Table 3).* A study has also shown that the use of the older St Gallen classification have led to the overuse of adjuvant chemotherapy. However, such overuse may be overcame by using Ki67 for LA cases thus recognizing HER2 negative LB, who are proven to benefit from chemotherapy (Goldhirsch *et al.*, 2011). The St Gallen classification in 2011 has recommended adjuvant chemotherapy for LB and not for LA in IBC NST. This is because the LB subtype proliferative index quantified by surrogate IHC marker has been proven to be associated with more aggressive cancer subtype and can be classified into LB despite HER2 marker being negative *(Table 5).*

1.1.5 Hormonal receptors

All breast carcinomas are characterized by expression of ER and PR receptors as well as the status of HER2. These, in relation to other clinicopathological parameters, defines treatment recommendations for each individual case (D.O. Shapochka, 2012). Approximately 70–80% of IBC NST are ER positive, and between 15–30% of cases are HER2 positive. Determining ER and HER2 status is considered as standard care for all invasive breast carcinoma as these biomarkers are predictive for response of patients to hormonal treatment and/or HER2-inhibiting medication (Wolff *et al.*, 2007; Hammond *et al.*, 2010).

1.1.6 Luminal A subtype

ER positive family was named luminal class since they have the same molecular signature strongly resembling luminal cells of breast duct (Taylor-Papadimitriou *et al.*, 1989). LA is the most common subtype (Kumar *et al.*, 2015). Whilst ER positive family, negative for HER2 is called LA, those expressing HER2 is called LB. It is important to differentiate these two subtypes because LA has been associated with a favourable prognosis (Perez-Rodriguez, 2015) and LB, less favourable prognosis (Sotiriou *et al.*, 2003).

1.1.7 Luminal B subtype

A study by (Cheang *et al.*, 2009) has shown that LB family is associated with higher histological grade (grade 3), compared to lower grade (grade 1 and 2). This subtype has a poorer prognosis (Tang *et al.*, 2015). Distinguishing LB subtype using Ki67 expression is of critical value since it would isolate a group of patients in early breast carcinoma that could benefit from adjuvant chemotherapy (Lim and Winer, 2011; Pavlakis *et al.*, 2012). New targeted therapy are also being developed specifically for LB subtype (Ben Tran, 2011).

1.1.8 HER2 Overexpressed subtype

Several studies demonstrated that HER2 overexpression is correlated with reduced survival. Overexpression is associated with relative resistance to tamoxifen therapy or alkylating agent (Wang and Hung, 2001). In contrast to previous data from Western countries that showed triple negative subtype to be the worst in term of prognosis, a study in South Korea showed that HER2 overexpressing breast cancers displayed the worst prognosis among the various subgroups, and not triple negative subtype. This finding proved that there is more to breast cancer of different subtypes in different population groups and the need for more study in local population as not all data demonstrated in Western population applies to Asians, and this might affect treatment and prognostication (Mattes *et al.*, 2015).

1.1.9 Triple negative subtype

Triple negative breast cancer (TNBC) is an aggressive clinical phenotype characterized by lack of expression (or minimal expression) of ER and PR as well as an absence of HER2 overexpression. TNBC is not amenable to treatment with hormone therapy or the anti-HER2 monoclonal antibody trastuzumab, and systemic treatment options are limited to cytotoxic chemotherapy. Unlike patients with ER/PR positive or HER2 overexpressing disease, systemic treatment options for patients with TNBC are limited to cytotoxic chemotherapy due to the lack of a molecular target (Siziopikou and Cobleigh, 2007; Nishimura and Arima, 2008; Cicin *et al.*, 2009). Despite its chemosensitivity, TNBC is still associated with a poor prognosis. The median time to death among patients with TNBC was also shorter than that with other subtypes (Cicin *et al.*, 2009).

1.1.10 Ki67

Recently, the immunohistochemical expression of Ki67 has replaced mitotic counting in assessing the proliferation of neoplastic cells. There is a lot of rising evidence on its importance as a prognostic and predictive marker of responsiveness to therapy and as a dynamic biomarker of treatment efficacy (O. Gottardi, 1993; Graef *et al.*, 2008; Miglietta *et al.*, 2013). In early breast carcinoma, high Ki67 is an independent factor for worse prognosis as

shown by significantly shorter overall and disease-free survival (Goldstein, 2004 ; Jung *et al.*, 2009; Zong *et al.*, 2014; Marrazzo *et al.*, 2015). High Ki67 was predictive of more benefit from adjuvant chemotherapy (Elzawahry *et al.*, 2013) even in cases without lymph node metastasis (Andre *et al.*, 2015). The prognostic role of Ki67 in early breast cancer was indicated in large sample study (Goldhirsch *et al.*, 2011). (Dowsett *et al.*, 2011) and (Nishimura *et al.*, 2010) mentioned the potential use of Ki67 for prognosis, predict responsiveness or resistance to endocrine or chemotherapy and as a dynamic biomarker of treatment efficacy. A study found that Ki67 expression is inversely related to their sensitivity to first line endocrine therapy (Graef *et al.*, 2008). The St Gallen International Expert Consensus on the primary therapy of early breast cancer 2011 defined a cut-off point of 14% for the distinction between luminal A and luminal B (HER2 negative) tumours (Dowsett *et al.*, 2011; András Vörös, 2014; Ono *et al.*, 2015).

1.2 Rationale of the study

Very few data regarding Sabah population is available, yet the numbers of breast cancer patients are increasing and they always presented late. This study involves population where presentation is late, thus association between Ki67 index and late presentation will be observed. Subtyping is important because of different treatment approach; Luminal B has a much worse prognosis than Luminal A, when taking Ki67 into consideration; it has a role of being an independent prognostic factor. We also wanted to see the significance of taking into account Ki67 in determining luminality in Sabah population, whether a significant percentage from the Luminal A subtype has high Ki67 index.

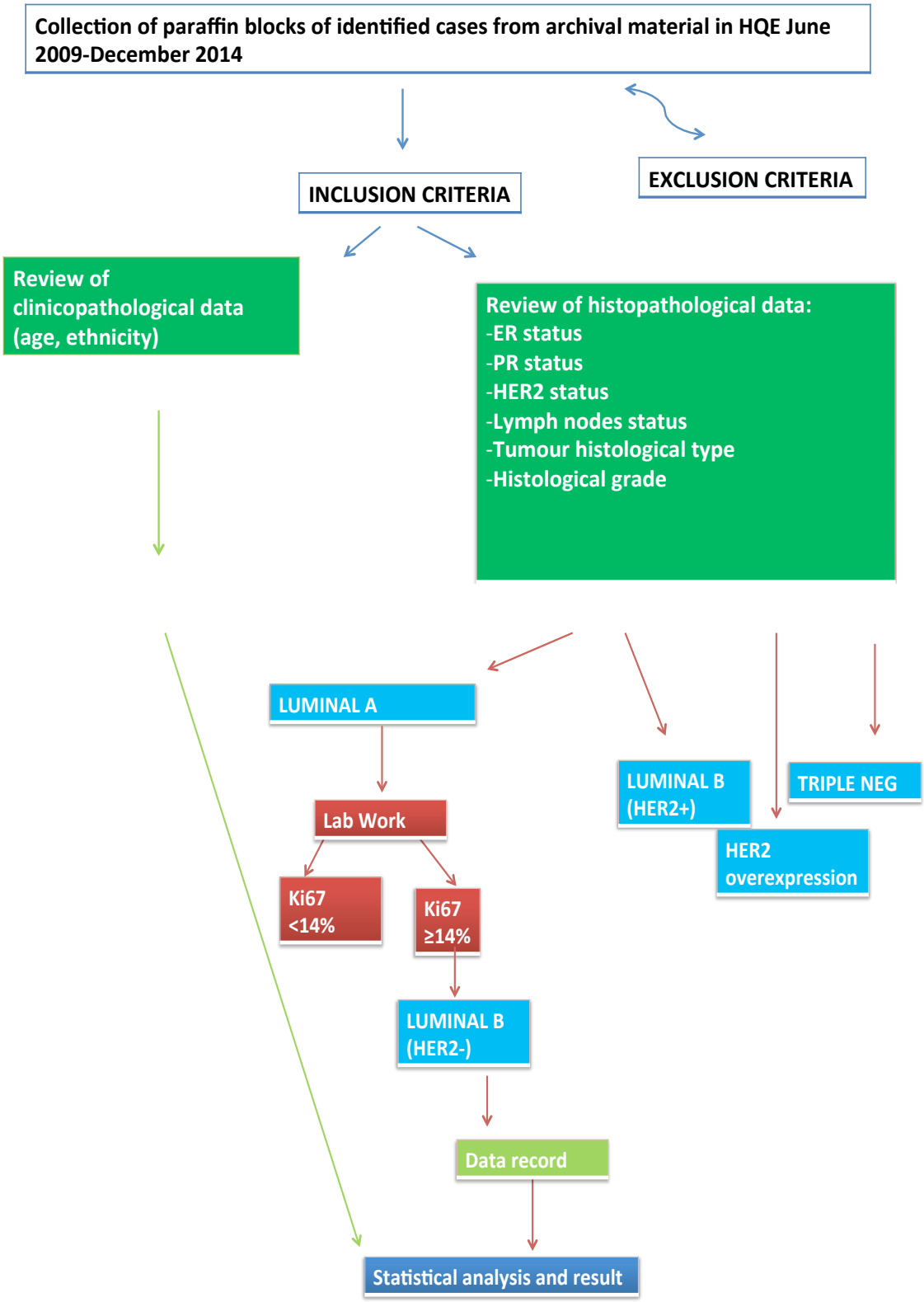
1.3 Objectives

Objective 1: To reclassify and compare luminal A & B IBC NST using Ki67 index.

Objective 2: To compare grade, stage and lymph node metastasis of luminal A and Luminal B IBC NST.

CHAPTER 2: STUDY PROTOCOL

2.1 Study design and method



2.2 Ethical approval letter (KKM)

JAWATANKUASA ETIKA & PENYELIDIKAN PERUBATAN
(Medical Research & Ethics Committee)
KEMENTERIAN KESIHATAN MALAYSIA
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Ruj. Kami : (3) KKM/NIHSEC/P14-365
Tarikh : 20 Jun 2014

Dr Muhd Afif Bin Mohd Yusof
Jabatan Patologi
Hospital Queen Elizabeth

Tuan,

NMRR-14-269-20213
CLASSIFICATION OF INVASIVE BREAST CARCINOMA ACCORDING TO ST GALLEN CLASSIFICATION 2011 WITH EMPHASIS ON KI-67 INDEX

Lokasi Projek : Hospital Queen Elizabeth

Dengan hormatnya perkara di atas adalah dirujuk.

2. Jawatankuasa Etika & Penyelidikan Perubatan (JEPP), Kementerian Kesihatan Malaysia (KKM) mengambil maklum bahawa projek tersebut adalah untuk memenuhi keperluan akademik Sarjana Patologi, Universiti Sains Malaysia.

3. Sehubungan dengan ini, dimaklumkan bahawa pihak JEPP KKM tiada halangan, dari segi etika, ke atas pelaksanaan projek tersebut. JEPP mengambil maklum bahawa kajian ini tidak melibatkan sebarang intervensi dan hanya melibatkan blok tisu parafin dalam mengumpul data kajian. Segala rekod dan data adalah SULIT dan hanya digunakan untuk tujuan kajian dan semua isu serta prosedur mengenai *data confidentiality* mesti dipatuhi. Kebenaran daripada Pengarah Hospital di mana kajian akan dijalankan mesti diperolehi terlebih dahulu sebelum kajian dijalankan. Tuan perlu akur dan mematuhi keputusan tersebut.

4. Adalah dimaklumkan bahawa kelulusan ini adalah sah sehingga **20 June 2015**. Tuan perlu menghantar *'Continuing Review Form'* selewat-lewatnya 2 bulan sebelum tamat tempoh kelulusan ini bagi memperbaharui kelulusan etika. Pihak tuan juga perlu mengemukakan laporan tamat kajian dan juga laporan mengenai "*All adverse events, both serious and unexpected*" kepada Jawatankuasa Etika & Penyelidikan Perubatan, KKM jika berkenaan. Borang-borang berkaitan boleh dimuat turun daripada laman web MREC (http://www.nih.gov.my/mrec)

Sekian terima kasih.

BERKHIDMAT UNTUK NEGARA

Saya yang menurut perintah,

(DATO' DR CHANG KIAN MENG)
Pengerusi
Jawatankuasa Etika & Penyelidikan Perubatan
Kementerian Kesihatan Malaysia

2.3 Ethical consideration

Confidentiality of the data was taken into account. Ethic approval was obtained from Ethics Approval; Medical Research & Ethics Committee NMRR (National Medical Research Register); NMRR-14-269-20213.

CHAPTER 3: MANUSCRIPT

3.1 Introduction

Breast cancer is the most common cancer in female worldwide (Peter Boyle, 2008) and is the number one cause of death due to cancer in Malaysian women (Benjamin Dak Keung Leong, 2007; Cheng Har Yip, 2006). The incidence varies among ethnicities in Malaysia, mostly affecting Chinese (Bhoo Pathy *et al.*, 2011). With recent progress in studies pertaining to the detailed aspects of the disease which include molecular testing, new classification has emerged that alter management and outcome of patient, therefore benefiting the patients (Goldhirsch *et al.*, 2011; Hortobagyi, 2012b). The introduction of genetic array testing has allowed breast cancer to be further subtyped by different methods (Sorlie, 2004; Molloy *et al.*, 2012). The most popular one is by approximation using immunohistochemistry staining (Maggie C. U. Cheang 2009). This is important due to the different response to therapy and the availability of specific targeted treatment using hormonal and chemotherapeutic agents (Boyle *et al.*, 2013b).

This study concentrated on the use of immunohistochemical definition of estrogen receptor (ER) and progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) oncogene, as well as Ki67 labelling index; a marker of cell proliferation, as the means of identifying tumour subtypes according to St Gallen expert consensus 2011(Goldhirsch *et al.*, 2011).

Most studies that have been done usually revolve around western population where detection of breast cancer is during the early stage. There is however a difference in hormonal status when the disease is at its more advanced stage. By concentrating on Malaysian population in Sabah, North Borneo, where detection and intervention is at a late stage, this study seeks to find out differences of hormonal status as compared to early stages. This study reclassified Luminal A (LA) and Luminal B (LB) subtypes of Invasive Breast Carcinoma of No Special Type (IBC NST) using Ki67 according to St Gallen International Consensus 2011 (Goldhirsch *et al.*, 2011). Associations between subtypes and other clinicopathological characteristics such as ethnicity, stage, histological grade and status of axillary lymph node were analysed.

This study aims to classify Invasive Breast Carcinoma of No Specific Type (IBC NST) according to St Gallen consensus 2011 with emphasis on Ki67 index. There are 2 specific objectives involved; 1) To reclassify and compare luminal A & B IBC NST using Ki67 index. 2) To compare grade, stage and lymph node metastasis of luminal A and Luminal B IBC NST (Hammond *et al.*, 2010).

3.2 Materials and Methods

This study is a cross sectional study design. It utilized cases of breast biopsies (core biopsies or excision biopsies) that were followed by mastectomy specimens only, with final histopathological diagnosis of IBC NST, for the period of June 2009-December 2014 in the Pathology Department, Hospital Queen Elizabeth, Kota Kinabalu. Only cases with biopsies followed by mastectomy were included. Search query was conducted using Lab Information System (LIS) at the Pathology Department, Hospital Queen Elizabeth, Kota Kinabalu, Sabah.

Cases that fulfilled the inclusion criteria but the corresponding paraffinised tissue blocks are not available, missing and inadequate for serial sections were excluded from the analysis for the objectives of this study. Biopsies that were not followed by mastectomy and mastectomies without prior biopsies were also excluded.

Clinicopathological parameters including age, ethnicity, tumour grade, tumour size (stage), lymph nodes involvement and ER and PR status, and HER2 expression were obtained from the histopathological reports (Tamaki *et al.*, 2010). In this study only the pathological stage (T), which is determined by tumour size was considered.

Independent pathologists of the Anatomic Pathology Department had examined these surgical specimens and formal pathology reports had been

issued within that period. Only suitable cases that met the inclusion criteria were chosen and the paraffin blocks were then retrieved from the archives.

3.2.1 Laboratory work

Standard immunoperoxidase procedures were followed for tissue sections obtained from each case. The selected single representative block for each case was sectioned to 4-micron thickness. Two sections were obtained from all LA cases (ER+ and or PR+, HER2-) according to data obtained from patient's record in LIS. This section was subjected to H&E and Ki67 staining. Ki67 staining was done using K2 antibody (Bond^{TM}) (Leica, 2009).

The obtained sections were placed on heater at 60°C for at least 1 hour to facilitate the adherence of tissue onto the slides. Leica Autostainer System performed the next steps automatically. For Ki67 IHC staining Bond^{TM} Ready-to-Use Primary Antibody Ki67 (K2) was utilised. The application was performed by Bond-Max Fully Automated IHC system (Leica) using according to the manufacturer's IHC protocol. The demonstration of human Ki67 nuclear antigen is achieved by first, allowing the binding of Ki67 (K2 antibody) to the section, and then visualizing this binding using the reagents provided in the detection system. The use of these products, in combination with an automated Bond system, reduces the possibility of

human error and variability resulting from individual reagent dilution, manual pipetting and reagent application (Leica, 2009).

3.2.2 Microscopic analysis

Using a bright-field microscope (Olympus CX31), microscopic evaluation of the slides immunostained for Ki67 was performed blinded from the clinicopathological data of the study subjects. H&E slides were examined to re-evaluate and to verify the diagnosis and location of tumour cells when compared to Ki67 slides. Ki67 proliferative index was reviewed by main researcher (supervisor) and co-supervisors at a separate time to avoid bias. Each case was divided into 2 groups; Ki67 equal or more 14% and Ki67 less than 14% (Dowsett *et al.*, 2011). ER, PR and HER2 hormonal qualitative status by IHC were obtained from the formal reports. The quantitative scores whether performed or not were disregarded.

3.2.3 Ki67

Slides stained with Ki67 were analysed according to Recommendations from the International Ki67 in Breast Cancer Working Group (Dowsett *et al.*, 2011). Only nuclear staining plus mitotic figures which were stained by Ki67 were incorporated into the Ki67 score that is defined as the percentage of positively stained cells among the total number of

malignant cells scored. When the staining is homogenous, at least three randomly selected high-power (×40 objective) fields were selected.

3.2.4 Statistical analysis

Data was entered and statistical analysis was conducted by using test on Statistical Package for Social Sciences (SPSS) program version 22. Univariate analysis using simple logistic regression was performed. McNemar's test was used for paired categorical analysis. A p-value of <0.05 was set to denote statistical significance.

3.3 Results

3.3.1 Clinicopathological data of the study subjects

One hundred and fifty eight cases of IBC NST, which met the inclusion criteria (cases with biopsy followed by mastectomy), were included in this study. Clinicopathological data of the study subjects were summarized in *(Table 6).* Distributions of the age, ethnicity and institutions were shown in (*Table 7)* and (*Figure 2)*, (*Figure 4)*, (*Table 8)* and (*Figure 3)*. Analysis for different subtypes and clinicopathological factors were summarized in *(Table 9)* and *(Figure 5).* Majority of the study subjects were natives. The mean age of the patients was 50.4 years with the age ranging from 28 to 78 years *(Figure 2).* This is comparable to data from peninsular Malaysia. All the cases included were those where biopsies were taken prior to mastectomies with axillary clearance. Almost half of the tumours (49.4%) had tumour size larger than 20mm but smaller than 50mm. Lymph node metastasis was found in majority of cases (64.6 %). More than half of the tumours (55.1%) were grade 3 tumours. ER, PR and HER2 status were positive in 74.7%, 62.7% and 45.6% of the cases respectively *(Table 6).*

Most cases were from Kota Kinabalu (62.7%; 99/158), followed by Sandakan and Tawau. Other cases are from Keningau, Lahad Datu, Labuan and Beaufort (*Table 8* and *Figure 3*). The main referral centre is in Hospital Queen Elizabeth, Kota Kinabalu where mastectomies were performed on a regular basis. Whereas in the other centres, it is carried out from time to time

by visiting surgeons. All the specimens were sent for examination to the Pathology Department of Queen Elizabeth Hospital in Kota Kinabalu.

The highest prevalence was observed among natives (78.5%; 124/158) followed by Chinese (18.4%; 29/158). Majority of cases were at late stage during presentation (stage 2 and 3; 94.7%; 148/158) and of high grade (grade 2 and 3; 93.7%; 148/158). Cases with lymph node metastasis were 64.6% (102/158).

3.2.2 Ki67 Proliferative Index and Luminal B Subtype

LA is the most common subtype (44%; 68/158), followed by LB (33%; 52/158) (*Table 9* and *Figure 5*). Only 37 LA cases were stained with Ki67 due to sample limitations. From these stained LA cases, 43% (16/37) showed Ki67 ≥14% *(Image 1)* (reclassified as LB subtype) (*Table 11*). Cases of Ki67 less than 14% remain as LA subtype *(Image 2).* The difference in number of cases between LA and LB with and without the use of Ki67 is statistically significant (<0.001) using McNemar's Test (*Table 12*). There was no significance when LA and LB subtypes with and without Ki67 staining were compared against grade, stage and lymph node status (*Table 13*).

3.4 Discussion and limitation

In general, breast cancer remains the most common cancer among Malaysian women, which accounted for 31% of newly diagnosed cancer cases in women registered in the National Cancer Registry (NCR) for the year 2003-2005. The risk differs among the major ethnic groups in Malaysia; about 1 in 16 Chinese, 1 in 17 Indian and 1 in 28 Malay women will develop breast cancer in their lifetime (NCR, 2007). In Malaysia, breast cancer is more common among Chinese as compared to Malay. However, in this study it was difficult to divide the study subjects strictly according to ethnicity. Due to the demographical situation in Sabah, which comprises of up to 36 ethnics, where mixed marriage is common (Suraya Sintang, 2011), all of the subjects that are not Chinese, Indian or Non-Malaysians were grouped together as natives. There is lack of information and standardisation when it comes to determining the ethnicity of a subject who is a result of interracial marriage. There is no concrete guidance in Malaysia whether to follow paternal or maternal ethnicity. Thus such subject was lumped together as natives. It is also doubtful whether dividing the subjects according to ethnicities will have any significance in term of determining the incidence of the disease, as studies tried to separate subjects according to race or ethnicities to find lifestyle and cultural influences on the aetiology or pathogenesis (Kurebayashi *et al.*, 2007). Whereby in Sabah, the population is more integrated in terms of lifestyle that is of any connection to the risk factors such as food, breast-feeding or contraception (B Norsa'adah, 2005).

In comparison with other studies, this study have focused mainly on the cases of IBC NST and only those cases where histopathological diagnosis was made by biopsy before being followed by mastectomy. Most studies failed to specify the samples used either biopsy or mastectomy specimen. In some centres in Malaysia hormonal receptors are tested on biopsy specimens whereas in others it is tested on mastectomies. Most literature reported cases in western countries. There are very few studies done on IBC NST cases in Sabah. Because most of IBC NST cases in Sabah are at a late stage, the cases presented earlier, mainly of Luminal A subtype that could have benefited from chemotherapy when categorized as Luminal B seems to be disregarded. As we are moving toward better medical care, earlier presentation of breast cancer cases are expected in the future.

Most of the results (grade, lymph node metastasis) shown were not statistically significant as anticipated at the beginning of the study *(Table 13).* Several factors may have contributed to this. As discussed, the limited sample size was a major setback. Only 37 cases were stained for subtyping. However, in comparison with the other studies done on larger samples, this study only collected cases where biopsy was performed prior to mastectomy. The majority of subjects presented in stage 2 and 3 (87%), this is when taking into account that these patients had a biopsy before mastectomy. The possible main reason behind this is late detection due to lack of access and awareness. However, there were cases of straightforward breast cancer that were diagnosed clinically and managed by mastectomy, without first