A RETROSPECTIVE STUDY COMPARING IMMUNOHISTOCHEMISTRY SUBTYPING AND FINDINGS ON F-18 FDG PET/CT IN PATIENTS WITH INVASIVE BREAST CANCER

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

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Declaration

I, Anica Venter, declare that this research report is being submitted for the degree of Master of Medicine in Nuclear Medicine at the University of Witwatersrand, Johannesburg. I declare that this report is original, my own personal work and not submitted previously for any examination or postgraduate degree at this or any other institution.

DR ANICA VENTER

On this 12th day of June 2025

Dedication

- I wish to dedicate this work to my loving husband Francois Knoetzen for your unconditional support and encouragement.
- I also dedicate this work to my newborn son, Frans Roelof Knoetzen, as well as to my loving parents, Roelf and Ronel Venter for always encouraging me to pursue my dreams.

Publications and presentations

This work has never been published or presented at a congress.

Abstract

INTRODUCTION: Breast cancer (BC) is a major global public health concern, and its diverse pathology makes management particularly complex. Molecular subtypes, identified through immunohistochemistry (IHC), along with metabolic activity and disease extent revealed by Fluorine-18 fluorodeoxyglucose positron emission tomography/computed tomography (F-18 FDG PET/CT), provide valuable insights. Exploring the relationship between IHC subtypes and F-18 FDG PET/CT findings can enhance personalized BC management.

AIM:

The study aimed to assess the correlation between IHC subtypes and F-18 FDG PET/CT findings in patients with invasive BC referred for staging, restaging, and response assessment at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) Nuclear Medicine department.

METHOD:

A retrospective analysis was conducted on histologically confirmed invasive BC patients who underwent F-18 FDG PET/CT between January 2016 and September 2022 at CMJAH. Patient demographics, IHC markers (ER, PR, HER-2, Ki-67%) on the initial histology, patterns of disease spread and SUVmax values of tumours on PET/CT were collected and analysed.

RESULTS:

This study included 136 BC patients. FDG PET/CT was predominantly indicated for restaging (71%), followed by response to therapy assessment (18%) and staging (11%), detecting disease in 102 (75%) cases. Although not statistically significant, bone metastases were prevalent in hormone receptor-positive subtypes, while triple negative breast cancer (TNBC) showed a predilection for lung metastases. Higher Ki67% and standardized uptake value maximum (SUVmax) values, a marker proliferation and tumour aggressiveness, were significantly associated with TNBC subtype with a mean Ki67% of 70% (range, 40-80%) and SUVmax of 13.5 (range, 7.2-19), while Luminal A tumours exhibited the lowest values with a mean Ki67% of 10% (range, 5-20%) and SUVmax of 6.8 (range, 4.3-11). P-values of 0.0001 for Ki67% and 0.0564 for SUVmax.

CONCLUSIONS:

The study highlights a significant correlation between IHC markers and FDG PET/CT imaging, particularly regarding the Ki67% and SUVmax, across different BC subtypes. These findings underscore the utility of FDG PET/CT not only in evaluating the disease extent—spanning local, nodal, and distant metastases—but also in complementing IHC analysis for a more comprehensive diagnostic approach, offering valuable insights that can guide clinical decision-making for personalized patient care. Future studies should incorporate prospective designs and novel PET tracers to overcome our current limitations.

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List of Abbreviations and Terminology

- ASCO: American society of clinical oncology
- BC: Breast cancer
- CEO: Chief executive officer
- CMJAH: Charlotte Maxeke Johannesburg Academic Hospital
- CNS: Central nervous system
- CT: Computed tomography
- CXCR4: Chemokine Receptor 4
- ESMO: European Society for Medical Oncology
- ER: Oestrogen receptor
- F-18 FDG PET/CT: Fluorine-18 fluorodeoxyglucose positron emission tomography/computed tomography
- FAPI: Fibroblast Activation Protein Inhibitor
- FES: F-18-fluoroestradiol
- FFNP: F-18-fluorofuranyl norprogesterone
- FISH: Fluorescence in Situ Hybridization
- GRPR: Gastrin-releasing peptide receptor
- HER-2/neu: Human epidermal growth factor 2
- HIV: Human immunodeficiency virus
- HOD: Head of department
- HREC: Human Research Ethics Committee
- IARC: International Agency of Cancer Research
- IHC: Immunohistochemistry
- LMICs: Low-and middle-income countries
- MRI: Magnetic resonance imaging
- NCCN: National Comprehensive Cancer Network
- NHLS: National Health Laboratory Service
- NPV: Negative predictive value
- NCR: National Cancer Registry
- PPV: Positive predictive value
- PR: Progesterone receptor
- PMSA: Prostate specific membrane antigen

- ROC: Receiver operating characteristics
- SA: South Africa
- SUV: Standardized uptake value
- SUVmax: Standardized uptake value maximum
- TNBC: Triple negative breast cancer
- WITS: University of the Witwatersrand

1. Chapter 1: Introduction and literature review

1.1 Introduction

Breast cancer (BC) is a major public health concern worldwide and its heterogenous and complex pathology makes the management thereof complex (1). Fortunately, the treatment of patients with BC has become more individualized. Numerous studies have shown associations between the uptake on Fluorine-18 fluorodeoxyglucose positron emission tomography/computed tomography (F-18 FDG PET/CT) and the clinicopathological features of the tumour, as well as the significance of the maximum standardized uptake value (SUVmax) in predicting the molecular subtype and its prognosis (1,2). Thus, combining the immunohistochemistry (IHC) and other diagnostic markers with the information gained from advanced and integrated imaging techniques, the diagnostic accuracy is improved, resulting in treatment and treatment response to be more precise (1,3).

The purpose of this study was to investigate the pattern of metastases observed on F-18 FDG PET/CT in patients with invasive BC and to correlate the findings with immunohistochemical markers on the initial histology, as well as to evaluate the potential of F-18 FDG PET/CT in predicting aggressiveness of the different tumour subtypes.

1.2 Background

1.2.1 Breast cancer epidemiology

Globally BC is the most diagnosed cancer in women. The burden of BC is on the rise, accounting for 1 in 8 cancer diagnoses. The incidence of BC in 2020, for both males and females combined, was over 2.3 million and it is estimated that by 2040, worldwide, the number of new cases will grow by more than 40% (4).

In 2020, it was estimated that 16% of females with BC died globally (4). In high income countries, the mortality has declined due to earlier detection and improved adjuvant treatment. In contrast, in South Africa (SA) and other low- and middle-income countries (LMICs), poor survival rates are linked to delayed medical presentation, inadequate infrastructure and funding, as well as the inequitable and unaffordable access to available resources (5). According to the 2019 figures by the National Cancer Registry (NCR) of SA, in females, a total of 10172 cases were diagnosed on histology, representing 23.22% of all female cancers.

In males, 184 new BC cases were diagnosed in 2019, representing 0.44% of all male cancers in SA (6).

1.2.2 Histological diagnosis and imaging in clinical practice

BC is a heterogeneous disease consisting of distinct biological subtypes. These subtypes are associated with the clinical outcome and response to treatment and can be determined by gene expression profiles or by IHC biomarkers from tissue specimens. In most centres, IHC marker analysis has generally been used as a surrogate for identifying BC subtypes due to the cost of DNA analysis (5).

IHC, introduced by Coons et al. in 1942, is a method that characterizes intracellular or cell surface proteins and was initially developed from immunofluorescence techniques (3,7). Single markers or panels of marker proteins are used in the characterization of tumour subtypes, as well as distinguishing metastatic tissue from primary tumour. These markers are also used to provide information on prognostication, predict treatment response and are used for the evaluation of remaining tumour post therapy (3).

The most frequently used IHC markers in BC are oestrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), and Ki-67, all of which are advocated in clinical practice (3).

According to the St. Gallen International BC consensus, there are five subtypes of BC. These subtypes are based on the presence of tumour cell surface receptors and the value of Ki-67, which is the biological potential for proliferation and division (5).

The subtypes are outlined as follow:

- Luminal A (ER and/or PR positive, HER-2 negative and Ki-67% < 20%)
- Luminal B HER-2 negative (ER and/or PR positive, HER-2 negative and Ki-67% > 20%)
- Luminal B HER-2 positive (ER and/or PR positive, and HER-2 positive)
- HER-2 non-luminal (ER or PR negative and HER-2 positive)
- Basal-like (ER or PR negative and HER-2 negative) (8–10)

In addition, tumours are classified ER positive or PR positive if > 1% staining, and tumours are classified HER-2 positive if they score 3+ by IHC. If the HER-2 score is 2+, tumours are

considered HER-2 positive if Fluorescence in Situ Hybridization (FISH) test shows HER-2 gene amplification (10).

After diagnosis, the next step is to stage or establish the extent of disease involvement (11). Modalities used for this purpose include ultrasound, mammography, and magnetic resonance imaging (MRI). Bone scintigraphy, computed tomography (CT) or MRI (chest and abdomen) are other modalities also used in the workup and staging of BC (12). FDG PET/CT is mainly implemented for the evaluation of distant disease involvement (12), specifically for patients with clinical stage IIB BC and above (13).

1.2.3 Patterns of metastases in breast cancer

It is estimated that in SA, 50-60% of females have locally advanced or metastatic disease at initial presentation (14). The most prevalent metastatic sites for BC include bone, lung, liver, brain, pleura, bone marrow, soft tissue and skin (15).

Hormone receptor positive BC has a more favourable prognosis. K Kast et al noted that the first site of metastasis is also a dependent factor of prognosis, with most favourable being bone and least favourable being brain metastases. Hormone receptor positive BC commonly metastasizes to bone initially, and hormone receptor negative BC usually have multiple sites of metastases in the viscera (16).

According to Bonacho T et al. basal-like subtype frequently have metastases to the brain and to lung. Luminal A and luminal B frequently have bone metastases. HER-2 enriched subtype is associated with liver metastases (15).

1.2.4 The role of F-18 FDG PET/CT

F-18 FDG, a radioactive glucose analog, is taken up by cells through glucose transporters. Intracellularly it is phosphorylated by hexokinase and becomes trapped at a rate that reflects glucose utilization, rendering it useful for detecting disease processes, such as malignancies. Compared to non-malignant cells, malignant cells have a higher expression of glucose transporters and intracellular hexokinase and phosphofructokinase, thus high glycolytic activity, easing tumour detection on FDG PET/CT. Staging, prognostication, treatment response evaluation, radiotherapy planning and detection of disease recurrence are all areas where FDG PET/CT plays a role (17,18).

For diagnosis and early disease assessment, FDG PET/CT's performance is suboptimal when compared to ultrasound, mammography, breast MRI and axillary nodal evaluation (with sentinel lymph node biopsy), limited by low spatial resolution, and therefore not routinely indicated in early diagnosis as per the National Comprehensive Cancer Network (NCCN) guidelines (17). However, its usefulness in localizing extraaxillary nodal disease and distant metastases becomes very significant and is mainly targeted to high-risk patients for extensive disease evaluation (12).

A whole-body FDG PET/CT has superior performance as compared to conventional imaging when used to detect occult distant metastases, except for brain metastases, where magnetic resonance imaging (MRI) is the preferred choice. Sensitivity and specificity of PET/CT is also greater than contrast-enhanced CT or bone scintigraphy in the detection of lytic or mixed bone metastases, and for identifying affected bone marrow (18).

1.2.5 The standardized uptake value (SUV)

The standardized uptake value (SUV) is a prognostic tool that semi-quantitatively measures the degree of F-18 FDG uptake or avidity in a lesion. Most commonly the maximum SUV value or SUVmax is used expressing the single voxel value that correlates with the most intense F-18 FDG uptake. Factors that influence the value include plasma glucose level, partial volume effects, as well as the injection time (19).

Numerous studies have shown that the level of glucose metabolism in a tumour is linked to its aggressiveness (20). FDG avidity is reduced in well-differentiated ER positive tumours as compared to ER negative tumours. This is the same for PR positive tumours as compared to PR negative tumours. FDG avidity is however substantially increased in triple negative breast tumours. In addition, when comparing luminal A tumours with luminal B tumours, FDG avidity is higher in luminal B tumours (21).

1.2.6 Pitfalls of F-18 FDG PET/CT

Patients undergoing FDG PET/CT imaging are prepared carefully to ensure optimal results and minimize potential pitfalls. This mainly includes fasting for a period of at least 4 hours, avoidance of strenuous exercise for 24 hours prior to the study, ensuring a blood glucose level of below 11 mmol/L prior to F-18 FDG administration, and keeping warm, silent and relaxed once the radiotracer has been injected (22,23). Imaging is typically performed one hour after

the injection, allowing adequate time for the radiotracer to distribute throughout the body (22).

Small tumour size, low tumour grade and proliferation, increased hormone receptor expression and lobular type on histology all decreases the sensitivity of FDG PET/CT imaging in BC. A spatial resolution of 5-6mm is a limiting factor and as mentioned already, FDG PET/CT is also inferior to sentinel lymph node biopsy in early-stage BC (18).

Benign breast disease, other malignancies e.g., lymphoma, solid tumour metastases and inflammation such as sarcoidosis or infections, also demonstrates increased FDG avidity and therefore focally increased glucose metabolism is not specific for BC. Reactive lymph nodes are frequently detected as it commonly results in increased metabolic activity. Therefore, information concerning recent vaccinations (e.g. to COVID-19) and HIV status is important when interpreting the images, to prevent reporting false positives (24).

1.3 Extended literature review

1.3.1 BC subtype and the patterns of metastases

BC typically spreads loco-regionally to ipsilateral axillary, internal mammary, or supraclavicular lymph nodes, while involvement of contralateral or cervical lymph nodes is classified as distant metastasis. Common metastatic sites include the skeleton, liver, lung, and brain. Factors like younger age at diagnosis and TNBC (also known as basal-like) increase the risk of distant metastases. Metastatic patterns vary by subtype: hormone receptor-positive (HR+) tumours favour bone, TNBC often involves visceral organs (especially lungs), and HER-2 enriched subtypes commonly metastasize to the lung, liver, and brain (25).

W Xiao et al. have reviewed the effect of molecular subtypes on specific metastatic sites. It is postulated that with better knowledge of the mode of metastases, adjuvant treatment and disease monitoring may be affected. In an analysis of 295 213 patients, it became clear that the probability of having site-specific metastases is reliant on the BC subtype (18).

In a study done by Savci-Heijink, CD. et al., on the metastatic behaviour of BC subtypes, 263 patients known with metastatic BC were identified and the association between immunophenotypic findings and site were assessed. The authors have found that the most typical site for distant metastasis are bone (70.6%), liver (54.5%), and lung (31.4%). In total,

76.8% of patients had visceral metastases. In patients with ER negative/Her-2 negative tumours, visceral and bone metastases were found in 81% and 55.2% respectively. Those with HER-2 positive tumours had visceral metastases in 77.4% and bone metastases in 69.8% of patients (26).

Furthermore, patients with ER positive, HER-2 negative, and a high Ki-67 had visceral metastases in 75.7% and bone in 87.8% of cases. Subjects with ER positive, HER-2 negative, and a low Ki-67 had visceral metastases in 76.9% and bone metastases in 73.1% of cases. This study concluded that there is an association between tumour subtype and patterns of distant metastases (26).

Abha Soni et al. reviewed 531 cases of BC with associated distant metastases. The clinical stage was based on imaging studies and the patients' demographic data with the pathological features, including IHC, of the primary tumour. Tumours were classified as luminal (ER positive and/or PR positive), HER-2 (ER negative, PR negative, HER-2 positive) and TNBC (ER negative, PR negative, HER-2 negative). Luminal BC was further separated into A (ER positive and/or PR positive, HER-2 negative) and B (ER positive and/or PR positive, HER-2 positive). TNBC was not further subclassified (27). In this study luminal A and B had a significant association with skeletal metastasis. Liver metastases were more common in HER-2 subtype and in the luminal subtypes, lung metastases were less frequent. Luminal A had less central nervous system (CNS) metastases as compared to HER-2 and TNBC subtypes, and luminal B were less associated with CNS metastases compared to HER-2 subtype. Lastly pleural metastases were less common in luminal A and B. Overall, the difference in site of metastases between luminal A and B was insignificant (27). The authors noted that there have been 5-30% discordant rates regarding receptor status between the primary and the metastatic tumours. This reflects tumour heterogeneity or dedifferentiation with time. The introduction of chemotherapy was also considered a factor that may have changed the metastatic patterns (27).

1.3.2 BC subtype and SUVmax

In a study done by Önner H et al. 124 patients with invasive ductal BC were investigated by looking at textural features, including SUVmax, extracted form FDG PET/CT and how it associates with IHC characteristics. It was found that ER-negative, PR-negative, HER-2-positive, triple-negative, high-grade, highly proliferative and advanced stage tumours were

more glycolytic and metabolically heterogenous, and it was concluded that adding SUVmax to the textural features, improves the prognostic value of the scan (19).

Similarly, Uğurluer et al. found in a study of 139 BC patients who had undergone a FDG PET/CT, that ER and PR negative, HER2 positive and TNBC had higher SUVmax values (28). A retrospective study by Koo et al. involving 548 patients with BC concluded that TNBC and HER-2 positive tumours had higher SUVmax values as compared to Luminal A tumours (29).

1.3.3 IHC biomarker profile discordance

Immunophenotypic disagreement/receptor conversion between primary BC and its recurrence have been noted by many groups. The frequency of this occurrence ranges from 10.2-32.4% for ER, 20.6-40.7% for PR and 1-43% for HER-2 receptors (30). These changes have also been noted to occur after neoadjuvant chemotherapy (30,31). The current guidelines by the National Comprehensive Cancer Network (NCCN) recommends biopsy of the metastatic site (25,30), as this further provides insight into treatment choice and prognosis (31).

The above studies show that BC subtype plays a role in the pattern of metastases in BC. It is also shown that metabolic activity can predict the BC subtype. This information could potentially be used to aid the development of better BC surveillance strategies and targeted personalized adjuvant treatment (27). However, in a centre where patients potentially present late, get lost to follow-up, default therapy (due to various reasons) and in view of the potential receptor conversion with treatment and time, the subtype as per initial histology may be misguiding.

1.4. Aim

To assess the relationship between IHC and F-18 FDG PET/CT findings in patients with BC referred to the Department of Nuclear Medicine, Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), for staging, restaging and response to therapy.

1.5. Study objectives

Primary objective

- To assess if there is a relationship between BC IHC and F-18 FDG PET/CT findings, and thus to determine if in our environment, if IHC can predict PET/CT findings

Secondary objectives

- To determine the indications for FDG PET/CT referral in patients with BC in our centre
- To assess the patterns of disease i.e., local, nodal, or distant sites of involvement on PET/CT scans and determine if there is an association with IHC
- To assess the correlation between SUVmax and BC subtype

2. Chapter 2: Materials and Methods

2.1. Study setting and period

This retrospective study was conducted at the Department of Nuclear Medicine, CMJAH, involving all patients with histologically proven BC who had a F-18 FDG PET/CT study over the period of 1 January 2016 to 30 September 2022. On average, a total of three F-18 FDG PET/CT scans for BC were done per working month at CMJAH.

2.2 Sample

A total of 136 patients with BC who have had a dedicated F-18 FDG PET/CT study at the Nuclear Medicine department, CMJAH, between January 2016 and September 2022 were included in the study.

2.2.1 Inclusion criteria

i. All patients with biopsy proven BC, who have had a dedicated F-18 FDG PET/CT between 1 January 2016 and 30 September 2022
ii. Male and female patients with BC
iii. All patients aged 18 years and above

2.2.2 Exclusion criteria

i. Patients in whom IHC results were not available
ii. Patients in whom the PET/CT report was not available
iii. Patients in whom invasive BC diagnosis has not been confirmed histologically
iv. Patients with equivocal IHC findings
v. Patients with another primary cancer, along with BC

2.3. Study design, methods and data collection

An audit was done on all patients with biopsy proven BC who were referred to the Nuclear Medicine department at CMJAH for dedicated F-18 FDG PET/CT imaging. Data including gender, age at diagnosis, breast side involved, the indication for the F-18 FDG PET/CT, therapy received, the patterns of disease involvement (i.e. local and chest wall, local and/or distant nodal, metastatic, including the site), as well as the highest SUVmax measured, were noted from the scan reports. The initial biopsy/surgery IHC results (including ER, PR, HER-2 and Ki-67% status) that were not documented on the scan reports were extracted from the National Health Laboratory Service (NHLS) Anatomical pathology database. The data were collected and captured onto an anonymous data sheet and then manually entered onto an Excel spreadsheet for analysis, all stored on a password protected laptop.

2.4 Source data and permission

FDG PET/CT reports were obtained from a secure password protected computer in the Department of Nuclear Medicine and where needed, IHC results were obtained from the NHLS Anatomical Pathology database, upon obtaining permission from the hospital Chief Executive Officer (CEO), Head of Department (HOD) of Nuclear Medicine and HOD of Anatomical Pathology division, and the Postgraduate committee of the University of the Witwatersrand (WITS), as well as ethics clearance from Human Research Ethics Committee (HREC).

2.4.1 Ethics

The study was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand, approval number M230410. This certificate is attached as Appendix A.

2.5 Data analysis and statistics

Subtype of BC was allocated based on the immunohistochemistry with a Ki-67 cut-off of 20% to distinguish between luminal subtypes. Luminal A subtypes have a Ki-67 value <20% and are HER-2 receptor negative, whereas Luminal B tumours have a Ki-67 index of 20% or above and/ or are HER-2 positive with any Ki-67%. To summarize, the five subtypes were allocated as follows:

- Luminal A (ER and/or PR positive, HER-2 negative and Ki-67% < 20%)
- Luminal B HER-2 negative (ER and/or PR positive, HER-2 negative and Ki-67% $\geq$ 20%)

- Luminal B HER-2 positive (ER and/or PR positive, and HER-2 positive, any Ki-67%)
- HER-2 non-luminal (ER or PR negative and HER-2 positive, any Ki-67%)
- Basal-like/TNBC (ER or PR negative and HER-2 negative, any Ki-67%) (8–10)

All data were analysed using STATA statistical software (version 18). Categorical variables, such as gender and subtype, were analysed using Fisher's exact test. For continuous variables, such as age and SUVmax, the median and interquartile range were reported. A p-value of < 0.05 was considered statistically significant. The Kruskal–Wallis test, followed by Benjamini–Hochberg adjustment for multiple comparisons, was used to assess differences in continuous variables across categorical groups.

3. Chapter 3: Results

The findings of this study are condensed in this chapter. Results are demonstrated using tables and figures of the statistical analysis done.

3.1 Patient demographics, FDG PET/CT imaging and tumour characteristics

Descriptive statistics, using frequencies (number of patients) and percentages, were computed to demonstrate the demographics and tumour characteristics of the patients for the different BC subtypes. A total of 136 patients were included in the study.

1. Table 3.1-1 Patient demographics and PET/CT characteristics

Patient and PET/CT characteristics		
Number of patients	**Frequency (n)** 136	**Percentage (%)** 100
Age median	**Median** 52	**IQR** [43-60]
Gender	**Frequency (n)**	**Percentage (%)**
Female	134	99
Male	2	1
Breast involved	**Frequency (n)**	**Percentage (%)**
Bilateral (at diagnosis)	13	10
Left	58	43
Right	65	47
Time elapsed between histological diagnosis and PET/CT (yrs)	**Median** 1.2	**IQR** [0.61-2.8]
PET/CT indication	**Frequency (n)**	**Percentage (%)**
Staging	24	18
Restaging	97	71
Response to therapy	15	11
Disease present	**Frequency (n)**	**Percentage (%)**
Yes	102	75
No	34	25

2. Table 3.1-2 Patient and tumour characteristics

Patient and tumour characteristics							
	Luminal A	Luminal B, HER2 negative	Luminal B, HER2 positive	HER2, non-luminal	TNBC	Total N(%)	p-value
Number of patients, n (%)	32(23.53)	19(13.97)	36(26.47)	1(0.74)	31(22.79)	136(100%)	0.3028*
Age median (IQR)	57(47.5-66)	49(39-59)	50(41-61)	52(44-58)	52(43-58)	52(43-60)	
Gender							
Female	30(94)	19(100)	37(100)	17(100)	31(100)	134(99)	0.173^
Male	2(6)	0	0	0	0	2(1)	
Breast involved							
Bilateral (at diagnosis)	2(6)	0	5(14)	1(6)	5(16)	13(10)	0.190^
Left	16(50)	9(47)	14(38)	11(65)	8(26)	58(43)	
Right	14(44)	10(53)	18(48)	5(29)	18(58)	65(47)	
Time elapsed between histological diagnosis and PET/CT	2.7(0.9-3.9)	1.6(1-4.2)	1.1(0.5-1.8)	0.96(0.5-1.6)	1(0.6-3.1)	1.2(0.61-2.8)	0.0576*
PET/CT indication							
Staging	4(13)	3(16)	5(14)	3(18)	9(29)	24(18)	0.447^
Restaging	22(69)	14(74)	29(78)	11(64)	21(68)	97(71)	
Response to therapy	6(18)	2(10)	3(8)	3(18)	1(3)	15(11)	
Disease present							
No	6(19)	7(37)	12(32)	3(18)	6(19)	34(25)	0.407^
Yes	26(81)	12(63)	25(68)	14(82)	25(81)	102(75)	

*=k-wallis ^=Fisher's exact test

The above two tables (Table 3.1-1 and -2) outline the patient demographics and tumor characteristics for different BC subtypes. The subtypes include Luminal A, Luminal B (HER2 negative and positive), HER2 non-luminal, and TNBC (Triple Negative Breast Cancer).

The median age across all subtypes is 52 years, with an interquartile range (IQR) of 43-60. There is no statistically significant difference in age between the subtypes (p=0.3028). Almost all patients were female (99%), apart from two, with no statistically significant difference in gender distribution across the subtypes (p=0.173).
Majority of the patients have unilateral breast involvement, with the right breast (47%) being slightly more common than the left (43%). 10% of patients had bilateral BC at diagnosis. There was no statistically significant difference in breast involvement across subtypes (p=0.190).

The median time between diagnosis and the FDG PET/CT scan was 1.2 years (IQR, [0.59-2.8]), (p=0.0576) indicating a trend towards differing times elapsed between histological diangosis and PET/CT across tumour types, with the most common indication for FDG PET/CT being restaging (71%), followed by treatment response assessment (18%) and then staging (11%). There is no statistically significant difference in FDG PET/CT indication across subtypes (p=0.447).

Disease was identified on FDG PET/CT in most patients (75%), with no statistically significant difference in disease presence across subtypes (p=0.407).

3.2 Therapy received

Treatment modalities available in our setting includes surgery, chemotherapy, hormonal therapy and occasionally immunotherapy.

3. Table 3.2-1 Treatment received per BC subtype

Treatment received per subtype							
	Total N(%)	**Luminal A**	**Luminal B, HER2 neg**	**Luminal B, HER2 pos**	**HER2 non-luminal**	**TNBC**	**p-value**
Surgery							
No	32(24)	7(22)	4(21)	7(19)	6(35)	8(26)	0.746
Yes	104(76)	25(78)	15(79)	30(81)	11(65)	23(74)	
Hormonal therapy							
No	97(71)	23(72)	11(58)	20(54)	14(82)	29(94)	0.001
Yes	30(22)	8(25)	6(32)	14(38)	0	2(6)	
Unknown	9(7)	1(3)	2(10)	3(8)	3(18)	0	
Radiation							
No	78(57)	15(47)	10(53)	25(68)	11(65)	17(55)	0.145
Yes	45(33)	14(44)	6(32)	11(30)	2(12)	12(39)	
Unknown	13(10)	3(9)	3(15)	1(2)	4(24)	2(6)	
Chemotherapy							
No	21(15)	9(28)	2(11)	6(16)	3(18)	1(3)	0.023
Yes	112(82)	23(72)	15(78)	31(84)	13(76)	30(97)	
Unknown	3(3)	0	2(11)	0	1(6)	0	

The above table (Table 3.2-1) summarizes treatment modalities received by patients with different breast cancer subtypes. The majority of patients across all subtypes have undergone surgery (76%). There is no statistically significant difference in surgery rates across subtypes (p=0.746). There is a statistically significant difference in the use of hormonal therapy across subtypes (p=0.001). This is expected as hormonal therapy is mainly used in Luminal A and B. There is no statistically significant difference in the use of radiation therapy across subtypes (p=0.145). A statistically significant difference in the use of chemotherapy across subtypes is noted (p=0.023), favouring Luminal B, HER2 positive and TNBC. Of note, there were no patients who received immunotherapy in this study, as there is no access to immunotherapy in the public sector from which this population is drawn.

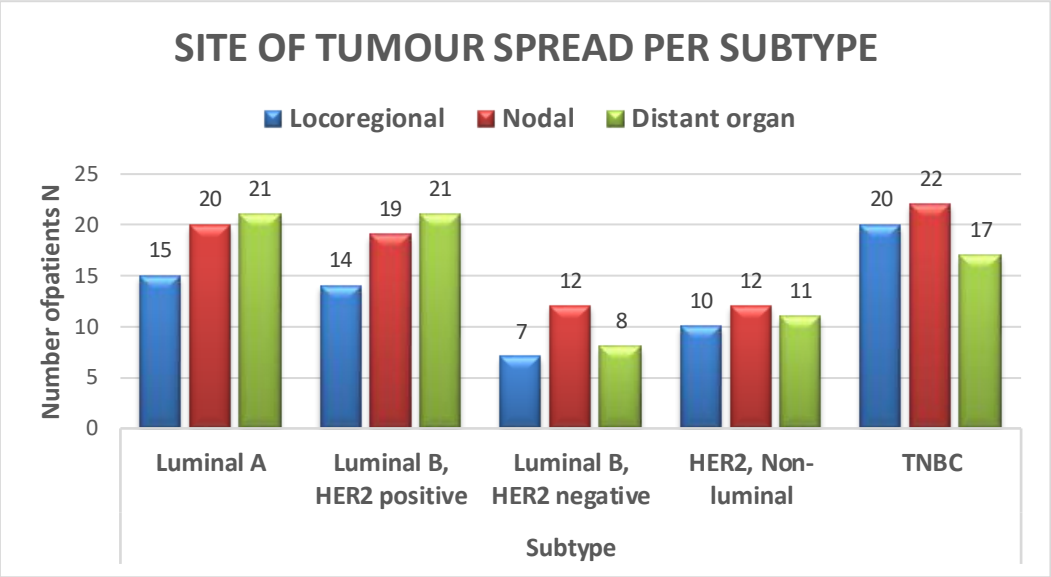
3.3 Pattern of disease spread/site of tumour metastases per subtype

4. Table 3.3-1 Site of tumour metastases per subtype

	Subtype					
	Luminal A	Luminal B, HER2 positive	Luminal B, HER2 negative	HER2, Non-luminal	TNBC	
Total number of patients per subtype N (%):	32(23.53)	37(27.21)	19(13.97)	17 (12.50)	31(22.79)	
Site of tumour involvement N (%)						**p-value***
Locoregional	15(46.88)	14(37.84)	7(36.84)	10(58.82)	20(64.52)	0.158
Nodal	20(62.50)	19(51.35)	12(63.16)	12(70.59)	22(70.97)	0.515
Distant organ	21(56.76)	21(56.62)	8(42.11)	11(64.71)	17(54.84)	0.544
Bone	14(43.75)	14(37.84)	7(36.84)	7(41.18)	9(29.03)	0.808
Lung	9(28.12)	4(10.81)	3(15.79)	5(29.41)	12(38.71)	0.071
Liver	6(18.75)	7(18.92)	0(0)	3(17.65)	3(9.68)	0.224
Brain	1(3.12)	3(8.11)	0(0)	2(11.76)	2(6.45)	0.583
Other	5(15.62)	6(16.22)	4(21.05)	4(23.53)	3(9.68)	0.714
*Fisher's exact						

Table 3.3-1 summarizes the pattern of disease spread across the subtypes and Figure 3.3-2 below presents this graphically for each subtype, i.e locoregional (involving the primary site in the breast and the chest wall), nodal (involving local i.e axillary and distal nodal i.e beyond axillary lymph nodes) and distant organ (involving brain, bone, lung, liver and other sites). Although not statistically significant, distant organ metastses are the most common in Luminal A and Luminal B HER2 positive subtypes, followed by TNBC. Nodal metastases are the most common in Luminal B HER2 negative, HER2 non-luminal and TNBC subtypes. Bone metastases were prevelent with Luminal A at 44%, Luminal B, HER2 positive at 38%, Luminal B, HER2 negative at 37%, HER2, non-luminal at 41% and TNBC at 29%. There is a trend (P=0.071) for differences in lung metastases across tumour types. Lung metastases were most prevenent in TNBC at 38%.

1. Figure 3.3-1 Regional site of tumour spread per BC subtype



2. Figure 3.3-2 Site of organ metastases per BC subtype

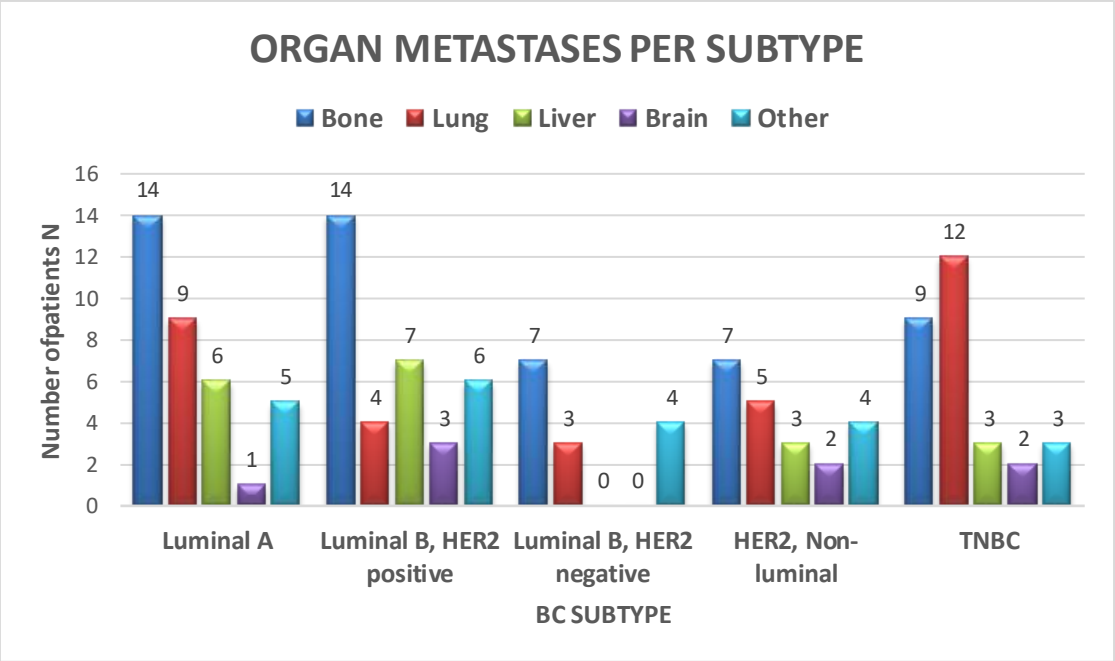


Figure 3.3-2 graphically presents the site of organ metastses for each subtype. Bone is the most common metastatic site across all subtypes, except for TNBC which has a prediliction for lung metastases.

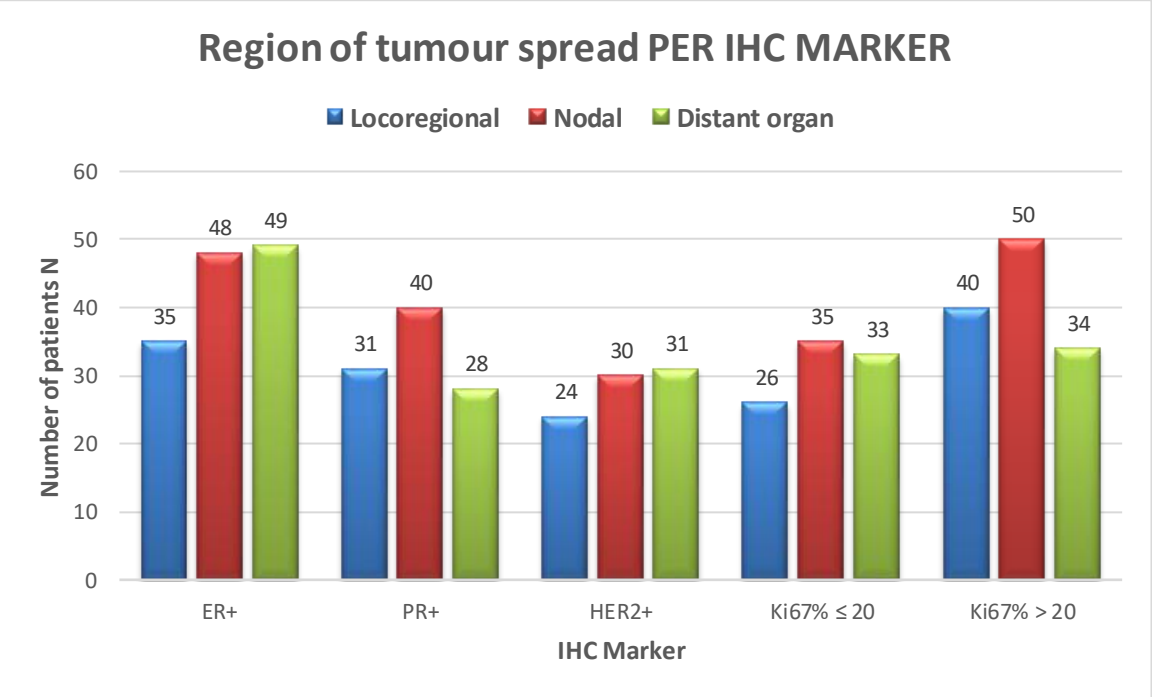
3.4 Metastatic site per IHC marker

5. Table 3.4-1 Site of tumour spread per IHC marker

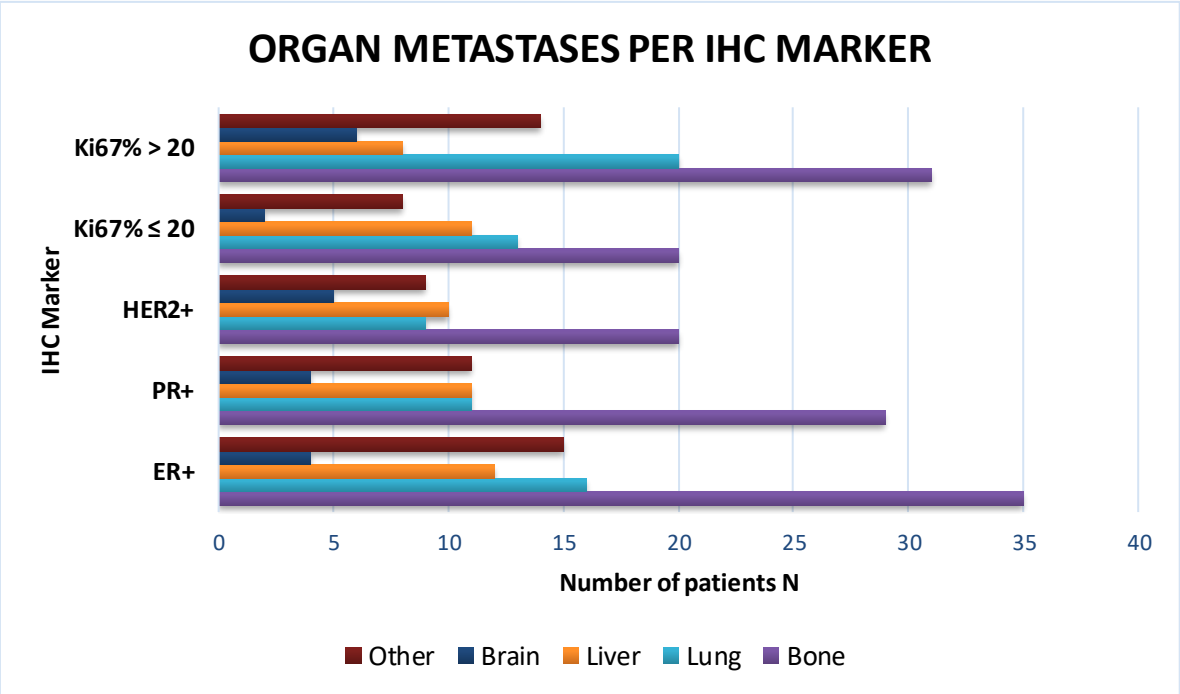
	Immunohistochemical marker				
Site of tumour involvement N (%)	**ER+**	**PR+**	**HER2+**	**Ki67% ≤ 20**	**Ki67% > 20**
Locoregional	35(41.67)	31(46.97)	24(45.28)	26(45.61)	40(45.61)
Nodal	48(57.14)	40(60.61)	30(56.60)	35(61.40)	50(63.29)
Distant organ	49(58.33)	28(42.42)	31(58.49)	33(57.89)	34(43.04)
Bone	35(41.67)	29(43.94)	20(37.74)	20(35.09)	31(39.24)
Lung	16(19.05)	11(16.67)	9(16.98)	13(22.81)	20(25.32)
Liver	12(14.29)	11(16.67)	10(18.87)	11(19.30)	8(10.13)
Brain	4(4.76)	4(6.06)	5(9.43)	2(3.51)	6(7.59)
Other	15(17.86)	11(16.67)	9(16.98)	8(14.04)	14(17.72)

Table 3.4-1 outlines the prevalence of metastases to the different sites per IHC marker, i.e. ER and PR positive, HER2 positive, Ki67% ≤ 20 and Ki67% >20. No statistically significant findings were made; however, hormone receptor positive tumours show a predilection for bone metastases with ER positive at 42%, PR positive at 44% and HER2 positive at 38%. Brain, lung and "other" metastases are more prevalent at a Ki67% 0f >20%. This is graphically represented in Figure 3.4-1 and Figure 3.4-2.

3. Figure 3.4-1 Regional site of tumour spread per IHC marker

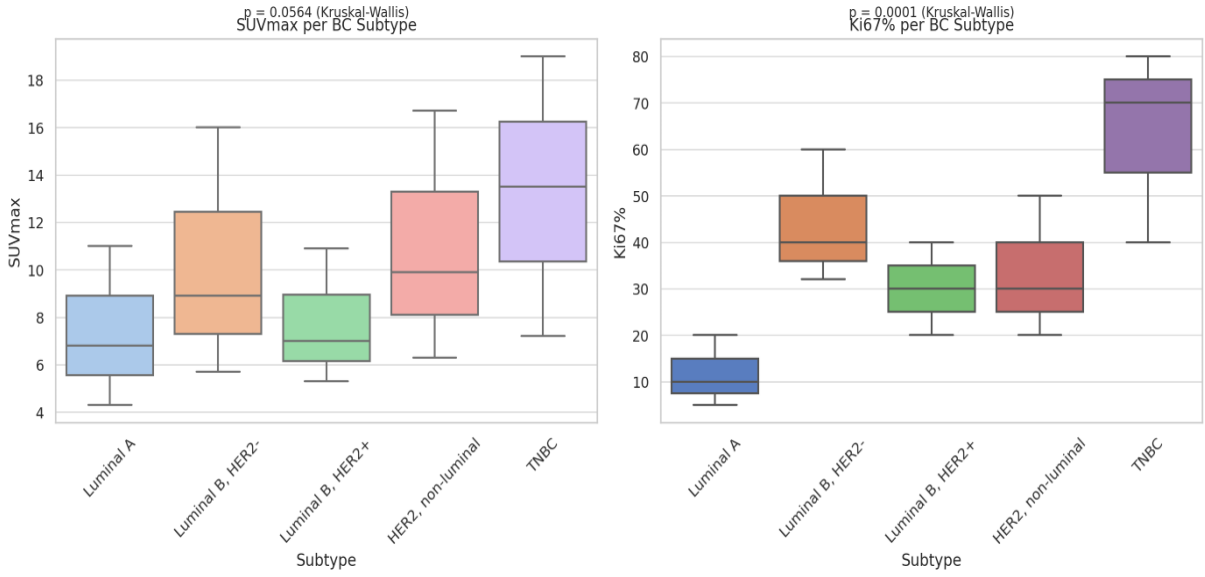


4. Figure 3.4-2 Site of organ metastases per IHC marker



3.5 SUVmax and Ki67% per BC subtype

6. Figure 3.4-3 SUVmax and Ki67% per BC subtype – Box and Whiskers plots



The box and whiskers plots illustrate the variation in SUVmax and Ki67% across different breast cancer (BC) subtypes. For SUVmax, Triple-Negative Breast Cancer (TNBC) exhibited the highest median value (13.5), indicating greater metabolic activity, while Luminal A showed the lowest (6.8). Although there was a visible trend of increasing SUVmax in more aggressive subtypes, the Kruskal-Wallis test yielded a p-value of 0.0564, suggesting this

difference approached but did not reach statistical significance. In contrast, Ki67%, a marker of cellular proliferation, showed a clear and statistically significant difference among subtypes (p = 0.0001). TNBC had the highest proliferation rate, as expected (median 70%), whereas Luminal A had the lowest proliferation rate (median 10%). These findings align with known biological behavior, where TNBC and HER2-enriched subtypes are more aggressive and proliferative than Luminal types.

4. Discussion

4.1. Results in context

The retrospective observational study explored the relationship between the BC subtypes and the findings on FDG PET/CT in patients with invasive BC. The primary aim was to determine whether the IHC based BC subtype can predict FDG PET/CT findings. The secondary objectives included auditing FDG PET/CT referral indications, patterns of disease spread, and exploring the correlation between SUVmax and BC subtypes. Important findings emerged, which merit further discussion in the context of current literature and clinical practice.

4.1.1 Patient demographics, PET/CT and tumour characteristics

Many studies highlight the bimodal age distribution of BC at diagnosis (32–34), interpreting it as two main etiologic groupings i.e. early onset with peak ages near 45 years, and late onset with peak ages near 65 years. Allot E et al. highlighted that higher ER-protein subtypes (e.g. Luminal A) showed a greater proportion of late-onset disease, vs basal-like subtype (TNBC) emerging earlier (32).

In our study, no statistically significant difference in age was appreciated between the subtypes, however, Luminal A subtype had a slightly later overall median age of diagnosis (median 57 years) versus TNBC (median 52 years). It is important to understand that in our setting, patients present late in the disease process. This could be because of various challenges including limited resources and availability of screening programmes and likely lack of understanding and awareness of implications.

Left sided BC is marginally more common than right sided BC with a ratio of 1.05 to 1.26 (35). However, without statistical significance, in our study, the right breast was slightly more

affected. Bilateral BC represents 1.4-12% of all breast cancers (36). 10% of our study population presented with bilateral BC.

The patient population of this study underwent FDG PET/CT at different time points in their management journey and for different indications, with disease identified in 75% of cases. The most common indication for FDG PET/CT being restaging (71%), followed by treatment response assessment (18%) and then staging (11%). Ideally, this study should have been conducted at the staging phase, prior to the initiation of therapy. Notably, only 11% of patients had staging scans in this study, making those FDG PET/CT findings more robust and less confounded by subsequent therapeutic interventions. The relatively low proportion of FDG PET/CT scans performed at staging in our setting suggests a missed opportunity to leverage PET/CT's sensitivity in detecting occult metastases. Incorporating FDG PET/CT earlier in the diagnostic workflow could enhance clinical decision-making by identifying metastatic disease and guiding therapy selection. While traditional imaging modalities such as mammography, ultrasound, and MRI remain the mainstay for initial staging, PET/CT plays a crucial role in evaluating distant metastases, particularly in advanced-stage disease (17).

The BC treatment guidelines from South Africa's (SA) National Department of Health closely align with those of American society of clinical oncology (ASCO) and the European Society for Medical Oncology (ESMO), recommending treatments consistent with ASCO standards. Although treatment modalities (hormonal therapy and chemotherapy) are available within SA's public healthcare system, their accessibility is often limited by high patient demand, provider shortages, and resource constraints.(37)
In this study, the therapy received prior to the FDG PET/CT was noted. Hormone receptor-positive tumours are typically treated with hormonal therapy, among other modalities, while tumours lacking hormone receptors, such as TNBC, do not benefit from hormonal therapy. This distinction explains the observed differences in treatment patterns in our study. Additionally, patients undergoing FDG PET/CT scans are often those with more advanced disease (stage IIB and above) (38) and are therefore more likely to have received systemic therapy as part of their treatment plan.

4.1.2 Patterns of disease spread and association with IHC

Invasive BC often metastasize to lymph nodes and systemic organs (39). This is represented in our study where majority of patients had nodal and distant organ metastases. This is also not unexpected, as in our clinical setting patients present late in the disease process, are

referred late for PET/CT or are scanned late due to limited resources and high patient volumes. Globally, around 5–10% of breast cancer (BC) patients are diagnosed at the metastatic stage, though this varies by region. In high-income countries, fewer than 8% present with metastatic disease at diagnosis, while in many low- and middle-income countries, this figure rises to 20–30% (40).

Analysis of metastatic patterns, although not statistically significant, revealed some notable trends across the subtypes.

Hormone receptor positive BC commonly metastasizes to bone initially (27) and this is shown in our study where bone metastases were the most common metastatic site for hormone receptor positive tumours. Hormone receptor negative tumours (TNBC or basal like BC) demonstrated a predilection for lung metastases (39%) in our study. These findings align with the known biology of BC subtypes and particularly the aggressive nature of TNBC, with a higher proliferation index, correlating with visceral metastases (19,27). Luminal B, HER2 positive is known to frequently metastasize to the liver (27) and was shown in 19% of patients in our study. HER2 positive, non-luminal commonly has multiple sites of metastases in the viscera (26,27) and in our study showed metastases to lung (29%), liver (18%), brain (12%), as well as soft tissue, pleura, adrenal gland and spleen.

It is noteworthy that FDG PET/CT is not the modality of choice for identifying brain metastases. This is due to the high FDG uptake in the brain (normal biodistribution) potentially masking/attenuating lesions. Contrast-enhanced MRI, including perfusion MRI, is the preferred imaging modality for detecting brain metastases in BC patients (41). In most South African public hospitals, CT brain scans should be considered a practical alternative to MRI due to their greater availability. This is an important consideration when interpreting the study's findings and underscores the need for multimodal imaging in comprehensive metastatic evaluation.

The Ki67%, a marker of cellular proliferation, serves as a well-known prognostic indicator (42). In this study this marker showed a statistically significant difference across the subtypes. Luminal A showed a mean Ki67% of 10%, versus Luminal B with a mean Ki67% of 30-40%. TNBC had a mean Ki67% of 70%. This is in accordance with the literature, with the higher the Ki67%, indicating tumour aggressiveness (42,43).

Despite the trends observed in this study, the lack of statistically significant differences in the site of metastases across subtypes and IHC markers reflects the potential influence of several factors. Pleural and soft tissue metastases were found in multiple subtypes, and visceral metastases were also found in the hormone receptor positive tumours. This underscores the heterogeneity of metastatic patterns in BC and highlights the study limitations discussed below. It could perhaps also be attributed to IHC discordance between the primary and metastatic tumours.

4.1.3 SUVmax and BC Subtypes

A key finding of this study is the correlation between SUVmax and BC subtypes. A noteworthy trend in SUVmax was found across BC subtypes ($p = 0.0564$), with TNBC demonstrating the highest SUVmax values, followed by HER2-positive tumours. These results align with previous studies, such as those by Uğurluer et al. and Koo et al., which also reported higher SUVmax values in TNBC and HER2-positive tumours. However, while SUVmax differs across subtypes at the group level, this does not necessarily imply that it provides additional clinically meaningful information in individual patients. Determining the added value of SUVmax—beyond established histological and staging information—would require further studies specifically designed to assess whether SUVmax contributes to prognosis, influences therapeutic decisions, or improves patient stratification. The Ki67 index, discussed earlier, further supports this observation, with TNBC showing the highest median Ki67% values (70%, range 40-80). These findings suggest that SUVmax could serve as a non-invasive surrogate marker for tumour aggressiveness and provide additional prognostic information, reinforcing the value of integrating SUVmax into clinical decision-making.

4.1.4 Correlation Between IHC and FDG PET/CT Findings

Associations between the uptake on FDG PET/CT and the clinicopathological features of the tumour, as well as the significance of SUVmax in predicting the molecular subtype and its prognosis, are well known (1,2).

Although not statistically significant, our results showed notable trends in disease patterns as discussed above. In addition, an important finding was the statistically significant correlation between the SUVmax and the BC subtype, highlighting its role in assessing tumour aggressiveness.

While IHC classifies BC based on molecular characteristics, FDG PET/CT helps correlate this information by assessing tumour burden and aggressiveness, detecting occult metastases, and monitoring treatment response. Together, these modalities bridge molecular and systemic perspectives, enabling a comprehensive approach to BC management.

4.2. Study limitations

Several limitations of this study warrant consideration.
The retrospective nature of the study introduces potential selection bias, and the single institution setting limits the generalizability of the findings. Not all eligible patients diagnosed with BC in our setting came for FDG PET/CT, as other modalities are often more accessible. This resulted in a small sample size, and possible selection bias. Additionally, the variability in the timing of FDG PET/CT scans relative to the histological diagnosis and the heterogeneity of BC itself, including receptor conversion over time or following treatment, further complicates the correlation between IHC and FDG PET/CT findings. Ideally, FDG PET/CT should have been performed at initial staging to provide more robust data on the natural course of disease.

Challenges in using FDG PET/CT should also be considered, including false positives due to inflammatory or infectious processes, errors in patient preparation and false negatives due to limited sensitivity for detecting small or low-grade tumours, as untreated disease provides a clearer picture of natural tumour behaviour.

4.3. Clinical implications and future directions

In clinical practice, this study reinforces the importance of integrating IHC subtyping with imaging findings to guide individualized management strategies. Additionally, subtype-specific patterns of disease spread can inform surveillance protocols and imaging choices. The significant correlation between SUVmax and aggressive subtypes such as TNBC suggests that FDG PET/CT may serve as a valuable adjunct in assessing tumour biology and prognosis.

Future research should focus on prospective studies with PET/CT performed for staging to validate the observed trends. The incorporation of novel PET tracers, such as FES for ER-positive tumours, FAPI or PSMA for specific BC subtypes should be studied as it may overcome some limitations and improve imaging specificity.

5. Conclusion

The study highlights a significant correlation between IHC markers and FDG PET/CT imaging, particularly regarding the Ki67% and SUVmax, across different BC subtypes. These findings underscore the utility of FDG PET/CT not only in evaluating the extent of disease involvement—spanning local, nodal, and distant metastases—but also in complementing IHC analysis for a more comprehensive diagnostic approach, offering valuable insights that can guide clinical decision-making for personalized patient care.

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Appendix A: Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Dr A Venter

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M230410

NAME: (Principal ~~Investigator~~)	Dr A Venter
DEPARTMENT:	School of Clinical Medicine Department of Radiation Sciences Division of Nuclear Medicine Medical School University
PROJECT TITLE:	*A retrospective study comparing immunochemistry subtypying and finding on F-18 FDG PET/CT in patients with invasive breast cancer*
DATE CONSIDERED:	2023/05/05
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Dr K Purbhoo and Professor M-D-T Vangu
APPROVED BY:	Dr M Vorster, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	2023/07/31 **EXPIRY DATE:** 2028/07/30

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

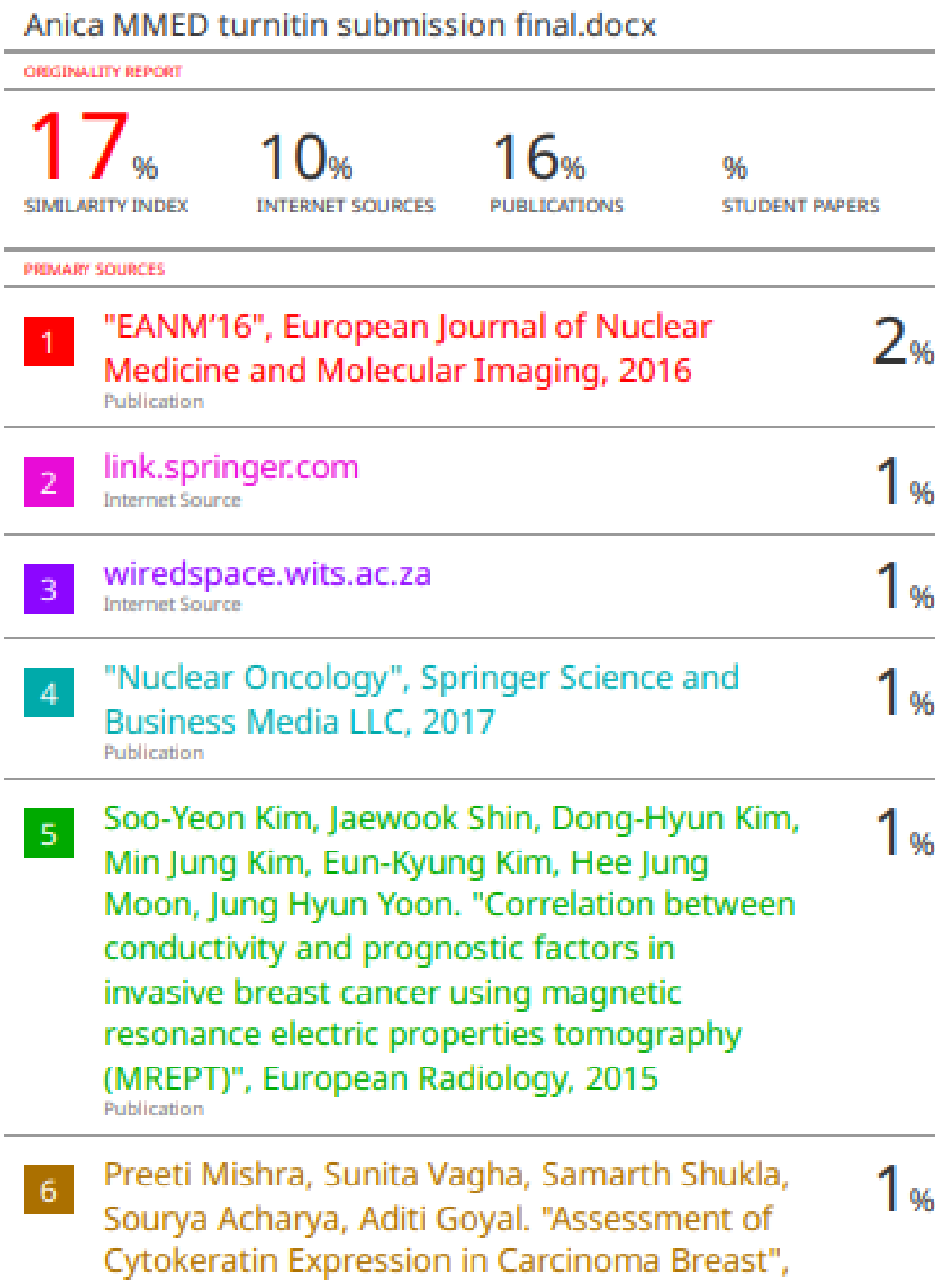
To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

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 submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **April** and therefore reports and re-certification will be due in the month of **April** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

11/08/2023
Date

Appendix B: Turnitin Report and Approval



Anica MMED turnitin submission final.docx

ORIGINALITY REPORT

17%	10%	16%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"EANM'16", European Journal of Nuclear Medicine and Molecular Imaging, 2016 Publication	2%
2	link.springer.com Internet Source	1%
3	wiredspace.wits.ac.za Internet Source	1%
4	"Nuclear Oncology", Springer Science and Business Media LLC, 2017 Publication	1%
5	Soo-Yeon Kim, Jaewook Shin, Dong-Hyun Kim, Min Jung Kim, Eun-Kyung Kim, Hee Jung Moon, Jung Hyun Yoon. "Correlation between conductivity and prognostic factors in invasive breast cancer using magnetic resonance electric properties tomography (MREPT)", European Radiology, 2015 Publication	1%
6	Preeti Mishra, Sunita Vagha, Samarth Shukla, Sourya Acharya, Aditi Goyal. "Assessment of Cytokeratin Expression in Carcinoma Breast",	1%

Appendix C: Note on referencing style

Please note that the referencing in this thesis is a modification of the Vancouver Referencing style, done according to the Faculty of Health Sciences Style Guide as set out by the Wits Health Sciences Library.

The information on this WHSL Vancouver Citation Style Guide for Theses, Dissertations and Research Reports is available from http://libguides.wits.ac.za/whsl-vancouver updated on 8 August 2017.

Appendix D: CEO approval

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG
ACADEMIC HOSPITAL

Enquiries: *Mr E Mosiamo*
Email: *edward.mosiamo@gauteng.gov.za*
Tel: *011 488 4206*
Ref: 1/7/2

To: ANICA VENTER

NHRD Ref: GP_202302_071

FINAL APPROVAL OF STUDY

STUDY TITLE:
A RETROSPECTIVE STUDY COMPARINIMMUNOHISTOCHEMISTRYSUBTYPING AND FINDINGS ON F-18 FDG PET/CT IN PATIENTS WITH INVASIVE BREAST CANCER.

Permission to conduct the above-mentioned study at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) is granted subject to the following:

- Charlotte Maxeke Johannesburg Academic Hospital will not in any way incur or inherit costs as a result of the said study.
- Your study shall not disrupt services at the study sites.
- Strict confidentiality shall always be observed.
- Informed consent shall be solicited from participants in your study.

Before commencing data collection, please liaise with the relevant Head/s of Department and / or Unit to confirm arrangements, and agree on dates and times that suit all parties.

Once the research is completed, kindly forward the results to the CMJAH research committee Secretariat on cmjahresearch@gauteng.gov.za

Approved

Prof. Mary Kawonga

Chairperson: CMJAH Research Committee

Signed on behalf of the CEO, CMJAH

Date: Jan 6, 2025

Appendix E: Data collection sheet

Gender: Male ☐ Female ☐ **Age at diagnosis:** _____

Breast side involved: Right ☐ Left ☐ Bilateral ☐

INDICATION FOR F-18 FDG PET/CT: (Tick)

Staging ☐ Restaging ☐ Treatment response ☐ Surveillance ☐

THERAPY RECEIVED BEFORE PET/CT SCAN: (Tick)

Surgery ☐ Hormonal therapy ☐ Immunotherapy ☐

Chemotherapy ☐ Radiation therapy ☐

IMMUNOHISTOCHEMISTRY: (value/score/%)

- ER _____
- PR _____
- HER-2 _____
- Ki-67% _____

Breast cancer Subtype: _____

FDG PET/CT FINDINGS:

Disease present: Yes ☐ No ☐

Site of disease: (Tick if present)

- Local (region of known primary): ☐
- Chest wall involved: ☐
- Loco-regional lymph nodes (axillary/internal mammary): ☐
- Distant nodal disease: ☐

Distant organ metastasis: (Tick)

Bone	Bone marrow
Lung	Soft tissue
Liver	Skin
Brain	Other (specify)
Pleura	