ANAEMIA AND RED BLOOD CELL TRANSFUSION AMONG BREAST CANCER PATIENTS AT TERTIARY CENTRES IN KELANTAN

BY

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DISCLAIMER

As a result, I declare that this research has been sent to Universiti Sains Malaysia for the degree of Master of Medicine (Transfusion Medicine). This dissertation has never been submitted for any degree to any other university. Therefore, I certify that this dissertation is my original work except for quotations, which have been fully acknowledged.

Date: 27/5/22 Dr Nur Aklina binti Ramli

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LIST OF ABBREVIATIONS

AC	Adriamycin, cyclophosphamide
AMDI	Advanced Medical and Dental Institute
BC	Breast cancer
CIA	Chemotherapy-induced anaemia
CKD	Chronic kidney disease
CRA	Cancer-related anaemia
FEC	Fluorouracil, epirubicin, cyclophosphamide
EPO	Erythropoietin
ER	Oestrogen receptor
ESAs	Erythropoiesis stimulating agents
Hb	Haemoglobin
HCT	Haematocrit
HER2	Human epidermal growth factor receptor 2
HRPZ (II)	Hospital Raja Perempuan Zainab (II)
HUSM	Hospital Universiti Sains Malaysia
JEPeM	Human Research Ethics Committee
MCH	Mean corpuscular haemoglobin

MCV	Mean corpuscular volume
MLR	Multiple logistic regression
MREC	Medical Research and Ethics committee
PR	Progesterone receptor
PRBC	Packed red blood cell
RBC	Red blood cell
SD	Standard deviation
SLR	Simple linear regression
TAC	Taxotere, adriamycin, cyclophosphamide
TRIM	Transfusion induced immune modulation
USM	Universiti Sains Malaysia

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ABSTRAK

Pengenalan: Anemia semasa kemoterapi adalah salah satu beban bagi pesakit kanser. Namun, data yang sedia ada mengenai prevalensi anemia serta rawatan untuk anemia seperti transfusi darah sering berbeza. Kajian ini bertujuan untuk mengenal pasti prevalensi anemia dan praktis transfusi darah di kalangan pesakit kanser payudara serta menentukan faktor penyebab anemia akibat kemoterapi.

Kaedah: Kajian kohort retrospektif ini dilakukan di Kelantan melibatkan 104 pesakit kanser payudara yang menjalani kemoterapi. *Chi-square* digunakan untuk perbandingan antara kumpulan yang mendapat anemia akibat kemoterapi atau tidak berdasarkan demografik pesakit, kriteria klinikal dan rawatan kanser payudara. *Simple* dan *multiple logistic regression* digunakan untuk menentukan hasil yang berkaitan.

Keputusan: Sebelum menjalani rawatan kemoterapi, 40.4% pesakit mempunyai anemia. Terdapat 30.8% telah menerima transfusi darah merah semasa kemoterapi dengan purata hemoglobin sebelum transfusi darah ialah 7.9 g/dL. Anemia akibat kemoterapi direkodkan sebanyak 54.8%. Walau bagaimanapun, tiada perbezaan signifikan dicatatkan berkenaan anemia akibat kemoterapi dari segi demografik pesakit, data klinikal dan rawatan kanser payudara.

Kesimpulan: Peratusan anemia yang tinggi dicatatkan di kalangan pesakit kanser payudara yang meningkat selepas menjalani kemoterapi. Lebih kurang 1/3 daripada pesakit memerlukan transfusi darah merah. Penilaian awal mengenai pesakit yang berisiko untuk anemia dengan penggunaan *erythropoietin* dan *iron* boleh mengurangkan prevalensi anemia akibat kemoterapi. Mengoptimumkan rawatan untuk anemia adalah penting, seterusnya meminimumkan akibat buruk dan membantu pesakit.

***Kata Kunci**: kanser payudara, anemia, transfusi sel darah merah, kemoterapi*

Conclusion: A high prevalence of anaemia was observed among BC patients at the time of diagnosis, even higher in patients receiving chemotherapy compared to a few studies. However, no significant predictor of CIA was found with demographic data, clinical characteristics or cancer treatment. Early evaluation of at-risk patients for anaemia with integrated use of erythropoietin and iron therapy can help to reduce CIA. Optimising anaemia treatment is essential for minimising the adverse consequences and improving patient outcomes.

Keywords: *breast cancer, anaemia, red cell transfusion, chemotherapy*

CHAPTER ONE: INTRODUCTION

1.1 Overview

This chapter covers a brief introduction to breast cancer (BC), the prevalence of anaemia and its contributing factors among BC patients. This chapter will also discuss the literature review of risk factors for anaemia and its adverse effects and the discussion on packed red blood cells (PRBC) transfusion practices in BC, including the risks and benefits. Research justifications and the objectives of the study will also be highlighted in this chapter.

1.2 Background of the study

Breast cancer (BC) is the most frequent solid tumour cancer in women, and it is one of the leading causes of cancer mortality worldwide. The Global Cancer Incidence Mortality and Prevalence (Globocan) had reported a total number of 2 088 849 (11.6%) new BC cases in 2018. Breast cancer was also responsible for 6.6% of cancer-related mortality among females (1). In Malaysia, it is reported that the incidence rate of BC was 28.6 per 100 000 population, accounting for 32.1% of all female cancers. The majority of those affected were between the age of 25 to 59, with the highest incidence in Chinese, followed by Indian and Malay (2). Cancer-related mortality from breast carcinoma also recorded an increasing trend from 3.8% in 2016 to 4.4% in 2017 (3).

The incidence of anaemia among BC patients is considerably high, adding a burden to oncology care. More than that, the aetiology of anaemia in cancer patients can be complex and multifactorial, such as direct tumour invasion or myelosuppressive chemotherapy. Different pathogenesis had been postulated, including overexpression of proinflammatory mediators, particularly interleukin-1, interleukin-6, and tumour necrosis factor-alfa (TNF- α) by cancer cells resulting in anaemia due to iron dysregulation, inhibition of erythropoiesis, impair erythropoietin (EPO) synthesis and reduction of RBC lifespan (4). These inflammatory cytokines were responsible for a high hepcidin level that eventually prevented iron reflux from macrophages, a condition known as functional iron deficiency, or anaemia of chronic inflammation, which is the commonest type of anaemia among cancer patients (5).

Multiple factors can contribute to anaemia, including nutritional deficiencies, ongoing blood losses, underlying medical illness such as chronic kidney disease, marrow infiltration or therapy-related anaemia (6). Therefore, a thorough workup for anaemia should be guided by the patient's symptoms and signs and supported by relevant laboratory investigations, for example, full blood count, reticulocytes, peripheral blood smear or iron studies. Anaemia of cancer is typically characterised by normocytic normochromic anaemia, reduced serum iron and transferrin saturation of less than 20% with raised serum ferritin above 100 ng/ml despite adequate iron stores in the bone marrow (7).

Anaemia occurs commonly among BC patients, especially in patients who underwent chemotherapy (8,9). As a result, anaemia and its symptoms are a major concern as they can impair the recovery process between the chemotherapy cycles, thus leading to poor quality of life. Nevertheless, there are no recent published data about the prevalence of anaemia and the requirement of PRBC transfusion in BC patients, particularly in Kelantan.

1.3 Literature review

Numerous studies had evaluated the prevalence and risk for developing anaemia among BC patients. A prospective study demonstrated a 58.7% incidence of anaemia in patients who underwent surgery followed by adjuvant multi-agent chemotherapy (10). A study by Jeffery *et al.* conducted on 310 BC patients who received adjuvant doxorubicin and cyclophosphamide showed that anaemia was markedly increased in the age group of 65 years or older following four cycles of chemotherapy (11). Another cross-sectional study by Pourali *et al.* concluded the anaemia prevalence of 43.1% in BC patients who were treated with cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF) regimen (12).

Treating anaemia is vital for cancer patients because anaemia can contribute to hypoxia, which makes cancer cells more resistant to chemotherapy and radiotherapy (13). Meanwhile, patients with pretreatment anaemia had a lower tumour response to the chemotherapy than non-anaemic patients (8). These findings were further supported in research by Zhang *et al.*, which concluded that anaemic patients had less overall survival compared to non-anaemic patients even at the similar stage of BC, with the mortality of 2.8-fold higher among the anaemic group (14). Furthermore, initial low haemoglobin (Hb) and persistent anaemia during chemotherapy also were associated with a remarkable reduction of 10-year survival among cancer patients (15).

The decision of PRBC transfusion is influenced by many factors and should be individualized. Factors to be considered include age, existing comorbidities and haemodynamic stability of the patients, anaemia-related symptoms such as fatigue or breathlessness, perioperative bleeding, pretransfusion Hb or type and cycle of chemotherapy (16). A retrospective study among 116 BC patients treated with four cycles of docetaxel and cyclophosphamide alternating with another four cycles of epirubicin and cisplatin showed that patients with low grade tumours or after completing two cycles of chemotherapy were at risk of requiring PRBC transfusion (17). Nevertheless, based on the Clinical Guidelines for the Rational Use of Blood and Blood Products by the National Blood Centre Ministry of Health Malaysia, a Hb level above 8 g/dL could be appropriate to control symptomatic anaemia during the marrow- suppressive treatment of cancer (18). The National Comprehensive Cancer Network (NCCN) 2018 made a recommendation regarding the PRBC in cancer and chemotherapy-induced anaemia (CIA), which the Hb should be maintained more than 7 g/dL in asymptomatic stable patients or as needed for recovery of symptoms in patients with symptomatic anaemia (19). The role of PRBC transfusion in cancer patients is mainly supportive treatment to rapidly improve tissue oxygenation by restoring the Hb and haematocrit level. Additionally, Hb levels above 10 g/dL were associated with better tissue oxygenation in BC patients, thus it was recommended to counteract the effect of hypoxia on the tumour and prevent treatment resistance to chemotherapy (20).

The association between the prechemotherapy Hb level with blood transfusion was observed in a few studies. An analysis of transfusion patterns in cancer patients determined that patients with pretreatment Hb of less than 8 g/dL received at least one unit of PRBC transfusion during their cancer treatment (21). Another prospective study determined that 82.1% of patients with prechemotherapy anaemia received PRBC transfusion following chemotherapy (22).

The practice of blood transfusion must outweigh its risks. Repeated allogeneic transfusion may increase the risk of red cells alloimmunisation in cancer patients. However, a low prevalence of RBC alloimmunisation was reported among solid tumour patients involving non clinically significant alloantibodies, for example, anti- Mi^a and anti- Le^a (23).

PRBC transfusion also facilitates tumour proliferation and invasion by inducing immune suppression (24). However, few studies suggested the immunomodulatory effect following RBC transfusion known as transfusion-induced immune modulation (TRIM). The mechanism of TRIM includes a decrease in the interleukin-2 secretion, suppression of natural killer cells, alteration of macrophage function and abnormal CD4 to CD8 ratios. These situations further reduce immune surveillance and facilitate malignant cell proliferation and dissemination (25). Several studies had investigated the association between PRBC transfusion and overall survival in solid tumours with a recent meta-analysis demonstrating that perioperative BC patients receiving blood transfusion had shorter overall survival than non-transfused patients (26).

Pharmacological approaches for cancer patients with anaemia include iron therapy or erythropoietin stimulating agents (ESAs). Consequently, the PRBC transfusion should be reserved for severe symptomatic anaemic patients whilst emphasising the alternatives to blood transfusion (27,28). According to the guidelines by Society for Medical Oncology (ESMO), intravenous (IV) iron was recommended for patients underwent chemotherapy who presented with Hb of 11 g/dL or Hb dropped more than 2 g/dL from baseline (29). Another prospective study also advocated the administration of IV iron to treat patients with CIA (30). Hence, transfusion alternatives such as iron therapy or ESAs are indicated for anaemia treatment rather than blood transfusion only.

1.4 Research justification

Breast cancer (BC) is the commonest type of cancer among women in Malaysia, with an increased mortality rate. Anaemia in cancer patients can be discovered either at the time of diagnosis or following treatment, which was markedly correlated with poor quality of life and disease progression. Despite all these, there are no recent published data about the prevalence of anaemia, especially on CIA among BC patients, particularly in Kelantan. Therefore, early identification of the group of patients most likely to become anaemic or worsening anaemic is crucial, thereby allowing earlier intervention to manage anaemia effectively.

Packed red blood cell transfusion (PRBC) requirement varied in an oncology setting. However, from the current observation, the decision of RBC transfusion mainly depends on the Hb level. Hence, the usage of red blood cells must be monitored and audited. Therefore, the findings of this study can provide useful data on the transfusion practice among BC patients and subsequently improve further blood transfusion service based on research evidence.

1.5 Research questions

- What are the demographic characteristics and prevalence of anaemia among breast cancer patients before the chemotherapy?
- What is the requirement of red blood cell transfusion among breast cancer patients undergoing chemotherapy?
- What are the factors that contribute to chemotherapy-induced anaemia in breast cancer patients?

CHAPTER TWO: OBJECTIVES

2.1 General objective

To study the anaemia and red blood cell transfusion practices among breast cancer patients who underwent chemotherapy in Hospital Universiti Sains Malaysia (HUSM) Kubang Kerian and Hospital Raja Perempuan Zainab (II), Kota Bharu (HRPZ II) at Kelantan.

2.2 Specific objectives

i. To describe the patient characteristics and prevalence of anaemia among breast cancer patients

ii. To determine the prevalence of red blood cell transfusion among breast cancer patients who underwent chemotherapy.

iii. To determine the association between patient characteristics, cancer characteristics and cancer treatment with chemotherapy-induced anaemia among patients with breast cancer.

2.3 Alternative hypothesis

There is a significant association between selected predictive factors with chemotherapy-induced anaemia among breast cancer patients.

2.4 Null hypothesis

There is no significant association between selected predictive factors with chemotherapy-induced anaemia among breast cancer patients.

CHAPTER THREE: METHODOLOGY

3.1 Study background

This chapter covers the methodology and ethical issues regarding the study on "Anaemia and red blood cell (RBC) transfusion among BC patients at tertiary centres in Kelantan".

3.2 Study design

A retrospective cross-sectional study was performed on 104 newly diagnosed BC patients between 1st January 2015 to 31st December 2016. These patients completed sixth cycle of chemotherapy until the year of 2017. About 58 and 46 patients were randomly selected from Hospital Universiti Sains Malaysia (HUSM) Kubang Kerian and Hospital Raja Perempuan Zainab HRPZ (II), Kota Bharu, respectively.

3.3 Study area

This study was conducted at HUSM Kubang Kerian and HRPZ (II), Kota Bharu, Kelantan. Both hospitals were the referral centres for the diagnosis and treatment of breast cancer in Kelantan. Meanwhile, HUSM Kubang Kerian is the only hospital offering radiotherapy services to oncology patients in Kelantan.

3.4 Study Population

3.4.1 Reference population: breast cancer patients above 18 years old

3.4.2 Target population: breast cancer patients who were referred to HUSM Kubang Kerian or HRPZ (II), Kota Bharu

3.4.3 Source population/sampling pool: breast cancer patients who underwent chemotherapy in HUSM Kubang Kerian or HRPZ (II), Kota Bharu.

3.4.4 Sampling frame: breast cancer patients who registered with HUSM Kubang Kerian or HRPZ (II), Kota Bharu and completed sixth cycle of chemotherapy until the year of 2017

3.5 Subject criteria

3.5.1 Inclusion criteria

Patients included in this study were females aged 18 years and above, newly diagnosed breast cancer patients in HUSM Kubang Kerian and HRPZ (II) Kota Bharu from 1st January 2015 until 31st December 2016 who underwent sixth cycle of chemotherapy.

3.5.1 Exclusion criteria

Patients with underlying benign breast disease, male breast cancer, or breast cancer patients with comorbidities such as end-stage renal failure, bleeding disorders or haemoglobinopathy, on medication like anticoagulant or antiplatelet, or pregnancy at the time of the diagnosis were excluded. Any incomplete data was also excluded.

3.6 Sample size

The sample size calculation was done following the specific objectives.

Objective 1

The sample size was calculated by using single proportion formula to determine the appropriate sample size. The estimated sample size for this objective was based on 5% precision, 95% confidence level, and significance level (α) 0.05, where 41% of breast cancer patients were anaemic before chemotherapy (12).

- $n = (z/\Delta)^2 p(1-p)$

n = required sample size

z = 1.96 for 95% confidence interval 95%

Δ = Precision (in proportion of one; if 10%, Δ =0.1)

p = Expected prevalence of anaemia in the breast cancer patients

- $n = (1.96/0.1)^2 \, 0.41 \, (1-0.41)$
- n= 92 with 10% dropout

The minimum sample size of 101 was required for objective 1

Objective 2

The sample size was calculated by using single proportion formula to determine the appropriate sample size. The estimated sample size for this objective was based on 5% precision, 95% confidence level, and significance level (α) 0.05, where 37.9% of breast cancer patients required PRBC transfusion throughout chemotherapy (13).

- $n = (z/\Delta)^2 p(1-p)$

- n = required sample size

z = 1.96 for 95% confidence interval 95%

Δ = Precision (in proportion of one; if 10%, Δ =0.1)

p= Expected proportion of breast cancer patients receiving red blood cell transfusion following chemotherapy

- $n = (1.96/0.1)^2\ 0.379\ (1-0.379)$
- n = 89 with 10% dropout

The minimum sample of 97 was required for objective 2.

Objective 3

The sample size was calculated by using double proportion formula to determine the appropriate sample size. The estimated sample size for this objective was based on 5% precision, 95% confidence level, and significance level (α) 0.05.

Two proportions:

n=p1(1-p1) + p2(1-p2)/(p1p2)2(zα + zβ)2

n=sample size

p1=proportion of the associated factor in high- risk group p2=proportion of the associated factor in low- risk group

$z\alpha$ = 1.96 for α = 0.05 (two tailed) or 2.58 for α =0.01 (two tailed)

$z\beta$ = 0.84 for 80% power or 1.28 for 90% power

n=calculated sample size

p1=proportion of breast cancer patients with high risk for chemotherapy induced anaemia

p2=proportion of breast cancer patients with less risk for chemotherapy induced anaemia

$z\alpha$ = 1.96 for α = 0.05 (two tailed)

$z\beta$ = 0.84 for 80% power or 1.28 for 90% power

n=0.36 (1-0.36) + 0.64 (1-0.64)2 / (0.36 -0.64)2 x (1.96 + 1.28)2

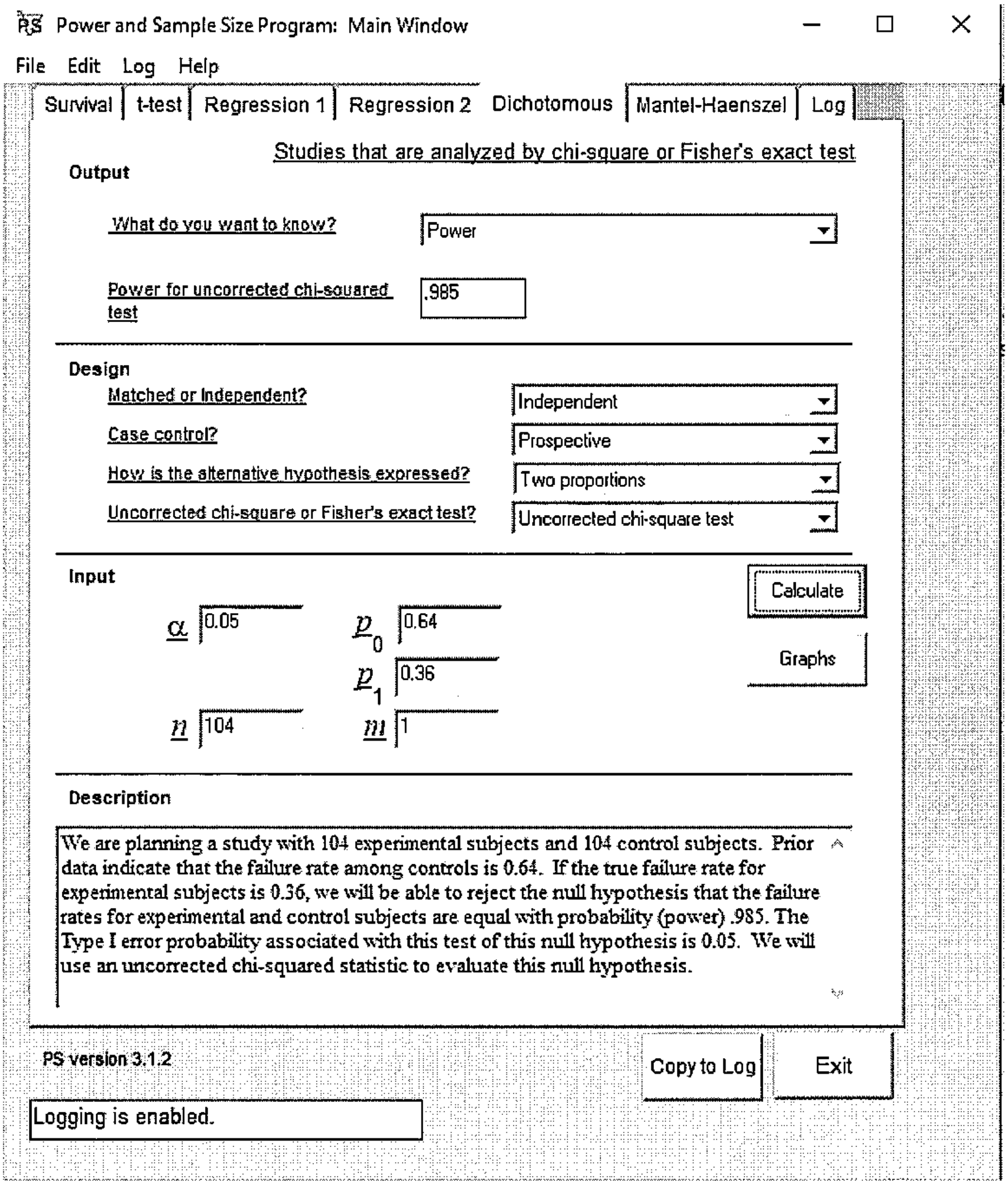
n=46 + 10% drop out = 51

51x2= 102

No of Reference Journal and author	Factor associated with chemotherapy induced anaemia	Significance level (α)	Power of study	p1	p2	Required sample size	Total sample size with 10% drop out
(12) L. Pourali *et al.*	Age	0.05	0.8	22.2	57.3	29	31
(12) L. Pourali *et al.*	Underlying comorbid	0.05	0.8	25	75	14	15

(12) L. Pourali *et al.*	Stage of tumour	0.05	0.8	66	44	37	40
(12) L. Pourali *et al.*	Types of chemotherapy	0.05	0.8	27	73	17	18
(12) L. Pourali *et al.*	Cycles of chemotherapy	0.05	0.8	50.9	10.9	20	22
(11) J. Krishner *et al*	Radiotherapy	0.05	0.8	36	64	46	51

The highest number of samples was 102 with 10% drop out according to radiotherapy and the number was chosen as the total number of samples for this study. The calculation was referred to the Lemeshow *et al* (33). Post-hoc power analysis using 104 as sample size resulted in 98.5% as shown in the image below.



3.7 Sampling method and subject recruitment

Convenience sampling was used as a sampling method for targeted population samples within the study period. A subject who met the inclusion criteria was chosen randomly based on the available data registry until the required sample size was achieved. The subjects were followed up until the completion of chemotherapy. The subjects were then grouped into mild, moderate, or severe anaemia based on the haemoglobin level before the initiation of chemotherapy.

3.8 Research tool

The patient proforma was prepared to ensure comprehensive and easy data collection to be reviewed. The data included were:

- Patient characteristics
    - Identification Card Number
    - Age, Race, Comorbid
    - Date of Admission, Date of Discharge
- Cancer characteristics
    - Initial Diagnosis and Date of diagnosis
    - Stage of breast cancer
    - Histopathological features
    - Histological tumour grade
    - Oestrogen status

- Progesterone status
- Human epidermal growth receptor 2 (HER2) status

- Cancer treatment was extracted from the patient's medical record.
    - Surgery

      Date of surgery, Types of surgery, Estimated blood loss
    - Chemotherapy

      Cycle and types of chemotherapy, Date of initiation, Date of completion
    - Radiotherapy

      Cycles and types of radiotherapy, Date of initiation, Date of completion
    - Hormonal therapy

      Types of hormonal therapy, Date of initiation

- Laboratory investigation
    - Haemoglobin Level (g/dL) (prechemotherapy and post chemotherapy)
    - Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) (prechemotherapy)
    - Iron studies if performed.

- Requirement of PRBC transfusion
    - Numbers of Packed RBC transfused
    - Date of Packed RBC transfusion

3.9 Data Collection Method

Comprehensive patients' data, including demographics data, characteristics of cancer, cancer treatment, laboratory investigations, and RBC transfusion, were obtained from the patient's medical record and extracted from the laboratory record at HRPZ (II) and MYTransfusi HUSM. First, the patients were identified retrospectively using the surgical department or medical record registration lists. After that, patients' clinical notes were traced manually, while the laboratory results and transfusion history were collected in the respective laboratory. Finally, the parameters were recorded in the patient's proforma form by the researcher.

3.10 Statistical Analysis

Data were analysed with Statistical Package for Social Sciences (SPSS) software version 26.0 for window-software (SPSS, Chicago, Illinois, USA). For descriptive statistics, continuous variables were presented as mean or medians with standard deviation (SD). The categorical variables were presented as frequencies and percentages (%). Finally, numerical data were presented as mean (SD) or median (IQR) based on their normality distribution.

Chi-Square test was used to compare the differences of distribution between two groups: chemotherapy-induced anaemia groups and without chemotherapy-induced anaemia groups for each discrete variable in the demographic data, clinical characteristic, and cancer treatment. Furthermore, the association between the dependent variable (chemotherapy-induced anaemia) and the independent variable (factors associated with chemotherapy-induced anaemia) were determined using logistic regression to predict the likelihood of the outcome.

Age of patients, race, underlying comorbidities, stage of cancer, histopathological grade, and features with cancer treatment, including the type of chemotherapy and any surgical intervention or radiotherapy before initiation of chemotherapy, was evaluated as a potential predictor for CIA.

Multiple logistic regression was used to identify statistically significant predictive factors with p-value less than 0.25 was further analysed to estimate a patient's risk of developing CIA. In addition, the C-statistic was used to measure the overall accuracy of the model, and the Hosmer Lemeshow (H-L) lack-of-fit test was used to assess the adequacy. P values below 0.05 were defined as statistically significant.

3.11 Operational definition

The clinical staging of BC was stratified according to the American Joint Committee on Cancer (AJCC) staging system. Stage I included T1, N0, M0, stage II included IIA (T0–1, N1, M0 or T2, N0, M0) and IIB (T2, N1, M0 or T3, N0, M0), stage III included IIIA (T0–2, N2, M0 or T3, N1–2, M0), IIIB (T4, N0–2, M0) and IIIC (any T, N3, M0). BC patients with distant metastasis are defined as Stage IV breast cancer (31). The patient's age included in this study is > 18-year-old as it is the cut-off age for paediatric admission in the hospital. Types of comorbidities were recorded for each patient.

Patients with at least 10% of tumour cells with oestrogen and/or progesterone receptors were considered oestrogen and/or progesterone positive (32). The other collected data include cancer treatment such as surgical treatment, chemotherapy and radiotherapy given, laboratory investigations and number of RBC transfusions. In addition, patients receiving chemotherapy or concomitant radiotherapy had follow-up data collected at the end of each chemotherapy cycle to a maximum of six cycles.

The baseline Hb was defined as the earliest recorded Hb level before the initiation of cycle one of chemotherapy. Anaemia was defined by the Hb level less than 12 g/dL in non-pregnant women, according to the World Health Organization (WHO) (34). Haemoglobin level was classified as a numerical variable and further categorised into normal haemoglobin, mild, moderate, and severe anaemia according to common toxicity criteria, National Cancer Institute (NCI) and the European Organization for Research and Treatment of Cancer (EORTC). Mild anaemia was defined as Hb levels between 11.9 g/dL to 10 g/dL Moderate anaemia was defined as the Hb between 10 g/dL to 8 g/dL. Severe anaemia was when the Hb was less than 8.0 g/dL (35,36). The average size and Hb content in a single red cell are further classified based on mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) level. Patients with normocytic normochromic had MCV within 80-100 fL and MCH range between 27-32 pg. Hypochromic microcytic was considered if MCV is less than 80 fL and MCH less than 27 pg, whereas the level above the normal range was classified as macrocytic.

Chemotherapy was given to all patients with the following regimens: FEC (Fluorouracil, Epirubicin, Cyclophosphamide) or TAC (Docetaxel, Adriamycin, Cyclophosphamide) or AC (Adriamycin, Cyclophosphamide). The subjects were followed up until day 14 after sixth cycle of chemotherapy.

Chemotherapy-induced anaemia was defined as patients with normal Hb before chemotherapy and developing anaemia or worsening of anaemia after completing the sixth cycle of chemotherapy. The total number of red blood cells received was defined as the unit of red blood cell transfusion during peri chemotherapy (before the procedure, during admission for chemotherapy and 14 days post-chemotherapy) as the patients came for prechemotherapy workup for full blood count on the second week after chemotherapy.

3.12 Ethical issue

Ethical approval was obtained from Human Ethics Committee of Hospital Universiti Sains Malaysia (ref no: USM/JEPeM/19120949) and the Medical Research and Ethics Committee (MREC) Ministry of Health Malaysia (NMRR No: 19-3471-51818). The medical record data was recorded anonymously so that the respective patients could not be identified either directly or indirectly with the data collected. The confidentiality of the subjects was strictly protected. The study data were only available to the research team and the authorities of relevant bodies (HUSM/MREC). The use or disclosure of protected health information involved no more than minimal risk to the privacy of individuals. The study data had been kept in the online storage device with an encrypted password hence only the research team could access the data. The data would be stored until at least 7 years. After that, they would be permanently deleted from the storage device. No data would be kept as hard copies or manually stored.