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Clinicopathological Features of Pregnancy-Associated Breast Cancer at Chris Hani Baragwanath Academic Hospital

Lisa Michelle Levenberg



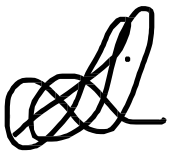
UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

A research report submitted to the Faculty of Health Sciences, University of Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in Surgery.

Johannesburg December 2024

DECLARATION

I, Lisa Michelle Levenberg, declare that this research report is my own original work. It is being submitted for the degree of Master of Medicine in Surgery at the University of Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any other degree or examination at this or any other University.



Signed on 10th day of December 2024 in Johannesburg

For the dedicated team at the Chris Hani Baragwanath Academic Hospital Breast Unit

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LIST OF ABBREVIATIONS

PABC: Pregnancy-Associated Breast Cancer

CHBAH: Chris Hani Baragwanath Academic Hospital

BC: Breast Cancer

LMIC: Low to middle-income country

ER: Oestrogen Receptor

PR: Progesterone receptor

HER2/neu: Human Epidermal Growth Factor Receptor-2

TNM: Tumour Node Metastasis
FISH: Fluorescence In-situ Hybridization

IQR: Interquartile Range

Ki67: Cell proliferation index

CT: Computed Tomography

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CLINICOPATHOLOGICAL FEATURES OF PREGNANCY-ASSOCIATED BREAST CANCER AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

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DISCLAIMERS

The authors declare no conflict of interest. No funding source to be declared.

ETHICS DECLARATION

Ethical approval was obtained from the Human Research Ethics Committee of the University of Witwatersrand (Ethics No. M210804) as well as the Chief Executive Officer of Chris Hani Baragwanath Hospital. Due to the retrospective nature of this study, no patient informed consent was obtained.

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ABSTRACT

Background: Pregnancy-associated breast cancer (PABC), defined as cancer diagnosed during pregnancy or up to one year postpartum, is typically associated with tumours displaying more adverse prognostic pathological features. It is unclear if this is due to the more aggressive tumour biology associated with early-onset breast cancer or influenced by the pregnancy itself. Given the existing paucity of data, especially in an African context, the aim of this study was to compare the clinicopathological features of patients presenting with PABC to their age-matched non-PABC controls in a high volume urban breast unit.

Methods: Records of reproductive age females diagnosed with breast cancer from 1 October 2006 to 31 October 2020 were retrospectively reviewed using the Chris Hani Baragwanath Academic Hospital Breast Unit electronic database. Cases of PABC were then identified, and non-PABC cases were selected as age-matched controls at a 2:1 ratio to each PABC case.

Results: A total of 1056 patients were eligible for inclusion, with 63 patients meeting criteria for PABC. After excluding 10 cases, 53 PABC cases were age-matched to 106 controls. The incidence of PABC was 1.5% with a median age of 36 years. Over 90% of patients presented with palpable breast masses. Pregnancy-associated breast cancer patients presented with significantly larger tumours (5.5 cm vs. 4.0 cm) at diagnosis with higher nuclear grades compared to age-matched controls. PABC patients also demonstrated more Oestrogen (40%vs.23%) and Progesterone Receptor (47%vs.28%) negativity compared to controls.

Conclusions: Pregnancy-associated breast cancer patients from our population presented with more adverse clinicopathological tumour characteristics than their age-matched controls.These findings align with existing literature and would more likely contribute to a worse prognosis in this patient group.

*Keywords***:** *Pregnancy-associated, breast cancer, clinicopathological features*

1. INTRODUCTION

Breast cancer (BC) is the commonest female cancer worldwide and the leading cause of cancer-related deaths in women[1]. In South Africa, a low- and middle-income country (LMIC), breast cancer accounted for the highest number of new cancer cases across genders, making it an important medical condition that contributes significantly to the burden on the healthcare system[2].

Pregnancy-Associated Breast Cancer (PABC) is defined as BC diagnosed during pregnancy or up to one year post-partum[3-6]. Although relatively rare, with an incidence of 0.2-3.8% of all BC cases, it remains the most frequently diagnosed malignancy during pregnancy[3-5]. The global incidence of BC is increasing as more women delay childbearing. This rise is independent of, but compounded by the fact that advancing age itself is a significant risk factor for developing BC[3,4,7]. The mean age at diagnosis of PABC ranges from 33-38 years[4,5]. African literature has pointed towards a relatively higher overall burden of PABC in these cohorts due to younger populations having greater multiparity when compared to Western populations[8,9]. This was suggested by the higher incidence of PABC reported by both Dusengimana[8] (10.3%) and Hou[9] (21.2%) in their African studies. Additionally, cultural and economic factors contribute to inadequate health education and poor adherence to contraception further impacting multiparity and increasing the likelihood of BC being associated with pregnancy[8,9].

Pregnancy and multiparity have been shown to confer a long-term protective effect against the development of BC. However, this effect is preceded by a transient period of augmented susceptibility with an increased risk of BC after the first pregnancy, persisting for as long as 5-10 years post-delivery[10-12]. Additionally, the risk of developing BC in the postpartum years is higher in first-time mothers over age 35, with the probability increasing the later the initial pregnancy occurs[10-13]. As a result, definitions of PABC are inconsistent in the literature ranging from 1-10 years after delivery, depending on the extent of this perceived risk[12]. However, the most widely agreed upon definition of PABC is BC occurring during pregnancy or up to one year post-delivery, which will be used to define PABC in this study.

Pregnancy-associated breast cancer presents similarly to BC in non-pregnant patients often with a palpable mass, overlying skin changes, or a bloody nipple discharge. According to the literature, a self-discovered breast mass was the most common presenting feature of PABC,

occurring in over 80% of cases[5,6,14]. Clinical examination of the breast during pregnancy and lactation is challenging due to physiologically increased breast density and nodularity. This is believed to lead to significant delays in identification and work-up of suspicious breast lumps and, subsequently, more advanced stage disease at diagnosis[3,6,13]. Wang et al.[5] described a ~8-month delay in diagnosis in their cohort of pregnant patients, reflecting the findings of comparable studies[5,6,15]. In sub-Saharan Africa, delays to diagnosis are exacerbated by system inadequacies and barriers to accessing healthcare. Donker et al.[16] analyzed these diagnostic delays and noted that, in African women, under-recognition of worrisome symptoms, together with fear and cultural beliefs, contribute to diagnostic delays.

Furthermore, PABC appears to demonstrate intrinsically more adverse tumour characteristics including larger, higher nuclear grade cancers with increased axillary node involvement and higher stage disease at diagnosis. In addition, these tumours have a greater propensity for Oestrogen (ER) and Progesterone Receptor (PR) negativity with Human Epidermal Growth Factor Receptor-2 (HER2/neu) positivity, features contributing to a worse overall prognosis[6,9,17,18]. The most common histological subtype in PABC is invasive ductal carcinoma occurring, in 75%-90% of cases[6,12-14], with multiple studies supporting these findings[6,9,17,18]. However, there are currently no published South African studies and only one African study from Rwanda, with a very small cohort, corroborating these conclusions[8]. It remains unclear whether these unfavourable tumour traits are due to the distinctively aggressive tumour biology anticipated in young BC patients or if they are independently influenced by the pregnancy.

However, the data is inconsistent in determining if these unfavourable clinicopathological features translate into worse outcomes in PABC patients[5]. A meta-analysis by Hartman et al.[18] in 2016 noted a higher risk of recurrence and death in PABC compared to age-matched controls, with O'Sullivan et al.'s[19] analysis showing similar results. However, Amant et al.'s[20] study demonstrated equivalent disease-free and overall survival in PABC and non-pregnant controls. These disparities in outcome may be the effect of small study cohorts and a lack of robust data owing to the relatively infrequent occurrence of PABC.

Overall, the diagnosis and management of PABC is complex and although there is increasing evidence in the literature, it remains poorly understood with limited high-quality studies[16]. There were no published papers in South Africa with a paucity of data on this condition in Africa. Therefore, this study aimed to investigate PABC in a local South African context by

evaluating the incidence, presentation and clinicopathological features of PABC to enhance our understanding and optimize diagnosis and management strategies.

2. STUDY OBJECTIVES

The aim of this study was to compare the clinicopathological features of PABC with non-PABC in age-matched controls at Chris Hani Baragwanath Academic Hospital (CHBAH) Breast Unit.

Eligible patients with confirmed breast cancer at the CHBAH Breast Unit from 1st October 2006 until 31st October 2020 were included in this analysis, with the study objectives being:

1) To determine the incidence of PABC in all patients of childbearing age diagnosed with breast cancer;
2) To describe the demographic characteristics of PABC patients compared to their age-matched controls;
3) To identify the presenting signs and symptoms in patients diagnosed with PABC vs non-PABC; and
4) To determine the clinicopathological characteristics of PABC, including tumour size and grade, stage of disease and receptor status, and compare these to those of non-PABC in age-matched controls.

3. METHODS

3.1 Study Design and Setting

This was a retrospective descriptive analysis of the CHBAH Breast Unit electronic database. After obtaining ethical approval for this study from the Human Research Ethics Committee of the University of Witwatersrand, and permission from the CHBAH management and the gatekeeper of the Breast Unit electronic database, female patients who met the eligibility criteria were included in the study.

3.2 Study Population and Data Collection

Inclusion criteria: Female patients of reproductive age, aged 18-45 years, with confirmed BC at CHBAH between 1st October 2006 and 31st October 2020.

Exclusion criteria: For the PABC group only: patients diagnosed with BC prior to pregnancy or more than one year postpartum.

The CHBAH electronic breast database was used to identify eligible women during this 14-year time period. Patients meeting the criteria for PABC were identified using specific keywords in an advanced terminology search on the database including: 'pregnant', 'pregnancy', 'breastfeeding', 'lactation', 'post-partum', 'delivery', 'gestation', 'baby','infant'. The principal investigator also evaluated included patient records to ensure PABC cases were not overlooked. Patients not meeting criteria for PABC were identified as controls for comparison. The study aimed to include 62 PABC patients, based on the literature-reported PABC incidence of 0.2-3.8%[3-5] and an estimated incidence of 1.5% in our total cancer registry of 4116 patients. To determine the clinicopathological characteristics of PABC (Objective 4), two control patients were age-matched precisely to each PABC case using STATA software.

3.3 Measuring Tool and Data Collection

Information from included patient records were captured on an MS Excel datasheet for further analysis. Variables extracted included: age at diagnosis, pregnancy/lactation status, presenting sign(s)/symptom(s), tumour size, nodal involvement (based on imaging findings), Tumour Node Metastasis (TNM) staging, Histological Grade (Nottingham Histologic Score System) and subtype, receptor status (ER/PR and HER2/neu), and Ki67%. To ensure accuracy in the size of the tumour recorded, the measured dimensions on the pathological specimen were used in patients who had upfront surgery, while the image-determined tumour size was utilized for those that underwent neoadjuvant therapy.

3.4 Statistical Analysis

Data analysis was performed using the STATA software with the assistance of professional statisticians. Statistical differences between comparable groups were calculated using Pearson's Chi-squared or Fischer's Exact test for categorical variables, and the Mann-Whitney test for continuous variables. A p-value of <0.05 was considered statistically significant.

4. RESULTS

A total of 1056 patients, from the BC registry of 4116 patients, satisfied criteria for inclusion during the defined study period. Of these, 63 patients met the case definition for PABC. Ten cases were excluded due to incomplete records of clinicopathological data, specifically the Ki67% and HER2/neu equivocal results without corresponding Fluorescence In-situ Hybridization (FISH) results. This yielded 53 PABC cases for evaluation. Each PABC case was then age-matched to two controls producing 106 age-matched non-PABC controls (see Figure 1). The incidence of PABC in this study was 1.5%. Notably, most cases of PABC (55.6%) in this study were detected during the final 3 years of the 14-year study period.

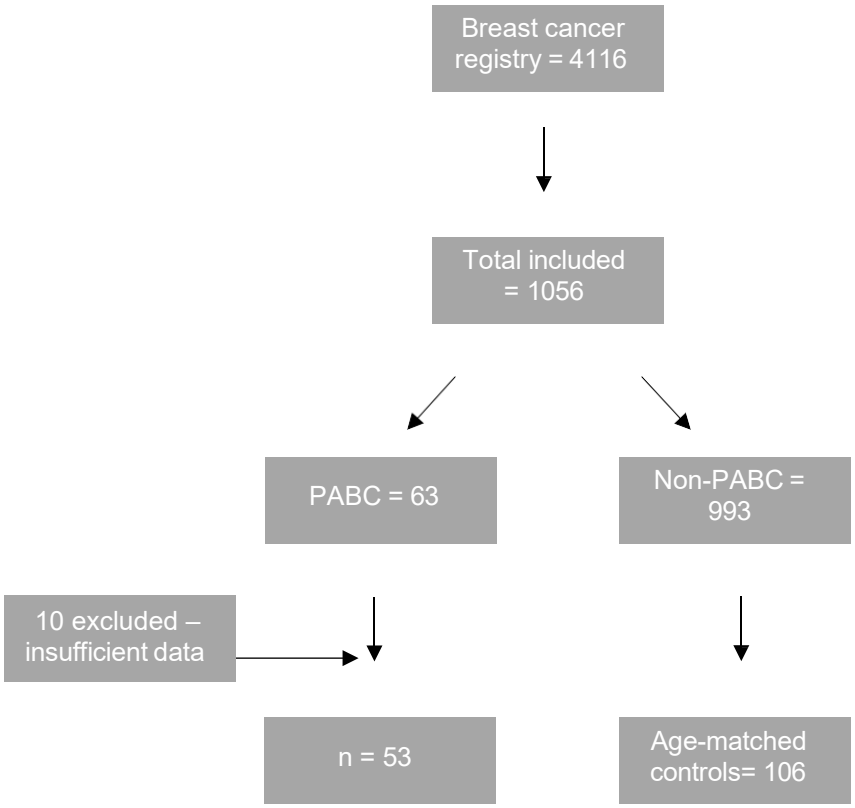


Figure 1: Flow Diagram of Study Participants

4.1 Demographic and clinical characteristics of the study population

Table I shows the demographic and clinical characteristics of the study population. The median age (Interquartile range, IQR) at diagnosis in the PABC group was 36 (32-39) years, identical to that of non-PABC control participants because of age-matching (*p*-value >0.99). Overall, the median age (IQR) at diagnosis of BC in all females of reproductive age (n=1056) was 39 (35-43) years.

Table I Demographic and Clinical Characteristics of PABC vs Non-PABC Age-Matched Controls

Parameter	PABC (n=53)	Age-matched controls (n=106)	*p*-value
Age median (IQR), years	36.0 (32.0- 39.0)	36.0 (32.0–39.0)	>0.99
Presenting sign – mass, n (%)	49 (92.5)	103 (97.2)	0.223
Tumour size median (IQR), cm	5.5 (3.8-5.5)	4.0 (3.0-5.5)	0.027
Node positivity, n (%)	41 (77.4)	88 (83.0)	0.39
De novo metastatic, n %	13 (24.5)	18 (17.0)	0.257
STAGE, n (%)			
I	2 (3.8)	5 (4.7)	0.151
II	11 (20.8)	40 (37.7)	
III	27 (50.9)	44 (41.5)	
IV	13 (24.5)	17 (16.0)	

Abbreviations: IQR, interquartile range; PABC, pregnancy-associated breast cancer.

From Table I, a palpable breast mass was the presenting sign in >90% of patients in both groups (*p*=value 0.22). Patients with PABC presented with significantly larger tumours of 5.5 cm at diagnosis compared to 4.0 cm in age-matched controls (*p*=0.03). Both groups had predominantly axillary node positive disease on presentation at 77.4% in the PABC group vs 83.0% in age-matched controls (*p*=0.39). In comparison, *de novo* metastatic disease was marginally more frequent in the PABC group at 24.5% vs 17.0%, respectively (*p*=0.29). Patients in the PABC group had significantly higher stage disease at diagnosis overall, with 22.2% vs 13.8% in those with Stage IV disease, respectively (*p*=0.018). However, the significance was lost when compared to age-matched controls. Likewise, early-stage BC was less prevalent in the PABC group compared to non-PABC controls for Stage I and II disease (see Table I and Figure 2).

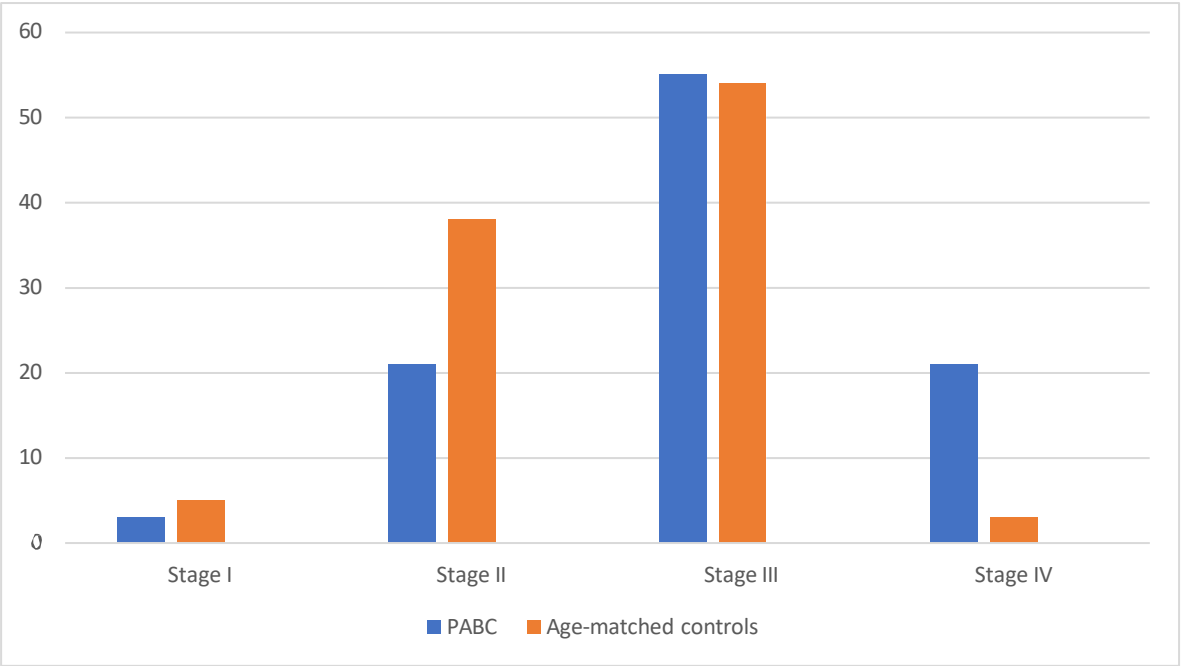


Figure 2: Comparison of Stage of Disease in PABC vs Non-PABC Age-Matched Controls (%)

4.2 Pathological Characteristics

The histological subtype was almost exclusively invasive ductal carcinoma occurring in 100% of PABC vs 97% of non-PABC patients (Table II). The PABC group exhibited significantly more Grade 2 and 3 tumours compared to age-matched controls (p=0.042), with no Grade 1 tumours identified in the PABC group. In addition, in PABC vs age-matched controls, significantly more ER-negative, at 40% vs 23% (p=0.025), and PR-negative, at 47% vs 28% (p=0.018), disease was observed respectively (see Figure 3 and Table II). Both groups demonstrated a preponderance for HER2/neu negative disease. No significant differences in the Ki67 index was found between the PABC vs age-matched control patients (p=0.116).

When comparing the clinicopathological features between the PABC group and the entire non-PABC study population (i.e. all patients aged 18-45 years with non-PABC BC, n=993), the significant differences identified in both the Grade and ER/PR hormone receptor status, no longer reached statistical significance (data not shown). This demonstrates the relevance of age-matching in this young BC population to enhance accuracy in analysis and interpretation of the results.

Table II Pathological Characteristics of PABC vs Age-Matched Controls

Parameter	PABC (n=53)	Age-matched controls (n=106)	*p*-value
Grade, n (%)			
1	0 (0.0)	10 (9.5)	
2	29 (54.7)	48 (45.7)	0.042
3	24 (45.3)	47 (44.8)	
Histological subtype, n (%)			
Invasive ductal	53 (100)	103 (97.2)	0.551
Other	0 (0.0)	3 (2.8)	
Hormone Receptor status, n (%)			
ER +	32 (60.4)	82 (77.4)	0.025
ER -	21 (39.6)	24 (22.6)	
PR +	28 (52.8)	76 (71.7)	0.018
PR -	25 (47.2)	30 (28.3)	
HER2 +	24 (45.3)	48 (45.3)	0.56
HER2 -	29 (54.7)	58 (54.7)	
Ki67%, median (IQR)	50 (25-70)	40 (25-60)	0.116

Abbreviations: IQR, interquartile range; ER, Oestrogen Receptor; HER, Human Epidermal Growth Factor Receptor-2; Ki67, cell proliferation index; PABC, pregnancy-associated breast cancer; PR, Progesterone Receptor.

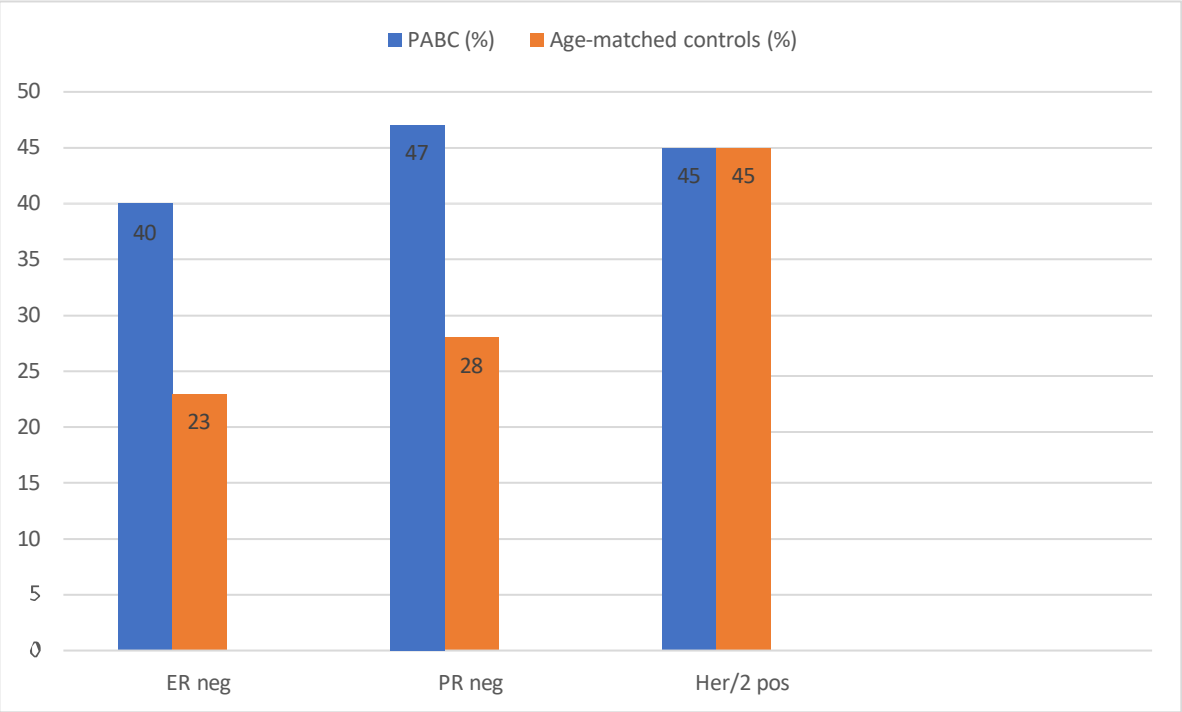


Figure 3: Comparison of Hormone Receptor Status in PABC vs Age-matched Controls

5. DISCUSSION

Our study was conducted at CHBAH, a tertiary referral center in the Soweto township and the biggest hospital in the Southern Hemisphere. CHBAH serves almost two million Soweto residents and a substantial catchment area across the Gauteng Province[20]. As a result, this study population is representative of the majority of South Africans living in low-resourced settings where barriers to healthcare are commonplace, and, further exacerbate diagnostic delays such as observed in PABC.

Additionally, patients were evaluated over a 14-year period producing a large study population that achieved the required PABC sample size, despite the condition's low incidence. The use of a substantial electronic database, where records are accurately documented and regularly updated by clinicians, patient-navigators, oncology nurses, research staff and information technology personnel, meant precise age-matching was feasible and this was performed with the assistance of a professional statistician to further aid accuracy.

5.1 Principal Findings

A total of 53 PABC cases eligible for further evaluation, with a median age of 36 years, which is consistent with previous studies[4,8,13]. Age-matching was utilized to eliminate age as a confounding factor and reduce its impact on the perceived aggressiveness of tumours in this patient group.

Pregnancy-associated breast cancer, in this single-center study, is a relatively rare entity with an incidence of 1.5% which aligns with the international literature-reported incidence of 0.2-3.8%[3-5]. Yet, this is likely an underestimation of the true frequency of PABC in this population, particularly, when compared to similar African studies[8,9]. In Dusengimana et al.'s[8] Rwandan study, 10.3% of women had PABC, while Hou et al.'s[9] Nigerian study documented a PABC incidence of 21.2%. However, broader criteria for inclusion were employed in both studies, with a wider definition of PABC in the Nigerian study (including patients up to two years post-partum), and an extended age range for females of reproductive age (18-50 years) in the Rwandan study[8,9]. The lower incidence of PABC in this study may be due to under-recognition of the association between pregnancy, lactation and BC by both patients and clinicians. This was particularly evident in this study prior to 2018 (12-year period) during which time only 44.4% of the total PABC cases were diagnosed. Yet, after 2018 during the last 3 years of the

14-year study period, 55.6% of all PABC cases were identified suggesting a growing awareness of PABC over time resulting in an increased detection rate.

The hypothesis that PABC presents with tumours that have more adverse prognostic clinicopathological characteristics, appeared to hold true in this study. The majority of patients in both the PABC and non-PABC age-matched control group presented with palpable breast masses, denoting more advanced, clinically detectable disease. However, patients in the PABC group presented with significantly larger tumours than their control group counterparts. In addition to generally unfavourable tumour biology, this finding may also be explained by the physiological changes occurring in the breast during pregnancy and lactation. Increased breast density and nodularity peripartum renders clinical examination challenging and, subsequently, suspicious breast masses may not be detected resulting in diagnostic delays and larger tumours at diagnosis[3,5,6,13-15]. This likely had an impact on the stage of disease noted in the PABC group in this study, where PABC patients presented with more Stage III and IV disease at diagnosis compared to non-PABC patients.

Further contributing to the more adverse pathological tumour traits in PABC, these patients were more likely to have tumours with a significantly higher nuclear grade as well as ER and PR negativity when compared to age-matched controls. These findings reflect international data, yet in contrast, the majority of patients in this study had HER2/neu negative tumours[17,18]. This may be due to inconsistent documentation of the FISH result. When tumours are HER2/neu equivocal on the initial biopsy, further characterization with a FISH test is required to determine the HER2/neu status. During the study period, the National Health Laboratory Service system underwent an update, and access to the previous database was unavailable, making it challenging to retrospectively trace missing data. Therefore, three PABC cases with equivocal HER2/neu results were excluded, potentially contributing to the difference in HER2/neu status seen in this study compared to existing literature.

Although PABC patients in this study presented with marginally increased *de novo* metastatic disease compared to non-pregnant controls, this did not reach statistical significance, in keeping with both African[8] and international literature[17]. One factor potentially influencing this, in our study, is the method by which staging of newly diagnosed PABC patients was determined. The European Society for Medical Oncology guidelines suggest staging should be performed with a Computed Tomography (CT) scan of the chest, abdominal imaging

(ultrasound, CT or MRI) and a bone scan in all patients with clinically positive axillary nodes, large tumours >5cm, aggressive tumour biology and clinical signs/ symptoms or laboratory values suggestive of metastatic disease[21]. However, due to the detrimental risk of radiation exposure to the fetus associated with a CT scan in PABC patients, in addition to limited access to cross-sectional imaging in our resource-constrained environment, CT is not routinely performed as a first-line investigation in this population. Therefore, pregnant patients underwent basic investigations for metastatic screening including blood tests, a liver ultrasound and a shielded chest X-ray at initial diagnosis which could have resulted in an under-estimation of *de novo* metastatic disease in this group.

5.2 Limitations

In this retrospective record review, identifying a PABC case at diagnosis was contingent on the attending clinician establishing an association with pregnancy or breastfeeding. If this was not recognized during the initial consultation, it may have been overlooked as a case of PABC and, therefore, not recorded as such in this study potentially, leading to an under-reporting of PABC. In addition, 10 PABC cases were excluded from the final analysis as the Ki67, an important marker of tumour proliferation with prognostic significance, was not reported by the laboratory. Prior to 2011, the laboratory did not routinely record the Ki67 value. Therefore, PABC cases detected during this period were ineligible for inclusion if the Ki67 was unavailable. Three of these excluded cases also had HER2/neu equivocal results, for which a FISH test could not be traced, further justifying their exclusion but, ultimately, contributing to a reduced sample size.

5.3 Lessons Learnt and Future Research

Given that PABC appears to demonstrate more adverse prognostic pathological features with more advanced stage disease, as demonstrated by this study, prompt diagnosis and improved management strategies are essential. This can be achieved through enhanced awareness among both clinicians and patients to ensure thorough investigation of new breast lumps in the peri-partum period. In addition, including mandatory fields for pregnancy and lactation in the electronic database will facilitate identification of PABC patients. Further studies should consider a prospective analysis and evaluation of treatment strategies between PABC and age-matched controls to fill current data gaps. However, this study has generated valuable clinicopathological data on PABC in a South African context which has not been published previously.

6. CONCLUSION

Pregnancy-associated breast cancer patients from our population tend to have larger tumours exhibiting more unfavourable characteristics. These factors are likely to adversely affect prognosis in these young patients. There is also a trend to more advanced stage of disease at diagnosis in the PABC group which may be attributed to the diagnostic delays in pregnant and lactating patients, in addition to the complex hormonal milieu of pregnancy potentially affecting tumour growth. However, larger prospective studies are required to assess the true association of these factors with PABC and enhance our understanding of this complex condition, thereby, enabling improved management strategies in our resource-constrained environment.

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APPENDIX 1: Plagiarism Declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Lisa Levenberg (Student number: 301375) am a student registered for the degree of Master of Medicine in the academic year 5.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date: 17 September 2024

APPENDIX 2: APPROVED PROTOCOL

Clinicopathological Features of Pregnancy-Associated Breast Cancer at Chris Hani Baragwanath Academic Hospital

Lisa Levenberg Student Number: 301375

Supervisors:

1. Dr N Murugan. Specialist Surgeon Breast Oncology, MBChB, FCS(SA)

2. Professor D Kruger. Associate Professor, Specialist Scientist in Clinical Medicine, Head of Research and Laboratories in Surgery, BSc, PGHE, PhD(UK)

3. Professor H Cubasch. Associate Professor of Surgery University of Witwatersrand, FCS(SA), Staatsexamen Germany, Breast Surgeon, Head of Breast Unit Chris Hani Baragwanath Academic Hospital

Summary

Pregnancy-associated breast cancer (PABC) refers to breast cancer diagnosed during pregnancy and up to one year postpartum. Although relatively uncommon, its incidence is increasing globally. PABC appears to be an aggressive cancer with adverse tumour characteristics. It remains unclear if these unfavourable tumour traits are the result of the distinctive tumour biology seen in young breast cancers, or if it is influenced by the pregnancy itself. Irrespective, pregnancy has an impact on the diagnosis and management of patients with PABC and, therefore, an enhanced understanding of this complex condition is required. There is limited research on PABC, particularly in an African context. This study will, therefore, aim to assess the clinicopathological features of PABC in our population and compare this to breast cancer in non-PABC age-matched controls.

Introduction

Breast cancer is the most commonly occurring female cancer worldwide and the primary cause of cancer-related female mortality[1]. This finding parallels South African data where the Global Cancer Observatory determined that breast cancer accounted for the highest number of new cancer cases across genders, highlighting it as a significant health problem[2]. Pregnancy-Associated Breast Cancer (PABC) defines breast cancer detected during pregnancy and up to one year post-partum[3]. Although relatively rare, breast cancer is the most frequently diagnosed malignancy in pregnancy. It affects approximately 1 in 3000 pregnant women with a reported incidence of 0.2-3.8% of all breast cancer cases and 15% of breast cancer cases in women younger than 35 years[3,4,5]. Global trends are showing a rising incidence with the increasing proclivity of women to delay childbearing until later in life when their breast cancer risk increases independently with advancing age[3]. The average age at diagnosis of PABC reportedly ranges from 33 to 38 years[5,6,7]. In African countries, the relative burden of PABC appears to be greater as a result of a younger general population with a higher parity and a younger age at presentation with breast cancer. These dynamics make it more likely to be associated with pregnancy[8,9]. Cultural and economic factors leading to inadequate health education and poor adherence to contraception likely influence and exacerbate this situation.

There are a number of risk factors that predispose to the development of breast cancer. Pregnancy and multiparity have a long-term protective effect on the development of breast cancer. However, this is initially preceded by a transient increased risk after the first pregnancy persisting as long as five to ten years post-delivery. After this period of augmented susceptibility, pregnancy once again confers protection against the development of breast cancer[10,11,12]. This risk is higher in first-time mothers over the age of 30-35 years where the later the initial pregnancy is, the higher and longer the probability of developing breast cancer in the postpartum years[13]. This has led to inconsistencies in the definition of PABC where studies use anywhere from one to ten years postpartum as the cut off depending on the extent of this perceived risk[12]. However, the most widely agreed upon definition, as mentioned previously, remains that of cancer occurring during pregnancy and one year post delivery. This will, therefore, be used in this study to define PABC.

PABC presents in the same way as breast cancer in non-pregnant patients with a palpable mass, overlying skin changes, or a bloody nipple discharge. A recent study by Wang et al demonstrated that 100% of their patients with PABC presented with a self-discovered breast mass[5]. This reflects other studies where a reported 82% to 95% of patients describe a self-discovered breast mass as their primary presenting complaint[6,14]. During lactation, a milk rejection sign may be indicative of an underlying malignancy with the infant refusing the cancer-containing breast[6]. However, clinical examination of the breast during pregnancy can be challenging because of increased breast density and nodularity. This can lead to a significant delay in diagnosis with a more advanced stage of disease at presentation[3,6,13]. In support of this, Wang et al.'s study further described a diagnostic delay of 7.84 months in their cohort of pregnant patients which is in keeping with prior papers that have described diagnostic delays of one to 13 months during pregnancy[5,6,15]. In Sub-Saharan Africa, these delays may be exacerbated by system inadequacies. In order to further dissect this, Donkor et al. analyzed the source of the greater diagnostic delays noted in African women with breast cancer and identified that a combination of under-recognition of worrisome symptoms together with fear, cultural beliefs and practices, and inadequate access to healthcare hinders early diagnosis and treatment[16]. Interestingly though, in a Rwandan study by Dusengimana et al. which assessed PABC, it was observed that patients did not experience significant delays in diagnosis and there was no difference in the stage of disease at presentation between pregnant and non-pregnant breast cancer patients[8]. Although this study provides useful data from an African context to allow comparison to our own setting, it is limited by the study's small sample size.

PABC appears to demonstrate adverse pathological features including larger, high grade tumours with an increased risk of axillary nodal involvement when compared to non-PABC counterparts. These cancers are predominantly Oestrogen Receptor (ER) and Progesterone Receptor (PR) negative with a Human Epidermal Growth Factor Receptor-2 (Her2/neu) positive predominance. The most common histological subtype is invasive ductal carcinoma which occurs in 75%-90% of all PABC cases[6,12,13,14]. Multiple studies support these conclusions, most notably the large study conducted by the Swedish group assessing the tumour characteristics of 778 patients with PABC[6,9,17]. Furthermore, the recent study published by O'Sullivan et al. also established that PABC is associated with high grade tumours with >70% classified as Stage II or higher at diagnosis with a greater propensity for ER and PR negativity compared to age-matched controls[18]. It is, however, unclear if these unfavourable tumour traits are the result of the distinctive tumour biology seen in young breast cancers independent of the pregnancy, or if it is influenced by the pregnancy itself and

should be considered as a separate entity. There are currently no known South African studies that have evaluated the clinicopathological characteristics of PABC. This will, therefore, be a primary aim of this study as understanding the tumour properties and patient prognosis in our PABC population will facilitate the determination of effective management strategies.

Consequently, these adverse clinicopathological features together with the delay in diagnosis, produce a more aggressive cancer with a poorer prognosis compared with age-matched controls[5]. A meta-analysis by Azim et al. assessing the outcome of PABC concluded that it carries a higher risk of recurrence and death compared to non-pregnant controls with the worst outcomes noted in those diagnosed in the post-partum period[11,13]. Similarly, Hartman et al.'s larger and updated meta-analysis of 41 studies reached the same conclusion[19]. Furthermore, a study conducted at Johns Hopkins University Hospital comparing 140 cases of PABC with 280 controls, determined that there was a higher hazard for recurrence and death in the pregnant case group, although this data did not reach statistical significance[18]. However, data in the literature seems to be conflicting regarding the prognosis of PABC[15]. Interestingly, some studies have demonstrated no difference in mortality between the PABC and non-PABC groups. In a European study, one of the largest studies to date, 447 patients with PABC derived from multinational cancer registries determined that disease-free and overall survival for patients with PABC compared to non-pregnant breast cancer patients were equivalent[20]. Earlier studies have corroborated this finding that PABC does not carry a worse prognosis when compared to non-pregnant patients. A 5-year-survival rate of 82% in both groups of patients was reported in Petrek et al.'s study[6,15]. This conflicting data may be the result of small cohorts in many of the studies on PABC owing to its relatively low incidence. Furthermore, these studies consist of case-controls, case reports and observational studies which are not as robust as randomized controlled-trials and call into question the validity of the results.

Management of patients with PABC requires a multidisciplinary team approach and careful balancing of the treatments required for the mother's survival and the potential risk of harm to the fetus. Treatment of PABC should, however, strive to meet the same standards achieved in treating non-pregnant patients with breast cancer and should not be delayed until after delivery. Surgery, involving a mastectomy with axillary lymph node dissection, is the definitive treatment of choice for PABC and is considered relatively safe during all three trimesters of pregnancy[6,7]. Breast-conserving surgery is a reasonable alternative; however, this necessitates post-operative radiation which is best avoided in pregnancy. Adjuvant treatment strategies include chemotherapy and radiation. Chemotherapy used in both the neoadjuvant and adjuvant setting is contraindicated in the first trimester when it may be associated with major malformations and fetal death[3,15]. This risk decreases from the second trimester when chemotherapy is relatively safe and an anthracycline-based regimen is generally preferred[3,7]. Radiotherapy also poses potential harm to the fetus including congenital malformations, growth retardation, childhood cancers, and fetal death[7]. If delivery is imminent treatment can be delayed until after the pregnancy. However, if radiotherapy is required earlier on in the pregnancy, it should not be postponed so as to minimize the risk of local recurrence[3,7].

PABC is a complex condition with multiple intricate and challenging issues. The increasing frequency, aggressive nature and ensuing impact on a key group of the population suggests that it requires meaningful attention. These patients are often the primary caregivers and form part of the economic workforce where poor outcomes have important psychosocial and financial consequences. Although there is an expanding collection of evidence assessing the

implications of PABC, it continues to be poorly understood with limited high-quality studies on optimal management[16]. In the African context, breast cancer patients in general have a higher risk of death compared to their counterparts in high income environments due to greater diagnostic delays experienced with insufficient breast cancer awareness programs resulting, in turn, in a more advanced stage of disease at diagnosis[8]. At the time of this protocol, there was no published data on PABC in South Africa with limited research assessing the burden of this condition in Africa. Therefore, this research is intended to generate data on PABC in a South African setting evaluating the incidence, presentation and clinicopathological features in order to enhance our understanding of this complex disease and optimize diagnosis and management strategies.

Study Objectives

Aim:
To evaluate the clinicopathological characteristics of patients with PABC.

Objectives
In patients with a confirmed breast cancer diagnosis attending the breast unit at Chris Hani Baragwanath Academic Hospital (CHBAH) from the inception of the breast unit's electronic database system on 1st October 2006 until the present (31 October 2020), the objectives of this study are:

- To determine the percentage of PABC in patients presenting with breast cancer to CHBAH.
- To describe the demographic characteristics of patients diagnosed with PABC at CHBAH.
- To identify the presenting signs and symptoms in patients diagnosed with PABC vs non-PABC patients.
- To determine the tumour characteristics in PABC including receptor status, tumour size and tumour grade, and compare these to those of non-PABC in age-matched controls.

Methods

Study Design
The study will be conducted in the form of a retrospective descriptive study.

Setting
The study will involve retrospectively reviewing the patient data from the existing database held and maintained by the breast unit at the Chris Hani Baragwanath Academic Hospital Breast Unit

Study Population
Inclusion Criteria:
All female patients of reproductive age (18-45 years) with confirmed breast cancer at CHBAH from 01 October 2006 until 31 October 2020.
Exclusion criteria:
For the PABC group only: patients diagnosed with breast cancer prior to pregnancy or more than one year postpartum.

Method of Sampling

This study will compare patients with PABC to age-matched non-PABC controls. The CHBAH electronic breast database system will be used to identify cases who meet the criteria from the introduction of this database in October 2006 over a 14-year period until current (October 2020). All women with confirmed breast cancer who are aged between 18 and 45 years will then be identified. Of this group, cases who meet the criteria for PABC will be isolated by entering the following keywords into the advanced search criteria on the database including: 'pregnant', 'pregnancy' 'breastfeeding', 'lactation', 'post-partum', 'delivery', 'gestation', 'baby', 'infant'. Patients who do not meet the criteria for PABC in this group will form the controls for comparison. The sample size will be based on the number of patients presenting that fulfill the inclusion criteria over the period specified. Based on the predicted incidence of 0.2-3.8% of PABC from prior studies with a cancer registry consisting of 4116 patients, the anticipated number of PABC patients at an estimated predicted incidence of 1.5% in our study population will be approximately 62 PABC patients. For objective 5, two controls will be matched to each PABC case by randomly selecting age-matched (± 2 years) non-PABC patients. Therefore, we estimate a total sample size of 186 patients (62 PABC, 124 non-PABC) for this last objective.

Measuring Tool & Data Collection

Each patient record captured on the electronic database will be analyzed and the information recorded on the data collection sheet as an MS Excel spread sheet. The data that will be recorded is as indicated below (See also Appendix):

- Age at diagnosis
- Patient-reported delay to diagnosis
- Size of tumour
- Nodal involvement
- Tumour Node Metastasis (TNM) staging
- Histological Grade according to the Nottingham Histologic Score System and subtype
- Receptor status/ subtype: ER, PR and Her2/neu
- KI 67%
- Gestation/ months post-partum at diagnosis

Data Protection

The electronic database can only be accessed with personal login credentials possessed by members of the breast unit team and researchers in order to protect the privacy and confidentiality of the patients. Data collection spreadsheets will remain on a password protected laptop and only the primary investigator and supervisors will have access to this.

Sources of Bias

Only patients that have been accurately recorded on the database as being pregnant, post-partum or lactating at diagnosis with breast cancer will be identified as potential study candidates. There may be individuals who would meet the criteria, however, their breast cancer diagnosis has not been documented to be associated with a pregnancy. This will result in an under-estimation of the incidence of PABC in this cohort of patients.

Data Analysis

Statistical analysis will be performed with the assistance of a professional statistician in order to ensure the validity of the results. Descriptive statistics will be used to summarize the data. Statistical difference between comparable groups will be calculated using Pearson's Chi-squared (X2) or Fischer's Exact test for categorical variables, and the Mann-Whitney-U test for continuous variables. A P-value of < 0.05 will be considered to be statistically significant. Statistica or STATA software will be used for statistical analysis.

Ethics

Ethics approval will be obtained from the Human Research Ethics Committee of the University of Witwatersrand, CHBAH management and the gatekeeper of the electronic breast unit database prior to conducting this study. All investigators in this study will preserve the confidentiality of the patient participants and all identifying data will be kept anonymous when collecting patient data with the use of patient numbers on data collection sheets. Only the principal investigator will have access to this identifying data. As this is a retrospective study, informed consent is not required from study participants, nor will clinical decisions and outcomes be affected by this research.

Timeline

	July-Sept 2020	Oct-Nov 2020	Dec-Jan 2020/21	Feb-April 2021	May-June 2021
Literature review					
Preparing protocol					
Protocol assessment					
Ethics application					
Data Collection					
Data analysis					
Writing up MMED					
Writing up paper					

Funding

The conducting of this study is not anticipated to incur any costs. Expenditures related to wifi data usage, transport and publishing will be covered by the principal investigator.

Problems

There are some deficiencies in the documentation of detailed patient information on the database, particularly from the early years of its creation. Furthermore, the database is updated during each patient interaction by both permanent breast consultants as well as rotating trainees and junior staff who are not as familiar with the database system. This may create inconsistencies in the completion of clinical data and insufficiencies in clinical information of potential study participants particularly relating to the documentation of an association of a breast cancer diagnosis with pregnancy and lactation, and the time from symptom onset to diagnosis with breast cancer. This could lead to exclusion of cases who meet the study criteria which may impact on the ability to obtain a statistically significant sample size.

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APPENDIX 3: Protocol Assessors Meeting and Faculty Approval Letter

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PROTOCOL ASSESSORS MEETING

Candidate Full Name: **Lisa Michelle Levenberg** Supervisors**: Cubasch/Murugan/Kruger**

Student Number: **301375** Date: **18th November 2020**

School / Department / Division: **Department of Surgery, Division of General Surgery**

Degree: **MMED**

Title: **Clinicopathological Features of Pregnancy Associated Breast Cancer at Chris Hani Baragwanath Academic Hospital.**

1. **Type of study** *(tick all that apply)*:

- Quantitative
- Qualitative
- Mixed Methods
- Laboratory
- Clinical ✓
- Other, please specify……..

2. **Is title of the study appropriate** (*preferably fewer than 20 words*)**?** Yes✓ No

Comments: The title of the study is appropriate

3. **Are the study objectives clear and linked to the research aim and title?** Yes No

Comments: Not entirely

- Objective 1- Please edit to say "percentage""
- Objectives state during October 2006 AND October 2020 – does she mean from and to? It appears so from her inclusion criteria. What is the reason for those specific dates?
- Objective 4- clarify if this is an intra-service delay or patient-reported delay

4. **Is the design of the study appropriate to meet the study objectives?** Yes No

Comments: Not entirely

- Exclusion criteria – as I understand it, the study aims to evaluate all breast cancers during that period and to extract the PABCs – which are well-defined, so I don't think the exclusion criteria are appropriate.

5. Are the proposed methods and tools appropriate to meet the research objectives? Yes✓ No

Comments: Methods and tools are appropriate

6. Is the study feasible within the resources of:

a) The applicant? Yes✓ No

b) The department? Yes✓ No

c) The time frame? Yes✓ No

7. If this is a PhD protocol assessment:

a) Is the content original? Yes No

b) Does the content show the scope and depth of a PhD? Yes No

Comments: _____

_____

_____

_____

Do you recommend:

i. Additional revision/amendment of the protocol? Please be specific on the recommendations being made:

- Careful proof reading to avoid grammatical errors.
- Swap references 17 and 18
- Swap references 19 and 20
- Check references as some have URLs and date accessed. Not necessary for properly indexed journals.
- In the data analysis section change 0.5 to 0.05
- Include year on timeline

ii. The appointment of the proposed Supervisor? Yes✓ No

Nominee/s: The assessors approve of the appointed supervisors

iii. The appointment of the proposed Co-Supervisor/s and/or additional co-supervisors? Yes No✓

Nominee/s: No additional co-supervisors to be appointed

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

11 February 2021
Person No: 301375
PAG

Dr LM Levenberg
Flat 6 Melrose Court
12 Tottenham Avenue
Melrose Estate
2196
South Africa

Dear Dr Lisa Levenberg

Master of Medicine in Surgery: Approval of Title

We have pleasure in advising that your proposal entitled *Clinicopathological Features of Pregnancy-Associated Breast Cancer at Chris Hani Baragwanath Academic Hospital* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

APPENDIX 4: Data Collection Sheet

Patient No	Age at diagnosis (years)	Pregnancy status		Tumour size (cm)	Node +/-	Mets =/-	Stage	Grade	Histo subtype	Hormone Receptor status			KI67%	Mass Y(1)/ N(2)	Year at diagnosis
		Pregnant (1)/ postpartum (2)	Trimester							ER	PR	Her2			

APPENDIX 5: Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Lisa Levenberg

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M210804

NAME: (Principal Investigator)	Dr Lisa Levenberg
DEPARTMENT:	Surgery Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Clinicopathalogical Features of Pregnancy-Associated Breast Cancer at Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	27/08/2021
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof H. Cubasch, Dr N. Murugan and Prof D. Kruger
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	20/10/2021

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 Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. 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.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **August** and will therefore be due in the month of **August** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 6: Turnitin Report

MMED journal draft update 3.docx

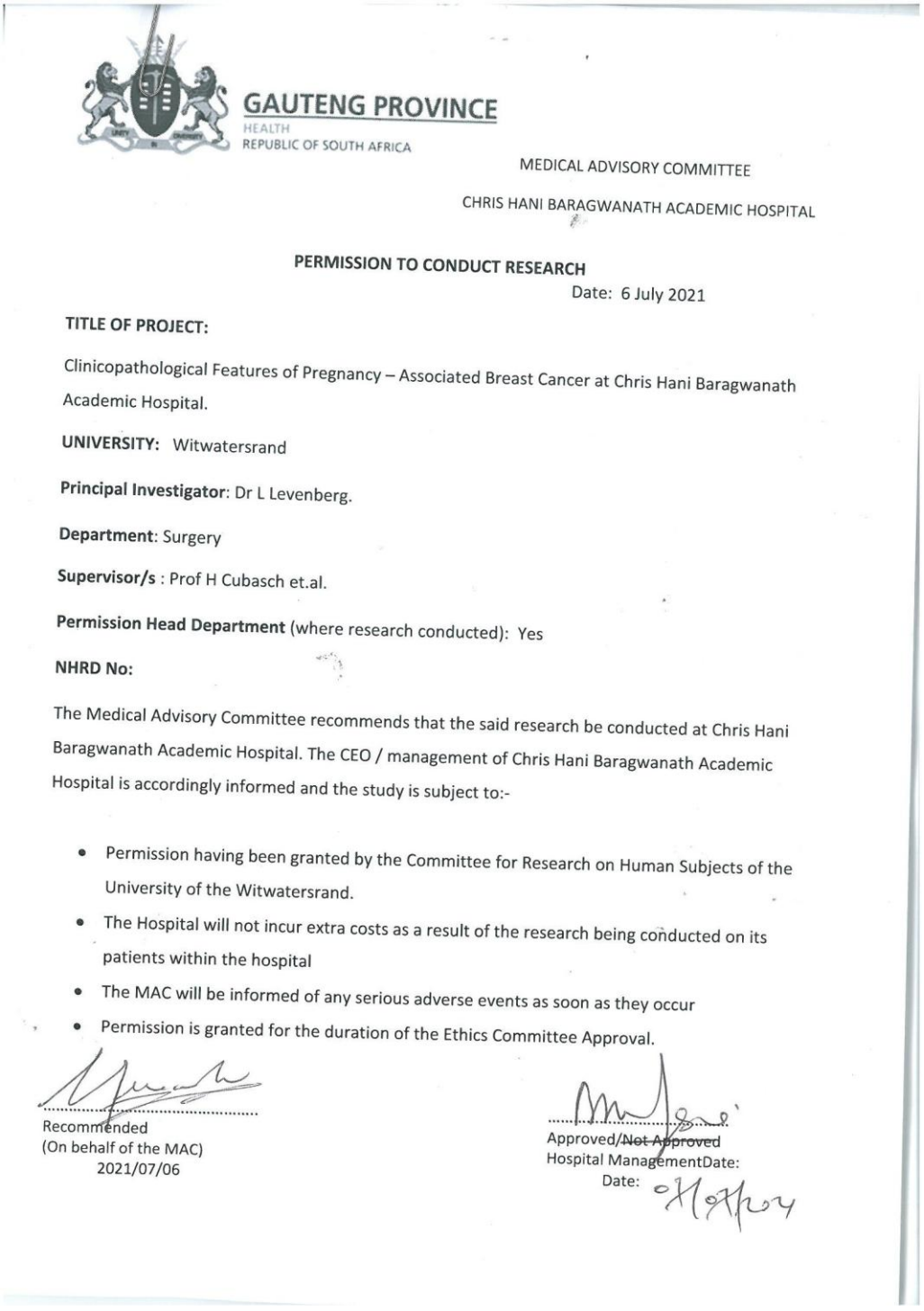
ORIGINALITY REPORT

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1	d.docksci.com Internet Source	1%
2	F. Cardoso, S. Kyriakides, S. Ohno, F. Penault-Llorca, P. Poortmans, I.T. Rubio, S. Zackrisson, E. Senkus. "Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up", Annals of Oncology, 2019 Publication	1%
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5	e-mjm.org Internet Source	<1%
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7	"Breast", Laboratory Investigation, 2008 Publication	<1%

APPENDIX 7: Letter of Approval to Conduct Research by Hospital Medical Advisory Committee



GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 6 July 2021

TITLE OF PROJECT:

Clinicopathological Features of Pregnancy – Associated Breast Cancer at Chris Hani Baragwanath Academic Hospital.

UNIVERSITY: Witwatersrand

Principal Investigator: Dr L Levenberg.

Department: Surgery

Supervisor/s : Prof H Cubasch et.al.

Permission Head Department (where research conducted): Yes

NHRD No:

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Academic Hospital. The CEO / management of Chris Hani Baragwanath Academic Hospital is accordingly informed and the study is subject to:-

- Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.
- The Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- The MAC will be informed of any serious adverse events as soon as they occur
- Permission is granted for the duration of the Ethics Committee Approval.

.....
Recommended
(On behalf of the MAC)
2021/07/06

.....
Approved/~~Not Approved~~
Hospital ManagementDate:
Date: 07/07/2021

APPENDIX 8: Letter of Approval Breast Unit Database





Department of Surgery

17th June 2021

To the Human Research Ethics Committee of the University of Witwatersrand,

This letter serves to confirm that I, Professor Herbert Cubasch, am the gatekeeper of the Chris Hani Baragwanath Academic Hospital Breast cancer database and patient clinical record system. In this capacity, I hereby grant permission for Dr Lisa Levenberg, as the principal investigator of the research project entitled '*Clinicopathalogical Features of Pregnancy-associated Breast Cancer as Chris Hani Baragwanath Hospital'*, to utilise these patient records and information for the purpose of conducting research.

Do not hesitate to get in touch should you require any further information. Yours Sincerely,

Professor Herbert Cubasch, FCS(SA) Specialist Surgeon Breast Oncology
Head of Breast Unit Chris Hani Baragwanath Hospital Department of Surgery
Chris Hani Baragwanath Academic Hospital Breast

APPENDIX 9: Journal Submission Guidelines – Journal Surgical Research

Authorship

All authors should have made substantial contributions to all of the following:

1. The conception and design of the study, or acquisition of data, or analysis and interpretation of data.
2. Drafting the article or revising it critically for important intellectual content.
3. Final approval of the version to be submitted.

All authors should agree to be accountable for all aspects of the work to ensure that the questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration of interests

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence or bias their work.

Authors with no competing interests to declare should select the option, "I have nothing to declare".

The resulting Word document containing your declaration should be uploaded at the "attach/upload files" step in the submission process. It is important that the Word document is saved in the .doc/.docx file format. Author signatures are not required.

Funding sources

Authors must disclose any funding sources who provided financial support for the conduct of the research and/or preparation of the article. The role of sponsors, if any, should be declared in relation to the study design, collection, analysis and interpretation of data, writing of the report and decision to submit the article for publication. If funding sources had no such involvement this should be stated in your submission.

List funding sources in this standard way to facilitate compliance to funder's requirements:

If no funding has been provided for the research, it is recommended to include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Informed consent and patient details

Authors must document in the manuscript that ethics committee approval and informed consent have been obtained for studies involving patients or volunteers (including organ/tissue donors). Key guidelines:

- Appropriate consents, permissions and releases must be obtained if case details, personal information and images of patients or any other individuals are included in a publication, even if anonymized.
- Patient and research subjects' names, initials, hospital or social security numbers, dates of birth or any other personal or identifying information should never be used, even where consent has been provided.

Written consents must be retained. They should not be provided to this journal unless this is specifically requested in exceptional circumstances, for example, when a legal issue arises. Only then should you provide copies of the consents, or evidence that all relevant consents were obtained.

Personal details of any patient must only be included in your article or in any supplementary materials (including all images and videos) in cases where written permission has been given by the patient (or, where applicable, the next of kin).

We advise you to review Elsevier's policy on patient consent prior to preparing your manuscript.

Writing and formatting

File format

We ask you to provide editable source files for your entire submission (including figures, tables and text graphics). Some guidelines:

- Save files in an editable format, using the extension .doc/.docx for Word files and .tex for LaTeX files. A PDF is not an acceptable source file.
- Lay out text in a single-column format.
- Use spell-check and grammar-check functions to avoid errors.

Title page

You are required to include the following details in the title page information:

- Article title. Article titles should be concise and informative. Please avoid abbreviations and formulae, where possible, unless they are established and widely understood, e.g., DNA).
- Author names. Provide the given name(s) and family name(s) of each author. The order of authors should match the order in the submission system. Carefully check that all names are accurately spelled. If needed, you can add your name between parentheses in your own script after the English transliteration.
- Affiliations. Add affiliation addresses, referring to where the work was carried out, below the author names. Indicate affiliations using a lower-case superscript letter immediately after the author's name and in front of the

corresponding address. Ensure that you provide the full postal address of each affiliation, including the country name and, if available, the email address of each author.

- Corresponding author. Clearly indicate who will handle correspondence for your article at all stages of the refereeing and publication process and also post-publication. This responsibility includes answering any future queries about your results, data, methodology and materials. It is important that the email address and contact details of your corresponding author are kept up to date during the submission and publication process.
- Present/permanent address. If an author has moved since the work described in your article was carried out, or the author was visiting during that time, a "present address" (or "permanent address") can be indicated by a footnote to the author's name. The address where the author carried out the work must be retained as their main affiliation address. Use superscript Arabic numerals for such footnotes.

Abstract

You are required to provide a concise and factual abstract which does not exceed 250 words. The abstract should briefly state the purpose of your research, principal results and major conclusions. Some guidelines:

- Abstracts must be able to stand alone as abstracts are often presented separately from the article.
- Avoid references. If any are essential to include, ensure that you cite the author(s) and year(s).
- Avoid non-standard or uncommon abbreviations. If any are essential to include, ensure they are defined within your abstract at first mention.

Keywords

You are required to provide 1 to 7 keywords for indexing purposes. Keywords should be written in English. Please try to avoid keywords consisting of multiple words (using "and" or "of").

We recommend that you only use abbreviations in keywords if they are firmly established in the field.

Math formulae

- Submit math equations as editable text, not as images.
- Present simple formulae in line with normal text, where possible.
- Use the solidus (/) instead of a horizontal line for small fractional terms such as X/Y.
- Present variables in italics.
- Denote powers of e by exp.
- Display equations separately from your text, numbering them consecutively in the order they are referred to within your text.

Tables

Tables must be submitted as editable text, not as images. Some guidelines:

- Place tables next to the relevant text or on a separate page(s) at the end of your article.
- Cite all tables in the manuscript text.
- Number tables consecutively according to their appearance in the text.
- Please provide captions along with the tables.
- Place any table notes below the table body.
- Avoid vertical rules and shading within table cells.

We recommend that you use tables sparingly, ensuring that any data presented in tables is not duplicating results described elsewhere in the article.

Figures, images and artwork

Figures, images, artwork, diagrams and other graphical media must be supplied as separate files along with the manuscript. We recommend that you read our detailed artwork and media instructions. Some excerpts:

When submitting artwork:

- Cite all images in the manuscript text.
- Number images according to the sequence they appear within your article.
- Submit each image as a separate file using a logical naming convention for your files (for example, Figure_1, Figure_2 etc).
- Please provide captions along with the artwork.
- Text graphics may be embedded in the text at the appropriate position. If you are working with LaTeX, text graphics may also be embedded in the file.

Artwork formats

When your artwork is finalized, "save as" or convert your electronic artwork to the formats listed below taking into account the given resolution requirements for line drawings, halftones, and line/halftone combinations:

- Vector drawings: Save as EPS or PDF files embedding the font or saving the text as "graphics."
- Color or grayscale photographs (halftones): Save as TIFF, JPG or PNG files using a minimum of 300 dpi (for single column: min. 1063 pixels, full page width: 2244 pixels).
- Bitmapped line drawings: Save as TIFF, JPG or PNG files using a minimum of 1000 dpi (for single column: min. 3543 pixels, full page width: 7480 pixels).
- Combinations bitmapped line/halftones (color or grayscale): Save as TIFF, JPG or PNG files using a minimum of 500 dpi (for single column: min. 1772 pixels, full page width: 3740 pixels).

Please do not submit:

- files that are too low in resolution (for example, files optimized for screen use such as GIF, BMP, PICT or WPG files).
- disproportionally large images compared to font size, as text may become unreadable.

Figure captions

All images must have a caption. A caption should consist of a brief title (not displayed on the figure itself) and a description of the image. We advise you to keep the amount of text in any image to a minimum, though any symbols and abbreviations used should be explained.

Provide captions in a separate file.

Article structure

Article sections

Divide your manuscript into clearly defined sections covering all essential elements. For example:

- Abstract
- Keywords
- Introduction
- Materials and methods
- Results
- Conclusions
- Artwork
- Tables with captions

Introduction

The introduction should clearly state the objectives of your work. We recommend that you provide an adequate background to your work but avoid writing a detailed literature overview or summary of your results.

Methods

The methods section should provide sufficient details about your materials and methods to allow your work to be reproduced by an independent researcher. Some guidelines:

- If the method you used has already been published, provide a summary and reference the originally published method.
- If you are quoting directly from a previously published method, use quotation marks and cite the source.
- Describe any modifications that you have made to existing methods.

Results

Results should be clear and concise. We advise you to read the sections in this guide on supplying tables, artwork, supplementary material and sharing research data.

Discussion

The discussion section should explore the significance of your results but not repeat them. You may combine your results and discussion sections into one section, if appropriate. We recommend that you avoid the use of extensive citations and discussion of published literature in the discussion section.

Conclusion

The conclusion section should present the main conclusions of your study. You may have a stand-alone conclusions section or include your conclusions in a subsection of your discussion or results and discussion section.

Glossary

Please provide definitions of field-specific terms used in your article, in a separate list.

Abbreviations

Abbreviations which are not standard in the field should be defined in a footnote on the first page of your article.

Abbreviations which are essential to include in your abstract should be defined at first mention in your abstract, as well as in a footnote on the first page of your article.

Before submission we recommend that you review your use of abbreviations throughout your article to ensure that it is consistent.

Appendices

We ask you to use the following format for appendices:

- Identify individual appendices within your article using the format: A, B, etc.
- Give separate numbering to formulae and equations within appendices using formats such as Eq. (A.1), Eq. (A.2), etc. and in subsequent appendices, Eq. (B.1), Eq. (B. 2) etc. In a similar way, give separate numbering to tables and figures using formats such as Table A.1; Fig. A.1, etc.

Footnotes

We advise you to use footnotes sparingly. If you include footnotes in your article, ensure that they are numbered consecutively.

You may use system features that automatically build footnotes into text. Alternatively, you can indicate the position of footnotes within the text and present them in a separate section at the end of your article.

Acknowledgements

Include any individuals who provided you with help during your research, such as help with language, writing or proof reading, in the acknowledgements section. Acknowledgements should be placed in a separate section which appears directly before the reference list. Do not include acknowledgements on your title page, as a footnote to your title, or anywhere else in your article other than in the separate acknowledgements section.

Funding sources

Authors must disclose any funding sources who provided financial support for the conduct of the research and/or preparation of the article. The role of sponsors, if any, should be declared in relation to the study design, collection, analysis and interpretation of data, writing of the report and decision to submit the article for publication. If funding sources had no such involvement this should be stated in your submission.

List funding sources in this standard way to facilitate compliance to funder's requirements:

It is not necessary to include detailed descriptions on the program or type of grants, scholarships and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, it is recommended to include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

References within text

Any references cited within your article should also be present in your reference list and vice versa. Some guidelines:

- References cited in your abstract must be given in full.
- We recommend that you do not include unpublished results and personal communications in your reference list, though you may mention them in the text of your article.
- Any unpublished results and personal communications included in your reference list must follow the standard reference style of the journal. In

substitution of the publication date add "unpublished results" or "personal communication."
- References cited as "in press" imply that the item has been accepted for publication.

Linking to cited sources will increase the discoverability of your research.

Before submission, check that all data provided in your reference list are correct, including any references which have been copied. Providing correct reference data allows us to link to abstracting and indexing services such as Scopus, Crossref and PubMed. Any incorrect surnames, journal or book titles, publication years or pagination within your references may prevent link creation.

We encourage the use of Digital Object Identifiers (DOIs) as reference links as they provide a permanent link to the electronic article referenced. See the example below, though be aware that the format of such citations should be adapted to follow the style of other references in your paper.

DOI link example (for an article not yet in an issue):
VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884.

Reference format

This journal does not set strict requirements on reference formatting at submission. Some guidelines:

- References can be in any style or format as long as the style is consistent.
- Author names, journal or book titles, chapter or article titles, year of publication, volume numbers, article numbers or pagination must be included, where applicable.
- Use of DOIs is recommended.

Our journal reference style will be applied to your article after acceptance, at proof stage. If required, at this stage we will ask you to correct or supply any missing reference data.

Reference style

Indicate references by adding a number within square brackets in the text. You can refer to author names within your text, but you must always give the reference number, e.g., "as demonstrated [3,6]. Barnaby and Jones [8] obtained a different result..... ".

Number references in the order they appear in your article.