PREDICTIVE FACTORS FOR NEUTROPENIA DURING CHEMOTHERAPY TREATMENT IN BREAST CANCER PATIENTS

By

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Dissertation Submitted In Partial Fulfilment Of The Requirements For The Degree Of Master Of Science (Transfusion Science)

ADVANCED MEDICAL AND DENTAL INSTITUTE

UNIVERSITI SAINS MALAYSIA

June 2017

DECLARATION

I hereby declare that I am the sole author of the thesis entitled "Predictive Factors for Neutropenia during Chemotherapy Treatment in Breast Cancer Patients". I declare that this thesis is being submitted to Universiti Sains Malaysia (USM) for the purpose of the award of Master of Science in Transfusion Science. This dissertation is the result of my own research under the supervision of Dr. Hasmah Hussin and Dr. Sharifah Azdiana Tuan Din, except for the quotation and citation which have been duly acknowledged. I also declare that this dissertation has not been previously or concurrently submitted to any other degree at USM or other institutions.

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ACKNOWLEDGEMENT

I would like to express my deepest gratitude to the people who have helped and supported me to the success of my dissertation. First of all, I would like to express my heartfelt gratitude to my supervisor, Dr. Hasmah Hussin for her persistent guidance, encouragement, and understanding throughout this study. Without her endless support and guidance, it would not be possible for me to complete the dissertation smoothly. Besides that, I would like to express my greatest appreciation to my co-supervisor, Dr. Sharifah Azdiana Tuan Din for her valuable advice, assistance, and encouragement.

I would also like to acknowledge and thank Puan Siti Meszaida Mohd Ehsan and Puan Masyitoh Binti Ismail from Medical Record of Advanced Medical and Dental Institute (AMDI) for their great helps and time spent in the retrieval of patient's medical records for my research purposes. I am heartily thankful for their patience and kind assistance.

I would like to extend my special thanks to Encik Nizuwan Azman, Dr. Noorsuzana Mohd Shariff and Dr. Rohayu Hami for the guidance given and time spent in guiding me to deal with biomedical statistic and data analysis.

I would like to express my sincere thanks to my beloved husband, Mr. Kang Hean Chew, children, Kang Zi Zoe and Kang Yee Huay and mother-in-law, Madam Ng Kim Ton for their endless love, understanding, and encouragement. Besides that, I would like to thank my parents, Mr. Ong Tong Hai and Madam Kok Kim Suan and my family members for their continuous support. Without them, I would not have been able to pass through a hard time during the completion of this dissertation and the study.

I would like to thank God for bestowing me the strength, courage, and determination to overcome all the obstacles during my study and completion of this dissertation.

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LIST OF ABBREVIATIONS

AANHPI	Asian Americans, Native Hawaiians, and Pacific Islanders
AC	Doxorubicin, Cyclophosphamide
AMDI	Advanced Medical and Dental Institute
ANC	Absolute Neutrophil Count
aOR	Adjusted Odd Ratio
ASCO	American Society of Clinical Oncology
B	Regression Coefficient
BMI	Body Mass Index
BRCA1	Breast Cancer genes 1
CI	Confidence Interval
CIN	Chemotherapy-Induced Neutropenia
CMF	Cyclophosphamide, Methotrexate and 5-Fluorouracil
DCIS	Ductal Carcinoma In Situ
D.O.B.	Date of Birth
EMR	Electronic Medical Record
EORTC	European Organization for Research and Treatment
ER	Estrogen Receptor
ESBC	Early Stage Breast Cancer
FEC	Fluorouracil, Epirubicin, Cyclophosphamide
FN	Febrile Neutropenia
G-CSF	Granulocyte-Colony Stimulating Factor
HER2	Human Epidermal Growth Factor Receptor 2
HIS	Hospital Information System

HR	Hormone receptor
INC-EU	the Impact of Neutropenia in Chemotherapy European
JEPeM	Jawatankuasa Etika Penyelidikan (Manusia)
Ki67	Ki67 protein, also known as MK167
LCIS	Lobular Carcinoma In Situ
LR	Likelihood Ratio
MLR	Multiple Logistic Regression
MNCR	Malaysian National Cancer Registry
NCCN	National Comprehensive Cancer Network
NHW	Non-Hispanic White
OR	Odd Ratio
PR	Progesterone Receptor
RCTs	Randomized Controlled Trials
ROC	Receiver Operating Characteristic
RR	Relative Risk
SD	Standard Deviation
SE	Standard Errors
SEER	Surveillance, Epidemiology and End Results
SLR	Simple Logistic Regression
SPSS	Statistical Package for Social Sciences
TNM	Tumour, Node and Metastasis staging system
UK	United Kingdom
US	United States
USM	Universiti Sains Malaysia
WHO	World Health Organization

ABSTRAK

Latar belakang dan objektif kajian: Kekurangan sel darah neutrofil ("neutropenia") akibat rawatan kemoterapi sering menyebabkan jangkitan yang teruk dan boleh mengancam nyawa. Ia boleh mengakibatkan pengurangan dos, penangguhan atau pemberhentian kemoterapi yang mungkin akan mengkompromikan hasil pesakit. Kajian ini bertujuan untuk menentukan kejadian kekurangan sel darah neutrofil dan faktor risikonya semasa rawatan kemoterapi pesakit kanser payudara.

Rekabentuk kajian: Ini adalah satu kajian retrospektif pusat tunggal yang dijalankan di Institut Perubatan dan Pergigian Termaju (AMDI), Pulau Pinang. Semua pesakit kanser payudara yang menerima rawatan kemoterapi dari Januari 2014 hingga Disember 2016 termasuk dalam kajian ini. Kelulusan etika telah diberikan oleh JEPeM USM. Perhubungan antara faktor demografi pesakit, faktor yang berkaitan dengan penyakit dan rawatan dengan kejadian kekurangan sel darah neutrofil telah dianalisis. Ujian "Chi-square", Ujian "Fisher's Exact", "Simple" dan "Multiple Logistic Regression" digunakan untuk data analisis.

Keputusan Kajian: Seramai seratus lima puluh lima pesakit telah dianalisis dalam kajian ini, dengan usia min (SD) 52.19 (9.46) tahun. Secara keseluruhannya, 85 pesakit (54.8%) menunjukkan kekurangen sel darah neutrofil dan sub-kategorinya mengikut keterukan kekurangen neutrofil, iaitu ringan (25.8%), sederhana (12.9%), teruk (9.0%) dan kekurangen sel darah neutrofil dengan demam (7.1%). Analisis deskriptif menunjukkan kebanyakan pesakit dalam kajian ini berusia 40 hingga 59 tahun (62.6%), berat badan berlebihan (67.1%), pascamenopos (60.6%), tiada sejarah kanser payudara dalam keluarga (85.8%), jenis patologi duktus (94.8%), jenis reseptor ER- atau PR- Positif (43.4%), kanser peringkat akhir (58.4%),

menjalani pembedahan mastektomi (79.3%), mendapat rawatan kemoterapi "Anthracycline-Taxane" (66.5%) dan menerima rawatan G-CSF (67.1 %). Semua pesakit kanser payudara adalah wanita dalam kajian ini. Dari "Simple Logistic Regression", pembolehubah yang mempunyai nilai ramalan untuk kekurangan sel darah neutrofil adalah indeks jisim badan (BMI) $\geq 23 kg / m^2$ atau kumpulan berat badan berlebihan ($p = 0.012$) dan tidak menerima G-CSF ($p = 0.018$). Dalam analisis "multivariable", BMI pesakit (B = -1.071, aOR = 0.34, 95% CI 0.16, 0.76, $p = 0.008$) dan status penerimaan G-CSF (B = -0.852, aOR = 0.43, 95% CI 0.22, 0.85, $p = 0.015$) adalah faktor ramalan yang berkaitan dengan kekurangan sel darah neutrofil semasa rawatan kemoterapi.

Kesimpulan: Kejadian kekurangan sel darah neutrofil di pusat kajian ini adalah setanding dengan pusat-pusat lain. Kajian kami telah mengenal pasti faktor ramalan berkaitan dengan kekurangan sel darah neutrofil dalam pesakit kanser payudara. Walau bagaimanapun, kajian lanjut melibatkan pelbagai pusat, kajian prospektif dan kohort yang lebih besar adalah sangat disyorkan untuk menubuhkan model ramalan tempatan untuk "neutropenia" dalam pesakit kanser payudara.

PREDICTIVE FACTORS FOR NEUTROPENIA DURING CHEMOTHERAPY TREATMENT IN BREAST CANCER PATIENTS

ABSTRACT

Background and objective: Neutropenia caused by chemotherapy treatment can frequently lead to severe infection and sometimes life-threatening. It may result in dose reductions, delays or discontinuation of chemotherapy which may subsequently compromise patient outcomes. The aim of this study was to determine the incidence of neutropenia and predictive factors for neutropenia during chemotherapy treatment in breast cancer patients.

Study design and methods: This was a single-centre, retrospective study conducted in the Advanced Medical and Dental Institute (AMDI), Penang. All breast cancer patients who received chemotherapy treatment from January 2014 to December 2016 were included into the study. Ethical approval was granted by the JEPeM USM. The association between patient's demographic factors, disease-related factors and treatment-related factors with incidence of neutropenia were analysed. Chi-square tests, Fisher's Exact Test, Simple and Multiple Logistic Regression Model were used for data analysis.

Results: One hundred and fifty-five patients were analysed in this study, with the mean (SD) age of 52.19 (9.46) years. In total, 85 patients (54.8%) manifested neutropenia and sub-categorisation of neutropenia based on severity, showed mild neutropenia (25.8%), moderate neutropenia (12.9%), severe neutropenia (9.0%) and febrile neutropenia (7.1%). Descriptive analysis showed that majority of patients in this study were aged 40 – 59 years (62.6%), overweight (67.1%), postmenopause (60.6%), no family history of breast cancer (85.8%), ductal pathology subtype

(94.8%), receptor subtype of ER- or PR- Positive (43.4%), late cancer stage (58.4%), underwent mastectomy surgery (79.3%), received Anthracycline-Taxane Chemotherapy Regimens (66.5%) and received G-CSF treatment (67.1%). All breast cancer patients were female in the present study. From Simple Logistic Regression, variables that have predictive value to neutropenia were body mass index (BMI) $\geq$ 23kg/ m^2 or overweight group (p = 0.012) and did not receive G-CSF (p = 0.018). In multivariable analysis, patient's BMI (B = -1.071, aOR = 0.34, 95% CI 0.16, 0.76, p = 0.008) and receive status of G-CSF (B = -0.852, aOR=0.43, 95% CI 0.22, 0.85, p = 0.015) were the significant associated predictive factors for neutropenia during chemotherapy treatment in breast cancer patients.

Conclusions: The incidence of neutropenia in this study centre was comparable with other centres. Our study had identified the associated predictive factors for neutropenia in breast cancer patients. However, further studies involved multi-centre, prospective and bigger cohort are highly recommended to establish local predictive model for neutropenia in breast cancer patients.

CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

According to World Cancer Research Fund International report, breast cancer was the most common cancer worldwide in women. In the year 2012, there was a total of 1.67 million new cases of breast cancer diagnosed, contributing to more than 25% of the total number of new cases diagnosed in women worldwide. This figure was excluding non-melanoma skin cancer (World Wide Data, 2012). According to World Health Organization Cancer Fact Sheet, total death worldwide for the year 2012 caused by breast cancer was 520,000 cases (WHO, 2012). In the United States, one case of breast cancer is diagnosed every two minutes and one woman will die of breast cancer every 13 minutes (Alteri et al., 2015). A recent study revealed that black women have higher mortality rate due to breast cancer than white women, even though they have a lower incidence of breast cancer. This condition believed is related to the higher prevalence of comorbidities, slower response to follow-up with treatment after an abnormal mammogram, lack of quality treatment, have higher body mass index and aggressive tumour characteristics among black women (Siegel, Miller & Jemal, 2016, Warner et al., 2015).

In Malaysia, breast cancer is the most common cancer among the female population. Based on Malaysian National Cancer Registry Report for the year 2007 till 2011, there was a total of 18,343 breast cancer cases reported, accounting for 17.7% of total cancer cases. A further breakdown of the data shown that there were 18,206 breast cancer cases in women, accounting for 32.1% of total cancer in women. From the aspect of ethnicity, the incidence of breast cancers was more common in Chinese (41.5%), followed by Indian (37.1%), then Malay (27.2%) and Others (14.3%) (Azizah et al., 2016).

Chemotherapy is one of the most commonly used methods of breast cancer treatment. In order to maximise the benefits of chemotherapy, it is vital to give dose intensity according to schedule. Based on previous studies, a patient will have better overall survival and disease-free survival if receive planned dose intensity (Budman et al., 1998). Development of neutropenia during chemotherapy treatment is not an unusual event and will subsequently affect patient's overall well-being status, such as exposure to infection, lead to delays in chemotherapy treatment, reduction in chemotherapy dose intensity and hospitalisation for broad-spectrum antibiotics administration. In some severe cases, chemotherapy treatment might be discontinued to avoid worsening the neutropenic condition. This may lead to poor prognosis of the patient (Chan et al., 2012).

Neutropenia is a condition whereby reduction in the blood absolute neutrophil count (ANC) below 1.5 x 10^9/L. Neutrophil, is a type of white blood cells that play an important role in our defensive system against invading pathogens. Neutrophil cells are produced in bone marrow, contributed to 60 – 70% of the total white blood cells (WBC) and have very short life in blood circulation, roughly 6 – 10 hours (Robert & Justin, 2011). Severe neutropenia condition will increase the risk and severity of infections caused by bacteria or fungus. There are 3 main categories of neutropenia based on severity, which are mild neutropenia (ANC: 1.0 – 1.5 x 10^9/L), moderate neutropenia (ANC: 0.5 – 1.0 x 10^9/L) and severe neutropenia (ANC: less than 0.5 x 10^9/L) (Robert & Justin, 2011). Febrile neutropenia (FN) is the development of fever in a neutropenic patient. A patient would be categorised as FN if his/ her absolute neutrophil count (ANC) is below 1.0 x 10^9/L and having a single oral temperature exceeding 38.3°C or a temperature of more than 38°C for at least 1 hour period (NCCN, 2016c).

Majority of cancer patients will develop neutropenia, mostly due to the myelosuppressive effect of chemotherapy treatment. Chemotherapy drugs or antineoplastic drugs will cause suppression to bone marrow, subsequently lead to decrease in neutrophil cells production and finally end up with neutropenia (Robert & Justin, 2011). For example, 78% of breast cancer patients treated with

CMF (Cyclophosphamide, Methotrexate and 5-Flurouracil) were developed neutropenia in a study done by Lyman et al. (Lyman, Abella & Pettengell, 2014). The manifestation of myelosuppression in patients who received chemotherapy includes neutropenia, thrombocytopenia and anemia. Some of the neutropenia are caused by infiltration of tumour cells into bone marrow while some are the side effect of radiation administration to bone marrow which suppresses the precursor cells proliferation in bone marrow (Lyman, Abella & Pettengell, 2014). Other etiologies for neutropenia include lymphoproliferative malignancies, autoimmune, hematological disorders, congenital, infections, older age, comorbidities, exposure of multiple cytotoxic chemotherapy and type of chemotherapy drugs (Lustberg, 2012, Hassan, Yusoff & Othman, 2011).

Neutropenia will increase patient's susceptibility to infection, thus causing sepsis, which subsequently may require antibiotic therapy, administration of growth factors and in serious cases, may need hospitalisation for urgent evaluation and administration of empiric broad-spectrum antibiotics (Freifeld et al., 2011). Severe neutropenia exposes patients to serious infection, which if prompt treatment is not given on time, it will lead to serious patient outcomes, include death (Lyman, 2009). Therefore, it is important to recognise neutropenia and provide prompt treatment on time to improve patient's prognosis. By identifying the risk factors of neutropenia in breast cancer patients, this will help physician/ clinician to predict neutropenia and take necessary preventive measures to reduce the incidence of neutropenia.

1.2 OBJECTIVES

1.2.1 GENERAL OBJECTIVE

The main objective of this study was to analyse the predictive factors for neutropenia during chemotherapy treatment in breast cancer patients in AMDI.

1.2.2 SPECIFIC OBJECTIVES

The specific objectives of this study were listed as below:

1. To determine the incidence of neutropenia in breast cancer patients in AMDI.

2. To determine the association between demographic factors and neutropenia in breast cancer patients.
   - Age
   - Gender
   - Body Mass Index (BMI)
   - Menopausal status
   - Family history of breast cancer

3. To determine the association between disease-related factors and neutropenia in breast cancer patients.
   - Pathology subtype
   - Receptor subtype
   - Stage of cancer

4. To determine the association between treatment-related factors and neutropenia in breast cancer patients.
   - Type of surgery
   - Chemotherapy agent
   - Usage of G-CSF

1.3 PROBLEM STATEMENT

Several studies had been conducted previously by different researchers to identify the predictive factors of neutropenia in breast cancer patients. Based on data from the INC-EU Prospective Observational European Neutropenia Study, Schwenkglenks et. al had examined 444 breast cancer patients for patient-specific and treatment-related factors that have an impact on the incidence of grade 4 Chemotherapy-Induced Neutropenia (CIN). They concluded that older age, low body weight, lower baseline white blood cell count, higher number of chemotherapy cycle, higher dosage intensity of doxorubicin, epirubicin, or docetaxel, having vascular-related comorbidity, and higher baseline bilirubin were the risk factors for grade 4 CIN (Schwenkglenks et al., 2011). Another study by Hosmer et al. which identified episodes of FN within first 28 days of chemotherapy has concluded advanced stage at diagnosis, number of co-morbidities, usage of myelosuppressive chemotherapy and received of chemotherapy treatment within 1 month of diagnosis were the independent predictor of FN (Hosmer, Malin & Wong, 2011).

Most of the studies were more focus on febrile neutropenia, but not the neutropenia in general. Besides that, there are limited studies in Malaysia which examine the factors that predict neutropenia in breast cancer patients who receiving chemotherapy treatment. Therefore, we desire to determine the incidence of neutropenia in breast cancer patients in this study centre and the data obtained could subsequently contribute to the prevalence of neutropenia among breast cancer patients in this country. On top of that, another aim of this study is to identify patient-related factors that are associated with increased risk of clinically significant neutropenic events in breast cancer patient during chemotherapy treatment. We hope that the information from this study could contribute to the development of local predictive model for neutropenia in breast cancer patients. Predictive models for neutropenia, especially febrile neutropenia would be informative for clinicians or physicians in clinical decision making, such as decision to direct or restrict the use of primary prophylactic G-CSF to the identified high-risk group.

CHAPTER TWO

LITERATURE REVIEW

This chapter provides a literature review on general knowledge and research did previously in the related studies, include breast cancer, neutropenia and risk factors associated with breast cancer, such as demographic factors, disease-related factors, and treatment-related factors.

Basically, breast cancer is cancer that develops from breast tissue, either from lobules tissue that made up milk production glands, ducts that connect the lobules to the nipple or the nearby lymphatic tissues. Cancer means changes of the pattern of body cell growth and is uncontrollable (Alteri et al., 2015). Based on Cancer statistics for Asian Americans, Native Hawaiians and Pacific Islanders (AANHPI) 2016, about 1 in 10 women will be diagnosed with breast cancer in her lifetime (Torre et al., 2016). Regarding the prevalence of breast cancer in Malaysia, the average lifetime risk for Malaysian population was 1 in 30 women according to Malaysian National Cancer Registry. The breakdown lifetime risk according to ethnicity is 1 in 22 for Chinese, 1 in 24 for Indian and 1 in 35 for Malay. It was demonstrated that Penang state has the highest incidence of breast cancer in the country, which was 50 incidences per 100,000. This may relate to the majority population in Penang state is Chinese (Azizah et al., 2016).

2.1 NEUTROPENIA AND RISK FACTORS

Incidences of neutropenia and FN risks increase with patient's underlying risk factors. Therefore, clinical practice guidelines recommend assessing the risk factors for estimating overall risk of FN (Aapro et al., 2010). NCCN guidelines (National Comprehensive Cancer Network) mentioned that the risk factors for causing severe neutropenia are patients with older age, such as more than 65 years old, previous history of chemotherapy or radiotherapy and poor performance status.

Besides that, cancer metastases to bone marrow, having certain comorbidities (e.g. renal or liver dysfunction) and pre-existing conditions (e.g. infection) also can contribute to severe neutropenia (NCCN, 2016c).

Based on previous studies, other factors that reported implicate the risk of FN include tumour type (e.g. colorectal, lung, breast, ovarian cancer), chemotherapy regimen and patient's previous experience of FN. Studies have proven that patients who had an episode of FN before will have higher chance to have subsequent episode of FN (NCCN, 2016b). Lyman et al. had examined individual risk of neutropenic complications in a prospective cohort study in the United States and reported, if a patient has low blood count before initiation of chemotherapy, particularly low absolute lymphocyte count (below 1.5×10^9 /L) and absolute neutrophil count (below 3.1×10^9 /L), the patient will have increased risk of FN. Besides that, they also reported that previous chemotherapy, abnormal liver and renal function tests, low white blood count, type of chemotherapy and planned dose delivery of more than 85% were independent risk factors for neutropenic complications (Lyman et al., 2011).

Another study in New York demonstrated elderly breast cancer patients will have a significant risk of FN development in subsequent chemotherapy cycle if their white blood count, absolute neutrophil count or hemoglobin were reduced from cycle 1 to cycle 2 chemotherapy (Hurria et al., 2005). A retrospective cohort study conducted in Singapore concluded that low body mass index (BMI) less than 23 kg/m^2 was associated with high risk of FN (Chan et al., 2012).

Furthermore, previous studies reported abnormal laboratory results were an indication of disease degree, the presence of comorbidities or effect of chemotherapy treatment which were the risk factors for FN. These abnormalities include low white blood cell counts (neutrophil or lymphocyte), low serum albumin or hemoglobin, increased bilirubin or lactate dehydrogenase and positive blood cultures report (Lyman, Abella & Pettengell, 2014).

2.2 DEMOGRAPHIC FACTORS

This study is assessing the contribution of demographic factors, such as age, gender, BMI, menopausal status and family history of breast cancer to the incidence of neutropenia in breast cancer patients.

Breast cancer incidence and death rates generally increase with age (Alteri et al., 2015). According to Cancer Statistics for AANHPI, breast cancer occurrence risk is higher for women aged 70 and older (1 in 21) compared with those aged below 49 years old (1 in 56) (Torre et al., 2016). However, according to Malaysian National Cancer Registry Report 2007 – 2011, breast cancer has the highest incidence for age group 25 – 59 years (40.7%), followed by age group 60 – 74 years (24.4%). For women above 75 years, breast cancer incidence (14.5%) was the second highest after colorectal cancer (21.3%) (Azizah et al., 2016). Previous studies had found that older age was the risk factor for FN and also FN-related hospitalisation (Lyman & Delgado, 2003). Patients with age more than 65 years were the high-risk group of neutropenia (Aapro et al., 2010). Studies also found that elderly patients with comorbidities were associated with high risk of FN (Hosmer, Malin & Wong, 2011).

Breast cancer predominantly happens in female than male. In the United States, breast cancer is the most common cancer among women, which is roughly 29% of total cancer cases (Alteri et al., 2015). In Malaysia, breast cancer contributing to 32.1% of all cancer types in women. However, men are generally at low risk for developing breast cancer, accounting for less than 1% of breast cancer cases in Malaysia (Azizah et al., 2016). Anyhow, men are more likely than women to be diagnosed with advanced-stage breast cancer, which likely due to decreased awareness and delayed detection (Alteri et al., 2015). There are limited data regarding the prevalence of neutropenia incidence in male breast cancer patients receiving chemotherapy compared with female breast cancer patients.

Previous studies reported that being overweight or obese will increase the risk of breast cancer after menopause. However, before menopause, being overweight modestly decreases the risk of breast cancer (Tretli, 1989). Breast cancer patients with low BMI were found have an increased risk of developing FN or FN-related hospitalisation (Chan et al., 2012). In contrast, higher BMI was found have less incidence of neutropenia (Jenkins, Elyan & Freeman, 2007) . On top of that, American Society of Clinical Oncology (ASCO) has recommended using full weight-based dosing of chemotherapy in obese cancer patient to achieve the better curative effect (Griggs et al., 2012). However, according to Cancer Statistics for AANHPI, Japanese women were reported to have higher survival rate compared with non-Hispanic white (NHW) due to lower body weight and healthy diet (Liu et al., 2013).

The World Health Organization (WHO) defines menopause as the permanent cessation of menstruation that results from loss of ovarian follicular activity. The average age of menopause is between 50 and 52 years, however, some happen after the age of 40, depend on the individual. Postmenopause are the years after menopause which is at least a year of amenorrhea (WHO, 1981). Studies discovered that there are correlations between body weight, menopausal status, and incidence of breast cancer. Women with higher body weight have 20 to 60 percent higher risk of postmenopausal breast cancer than slimmer women (Ahn et al., 2007). Previous studies also demonstrated that premenopausal breast cancer was related to high-frequency aggressive cancers with poor prognosis. Premenopausal breast cancer has a high prevalence of tumours with high histologic grade, elevated cell proliferation rate, belong to later tumour stage with larger size and lymph node involvement (Talley et al., 2002). Most premenopausal breast cancer is estrogen-independent, ER/ PR-negative tumours predominate, especially in younger women (Nixon et al., 1994).

On the other hand, postmenopausal breast cancer was reported seldom present as aggressive cancers but frequently slow progressing cancers that increase with age. Normally, postmenopausal breast cancer was detected in earlier tumour stage which tumours are relatively

small with node metastases, they have a low prevalence of poorly differentiated or rapidly proliferating tumours. Furthermore, postmenopausal breast cancer is estrogen-dependent and predominant ER/PR-positive tumours (Talley et al., 2002). Few previous studies reported menopausal status did not show significant association to incidence of neutropenia (Chan et al., 2012, Chen et al., 2014, Altwairgi, Hopman & Mates, 2013).

Women, as well as men, are at higher risk of developing breast cancer if having a family history of breast cancer, especially in a first-degree relative, such as mother, sister, daughter, father, brother or son. The risk will increase if more than one first-degree relative affected with the disease. Comparison has been done between women without a family history and those have, it was discovered that the risk of breast cancer is about 2 times higher for women with one first-degree female relative, nearly 3 times higher for women with two relatives, and nearly 4 times higher for women with three or more relatives (Collaborative Group, 2001). If the related relative was diagnosed with breast cancer at a young age, then the risk is even higher. Breast cancer risk is further increased if there is a family history of ovarian cancer. This may affect both men and women (Collaborative Group, 2001). There is very limited study regarding the association between family history of breast cancer and incidence of neutropenia.

2.3 DISEASE-RELATED FACTORS

Disease-related factors, such as pathology subtype, receptor subtype and stage of cancer were assessed in this study to determine their influence on neutropenia incidence in breast cancer patients.

According to American Cancer Society, breast cancer is mainly categorised into Ductal Carcinoma In Situ (DCIS), Lobular Carcinoma In Situ (LCIS) and Invasive breast cancer. DCIS is considered as a non-invasive form of breast cancer. It is the condition whereby abnormal cells

substitute the normal epithelial cells of the breast ducts and subsequently extent the ducts and lobules. DCIS is the most common type of in situ breast cancer and may or may not progress to invasive cancer. LCIS also was known as lobular neoplasia, refers to cells that grow within the lobules of the breast. It is associated with increased risk of developing invasive cancer. However, LCIS is less common and accounts for about 13% of in situ breast cancer. Invasive breast cancer means cancer with the capability to invade the wall of ducts, glands and to the neighboring breast tissues. Prognosis of invasive breast cancer is influenced by stage of the disease (Alteri et al., 2015). According to Cancer Statistics for AANHPI 2016, invasive breast cancer was the most frequently diagnosed cancer and is the second leading cause of mortality in AANHPI women (Torre et al., 2016). Few previous studies had reported that pathology subtype was not associated to the incidence of neutropenia (Chen et al., 2014, Altwairgi, Hopman & Mates, 2013).

Molecular subtypes of breast cancer are determined using biological markers, such as hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2). HR used to determine the presence or absence of hormone (estrogen or progesterone) and reported as (HR+/HR-). However, HER2 used to determine the growth-promoting hormone and reported as (HER2+/HER2-) (Anderson, Rosenberg & Katki, 2014).

Molecular subtypes have been categorised into 4 groups below:

i. Luminal A (HR+/HER2-)

Majority breast cancers (74%) express the estrogen receptor (ER+) and/ or the progesterone receptor (PR+), but not HER2 (HER2-). Characteristics of Luminal A type cancers are slow-growing, less aggressive than other subtypes and have most favorable prognosis (especially in the short term). The expression of hormone receptors is predictive of a favorable response to hormonal therapy (Anderson et al., 2014, Blows et al., 2010).

ii. Triple negative (HR-/HER2-)

Triple negative means ER-, PR-, and HER2- and the prevalence is about 12% of breast cancers. In the US, these cancers have double incidence in black women than white women. Premenopausal women and those with the BRCA1 gene mutation are commonly discovered with triple negative molecular subtypes. These type of breast cancers have a poorer short-term prognosis than other breast cancer types, mainly due to lacking targeted therapies for these tumours (Blows et al., 2010, Adrada et al., 2014).

iii. Luminal B (HR+/HER2+)

Luminal B breast cancers are positive for both HR (ER+ and/or PR+) and HER2. HER2+ means having high positivity for Ki67, which is the indicator of actively dividing cells in large scale. Its prevalence is about 10% of breast cancers. Luminal B breast cancers tend to be higher grade and more aggressive than luminal A breast cancers (Parise & Caggiano, 2014).

iv. HER2-overexpression (HR-/HER2+)

About 4% of breast cancers do not express hormone receptors but produce excess HER2.
This type of cancer shows aggressive growing and spreading than other breast cancers and are associated with poorer short-term prognosis compared to ER+ breast cancers (Blows et al., 2010).

Few previous studies reported receptor subtype was not the predictors for neutropenia (Chan et al., 2012, Altwairgi, Hopman & Mates, 2013). However, Chen et al. revealed that HER2- status was the predictive factor for neutropenia while ER- and PR- status were not the predictors (Chen et al., 2014).

Generally, TNM classification is the most commonly used staging system for tumour staging. Tumour is classified based on tumour size, distance of spread within the breast and to adjacent tissues (T), to the nearby lymph nodes (N), and the presence or absence of distant metastases (M) (Stephen et al., 2010). After T, N, and M have confirmed, determination of the stage of cancer

will be using coding of 0, I, II, III, or IV, whereby Stage 0 means in situ, Stage I means the early stage of invasive cancer, and Stage IV means the most advanced disease (NCCN, 2016a).

On top of that, the Surveillance, Epidemiology and End Results (SEER) Summary Stage system is another frequently used system in cancer registry data, public health research and in planning (Young et al., 2000). The SEER Summary Stage system categorise cancers into local, regional, and distant stage. Local stage refers to a confined type of breast cancer, which equal to the stage I and some stage II cancers in the TNM staging system. Regional stage refers to tumours that have spread to surrounding tissue or nearby lymph nodes, which is equal to stage II or III cancers, depending on size and lymph node involvement. However, distant stage refers to metastasis of cancers to distant organs or lymph nodes above the collarbone. This stage of cancer is equal to some stage IIIc and all stage IV cancers (Anderson, Rosenberg & Katki, 2014).

A more advanced stage of cancer at diagnosis predicted a lower survival rate of patients. Previous studies demonstrated 5-year relative survival for localized disease is 99%, the regional disease is 85%, and distant-stage disease is 38% (Howlader et al., 2016). A larger tumour size at diagnosis also indicated a decrease in survival. Advanced stage of diseases, especially those cancers which have spread to bone marrow are related to the incidence of FN. Advanced stage of cancer at diagnosis, include stage II, stage III and stage IV was predictive factors for FN (Hosmer, Malin & Wong, 2011).

2.4 TREATMENT-RELATED FACTORS

Treatment-related factors, such as the type of surgery, chemotherapy agent and usage of G-CSF (Granulocyte Colony Stimulating Hormone) were assessed in this study to determine their contribution to the incidence of neutropenia in breast cancer patients.

There are mainly two types of surgery for breast cancer, which are breast-conserving surgery and mastectomy. Breast-conserving surgery sometimes also called as lumpectomy, quadrantectomy, partial mastectomy, or segmental mastectomy. In this surgery, only part of the breast containing the cancer is removed. The goal is to remove cancer as well as some surrounding normal tissue. Anyhow, breast-conserving surgery has a higher risk of developing local recurrence of cancer than mastectomy. A mastectomy is a surgery to remove a breast. In the past, a radical mastectomy with complete removal of the breast was the standard treatment for breast cancer. Anyhow, the advances of surgical over the past two decades have given women more options than ever before (van Tienhoven et al., 1999). Neoadjuvant chemotherapy or chemotherapy for breast-conserving patients was found have a higher risk of causing neutropenia in breast cancer patient (Chen et al., 2014).

Previous prospective clinical trials have demonstrated that chemotherapy regimens may cause neutropenia. The risk of chemotherapy regimens in causing FN are classified into high, intermediate and low risk (Crawford et al., 2009) . According to current guidelines, chemotherapy regimens are considered as high risk in causing FN, if more than 20% of chemotherapy-naïve patients have developed FN during clinical trials (Aapro et al., 2010). However, previous clinical trials have proven the majority of the chemotherapy regimens for adult solid cancers are rated as intermediate risk category in causing FN (Culakova et al., 2015).

Referring to NCCN Clinical Practice Guidelines in Oncology, chemotherapy regimens are categorised as high risk if causing > 20% of FN rate during trials, whereas the intermediate risk is 10 - 20% chemotherapy-naïve patients develop FN during clinical trials. High-risk category of breast cancer chemotherapy regimens include Docetaxel + Trastuzumab, dose-dense AC-T (Doxorubicin, Cyclophosphamide, Paclitaxel), AT (Doxorubicin, Paclitaxel), AT (Doxorubicin, Docetaxel) and TAC (Docetaxel, Doxorubicin, Cyclophosphamide) (Crawford et al., 2009).

On the other hand, intermediate risk category of breast cancer chemotherapy regimens include Docetaxel every 21 days, Epirubicin, Epirubicin + sequential Cyclophosphamide + Methotrexate + 5-Fluorouracil, CMF classic (Cyclophosphamide, Methotrexate, Fluorouracil), AC (Doxorubicin, Cyclophosphamide) + sequential Docetaxel (Taxane portion only), AC + sequential Docetaxel + Trastuzumab, FEC (Fluorouracil, Epirubicin, Cyclophosphamide) + sequential Docetaxel, Paclitaxel every 21 days and Vinblastine (Crawford et al., 2009).

Previous studies demonstrated that anthracyclines (e.g. Doxorubicin), taxanes (e.g. Docetaxel), alkylators (e.g. Cyclophosphamide) and topoisomerase inhibitors (e.g. Etoposide), Gemcitabine and Vinorelbine are myelosuppressive and increase the risk of neutropenia. Previous history of had chemotherapy treatment before and treated with more than three types of chemotherapy agents shown a higher risk for FN (Lyman et al., 2011, Pettengell et al., 2008). Taxanes and high dosage of anthracycline in chemotherapy regimens were related to increased risk of FN (Debled et al., 2007).

Granulocyte-colony stimulating factor (G-CSF) is used in a neutropenic patient to regulate the production, maturation, and function of neutrophil cells (Renwick, Pettengell & Green, 2009). G-CSF can improve production of myeloid cells in bone marrow and increase circulating neutrophil by reducing stem cell maturation time to adult neutrophil. This subsequently improves neutrophil recovery, reduce patient's fever duration and shorten the stay in hospital (Rapoport, 2011). The American Society of Clinical Oncology (ASCO) and the European Organization for Research and Treatment of Cancer (EORTC) have established guidelines on the G-CSF usage, either as a prophylactic and therapeutic purpose in a clinical setting (Smith et al., 2006).

According to the EORTC guidelines, to ensure better patient outcomes or survival benefits, it is recommended to use prophylaxis G-CSF in patients with more than 20% risk of developing FN and when involved high dosage chemotherapy treatment (Aapro et al., 2010). In addition, several controlled clinical trials and meta-analysis reported there is a reduction in FN incidences in

patients who received primary prophylaxis of G-CSF with chemotherapy treatment (Kuderer et al., 2007). Randomized control trials (RCTs) has established the safety and efficacy of prophylactic usage of G-CSF in a cancer patient, including breast cancer patient (Lyman et al., 2015). Jenkins et al. have revealed that breast cancer patients who received prophylactic G-CSF with antibiotics had a lower incidence of FN and most evident in the first three cycles of chemotherapy than those did not (Jenkins, Scaife & Freeman, 2012). However, a study conducted at the Cancer Centre of Southeastern Ontario, Canada reported that FN incidence was still observed in breast cancer patients who had received prophylaxis G-CSF before initiation of chemotherapy treatment. The authors of the study explained that older age, taxane-based chemotherapy and filgrastim were observed linked to high FN risk despite prophylaxis G-CSF were given (Altwairgi, Hopman & Mates, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY DESIGN

This was a retrospective record review, which involved data collection of patient information from medical records.

3.2 STUDY LOCATION

This study was conducted in the Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia (USM), Bertam, Penang. AMDI is a clinical service based research centre and referral centre for oncological services, which providing chemotherapy, radiotherapy, surgery services and provide in-patient services for cancer treatment.

3.3 STUDY DURATION

This study involved breast cancer patients who received chemotherapy treatment in AMDI from January 2014 to December 2016 (3 years).

3.4 STUDY POPULATION

The target population was all the breast cancer patients who received chemotherapy treatment in Oncology Clinic in AMDI. The sampling frame was referring to the new cancer statistics list of

Oncology Clinic AMDI from January 2014 to December 2016. The universal sampling method was used for data collection.

3.4.1 INCLUSION CRITERIA

Patients included in this study must fulfill criteria below:

i. All the new breast cancer cases treated with chemotherapy in AMDI.

   New breast cancer case refers to the new case of breast cancer in AMDI's Oncology Clinic registration list with a new hospital registration number.

ii. All cycles of chemotherapy.

   Chemotherapy is typically given in cycles, which is a treatment followed by a period of rest. A cycle can last for one or more days but is usually few weeks long. A course of chemotherapy consisted of multiple cycles depend on types of chemotherapy agents but generally, is comprised of four to six cycles. For this study, all the cycles of chemotherapy are taken into consideration.

3.4.2 EXCLUSION CRITERIA

The patients with the following criteria were excluded from the study:

i. Repeat or recurrent breast cancer cases.

   Repeat or recurrent breast cancer case refers to a patient with breast cancer treated in AMDI previously, but now having disease relapse and seeking for treatment in AMDI's Oncology Clinic. The patient was registered with an old hospital registration number.

ii. A patient who is on concurrent chemotherapy and radiotherapy treatment.

Concurrent chemotherapy and radiotherapy treatment refer to patients who received the administration of chemotherapy agents at the same time with radiotherapy treatment.

iii. Patient whose baseline Absolute Neutrophil Count (ANC) less than 1.5 x 10^9/L (before initiation of chemotherapy treatment).

If a patient's Full Blood Count/ Complete Blood Count report showed an ANC less than 1.5 x 10^9/L prior the initiation of chemotherapy treatment, then the patient will be excluded from this study.

3.5 SAMPLE SIZE CALCULATION

The sample size for this study was calculated based on the one sample proportion formula below:

$$n = \frac{Z^2\, p\,(1-p)}{d^2}$$

Z = 0.05 (95% CI)
d = precision (5%)
p = proportion based on literature review result
power of study : 80%

The sample size was calculated based on the proportion value (p) from a study done by Altwairgi et al. (Altwairgi, Hopman & Mates, 2013).

Sample size calculation as below:

Objective 1 : To determine the incidence of neutropenia in breast cancer patients in AMDI.

- This is a descriptive analysis, therefore no sample size calculation requirement.
- Will be based on all samples of the study.

Objective 2 : To determine the association between demographic factors and neutropenia in breast cancer patients.

- Sample size calculation:

  p = 0.18, cited from paper Altwairgi et al., 2013; d = 0.07

  n = 116

- Therefore, with 10% drop-out rate, sample size = 128 samples

Objective 3 : To determine the association between disease-related factors and neutropenia in breast cancer patients.

- Sample size calculation:

  p = 0.88, cited from paper Altwairgi et al., 2013; d = 0.055

  n = 134