

# **PREVALENCE AND TYPE OF ANAEMIA IN RECEPTOR NEGATIVE BREAST CANCER**

This research report was compiled to meet the requirements for a Master of Medicine in Surgery degree. The research report was submitted to the Department of Surgery at the University of the Witwatersrand.

Johannesburg, 2015

## **DECLARATION**

This research report is my original, personal and unaided work.

The report will be submitted for the degree of Master of Medicine in Surgery (MMed) at the University of the Witwatersrand in Johannesburg.

I further declare that this research report has not been previously submitted for any degree or examination at any other university.

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\_\_\_\_\_ 2016, Johannesburg.

## **PREFACE**

Breast cancer is the most common cancer in women worldwide, with an incidence of approximately one million cases every year and is the main cause of cancer related deaths in women. Triple negative breast cancer is known to be aggressive; have a worse prognosis and is associated with lympho-vascular invasion, higher recurrence rate and metastasis. The reason behind this aggressiveness is not completely understood. Anaemia is common in breast cancer patients and may be a result of the disease process, complication of treatment or pre-existing co-morbidities. We would like to determine the prevalence and type of anaemia in patients with triple negative breast cancer at Charlotte Maxeke Johannesburg Academic Hospital. If anaemia is identified early and corrected, it will positively impact on the outcome in terms of a clinical perspective and quality of life. Hence I endeavoured to determine the type and prevalence of anaemia in triple negative breast cancer patients. The recommendation to create awareness of anaemia and the correction of anaemia in this group will make a significant change in the management plan of the patients.

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## **LIST OF ABBREVIATIONS**

HAART - Highly Active Antiretroviral Treatment

Hb - Haemoglobin

Her2 – Human epidermal growth factor receptor 2

HIV - Human Immunodeficiency Virus

MCV - Mean Corpuscular Volume

MMED – Master of Medicine

NHLS - National Health Laboratory Services

RPBC - Receptor Positive Breast Cancer

TNBC - Triple Negative Breast cancer

TNM – Tumour Nodes Metastasis

VCT - Voluntary Counselling and Testing

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## **ABSTRACT**

### **Background**

Breast cancer is the most common cancer in women worldwide, with an incidence of approximately one million cases every year and is the main cause of cancer related deaths in women [1-3]. Breast cancers which are considered to be aggressive are triple negative breast cancers [4]. Triple negative breast cancer is known to have a worse prognosis and is associated with lympho-vascular invasion, higher recurrence rate and metastasis [4]. The reason behind this aggressiveness is not completely understood. Anaemia is common in breast cancer patients and may be a result of the disease process, complication of treatment or pre-existing co-morbidities [5-8]. Clinically significant anaemia is defined as a Haemoglobin level less than 10g/dL, and is accompanied by the symptoms and signs of anaemia such as fatigue, dizziness and pallor [9]. This degree of anaemia can result in a delay of initiating therapy as well as delay to correct the anaemia before initiating treatment such as neoadjuvant chemotherapy and surgery [10]. Furthermore, patients with anaemia with an Hb<10g/dL are known to blunt response to chemotherapy and radiotherapy due to tissue hypoxia [6]. The aim of this study is to determine the prevalence and type of anaemia in patients with triple negative breast cancer at Charlotte Maxeke Johannesburg Academic Hospital.

### **Aim**

To determine the prevalence and type of anaemia in patients with triple negative breast cancer at Charlotte Maxeke Johannesburg Academic Hospital.

## **Objectives**

1. To study the demography of patients presenting with breast cancer.
2. To determine the prevalence of triple negative breast cancer.
3. To determine prevalence and type of anaemia in patients with breast cancer and whether this is affected by receptor status.

## **Method**

This was a retrospective review of records of patients from the Breast and Endocrine Unit at Charlotte Maxeke Johannesburg Academic Hospital over 2002-2012 (11 year period). Once ethical approval was granted, data was obtained from the National Health Laboratory Services records of all breast cancer patients for the period 2002-2012 (11 year period) at Charlotte Maxeke Johannesburg Academic Hospital. Patients demography, histological subtypes and profile was recorded as well as haemoglobin and mean corpuscular volume (Appendix 1).

The data was recorded in Excel spreadsheets and the proportion of triple negative breast cancer patients with or without anaemia at the time of diagnosis (within 3 months) was compared using the chi-squared statistical tests to determine if a correlation exists. Age categories were expressed using the data sets of average (including standard deviation) and range. Gender was calculated as percentages and expressed as a ratio.

## **Results**

Records of 520 patients were found; of which 94 patients were excluded. The average age was  $55 \pm 13.3$  years (range 18-91). The female to male ratio was 109:1. Fifty two patients had triple negative breast cancer (TNBC) and 374 patients had receptor positive breast cancer (RPBC). Of these, only 246 patients had a haemoglobin level matched at time of diagnosis (within 3 months) that is 29 patients in the TNBC group and 217 patients in the RPBC group. Approximately 26% (65/246) of patients with haemoglobin level matched at time of diagnosis (within 3 months) had anaemia at the time of presentation.

Anaemia was found to be more significant and prevalent in the TNBC group as 55.2% (16/29) of the TNBC group had anaemia whereas 22.6% (49/217) of the RPBC group patients were found to have anaemia at presentation. In the TNBC group, 16 of 29 patients had anaemia and microcytic and normocytic anaemia were equally common. Whereas, in the RPBC group, 49 of 217 patients had anaemia and normocytic anaemia was the most common i.e. 51% (25/49 patients).

## **Conclusion**

Anaemia was found to be more common in patients with TNBC compared to RPBC. The predominant types of anaemia found in TNBC patients are microcytic and normocytic and may suggest different mechanisms. Within the TNBC group, microcytic and normocytic and macrocytic anaemia were 7/16 (43.75%); 7/16 (43.75%); and 2/16 (12.5%) respectively. Whereas, within the RPBC group, microcytic and normocytic and macrocytic anaemia were 22(44.89%); 25 (51.02%) and 2 (0.4%) respectively.

# **CHAPTER 1**

## **1. Introduction**

Breast cancer is the most common cancer in women worldwide, with an incidence of approximately one million cases every year and is the main cause of cancer related deaths in women [1-3]. Triple negative breast cancers are known to be aggressive [4]. Triple negative breast cancer is known to have a worse prognosis and is associated with lympho-vascular invasion, higher recurrence rate and metastasis [4]. The reason behind this aggressiveness is not completely understood. Anaemia is common in breast cancer patients and may be a result of the disease process such as bone marrow infiltration, complication of treatment or pre-existing co-morbidities and as a result of iron metabolism and utilisation [5-8, 11]. Cytokine mediated systemic inflammatory response which occurs in cancer patients has been implicated to cause anaemia [11].

Clinically significant anaemia is defined as a Haemoglobin level less than 10g/dL or when accompanied by the symptoms and signs such as fatigue, dizziness and pallor [9]. This degree of anaemia can result in a delay of initiating therapy such as neoadjuvant chemotherapy and surgery [10]. Furthermore, patients with anaemia with an Hb<10g/dL is known to blunt response to chemotherapy and radiotherapy due to tissue hypoxia [6].

The aim of this study was to determine the prevalence and type of anaemia in patients with triple negative breast cancer at Charlotte Maxeke Johannesburg Academic Hospital.

## 1.1. Literature Review

Breast cancer is the most common cancer worldwide with an incidence of approximately one million cases every year [1, 2]. It is the main cause of cancer related deaths in women, however the incidence rates differ worldwide [3]. Prognostic factors include tumour size, histological grade and subtype, mitotic index of proliferation, lymph node status, hormone receptor status and lympho-vascular infiltration [12]. In South Africa data regarding incidence rates of breast cancer including triple negative breast cancer, risk assessment and symptomology remains under-reported.

Triple negative breast cancer is defined as a breast malignancy with the absence of all of the following receptors: oestrogen, progesterone and human epidermal growth factor-2 [13, 14]. Receptor positive breast cancer refers to a breast malignancy with the presence of one or more of the following receptors: oestrogen, progesterone and human epidermal growth factor-2 (Table 1). The global incidence of triple-negative breast cancer ranges between 10.7-29% of all breast cancers [14-25]. This refers to about 200,000 new cases annually [14]. However, Ly et al (2012) reported a higher incidence of 46 % in Mali [26]. Triple negative breast cancer is reported to be more common in younger women [13, 27-29]. Furthermore, it is more prevalent in black women when compared with white women [18,25,28-29].

Table 1: Receptor status

|                              |                                                           |
|------------------------------|-----------------------------------------------------------|
| <b>Oestrogen receptor</b>    | Positive                                                  |
|                              | Negative                                                  |
|                              | Unknown/ not documented on NHLS histopathological reports |
| <b>Progesterone receptor</b> | Positive                                                  |
|                              | Negative                                                  |
|                              | Unknown/ not documented on NHLS histopathological reports |
| <b>Her2 receptor</b>         | Positive                                                  |
|                              | Negative                                                  |
|                              | Unknown/ not documented on NHLS histopathological reports |

Triple negative breast cancer is typically more aggressive than other types of breast cancer and consequently is associated with a poorer prognosis i.e. a worse five year and 10 year overall survival rate [4, 30]. Additionally, TNBC had a higher rate of recurrence after treatment [24, 31]. Interestingly, the patients with TNBC are likely to have tumour with aggressive markers such as high histopathological grade (Table 2) [32]. TNBC is also associated with a higher risk of metastasis [18]. Although there are modifications to the Bloom-Richardson Grading System (1957), the National Health Laboratory Services histopathological reports had made reference to the Bloom-Richardson Grading System (1957), thus this grading system has been included in this research report (Table 2).

Table 2: The Bloom-Richardson Grading System (1957):

| Grade                   | Points |
|-------------------------|--------|
| Low (Grade I)           | 3-5    |
| Intermediate (Grade II) | 6-7    |
| High (Grade III)        | 8-9    |

The next group of breast cancer patients who are expected to fair badly are patients who have oestrogen receptor negative tumours [1,2]. Oestrogen receptor negative breast cancer patients were found to have a worse outcome than their oestrogen receptor positive counterparts [1, 2]. A cancer that is positive for oestrogen and progesterone while testing negative for human epidermal growth factor-2; stage for stage would be expected to have a better prognosis [33].

Environmental factors associated with poor prognosis in breast cancer patients include socioeconomic status, obesity and unhealthy lifestyle [12]. Anaemia impacts the prognosis of patients with breast cancer. The European Cancer Anaemia Survey described a significant correlation between low haemoglobin level and poor performance status in breast cancer patients [34].

Anaemia is defined by the World Health Organization as a haemoglobin level less than 12.0 g/dL for non-pregnant women (>15yrs) and less than 11.0 g/dL for pregnant women [35]. The global prevalence of anaemia is 24.8% and non pregnant women account for 30.2% of the global prevalence [35].

Anaemia is classified as macrocytic, normocytic or microcytic [36]. Microcytic anaemia refers to a mean corpuscular volume (MCV) less than 82 femtolitres and may be due to dietary restrictions such as iron deficiency, thalassemia and anaemia of chronic disorders where the cancer interferes with the metabolism and utilisation of iron [36]. Normocytic anaemia is defined as a MCV that ranges between 82-98 femtolitres and may possibly be a result of cytokine mediated inflammatory response in cancer. Macrocytic anaemia is defined as a MCV greater than 98 femtolitres and is commonly associated with vitamin B12 and folate deficiency, antiretroviral therapy, chemotherapy and chronic illness such as leukaemia and alcoholism [36].

Surgery is the mainstay of treatment of breast cancer [37]. Depending on the stage at presentation and histological findings some patients with breast cancer require adjuvant or neo-adjuvant chemotherapy and/or radiation [37]. Some patients with cancer will present with anaemia that can be detected before, during or after treatment [5]. Suggested underlying reasons for anaemia in cancer in general include problems in iron metabolism as a result of sequestration of iron by the reticulocyte endothelial system, low levels or resistance to erythropoietin and bone marrow infiltration [11]. Additionally, patients with breast cancer may already have co-morbidities such as diabetes, hypertension, human immunodeficiency virus (HIV) [38]. South Africa has a HIV epidemic and many of the patients are HIV positive and may be on Highly Active Antiretroviral Treatment (HAART) [38]. Both HIV and the HAART drugs are associated with anaemia [8, 35] which may be due to bone marrow failure or infiltration [39].

The presence of anaemia in breast cancer patients may delay treatment such as mastectomy when Hb<7g/dL or Hb<10g/dL in cardiac or elderly patients [40]. In post mastectomy breast cancer patients who are undergoing chemotherapy or radiation therapy, anaemia has been implicated as a significant co-factor causing fatigue [6].

This aim of the study was to evaluate the prevalence and type of anaemia in patients with triple negative breast cancer who were managed at the Breast and Endocrine Unit at Charlotte Maxeke Johannesburg Academic Hospital. A more detailed consideration of the epidemiology of breast cancer will have major health implications for risk assessment, prevention; diagnosis, management, healthcare resource allocation and policy planning.

Most importantly, the results of this study will contribute to existing knowledge which will benefit patient management and improve patient outcomes in the South African setting. In addition, this study will provide a baseline for future comparative studies within South Africa and Africa.

## **CHAPTER 2**

### 2. Statistics and Research Methodology

#### 2.1 Hypothesis

There is a significant difference in the prevalence and type of anaemia between the triple negative receptor breast cancer patients and receptor positive breast cancer patients.

#### 2.2 Aim

To determine the prevalence and type of anaemia in patients with triple negative breast cancer at Charlotte Maxeke Johannesburg Academic Hospital.

#### 2.3 Objectives

1. To study the demography of patients presenting with breast cancer.
2. To determine the prevalence of triple negative breast cancer.
3. To determine prevalence and type of anaemia in patients with breast cancer and whether this is affected by receptor status.

#### 2.4 Design

This was a retrospective study.

## 2.5 Setting

Breast Clinic and Breast and Endocrine Unit at Charlotte Maxeke Johannesburg Academic Hospital situated in Parktown, Johannesburg, Republic of South Africa.

## 2.6 Study Population

National Health Laboratory Services (NHLS) records of patients diagnosed with breast cancer at Charlotte Maxeke Johannesburg Academic Hospital.

## 2.7 Period

All NHLS records from January 2002 until December 2012 (11 year period).

## 2.8 Inclusion and exclusion criteria

Inclusion criteria was all newly diagnosed breast cancer patients.

The exclusion criteria comprised of patients who:

- were previously operated at other hospitals.
- were previously diagnosed with a primary malignancy other than breast cancer and presented with a second primary malignancy.
- presented with recurrence of breast cancer within 2002-2012. These patients had an initial mastectomy or wide local excision prior to 2002.

## 2.9. Ethical Considerations

This study was approved by the Postgraduate Committee of the University of the Witwatersrand and the Human Research Ethics Committee (HREC) of the University

of the Witwatersrand - M130439 (Appendix 1). Written permission was obtained from the Research Review Board of Charlotte Maxeke Johannesburg Academic Hospital and the Head of the Department of Pathology (Professor Hale) at National Health Laboratory. This study was conducted at a Master of Medicine (MMED) study level with the intention of publication and possible presentation at conferences.

#### 2.10 Data Collection

Once ethical approval was granted, data was obtained from the National Health Laboratory records of all breast cancer patients for the 11 year period of 2002-2012 at Charlotte Maxeke Johannesburg Academic Hospital. Patient demography, histological features and receptor was recorded as well as haemoglobin and mean corpuscular volume (Appendix 2). If more than one histopathological report was obtained, the initial report i.e. the first report diagnosing breast cancer and a matched Hb at the time of diagnosis (within 3 months) of breast cancer was used. If multiple Hb level results were retrieved, the one at the time of diagnosis (within 3 months) of breast cancer was used.

#### 2.11 Data Analysis

The data was recorded in Excel spreadsheets and the proportion of triple negative breast cancer patients with or without anaemia at the time of diagnosis (within 3 months) was compared using the chi-squared statistical tests to determine if a correlation exists. Age categories were expressed using the data sets of average (including standard deviation) and range. Gender was calculated as percentages and expressed as a ratio.

## **CHAPTER 3**

### **3. Results**

#### **3.1 Demography**

A total of 520 records were found and based on the exclusion criteria, 94 records were excluded for the following reasons (see Figure 1):

- Four patients were excluded as they were previously operated at other hospitals.
- Fifteen patients were previously diagnosed with a primary malignancy other than breast cancer.
- Sixty one patients presented with recurrence of breast cancer within 2002-2012. These patients had an initial mastectomy or wide local excision prior to 2002.
- Additionally 14 records were excluded as their receptor status was unknown.

The remaining 426 records were further subdivided into TNBC (52 patients) and RPBC (374 patients) (see Figure 1). Of these, only 246 patients had a haemoglobin level matched at time of diagnosis (within 3 months), that is 29 patients in the TNBC group and 217 patients in the RPBC group. Twenty three TNBC patients and 157 RPBC patients had insufficient data regarding haemoglobin level at time of diagnosis (within 3 months).

Approximately 26% (65/246) of patients with haemoglobin level matched at time of diagnosis (within 3 months) had anaemia at the time of presentation. Anaemia at time of diagnosis (within 3 months) was found in 55.2% of the TNBC group and 22.6% of the RPBC group (see Figure 1).

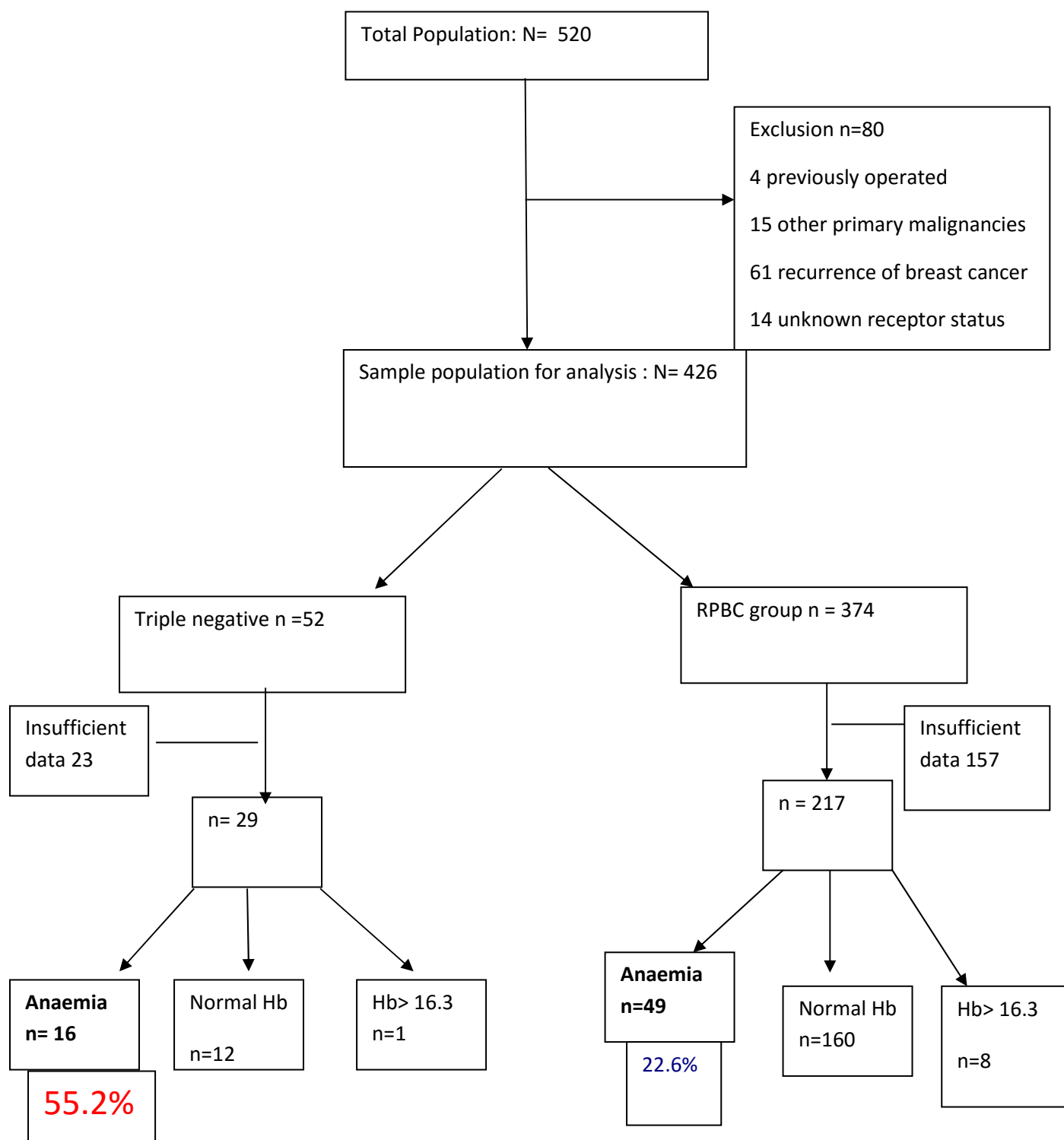


Figure 1: Comparison of TNBC and RPBC groups.

In the TNBC group, 55.2% had anaemia versus the RPBC group (22.6%).

Anaemia was thus more than double in the TNBC group.

Table 3: Comparison of age, receptor status and anaemia

| <b>Parameters</b> | <b>TNBC n=52</b> mean +/-<br>standard deviation. | <b>RPBC n=374</b>                | <b>pValue</b> |
|-------------------|--------------------------------------------------|----------------------------------|---------------|
| <b>Mean Age</b>   | 53 ± 11.0 years<br>[32-75 years]                 | 55 ± 13.5 years<br>[18-91 years] | 0.17          |
| <b>Hb Average</b> | 11.7 ± 2.4g/dL<br>[7.6-16.4g/dL]                 | 13.2 ± 2.0g/dL<br>[7.2-20.3g/dL] | 0.0003        |

### 3.1.1 Age distribution of sample population

The average age of the sample population (N=374) was  $55 \pm 13.3$  years (range 18-91 years). The average age in the TNBC was  $53 \pm 11.0$  years (range 32-75 years) and  $55 \pm 13.5$  years (range 18-91 years) in the RPBC group (Table 3). The patients in the RPBC group presented at a younger age compared to those in the TNBC group. Four of the 426 patients had no age recorded on the NHLS database.

### 3.1.2. Gender distribution

In the sample population of 426 patients, the female : male ratio was 109:1. The distribution was similar among both the TNBC and RPBC groups.

## 3.2 Histological features of tumour

### 3.2.1 Histological type of tumour

Invasive ductal carcinoma was the most common histological type in both TNBC and RPBC groups; at 96% and 88% respectively.

### 3.2.2 Histological grade of tumour

The grading system was categorised according to the Bloom-Richardson Grading System (1957) (Table 2). A high histological grade was more common in the TNBC group at 71.2 %. In the RPBC group, moderate histological grade was more common at 55.3%.

### 3.3 Receptor status

Fifty two patients had triple negative breast cancer (TNBC) ie oestrogen, progesterone and her 2 receptor negative (Table 4).

Table 4: Breakdown of breast cancer by receptor status (N=426).

| <b>Receptor status</b> | <b>Oestrogen receptor</b> | <b>Progesterone receptor</b> | <b>Her 2 receptor</b> | <b>Number of patients</b> | <b>Percentage of sample population<br/>n =426</b> |
|------------------------|---------------------------|------------------------------|-----------------------|---------------------------|---------------------------------------------------|
|                        | Negative                  | Negative                     | Negative              | 52                        | 12.2%<br>(52/426)                                 |
|                        | Positive                  | Positive                     | Positive              | 95                        | 22.3%                                             |
|                        | Positive                  | Positive                     | Negative              | 138                       | 32.4%                                             |
|                        | Positive                  | Negative                     | Positive              | 28                        | 6.6%                                              |
|                        | Negative                  | Positive                     | Positive              | 2                         | 0.5%                                              |
|                        | Positive                  | Negative                     | Negative              | 35                        | 8.2%                                              |
|                        | Negative                  | Positive                     | Negative              | 11                        | 2.6%                                              |
|                        | Negative                  | Negative                     | Positive              | 52                        | 12.2%                                             |
|                        | Negative                  | Negative                     | Unknown               | 3                         | 0.7%                                              |
|                        | Negative                  | Positive                     | Unknown               | 1                         | 0.2%                                              |
|                        | Positive                  | Positive                     | Unknown               | 8                         | 1.9%                                              |
|                        | Positive                  | Negative                     | Unknown               | 1                         | 0.2%                                              |
| <b>Total</b>           |                           |                              |                       | 426                       |                                                   |

The remainder of the patients (374) had receptor positive breast cancer (RPBC) i.e. oestrogen and/or progesterone and/or her 2 receptor positive (Table 4). Fourteen of the 440 NHLS records that were reviewed has unknown receptor status and were thus excluded.

### 3.4 Analysis of haemoglobin results

#### 3.4.1 Haemoglobin level

Table 5: Haemoglobin distribution of TNBC and RPBC groups

| <b>Hb level g/dL</b>    | <b>TNBC n=29 (%)</b><br><b>(44 records were excluded)</b> | <b>RPBC n=217 (%)</b><br><b>(44 records were excluded)</b> | <b>pValue</b> |
|-------------------------|-----------------------------------------------------------|------------------------------------------------------------|---------------|
| <b>&lt; 10g/dL</b>      | 8(27.6%)                                                  | 12(5.6%)                                                   | 0.0001        |
| <b>10.1 – 12 g/dL</b>   | 8(27.6%)                                                  | 37(17.1%)                                                  | 0.19          |
| <b>12.1 – 16.3 g/dL</b> | 12 (41.4%)                                                | 160 (73.6%)                                                | 0.0107        |
| <b>&gt; 16.3 g/dL</b>   | 1 (3.4%)                                                  | 8 (3.7%)                                                   | 0.95          |
| <b>Total</b>            | 29 (100%)                                                 | 217 (100%)                                                 |               |

The haemoglobin distribution amongst the TNBC and RPBC groups are outlined in Table 5. In the TNBC group (52 patients), 31% had a Hb < 12.0; 23% had a normal Hb (12.1-16.3), and 2% had a Hb >16.3. In the TNBC group, 44% of Hb levels were not found on the NHLS database at the time of diagnosis (within 3 months) of breast cancer. In the RPBC group, 13% had a Hb < 12.0; 41% had a normal Hb (12.1-16.3), and 2% had a Hb >16.3. In the RPBC group, 44% of Hb levels were not found on the NHLS database at the time of diagnosis (within 3 months) of breast cancer.

### 3.4.2 Mean corpuscular volume and types of anaemia

Table 6: Mean Corpuscular Volume distribution of TNBC and RPBC anaemic patients.

| <b>TNBC number of patients with anaemia n =16</b> | <b>MICROCYTIC Anaemia<br/>MCV &lt;82 Femtolitres</b> | <b>NORMOCYTIC Anaemia<br/>MCV 82 - 98 femtolitres</b> | <b>MACROCYTIC Anaemia<br/>MCV &gt;98 femtolitres</b> |
|---------------------------------------------------|------------------------------------------------------|-------------------------------------------------------|------------------------------------------------------|
| Total                                             | 7                                                    | 7                                                     | 2                                                    |
| Percentage                                        | 43,75%                                               | 43,75%                                                | 12.5%                                                |
| <b>RPBC number of patients with anaemia n =49</b> | <b>MICROCYTIC Anaemia<br/>MCV &lt;82 Femtolitres</b> | <b>NORMOCYTIC Anaemia<br/>MCV 82 - 98 femtolitres</b> | <b>MACROCYTIC Anaemia<br/>MCV &gt;98 femtolitres</b> |
| Total                                             | 22                                                   | 25                                                    | 2                                                    |
| Percentage                                        | 44,89%                                               | 51.02%                                                | 0.4%                                                 |

In the TNBC group (see Table 6), 16 of 29 patients had anaemia and microcytic and normocytic anaemia were equally common 43,8% (7/16 patients). In the RPBC group (see Table 6), 49 of 217 patients had anaemia; normocytic anaemia was the most common i.e. 51% (25/49 patients).

## **CHAPTER 4**

### **4. Discussion**

The main finding of this study is that anaemia is more prevalent and more significant (i.e. Hb < 10g/dL) in TNBC patients compared to RPBC patients. Interestingly, microcytic and normocytic anaemia were equally common in the TNBC patients.

#### **4.1 Demography**

Majority of patients were female (99%) with a female: male ratio was 109:1. This is in keeping with global trends [41]. The distribution was similar among both the TNBC and RPBC groups.

In the general population, the age of onset of breast cancer in 95% of new cases occurred in women 40 years or older [41]. The mean age of the study group (N=426) was 55 years; this is similar to the data recorded in another study recently performed at Chris Hani Baragwanath Hospital where the mean age of population was 55 years [42]. The mean age in the TNBC group and RPBC group were 53 years and 55 years respectively. TNBC is reported in studies to be a disease of young women and it is of interest that the TNBC patients in this study were middle-aged [13, 27-29]. Furthermore, there was no statistically significant difference in age at presentation between the TNBC and RPBC groups.

A recent study performed in Soweto, reported a rate of HIV probability of 19.7 % in breast cancer and HIV status was not associated with stage of tumour or molecular subtype i.e. receptor status [39]. Murugan et al, revealed that 90% of patients in their general study population were black [42]. International studies have shown that TNBC is more common in black women when compared with white women [18, 25, 28-29]. As the current study was a retrospective review of NHLS histopathological reports, information regarding race and HIV status was missing in the majority of the reports. It was therefore not included for data analysis.

#### 4.2 Histological type and grade

Invasive ductal carcinoma was the most common histological subtype in both TNBC and other group; at 96% and 88% respectively. In the sample population (N=426), other histological subtypes were ductal carcinoma in situ (4.7%), invasive lobular carcinoma (3.6%), mixed invasive lobular and ductal carcinoma (4.7%). Interestingly, the incidence of invasive lobular carcinoma was lower than that reported in the literature [43]. A high histological grade was more common in the in the TNBC group at 71.2 % which is in keeping with the current literature [17].

Although the poor prognostic factors of TNBC include lymphovascular invasion [17], information in the current study was incomplete in the majority of NHLS records. It would be of interest to assess the differences of lymphovascular invasion in the TNBC and RPBC groups.

### 4.3 Receptor status

In this study, the prevalence of TNBC was 12.2% which is significantly lower than reported in two South African studies by Cubash et al and Murugan et al who reported a prevalence of 21.5% and 20.7 % respectively [38, 42].

### 4.4 Anaemia and breast cancer

Anaemia is defined by the World Health Organization as a haemoglobin level less than 12.0 g/dL for non-pregnant women (>15yrs) and less than 11.0 g/dL for pregnant women [35]. The global prevalence of anaemia is 24.8% and non pregnant women account for 30.2% of the global prevalence [35].

In the study population, approximately 26.0% (65/246) of patients with haemoglobin level matched at time of diagnosis (within 3 months) had anaemia at the time of presentation. Anaemia was found to be more significant and prevalent in the TNBC group as 55.2% of the TNBC group had anaemia whereas 22.6% of the RPBC group patients were found to have anaemia at presentation.

Some patients with breast cancer may already have co-morbidities such as diabetes, hypertension, human immunodeficiency virus (HIV) [38]. South Africa has a HIV epidemic and many of the patients are HIV positive and may be on Highly Active Antiretroviral Treatment (HAART) [38]. Both HIV and the HAART drugs are

associated with anaemia [8, 35]. It may be due to bone marrow failure or infiltration; and may be chronic and severe [39].

Although iron studies were not done in this study, it is possible that poor dietary intake as seen in patients from poor social economic backgrounds; and possible influence of cancer on bone marrow metabolism may be contributing factors towards iron deficiency anaemia [44, 45]. It is thus evident that anaemia may be multifactorial. A prospective study is thus recommended in order to determine the cause of anaemia in study population.

The European Cancer Anaemia Survey (ECAS) has demonstrated that in patients with various cancer types, 39% had anaemia at enrolment and 68% developed anaemia during the 6 month survey period [46]. Anaemia has been reported to occur in breast cancer patients [5] and some patients with cancer will present similar to patients with anaemia of chronic disease [5]. Anaemia in cancer can be detected before, during or after treatment [5]. Some of the suggested underlying reasons for anaemia in cancer are problems in iron metabolism such as sequestration of iron by the reticulocyte endothelial system, low levels or resistance to erythropoietin and bone marrow infiltration [11].

Surgery is the mainstay of treatment of breast cancer [37] . Depending on the stage at presentation and histological findings some patients with breast cancer will require adjuvant or neo-adjuvant chemotherapy and/or radiation [37]. Surgery is the

mainstay of treatment and some patients may require adjuvant or neo-adjuvant chemotherapy and radiation [37]. The presence of anaemia in breast cancer patients may delay treatment such as mastectomy when Hb<7g/dL in fit patients or Hb<10g/dL in cardiac or elderly patients [40].

Patients with TNBC as compared to the RPBC group are likely to present with significant anaemia, that is Hb level <10g/dL. These patients would either require pre-intervention blood transfusion or risk worsening of anaemia post chemotherapy which is essential in the management of TNBC patients [47]. There is evidence that the pre-treatment state of a cancer patient may impair that patient's response to chemotherapy [10]. Anaemia is one of the risk factors known to decrease the response to chemotherapy [10]. Additionally, anaemia has been implicated in poor wound healing and dehiscence [48].

Anaemia is classified as macrocytic, normocytic or microcytic [36]. Microcytic anaemia refers to a mean corpuscular volume (MCV) less than 82 femtolitres and may be due to dietary restrictions such as iron deficiency, thalassemia and anaemia of chronic disorders where the cancer interferes with the metabolism and utilisation of iron [36]. Normocytic anaemia is defined as a MCV that ranges between 82-98 femtolitres and may possibly be a result of cytokine mediated inflammatory response in cancer. Macrocytic anaemia is defined as a MCV greater than 98 femtolitres and is commonly associated with vitamin B12 and folate deficiency, antiretroviral therapy, chemotherapy and chronic illness such as leukaemia and alcoholism [36].

In the TNBC group, 16 of 29 patients had anaemia and microcytic and normocytic anaemia were equally common. In the RPBC group, 49 of 217 patients had anaemia and normocytic anaemia was the most common (51.0%). A prospective study is thus recommended to determine the cause of anaemia and the type of anaemia.

A fundamental principle in breast cancer is that cytokines may cause an inflammatory process that causes inflammatory induced anaemia. Cytokine impairment of erythropoietin occurs as a result of the inflammatory process where Tumour Necrosis Factor  $\alpha$ , Interleukin-1 and Interleukin-6 are released. Tumour Necrosis Factor  $\alpha$  results in reduced lifespan of the red blood cell. Tumour Necrosis Factor  $\alpha$  and Interleukin-1 impair erythropoietin production by the kidney. Interleukin-6 worsens anaemia by plasma volume expansion and increases hepcidin secretion which inhibits iron absorption and release from macrophages [11]. Knowledge of the cytokine profile of TNBC may assist in the understanding as to why TNBC is known to be aggressive and have a worse prognosis. This may form a basis for further studies where targeted molecular therapy maybe be developed to dampen or stop the cytokine inflammatory process which has been postulated to cause anaemia.

#### 4.5 Impact of anaemia on the outcome of other cancers

Anaemia is common in patients with right sided colon cancer [49]. The presence of anaemia in at risk patients alerts clinicians to the possibility of colorectal cancer [49]. However, colorectal cancer patients who receive blood transfusions have worse outcomes [49]. Peri-operative blood transfusion was associated with an increased relative risk of death [49]. Transfusion trigger protocols advocate transfusing patients whose hemoglobin level is less than 7g/dL or 10g/dL in cardiac patients [40]. Breast cancer patients commonly have anaemia of chronic disease secondary to the disease itself or bone marrow infiltration [39]. These patients may require blood transfusion pre-operatively or before chemotherapy and this may delay treatment and may also be prone to developing anaemia after chemotherapy.

#### 4.6 Limitations

The limitations of the study were as a result of the unavailability and quality of the NHLS records, many of which were incomplete. The lack of categorising of patients into racial groups makes it difficult to analyse the results. Majority of the patients in the study did not have any record of an HIV test result on the NHLS database, that is 82% patients in the triple negative breast cancer group and 86.59% patients in the other group. The lack of this information made it impossible to comment on any correlations between TNBC and HIV status or race. Additionally, information on other co-morbidities were not available on the NHLS database.

The haemoglobin level that was used was found on the NHLS database at the time of the initial diagnosis. This means that the matched haemoglobin was done at the time of diagnosis (within 3 months) of breast cancer. If there was no Hb level done at the time of diagnosis (within 3 months), this was categorised as insufficient or inadequate data and was not included in the calculation of the type and prevalence of anaemia.

The reason for excluding the Hb level if it was not done at the time of diagnosis (within 3 months) is that the patient may have been exposed to chemotherapy, radiotherapy or surgical procedures. This was a retrospective study and the histopathological results together with the haemoglobin level at the time of diagnosis (within 3 months) were obtained. It must be acknowledged that the histopathological reports may not have a fully comprehensible history. It is acknowledged that factors such as chemotherapy, radiotherapy or surgical procedures may influence the Hb level. In this study, it was assumed that chemotherapy would only be started once the diagnosis was confirmed on histology. This study involves reviewing patients on their first presentation and a matched Hb level at this time. Twenty three patients in the TNBC group and 157 patients in RPBC had insufficient data and these records were removed from the calculation of the type and prevalence of anaemia. After removing these patients, 16 of 29 of TNBC group patients had anaemia that is - 55,2% and 49 of 217 of other group had anaemia that is 22,6%.

#### 4.7 Risk of Bias

This is a a very small subset of patients, those who came directly to the Breast Clinic and Breast and Endocrine Unit at Charlotte Maxeke Johannesburg Academic Hospital as a first presentation where their cancer was diagnosed and a haemoglobin level was done at that time. Patients who first went to other hospitals and were referred to CMJAH Breast Clinic were excluded.

The records were accessed through the NHLS database using a programme called “Snomed” which created a list of all breast specimen samples that were examined at the NHLS between January 2002 – December 2012 (11 year period). Only, the histopathological reports of those specimens that came from Breast Clinic and Breast and Endocrine Unit at Charlotte Maxeke Johannesburg Academic Hospital were then examined. If duplicate samples were encountered, for example a core biopsy and a mastectomy sample for the same patient, the initial core biopsy was used. This amounted to 520 histopathological reports, of which 80 reports were excluded based on the exclusion criteria. Additionally, fourteen records were excluded as the receptor status was unknown.

The number of patients used to calculate the prevalence and type of anaemia was even smaller due to missing data and records that did not have a matched Hb level at the time of diagnosis (within 3 months) of breast cancer. Thus the sample size was reduced to 246 patients when those who did not have a matched Hb level at the time of diagnosis (within 3 months) of breast cancer were further eliminated. Thus a selection bias due to incomplete reporting data may be present.

## **CHAPTER 5**

### **5. Conclusion**

In conclusion, the main findings of this study are:

1. More than 50% of the TNBC patients were anaemic when diagnosed with breast cancer.
2. The prevalence of anaemia in the TNBC patients was greater than that of the RPBC patients.
3. The anaemia in the TNBC patients was more likely to be clinically significant (Hb <10g/dL) than that of the RPBC patients.
4. The predominant types of anaemia found in TNBC patients are microcytic and normocytic and may suggest different mechanisms. Within the TNBC group, microcytic and normocytic and macrocytic anaemia were 7/16 (43.75%); 7/16 (43.75%); and 2/16 (12,5%) respectively.
5. Within the RPBC group, microcytic and normocytic and macrocytic anaemia were 22(44,89%); 25 (51.02%) and 2 (0.4%) respectively.

Correcting anaemia before any treatment of breast cancer is commenced is likely to improve the response to and the outcome of that treatment. This may be of particular importance in patients suffering from the more aggressive types of breast cancer.

## **6. CHAPTER 6**

### **6.1 Recommendations**

#### **6.1.1. Implications for practice**

It is recommended that a routine Hb level and MCV to be done at the time of initial diagnosis of breast cancer. If microcytic anaemia is present, iron studies have to be performed. Anaemia should be corrected prior to chemotherapy or surgery.

#### **6.2. Implications for research**

The study provides a stimulus to undertake other comparative studies of iron status and iron deficiency in breast cancer patients in South Africa and Africa. A prospective study of prevalence and causes of pre-treatment anaemia in breast cancer is indicated.

Knowledge of the cytokine profile of TNBC may assist in the understanding as to why TNBC is known to be aggressive and have a worse prognosis. This may form a basis for further studies where targeted molecular therapy maybe be developed to dampen or stop the cytokine inflammatory process which has been postulated to cause anaemia.

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**Appendix 1: Human Research Ethics Committee (HREC) of the University of the Witwatersrand  
Certificate (M130439)**



R14/49 Dr Amisha Maraj

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**  
**CLEARANCE CERTIFICATE NO. M130439**

**NAME:** Dr Amisha Maraj  
**(Principal Investigator)**

**DEPARTMENT:** Department of Surgery  
CM Johannesburg Academic Hospital

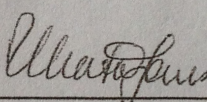
**PROJECT TITLE:** Prevalence and Type of Anaemia in Patients  
with Receptor Negative Breast  
Cancer

**DATE CONSIDERED:** 26/04/2013

**DECISION:** Approved unconditionally

**CONDITIONS:**

**SUPERVISOR:** Prof A Mannell

**APPROVED BY:**   
Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

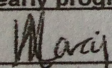
**DATE OF APPROVAL:** 26/04/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

  
Principal Investigator Signature

Date 17/5/13

**PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES**

## Appendix 2: Data collection sheet

Patient series number \_\_\_\_\_

### Demographics

|            |           |
|------------|-----------|
| Age        |           |
| Race       |           |
| Gender     |           |
| HIV status | CD4 count |

### Staging, Histological subtype and Profile

|                                           |                          |                       |                   |               |
|-------------------------------------------|--------------------------|-----------------------|-------------------|---------------|
| <b>Stage:</b>                             | 1                        | 2                     | 3                 | 4             |
| <b>Nodal involvement</b>                  | Yes                      | No                    |                   |               |
| <b>Metastatic disease</b>                 | Yes. Site:               | No                    |                   |               |
| <b>Histological subtype</b>               | Ductal carcinoma in situ | Ductal carcinoma      | Lobular carcinoma | Others: _____ |
| <b>Oestrogen</b>                          | Positive                 | Negative              |                   |               |
| <b>Progesterone</b>                       | Positive                 | Negative              |                   |               |
| <b>Her2 Receptor</b>                      | Positive                 | Negative              |                   |               |
| <b>Histological grade differentiation</b> | Low                      | Intermediate/Moderate | High              |               |
| <b>Lymphovascular invasion</b>            | Yes                      | No                    |                   |               |

|             |                    |                                |
|-------------|--------------------|--------------------------------|
| <b>Date</b> | <b>Haemoglobin</b> | <b>Mean Corpuscular Volume</b> |
|             |                    |                                |
|             |                    |                                |