PATIENT AND TUMOUR FACTORS AFFECTING THE HISTOLOGY OF SENTINEL LYMPH NODE BIOPSY IN BREAST CANCER PATIENTS AT UNIVERSITAS ACADEMIC HOSPITAL, BLOEMFONTEIN

BY

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SUBMITTED IN FULFILMENT OF THE REQUIREMENTS IN RESPECT OF THE MASTER'S DEGREE MMED IN THE DEPARTMENT OF SURGERY IN THE FACULTY OF HEALTH SCIENCE AT THE UNIVERSITY OF THE FREE STATE

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31 JULY 2020

DECLARATION OF AUTHORSHIP

I declare that the coursework Master's Degree mini-dissertation and interrelated publishable article that I herewith submit for the degree in MMed (Surgery) at the University of the Free State are my own independent work and that I have not previously submitted it for a qualification at another institution of higher education. Where help was sought, it has been acknowledged.

I hereby declare that I am aware that copyright of this mini-dissertation is vested in the University of the Free State.

I hereby declare that all royalties in relation to intellectual property that was developed during the course of and/or in connection with the study at the University of the Free State will accrue to the University.

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DR RS LETSOARA **DATE**

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- My supervisor, Dr N Pearce, Department of Surgery, University of the Free State for guidance with regards to the research fundamentals as they relate to the field of General Surgery, encouragement to uphold high standards of professional conduct, critical and constructive commentary on the study design and interpretation of results.

- Prof. G. Joubert, Head: Department of Biostatistics, University of the Free State for providing analyzed input on the design of the study.

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Abstract

Introduction: SLNB has become accepted standard staging tool in clinically node negative breast cancer patients.

Aim(s): Primary aim was to determine factors (demographics and tumor factors) affecting histology of SLNB. Secondary aim was to determine the histological characteristics of the SLNB and final histological status of the axilla if SLNB was positive.

Methods: Retrospective analytical study of patients who underwent mastectomy or lumpectomy with SLNB from 2007 to 2016 (n=60) at Universitas Academic Hospital.

Results:

***Demographics**:* Age ranged from 36 to 90 years and its association with positive SLNB histology was close to statistically significant (p-value 0.0242).
Tumour factors: Location of the tumour, type of tumour, size of tumour all with p-value of 1.000, histological type (p-value 0.7464), grade (p-value 0.6244), lymphovascular infiltration (p-value 1.000) and hormonal receptors status (ER-positive p-value 0.6434, PR-positive p-value 0.7290, HER-positive p-value 0.2341 and ER/PR/HER-positive p-value 1.000) did not have statistically significant association with positive SLNB histology. There was a statistically significant association between positive SLNB histology and final histology of the axilla (p-value <0.001).

Conclusion: The study did not prove our hypothesis that, tumour size, site, grade, lymphovascular infiltration and hormone receptors will affect histology of SLNB. Age nearly affected histology of SLNB. Small sample size and incomplete lymphovascular infiltration reporting might have affected results. There was statistically significant association between histology of SLNB and histology of the axilla.

Keywords

Patient and tumor factors, SLNB and histology of axilla

List of Abbreviations

T: Tumor

ER: Oestrogen receptors

PR: Progesterone receptors

HER-2: Human epidermal growth factor receptor 2

SLN: Sentinel lymph node

SLNB: Sentinel lymph node biopsy

DCIS: Ductal carcinoma in situ

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CHAPTER 1

PATIENT AND TUMOUR FACTORS AFFECTING THE HISTOLOGY OF SLNB IN BREAST CANCER PATIENTS AT UNIVERSITAS HOSPITAL, BLOEMFONTEIN

INTRODUCTION

Sentinel lymph node (SLN) is the first node(s) to receive lymphatic drainage from the tumour, thus theoretically will be the first site of lymphatic metastases. The first introduction of sentinel lymph node biopsy (SLNB) was by Cabanas in penile cancer and followed by Morton in melanoma[1]. SLNB pioneered by Giuliano in breast surgery gained popularity through the 1990s[2] and has been accepted as standard of care in clinically node negative breast cancers (T1-T3) for deciding whether axillary dissection should be performed or not.

Clinical trials comparing SLNB and axillary lymph node dissection show no significant difference in SLN negative patients in terms of survival, recurrence of a tumour and that SLNB can accurately predict the status of the axilla. As much as 75% of patients undergoing SLNB are node negative, thus many patients can be spared the unwanted side effects of axillary dissection like nerve injury, shoulder immobility, seroma formation and lymphoedema[3, 4].

Studies have been done to look for patients and tumour characteristics that can be used to predict histology of SLNB and thus the axillary involvement with varying degrees of proven relationship. There is a consensus that tumour size, grade and lymphovascular infiltration are directly related with positive SLNB[5,9,12].

BACKGROUND

Patient factors

Age

Chen *et al.* found that age is not an independent risk factor for positive SLNB histology[5] with Jaka *et al.* finding similar results[6]. Some studies quote age as an independent predictor of SLNB positivity as was demonstrated by Gangi *et al.* where patients younger than 50 years were at risk of lymph node metastases and therefore positive SLNB histology[7].

Race

No data can be found in South Africa with regards to studies that evaluate association of race and SLNB in breast cancer. Comparing to the rest of the world this is also true. However Gann *et al.* reported 35% to 40% increased risk of lymph node metastasis in African-American women and Hispanic women and suggest that women in these groups develops more biologically aggressive breast carcinomas for reasons not yet known[9].

Tumour factors

Size (T-status)

Tumour size is an independent predictor of positive SLNB. As the size of tumour increases so is the ability for it to spread. In big studies the frequency of positive SLNB is 10% in T1mic , 9-13% in T1a, 13-19% in T1b, 26-29% in T1c, 39-59% in T2, and 80% in T3 tumours [7, 8, 10, 11,15].

Site

Upper inner quadrant tumours have lower frequency of axillary metastases and researchers query alternative route of spread to internal mammary lymph nodes[11]. Yoshihura *et al.* found that tumour location was the second most powerful predictor of axillary lymph nodes involvement and therefore positive SLNB histology as lateral

and retro-areolar tumours were associated with higher frequency of axillary lymph node metastases (p-value of 0.0019)[8,15].

Multifocal cancer

Veronesi *et al.* suggested that SLNB is not suitable for multifocal breast cancers as each lesion might have its own lymphatic drainage, which can't be connected. Therefore a negative SLNB for one lesion cannot assure negative results for others[12]. However Chinese Anti-Cancer Association Committee of breast cancer (CACA-CBCS) still considers multifocal cancer breast cancer as an indication for SLNB[11]. A meta-analysis on 996 cases of multifocal/multi-center breast cancer revealed success rate of 92.0-100% and a false negative rate of 0-25% in SLNB which is close to unifocal breast cancer[13].

Histological type

Infiltrating ductal and lobular carcinoma have higher frequency of positive SLNBs when compared to other histological subtypes (tubular, mucinous and medullary). However these tumours are rare and most studies are too small to draw any concrete conclusions. Studies quote the incidence of 17% for tubular cancers, 6% for mucinous cancers, and 21% for pure medullary cancers of positive SLNB[7, 14].

Grade

Chen *et al.* and Jaka, *et al.* did not find statistical correlation between grade and SLNB status[5,7]. Bevilacqua *et al.* quotes that histological grade can play a role in predicting the status of axilla, with the incidence of positive SLNB histology for grade I being 10% and for grade III 39%[15].

Lymphovascular infiltration

As with tumour size, most studies have shown lymphovascular infiltration as an independent predictor of SLNB metastasis and has been proven in many studies. Cancers showing lymphovascular infiltration have 57% chance of being node positive compared to 23% for cancers without lymphovascular infiltration[8, 15].

Oestrogen receptors / Progesterone receptors (ER/PR)

Positive expressions of ER and PR receptors by tumours have previously shown to be significantly correlated with histological grade, mitotic score and nuclear pleomorphism. Therefore it can be assumed that ER and PR positive tumours will metastasize to the axilla early and affect histology of SLNB[16]. Winstanley *et al.* found no significant association between progesterone or oestrogen receptors and SLNB status[17]. Pourzand *et al.* demonstrated that there was statistically insignificant association between positive oestrogen receptor tumours and histology of SLNB[18]. Similar results were found for progesterone receptors[18]. Bevilacqua *et al.* found ER/PR positive tumours had higher frequency of SLNB metastases when compared to ER/PR negative tumours, with small but significant increase of 2% for ER and 4.6% for PR receptors[15].

HER-2 (Human Epidermal Growth Factor-2)

There is no consensus with regards to the association of HER and axillary lymph node status. Jaka *et al.* and Chen *et al.* showed no significant correlation between HER receptor status and positive SLNB[5,6]. Crabb *et al.* specified HER-2 as an independent predictor of nodal involvement in a retrospective analysis of 3441 early breast cancer patients. Gulben *et al.* showed similar results[19 ,20]. To try to clarify the conflict some authors speculated that HER2-positive tumours are more aggressive and therefore tend to spread hematogenously rather than lymphatically[14].

Histology of SLNB

Histology of SLNB is reported as follows:

- Negative: No histological and immunochemical evidence of tumour in the lymph node[21]
- Macrometastasis: Tumour deposits of more than 2 mm (pN1)[21]
- Micrometastasis: Range in size from 0.2mm to less than/equal to 2 mm, or consist of more than 200 carcinoma cells in a single lymph node section (pN1mi) [21].
- Isolated cancer cells Isolated: Isolated tumour cells are single cells or cell clusters each less than 0.2 mm in size and amounting to less than 200 carcinoma cells in one lymph node section [pN0 (i+)], regardless of method of detection[22]

- Number of SLNBs: Observation by Men Yi and colleagues showed that removing up to 5 SLNB is sufficient to identify metastatic carcinoma in 99% of patients. However there are several patient and tumour characteristics that influence the number of SLNBs that needs to be removed[22].
- Histology of axilla: In as much as 50% of SLNB biopsies, SLNB is the only positive node in the axilla as was demonstrated by Jaka *et al.* where 20 of 37 cases of SLNB had SLNB as the only positive node[5,7]. Thus many patients still undergo axillary lymph node clearance with no additional therapeutic or staging benefit[11, 14]
- SLNB technique: Historically, the colorimetric detection of lymph nodes by the trypan blue (or equivalent) preceded the use of radioisotopes. For its rapid migration kinetics, About 1-4 ml of the dye is injected intra-operatively either into or around the tumour, or superficially into the section of areolar tissue that correlates with the index quadrant, 5 to 10 minutes before surgery. Injection of the radio-isotope occurs between 2 and 24 h before surgery. Radioactive nanocolloid (e.g. Technetium-99m) is injected preoperatively, with the assistance of radiological imaging either around the tumour, or into the overlying skin. The advantage of vital dyes is their "real time" efficiency in guiding the gamma probe to the sentinel node. This reduces exploratory dissection and tissue plane disruption and leads to a surgical exploration much less aggressive than with isotope technique alone, as dissection is guided by hot and blue node. After the introduction of the isotope method, some teams have remained faithful to the colorimetric method while others are converted to the exclusive use of the radiocolloids; but the majority of teams use the combination of the two techniques. The reported technical success rates for SLNB are 69%-99% for radioisotope alone, 67%-93% for blue dye alone and 90%-100% for the combined method[21]
- In our institution dual method is used with addition of SPECT/CT to help further locate the SLN.
- Histopathological analysis: The histopathological analytical methods for excised SLNBs have been improved in order to increase staging accuracy and reduce false-negative rates. The relevant SLNB pathology must include multisection staining with haematoxylin and eosin. Immunohistochemical analysis with antibodies to

cytokeratin is also commonly used for haematoxylin and eosin-negative cases and may facilitate the detection of small deposits, thereby enhancing the screening of SLNBs sections. The majority of SLNB cases can be classified as positive or negative based on the presence or absence of macrometastasis. The 2003 edition of the TNM classification uses 2.0 mm as the cut-off size that distinguishes between micro- and macrometastasis. The cut-off value for isolated tumour cells or so-called submicrometastasis is 0.2 mm[21].

- In our hospital setting haematoxylin and eosin staining is used to evaluate the multisection of the SLNB, additional immunohistochemical staining is rarely performed.

OBJECTIVES

Primary aims were to determine patient and tumour factors affecting histology of axillary SLNB in breast cancer at Universitas Academic hospital. Such study has never been done in our institution. Secondary aims were to determine the histological characteristics of positive SLNB and final histological status of the axilla if SLNB was positive.

HYPOTHESIS

The hypothesis of the study was that tumour size, site, grade, lymphovascular infiltration will affect histology of SLNB as well as positive hormone receptors.

ETHICS

HSREC 31/2017(UFS-HSD2017/0183)

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CHAPTER 2

PATIENT AND TUMOUR FACTORS AFFECTING THE HISTOLOGY OF SLNB IN BREAST CANCER PATIENTS AT UNIVERSITAS HOSPITAL, BLOEMFONTEIN

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ABSTRACT

Introduction: SLNB has become accepted standard staging tool in clinically node negative breast cancer patients.

Aim(s): Primary aim was to determine factors (demographics and tumor factors) affecting histology of SLNB. Secondary aim was to determine the histological characteristics of the SLNB and final histological status of the axilla if SLNB was positive.

Methods: Retrospective analytical study of patients who underwent mastectomy or lumpectomy with SLNB from 2007 to 2016 (n=60) at Universitas Academic Hospital.

Results:
Demographics*:* Age ranged from 36 to 90 years and its association with positive SLNB histology was close to statistically significant (p-value 0.0242).
Tumour factors: Location of the tumour, type of tumour, size of tumour all with p-value of 1.000, histological type (p-value 0.7464), grade (p-value 0.6244), lymphovascular infiltration (p-value 1.000) and hormonal receptors status (ER-positive p-value 0.6434, PR-positive p-value 0.7290, HER-positive p-value 0.2341 and ER/PR/HER-positive p-value 1.000) did not have statistically significant association with positive SLNB histology. There was a statistically significant association between positive SLNB histology and final histology of the axilla (p-value <0.001).

Conclusion: The study did not prove our hypothesis that, tumour size, site, grade, lymphovascular infiltration and hormone receptors will affect histology of SLNB. Age nearly affected histology of SLNB. Small sample size and incomplete lymphovascular infiltration reporting might have affected results. There was statistically significant association between histology of SLNB and histology of the axilla.

INTRODUCTION

SLNB (SLN) is the first node(s) to receive lymphatic drainage from the tumour, thus theoretically will be the first site of lymphatic metastases. The first introduction of sentinel lymph node biopsy (SLNB) was by Cabanas in penile cancer and followed by Morton in melanoma[1]. SLNB pioneered by Giuliano in breast surgery gained popularity through the 1990s[2] and has been accepted as standard of care in clinically node negative breast cancers (T1-T3) for deciding whether axillary dissection should be performed or not.

Clinical trials comparing SLNB and axillary lymph node dissection show no significant difference in SLN negative patients in terms of survival, recurrence of a tumour and that SLNB can accurately predict the status of the axilla. As much as 75% of patients undergoing SLNB are node negative, thus many patients can be spared the unwanted side effects of axillary dissection like nerve injury, shoulder immobility, seroma formation and lymphoedema[3, 4].

Studies have been done to try to look for both patients and tumour characteristics that can be used to predict SLN and thus the axillary involvement with varying degrees of proven relationship. There is a consensus that tumour size, grade and lymphovascular infiltration are directly related with positive SLN[5,9,12].

Our hospital is a referral centre for Free State, Northern Cape and Lesotho and considerable number of SLNB are done here and there is no study that has looked at factors affecting histology of SLNB in our hospital.

BACKGROUND

Patient factors

Age

Chen *et al.* found that age is not an independent risk factor for positive SLN histology[5] with Jaka *et al.* finding similar results[6]. Age was found as an independent predictor of SLN positivity as was demonstrated by Gangi *et al.* where patients younger than 50 years were at risk of lymph node metastases and therefore positive SLNB histology[7].

Race

There is limited data on the effect of race on histology of SLNB. Gann *et al.* reported 35% to 40% increased risk of lymph node metastasis in African-American women and Hispanic women and suggest that women in these groups develops more biologically aggressive breast carcinomas for reasons not yet known[9].

Tumour factors

Size (T-status)

Tumour size is an independent predictor of positive SLNB. As the size of tumour increases so is the ability for it to spread[7, 8, 10, 11,15].

Site

Upper inner quadrant tumours have lower frequency of axillary metastases and researchers query alternative route of spread to internal mammary lymph nodes[11]. Yoshihura *et al.* found that tumour location was the second most powerful predictor of axillary lymph nodes involvement and therefore positive SLNB histology as lateral and retro-areolar tumours were associated with higher frequency of axillary lymph node metastases (p-value of 0.0019)[8,15].

Multifocal cancer

Veronesi *et al.* suggested that SLNB is not suitable for multifocal breast cancers as each lesion might have its own lymphatic drainage, which can't be connected. Therefore a negative SLN for one lesion cannot assure negative results for others[12]. Other centers still considers multifocal cancer breast cancer as an indication for SLNB[11]. A meta-analysis(n=996) of multifocal/multi-centeric breast cancer revealed success rate of 92.0-100% and a false negative rate of 0-25% in SLNB which is close to unifocal breast cancer[13].

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As with tumour size, most studies have shown lymphovascular infiltration as an independent predictor of SLNB metastasis . Cancers showing lymphovascular infiltration have 57% chance of being node positive compared to 23% for cancers without lymphovascular infiltration[8, 15].

Oestrogen receptors / Progesterone receptors (ER/PR)

Positive expressions of ER and PR receptors by tumours have previously shown to be significantly correlated with histological grade, mitotic score and nuclear pleomorphism. Therefore it can be assumed that ER and PR positive tumours will metastasize to the axilla early and affect histology of SLNB[15,16]. Other studies found no significant association between progesterone or oestrogen receptors and SLN status[17,18]. Similar results were found for progesterone receptors[18].

HER-2 (Human Epidermal Growth Factor-2)

There is no consensus with regards to the association of HER and axillary lymph node status. Jaka et al. and Chen et al. showed no significant correlation between HER receptor status and positive sentinel lymph node[5,6]. Crabb et al. specified HER-2 as an independent predictor of nodal involvement in a retrospective analysis of 3441 early breast cancer patients. Gulben et al. showed similar results[19 ,20]. To try to clarify the conflict some authors speculated that HER2-posetive tumours are more aggressive and therefore tend to spread hematogenously rather than lymphatic spread[16].

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- Isolated cancer cells Isolated: Single cells or cell clusters less than 0.2 mm in size and amounting to less than 200 carcinoma cells in one lymph node section[21].
- Number of SLNs: Removing up to 5 SLN is sufficient to identify metastatic carcinoma in 99% of patients[22].
- Histology of axilla: In as much as 50% of SLN biopsies, SLN is the only positive node in the axilla, thus many patients still undergo axillary lymph node clearance with no additional therapeutic or staging benefit[11, 14].
- SLN technique: Colorimetric detection by the trypan blue (or equivalent) and radioactive nanocolloid (e.g. Technetium-99m) are used to detect SLNB (blue and hot node). The reported technical success rates for SLNB is 90%-100% for the combined use[21].
- In our institution a similar method is used with addition of SPECT/CT to help further locate the node.
- Histopathological analysis: The relevant SLN pathology must include multisection staining with haematoxylin and eosin. Immunohistochemical analysis with antibodies to cytokeratin is also commonly used for haematoxylin and eosin-negative cases to facilitate the detection of small deposits[21].
- In our institution multisection haematoxylin and eosin staining is used to evaluate the SLN, additional immunohistochemical staining is rarely performed.

OBJECTIVES

Primary objectives were to determine patient and tumour factors affecting histology of axillary SLN in breast cancer at Universitas Academic Hospital. Secondary objectives were to determine the histological characteristics of positive SLN and final histological status of the axilla if SLNB was positive. Such study has never been done in our institution.

The hypothesis was that tumour size, site, grade and lymphovascular infiltration will affect histology of SLN as well as positive hormone receptors.

METHODS

The study was approved by ethics committee of the University of the Free State HSREC 31/2017(UFS-HSD2017/0183).

Retrospective analytical study was done by means of review of patient files, Meditech notes and histological records from DISA and Labtrak. The first ten patients were used in the pilot study to modify the data sheet but were included in the main study. Histological report of the SLNB was the only factor that was changed on the data sheet as our reports only stated SLNB as either positive or negative.

All patients who had mastectomy/lumpectomy with SLNB between 2007 and 2016 at Universitas Academic Hospital, breast and endocrine unit were included in the study except those on the exclusion criteria- male and patients with DCIS(Ductal carcinoma in situ). After going through the data 2 patients were excluded (one male and one DCIS) leaving only 60 patients for the study.

The data was captured on the data sheet and excel spreadsheet which served as second copy. The data that was captured included patient demographics and tumour factors as alluded in the literature review.

Results were summarized by frequencies and percentages (categorical variables) and means, standard deviations or percentiles (numerical variables). Associations were investigated by using contingency tables with appropriated hypothesis testing. P-value of 0.05 was statistically significant.

RESULTS

There were 60 patients enrolled in the study after exclusion of two (cf. Figure 1). The age ranged from 36 to 90 years with median age of 63.5 years. Patients were divided into different age groups (cf. Figure 2). The study had 53.3% (n=32/60) blacks, 40.0% (n=24/60) white, and 6.7% (n=4/60) coloured patients (cf. Figure 3). Most patients 72.7% (n=43/60) had mastectomy and while 28.3% (n=17/60) had lumpectomy with SLNB. Five percent of patients (n=3/60) had pre-operative chemotherapy.

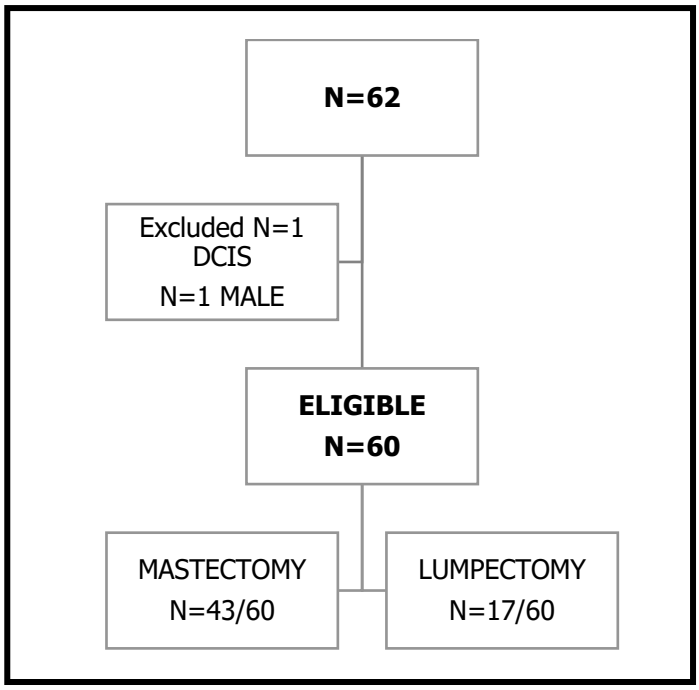

Figure 1: Number of cases in the study

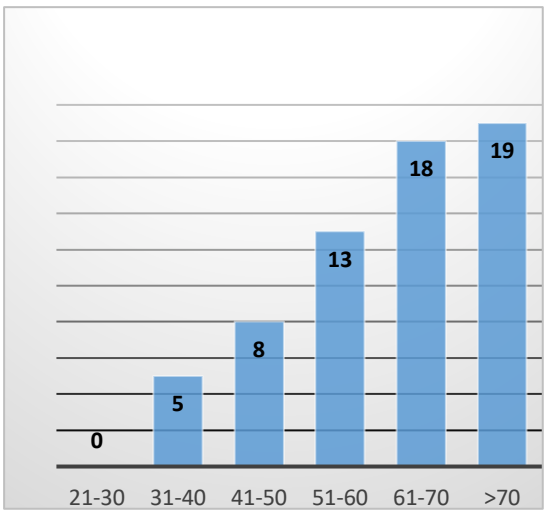

Figure 2: Age groups in decades

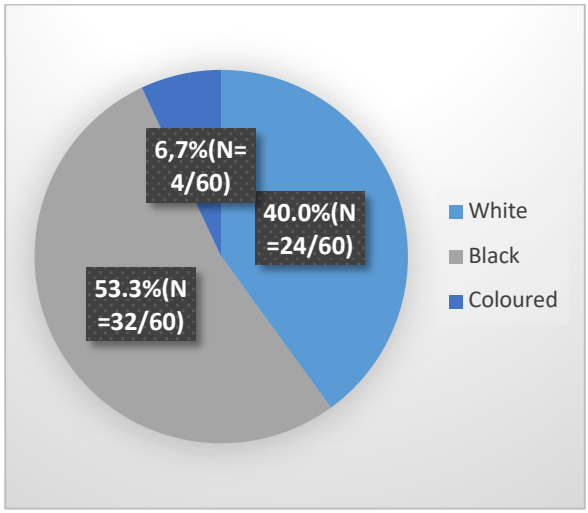

Figure 3: Race distribution

Age of less than 50 years had close to statistically significant association with positive SLNB with p-value of 0.0970 when compared to patients more than 50 years old. Race didn't have statistically significant association with the histology of SLNB with a p-value of 0.7935 between white and other racial groups. Pre-operative chemotherapy had no statistically significant association with the histology of SLNB (p-value of 0.2678).

Figure 4 demonstrates the location of tumour with most tumours found in superior medial and least tumours found in inferior lateral.

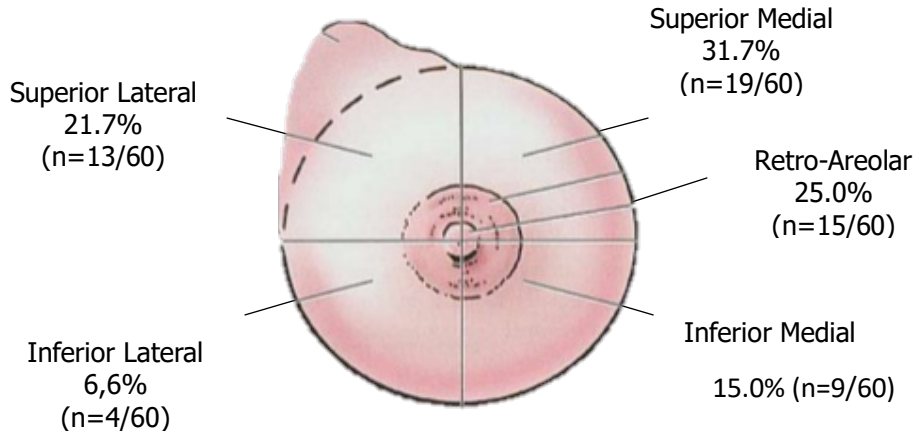

Figure 4: Tumour location

Comparing the effect of different location of the tumour to the histology of SLN in relation to superior lateral quadrant versus other quadrant there was no statistically significant association between site and histological results of the SLNB with p-value of 1.000.

The size of tumour ranged from 5mm to 75mm with the mean size of 25.8mm. The primary tumour sizes were divided according to tumour size of American Joint Committee on Cancer, T1 had 50.0% of patients (n=30/60), T2 had 46.7% (n=28) and finally T3 had 3.3% (n=2/60). With regards to tumour size, T1 group had 30.0% (n=9/30) positive SLNB, T2 and T3 group had 50.0% (n=14/28) and (n=1/2) positive SLNB respectively, however there was no statistically significant association between size of the tumour and SLNB results (T1 vs T3 p-value 0.6244).

Histologically 91.7% (n=55/60) of the primary tumours removed were unifocal tumour with 8.3% (n=5/60) being multifocal tumour. In unifocal tumours 40.0% (n=22/55) had positive SLNB and the same was found for multifocal tumours 40.0% (n=2/5). There was no statistically significant association between the morphology of the tumour and histology of SLN as the p-value was 1.000 (unifocal vs multifocal).

Figure 5 demonstrates the histological types of tumours removed. In the other carcinoma group (n=10/60) mucinous carcinoma made up 40.0% (n=4/10) patients, tubular and tubular mixed were 30.0% (n=3/10) and 10.0% (n=1/10) respectively and lastly micropapillary and medullary type with one case each (cf. Figure 5).

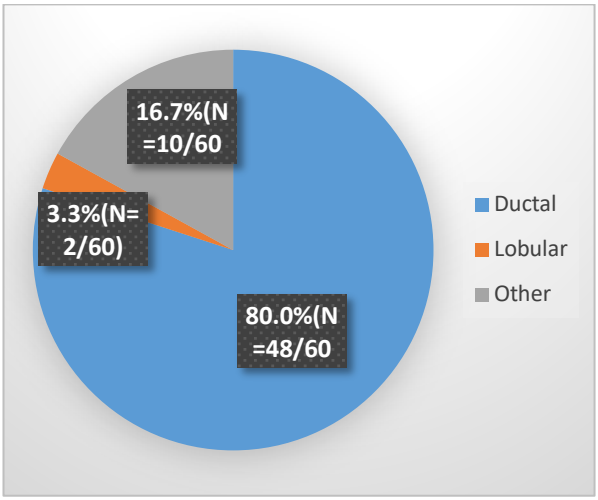

Figure 5: Histological type

There was statistically insignificant association when it comes to the association of the histological type of a primary tumour and histology of the SLN as p-value of 0.7464 between infiltrating ductal carcinoma and infiltrating lobular carcinoma plus other subtypes.

All 48 patients of infiltrating ductal carcinoma and 1 mucinous carcinoma were graded in the study (cf. Figure 6). In the case of graded ductal carcinoma the histological grade of the tumour did not have statistically significant association with the histological status of SLNB (p-value of 0.3794, grade I vs grade III).

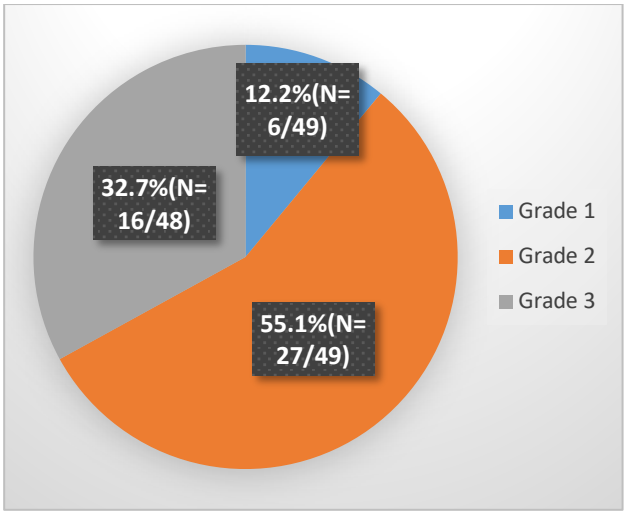

Figure 6: Histological grade

Lymphovascular infiltration was not evaluated in 28/60 patients for unknown reasons, on 32 patients where it was evaluated 68.8% (n=22/32) of the tumours had lymphovascular infiltration and 31.2% (n=10/32) had none. Lymphovascular infiltration did not have a statistically proven association with the histology of the SLNB (p-value of 1.000).

Table 1 represents hormonal receptor status of the tumour. For all the hormone receptors there was no statistically significant association between the status of receptor (positive) and histology of the SLN as shown in table 1. Combined ER/PR as well as ER/PR/HER statuses did not have statistically significant association with histology of SLNB.

Table 1: Receptor status vs histology of SLNB

HORMONE RECEPTOR STATUS VS HISTOLOGY OF SLNB		
ER Positive	93% (N=56/60)	P-value 0,6434
PR Positive	85% (N=51/60)	P-value 0,7290
HER Positive	26,7% (N=16/60)	P-value 0,2341
ER/PR Positive	85.0% (N=51/60)	P-value 0,2736
Triple Positive	16,7% (N=10/60)	P-value 1,000
Triple Negative	1,7% (N=1/60)	P-value 1.000

The average of 2.5 SLNs biopsies were taken with minimum of 1 node to maximum of 15 nodes taken for biopsy- although 15 qualifies as axillary clearance, on histological report they were reported as such. Histological reports in our study did not make distinction between

isolated tumour cells, micrometastasis or macrometastasis as anticipated, histological results of the axilla were reported as either positive or negative.

Forty percent (n=24/60) of biopsies taken were positive and 60% (n=36/60) were negative. Forty five point eight percent of biopsies (n=11/24) in the positive SLN group involved other nodes in the axilla on further axillary dissection. The remaining 54.2% (n=13/24) of positive SLN group did not have other nodes involved on axillary dissection. There was a significant association between histology of SLNB and histology of other nodes in the axilla with a p-value <0.001.

In the positive SLNB group 18 out 24 patients had less than 3 SLNs biopsy taken and 50.0% (n=9/18) of these patients had SLN(s) as the only lymph node(s) involved. In the remaining 6 out of 24 positive group more than 3 nodes were taken as a SLNB and 66.7% (n=4/6) had only SLNs as the only nodes involved. However there was no statistically significant association between the number of nodes removed if positive and the involvement of other nodes in the axilla (p-value 0.6494).
Axillary dissection was not done if SLNB histology was negative.

DISCUSSION

Since its introduction in breast cancer surgery by Giuliano in the 1990s, SLNB has become an accepted standard method of care in early clinically node negative breast cancers (T1-T3). With minimal contraindications SLNB can adequately predict the histological status of the axilla and therefore spare up to 75% of patients the unwanted side effects of axillary lymph node dissection[2,3]. Currently there is an increased interest in factors that can adequately predict the histological status of SLNB and if possible avoid it in patients with low risk for axillary lymph node involvement[2].

The overall number of patients in our study over 10 years period was 60 for a unit that does an average of 3 mastectomies per week, which was lower than other studies done in Western world where they usually see more than 100 patients in periods of 5 years. In their studies they also include male patients and patients with DCIS which were excluded in our study[14 ,23]. Although our hospital has established multidisciplinary breast oncology clinic for years, lack of standard patient referral for treatment plan before surgery might have overall numbers of patients. The most common age group in our study was 61-70 (n=18/60) and >70(n=16/60), the mean age was 61.5 which was the same as in the study done by La Verde *et al.*[3] There is conflicting evidence of whether age is a predictor of histology of SLNB with some authors like Chen *et al.* supporting this and other disputing this- Gangi *et al.*[5,6] When using 50 years as cut off between young and old we found that patients less than 50 years had higher chances of positive SLNB. Similar results were found by Rivadeneria *et al.* in analysis of 900 patients[24].

Black patients made up 53.3% of the study population followed by white patients at 40% and coloureds at 7%. Few studies are available comparing the relationship of race and histology of SLN, however Gann *et al.* reported 35% to 40% increased risk of lymph node metastasis in African-American women and Hispanic women and suggest that women in these groups develop more biologically aggressive breast carcinomas for reasons not yet known and therefore tend to metastasize early to the axilla[9].

In the series by La Verde 84.6% (n=292/345) of patients underwent quadrectomy, 12.8% (n=44/345) underwent mastectomy and the remaining 2.6% (n=29/345) underwent lumpectomy[23]. Comparing to them our patients had more mastectomies 72.7% (n=43/60) than

breast conserving surgery 28.3% (n=17/60) which is similar to the study done in India by Chakroborty *et al.*[26] No comment can be made on the reasons for high numbers of mastectomies versus lumpectomies as the study is retrospective and the choice of surgery depends on multiple factors, amongst others patient choice, tumour size, breasts size and surgeon experience.

In our study only 5% (n=3/60) had pre-operative (neoadjuvant) chemotherapy, the lower number can be attributed to the fact that most patients with early breast cancer don't get neo-adjuvant chemotherapy and that although multidisciplinary oncological approach was present for more than 30 years not all patient were referred compared to now as all patients have to be seen at multidisciplinary meeting for treatment plan[33]. In our study neoadjuvant chemotherapy did not have statistically significant association with histology of SLNB (p-value 1.000). Several metanalyses including more than 5000 patients comparing upfront SLNB and neoadjuvant first report SLN detection of 90-97% and false negative rates of 7-12%. NSABP B-18 and NSABP B-27 showed significant reduction in node positivity in patient who underwent neoadjuvant versus surgery first (p-value <0.001)[31,32].

The breast is divided into five quadrants and site of the tumour is described according to the quadrant where it is found. In our study the most common site where tumours were found were superior medial, retro-areolar and superior lateral respectively. In a large series (n=5331) by Bevilacqua *et al.* the lateral quadrants and retro-areolar were commonest site to find the tumour which is the same to our study[16]. In our study we found that tumour location did not have any effect on the histology of SLN (superior lateral vs other quadrants p-value of 1.000). Yoshihara *et al.* showed that lateral and retro-areolar tumours have high frequency to metastasize to the axilla[8]. The study by Bevilacqua showed that 25.4% (143/564) of superior medial tumours (n=564/3786) had positive SLNB compared to 33.7% (726/2154) of superior lateral tumours (n=2154/3786) with positive SLNB, compared to other quadrants the results were similar. The authors concluded that superior medial tumours have less frequency to metastasize to the axilla compared to other quadrants because of possible alternative route to internal mammary glands and thus tumour site does affect histology of SLN[15]. In the study of 103 patients done by Chandrasaker *et al.* looking at different variables affecting the histology SLN, tumour site did not have statistically significant association with histology of SLNB[26].

Multifocal tumor was found in 8.3% (n=5/60) and this was roughly similar to the result found by Goyal at el 8.9% (75/842)[27]. The concern with multifocal tumours is that they may have

different lymphatic drainage and therefore render SLNB unreliable, however Goyal *et al.* quoted SLN detection of >95% and false negative results of only 9% in multifocal tumours[27]. In our study we also found that the morphology of the primary tumour did not have any significant association with the histology of the sentinel node biopsy (unifocal vs multifocal p-value 1.000). Most studies showed that morphology of tumour had no effect on the histology of SLNB[23, 26,27].

Most studies have shown that as the size of the tumour increases so is its ability to metastasize to the axilla and therefore size of the tumour increases can be used to predict the histology of the axilla[23,14,26,29]. In our study most patients presented with T1 and T2 tumours 96.7% (n=58/60). There was no statistically significant association between the size of the tumour and the histology of SLNB (T1 vs T3 p-value 0.6244). Ashturkar *et al.* found similar results with the similar study performed in India in 2010[14].
The most common type of breast cancer in our study was infiltrating ductal carcinoma with 80.0% (n=48/60) which was similar to all international studies. Infiltrating lobular carcinoma was rare in our study with only 3% (n=2/60) and with other rare tumours groups at 17.0% (n=10/60). These numbers were too small to draw any conclusion. When comparing the effect of histological type of breast cancer on the histology of SLN there was no statistically significant association between histological type of cancer and histology of the SLNB. This was also demonstrated by Jaka *et al.* and La Verde *et al.* [6 ,23]

The most common grade in our study was grade II (n=27/58), followed by grade II (n=16/48), same distribution was quoted by Chakraboty *et al.* and Guiterezz *et al.* [25,29]. Similarly as with Jaka *et al.* we did not find any statistically significant association between histological grade of the tumour and histology of SLNB[6]. In the study by La Verde *et al.* higher histological grade was associated with positive SLNB[23].

Most studies have reported lymphovascular infiltration as an independent risk factor for lymphatic metastases and therefore positive SLNB histology[8,15,23]. Despite this, our study didn't show any statistically significant association between lymphovascular infiltration and histology of SLNB. In a study of 81 patients by Kondov *et al.* looking at different factors affecting histology of SLNB, tumour size and HER receptors were only important factors predicting the histology of SLNB[28].

All the patients in our study were evaluated for hormonal receptor status and an overall 93.3% and 85% of patients had positive ER and PR receptors which compares well to other studies where results are always above 80%[15,28]. There was no statistically significant association between ER/PR status and histology of the SLN as was shown by both small and big studies[4,15]. HER positive tumours were found in 26.7% (n=16/60) of tumours and of this HER positive tumours 25.0% (n=4/16) had positive SLN which is roughly the same as found by Chakraborty *et al*[25]. When examining the relationship between the status of HER receptors to the histology of SLNB there was no statistically significant association between the two. However univariate analysis by Kondov *et al.* found that positive expression of HER receptors influenced positivity of SLNB histology[28]. Finally combined receptor status did not have any association with the histology of the axilla as was also found by Chakraborty *et al*[25].

In our study an average of 2.5 SLNs were removed which is one lymph node higher when compared to 1.5 removed by La Verde[23]. The number of positive SLNs and therefore positive axilla was 40.0% (n=24/60) which is similar in study done by Postaci *et al.*, 37.6% (n=59/157)[14] and therefore saved 60.0% (n=36/60) unnecessary axillary clearance. In our study 45.8% (n=11/24) of positive biopsies involved other nodes in the axilla on further axillary dissection. Metanalysis done by Kim T *et al.*, found similar results that up to 48% of patients will have other nodes that are positive in the axilla which warrants further axillary clearance[30]. As with other studies the histology of SLNB had statistically significant association with the final histology of the axilla[16,23, 30].

CONCLUSION

The study did not prove our hypothesis that tumour size, site, grade, and lymphovascular infiltration will affect histology of SLNB, even though multiple bigger studies have proved that the above is true. Although we hypothesized that receptor status will have an effect on the histology of SLNB our study failed to prove it. The only variable that almost affected histology of SLNB in the study was age. Small sample size, and considerable amount of patients in which lymphovascular infiltration was not reported might have affected the results. The histology of SLNB adequately predicted histology of the axilla.

Lack of standard patient referral affected the number of patients accrued for the study. Different histological reports and missing information also had effect on our results. With all breast cancer patients now required to be referred to multidisciplinary oncological clinics for treatment plan and standard histological reporting adopted accurate data will be kept which will address our research shortcomings should similar studies pursued.

AUTHOR CONTRIBUTIONS

RS Letsoara was the principal investigator. NE Pearce was the supervisor for this research project. Both authors worked together on the protocol, and analysed the data. RS Letsoara wrote the first draft manuscript, both authors modified and approved the final version.

ACKNOWLEDGEMENTS

The authors are grateful for contributions from Prof J Joubert (Department of Biostatistics, University of the Free State).

CONFLICT OF INTEREST

None.

Author funding sources

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APPENDIX A: HSREC letter of approval

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

28 November 2019

Dear **Dr R Letsoara**

Ethics Clearance: **Patient and Tumour Factors Affecting the Histology of Sentinel Lymph Node Biopsy at Universitas Academic Hospital, Bloemfontien**
Principal Investigator: **Dr Rakauoane Letsoara**
Department: **Surgery Department (Bloemfontein Campus)**

APPLICATION APPROVED

Please ensure that you read the whole document.

With reference to your application for ethical clearance with the Faculty of Health Sciences, I am pleased to inform you on behalf of the Health Sciences Research Ethics Committee that you have been granted ethical clearance for your project.

The HSREC approved the above project with note to why the previously stipulated conditions of conditional approval were met late. This is seen as an administrative issue, and not an ethical issue.

Your ethical clearance number, to be used in all correspondence is: **HSREC 31/2017.** The RIMS record number is **UFS-HSD2017/0183.**

The ethical clearance number is valid for research conducted for one year from issuance. Should you require more time to complete this research, please apply for an extension.

We request that any changes that may take place during the course of your research project be submitted to the HSREC for approval to ensure we are kept up to date with your progress and any ethical implications that may arise. This includes any serious adverse events and/or termination of the study.

A progress report should be submitted within one year of approval, and annually for long term studies. A final report should be submitted at the completion of the study.

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences. For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za. Thank you for submitting this proposal for ethical clearance and we wish you every success with your research.

Yours faithfully

DR SM LE GRANGE
CHAIRPERSON: HEALTH SCIENCES RESEARCH ETHICS COMMITTEE

Health Sciences Research Ethics Committee
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APPENDIX B: FSDOH letter of approval

health
Department of Health
FREE STATE PROVINCE

10 May 2017

Mr. RS Letsoara
Dept. of Surgery
Faculty of Health Science
UFS

Dear Mr. RS Letsoara

Subject: Patient and Tumor factors affecting the histology of sentinel lymph node biopsy in breast cancer patients at Universitas Academic Hospital, Bloemfontein.

- Please ensure that you read the whole document, Permission is hereby granted for the above – mentioned research on the following conditions:
- Participation in the study must be voluntary.
- A written consent by each participants must be obtained
- Serious adverse events to be reported and/or termination of the study.
- Ascertain that your data collection exercise neither interferes with the day to day running of Universitas Hospital nor the performance of duties by the respondents or health care workers.
- Confidentiality of information will be ensured and please do not obtain information regarding the identity of the participants.
- Research results and a complete report should be made available to the Free State Department of Health on completion of the study (a hard copy plus a soft copy).
- Progress report must be presented not later than one year after approval of the project to the Ethics Committee of the University of Free State and to Free State Department of Health.
- Any amendments, extension or other modifications to the protocol or investigators must be submitted to the Ethics Committee of the University of Free State and to Free State Department of Health.
- **Conditions stated in your Ethical Approval letter should be adhered to and a final copy of the Ethics Clearance Certificate should be submitted to** scheclats@fshealth.gov.za **before you commence with the study**
- No financial liability will be placed on the Free State Department of Health
- Please discuss your study with the institution managers/CEOs on commencement for logistical arrangements
- Department of Health to be fully indemnified from any harm that participants and staff experiences in the study
- Researchers will be required to enter in to a formal agreement with the Free State department of health regulating and formalizing the research relationship (document will follow)
- You are encouraged to present your study findings/results at the Free State Provincial health research day
- Future research will only be granted permission if correct procedures are followed see http://nhrd.hst.org.za

Trust you find the above in order.

Kind Regards

Dr D Motau
HEAD: HEALTH
Date: 11/05/2017

Head : Health
PO Box 227, Bloemfotein, 9300
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APPENDIX C: HOD surgery letter of approval

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

16 February 2017

TO WHOM IT MAY CONCERN

RE : DR S LETSOARA STUDENT NO. 2005082819

This is to certify that I am aware of Dr S Letsoara research project and approved it.

He is requesting approval from the Ethics Committee to commence with data collection and to proceed with the research.

Yours sincerely,

DR N.E. PEARCE

HEAD: DEPARTMENT OF SURGERY

FACULTY OF HEALTH SCIENCE

UNIVERSITY OF THE FREE STATE

BLOEMFONTEIN

Head of Department/Departementshoof: Prof RS du Toit
Prof in Surgery/Prof in Chirurgie: Prof R Barry
Consultants/Konsultante: Prof SJA Smit, CA Loubser, RG Botha, SM le Grange, CG Troskie, E Arko-Cobbah, DP Menge, M Crots, NE Pearce, CT Snowdown, VC Simmons, JL Ras

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APPENDIX D: Biostatistics approval letter

14 February 2017

For attention: Health Sciences Research Ethics Committee, UFS

Title of project:

Patient and tumour factors affecting the histology of sentinel lymph node biopsy in breast cancer patients at Universitas Academic Hospital, Bloemfontein

Researcher:

Dr RS Letsoara, Dept of Surgery

I hereby confirm that I provided inputs on the protocol and approve the protocol with regards to: study design, sampling method, measurement, measuring instruments and statistical analysis.

Yours faithfully

G Joubert

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APPENDIX E: HSREC approved protocol

PATIENT AND TUMOUR FACTORS AFFECTING THE HISTOLOGY OF SLNB IN BREAST CANCER PATIENTS AT UNIVERSITAS HOSPITAL, BLOEMFONTEIN

Researchers

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INTRODUCTION

SLNB is the first node(s) to receive lymphatic drainage from the tumour, thus theoretically will be the first site of lymphatic metastases. The first introduction of SLNB was by Cabanas in penile cancer[1] and followed by Morton in melanoma. SLNB pioneered by Giuliano in breast surgery has gained popularity through the 1990s[2] and has been accepted as standard care in clinically node negative breast cancers (T1-T3) for deciding whether axillary dissection should be performed or not. Controversy still exist on whether should subclinical lymph nodes after neoadjuvant should be observed, resected or subjected to SLNB[1,3].

Clinical trials comparing SLNB and axillary lymph node dissection show no significant difference in SLNB negative patients in terms of survival, recurrence of a tumour and that SLNB can accurately predict the status of the axilla. As much as 75% of patients undergoing sentinel lymphnode biopsy are node negative, thus many patients can be spared of unwanted side effects of axillary dissection like nerve injury, shoulder immobility, seroma formation and lymphedema [3, 4].

There are numerous instances where SLNB is relatively and absolutely contraindicated. Inflammatory breast cancer is a relative contraindication to sentinel lymph biopsy. Subdermal lymphatic invasion may inhibit migration of lymphatic mapping substrate, decreasing the accuracy in this patient population that has a 55% to 85% incidence of clinically node-positive disease. While using radiolabelled sulphur colloid is safe and accurate in pregnancy, methylene blue and isosulfan blue are contraindicated in pregnancy because of risk of teratogenicity and anaphylaxis. Routine axillary staging is not required for pure DCIS unless a total mastectomy or

an excision that may compromise future performance of SLNB is planned. Sentinel lymph biopsy is possible in patients who had diagnostic or excisional breast biopsy, however there is no adequate data for recommendation when it comes to breast augmentation or reduction surgery. As for previous axillary surgery SLNB is contraindicated[5].

Studies have been done to try to look for both patients and tumour characteristics that can be used to predict SLNB and thus the axillary involvement with varying conclusions. However there is a consensus that tumour size, grade and lymphovascular infiltration are directly related with positive SLNB.

BACKGROUND

Patient factors

- Age

Chen et al found that age is not an independent risk factor for positive sentinel lymphnode[6]. Jaka et al found the same results too[7]. Some studies quote age as an independent predictor of SLNB positivity as was demonstrated by Gangi et al where patients younger than 50 years were at risk of lymph node metastases[8]. Most studies have indicated that the younger the age of the patient, the more aggressive tumour is and thus the higher the chance of lymphatic metastases[9].

- Race

No data can be found in South Africa with regards to studies that evaluate association of race and SLNB in breast cancer. Comparing to the rest of the world this is also true. However Gann et al reported 35% to 40% increased risk of lymph node metastasis in African-American women and Hispanic women and suggest that women in these groups develops more biologically aggressive breast carcinomas for reasons not yet known[10].

Tumour factors

- Size(T-status)

Tumour size is an independent predictor of positive SLNB. As the size of tumour increases so is the ability for it to spread. In big studies the frequency of positive SLNB is 10% in T1mic , 9-13% in T1a, 13-19% in T1b, 26-29% in T1c, 39-59% in T2, and 80% in T3 tumours [8, 11, 12 and 16].

- Site

Upper inner quadrant tumours have lower frequency of axillary metastases and researchers query alternative route of spread to internal mammary lymph nodes[12]. Yoshihura et al says lateral and retro-areolar tumours are associated with higher frequency of axillary lymph node metastases[9]. However most studies have shown

over and over that there is no correlation between sentinel lymphnode positivity and tumour site.

- Multifocal cancer

Veronesi et al suggested that SLNB is not suitable for multifocal breast cancers as each lesion might have its own lymphatic drainage, which can be connected. Therefore a negative sentinel lymphnode for one lesion cannot assure negative results for others[13]. However Chinese Anti-Cancer Association Committee of breast cancer (CACA-CBCS) still considers multifocal cancer breast cancer as an indication for sentinel lymphnode biopsy. A meta-analysis on 996 cases of multifocal/multi-center breast cancer revealed success rate of 92.0-100% and a false negative rate of 0-25% in SLNB which is close to unifocal breast cancer[14].

- Histological type

Infiltrating ductal and lobular carcinoma have higher frequency of positive SLNBs when compared to other histological subtypes (tubular, mucinous and medullary). However these tumours are rare and most studies are too small to draw any concrete conclusions. Studies quote the incidence of 17% for tubular cancers, 6% for mucinous cancers, and 21% for pure medullary cancers of positive SLNB[8, 15].

- Grade

Chen et al and Jaka R C et al did not find statistical correlation between grade and SLNB status[6, 7]. However studies have shown that histological grade can play a role in predicting the status of axilla, for grade I the incidence is 10% and grade III is 39%[16].

- Lymphovascular infiltration

As with tumour size, most studies have shown lymphovascular infiltration is an independent predictor of sentinel lymphnode metastasis and has been proven in many studies. Cancers showing lymphovascular infiltration have 57% chance of being node positive compared to 23% for cancers without lymphovascular infiltration[16].

- Estrogen receptors/ Progesterone receptors (ER/PR)

Positive expressions of ER and PR receptors by tumours have previously shown to be significantly correlated with histological grade, mitotic score and nuclear pleomorphism. Therefore it can be assumed that ER and PR positive tumours will metastasize to the axilla early[17], but most studies have shown low risk of axillary lymphnode metastasis. Winstanley et al found no significant association between progesterone or oestrogen receptors and lymph node status[18]. This was also demonstrated by A Pourzand et al where 34 out of 57 oestrogen positive patients (59.6) had lymph node involvement and 29 out of 48 of receptor negative patients (60.4) had involved nodes and the difference was statistically insignificant[19]. The

same was found for progesterone receptors where the difference was statistically insignificant, 57.1 % and 64.2% patients had lymph node involvement for progesterone positive and negative respectively[9]. Bevilacqua et al found ER/PR positive tumours had higher frequency of sentinel lymphnode metastases when compared to ER/PR negative tumours, with small but significant increase of 2% for ER and 4.6 for PR receptors[16].

- HER-2(Human epidermal growth factor-2)

There is no consensus with regards to the association of HER and axillary lymph node status. Jaka et al and Chen et al showed no significant correlation between HER status and positive SLNB[6, 7]. Crabb et al specified HER-2 as an independent predictor of nodal involvement in a retrospective analysis of 3441 early breast cancer patients. Gulben et al showed similar results[20, 21]. To try to clarify the conflict some authors speculated that HER2-posetive tumours are more aggressive and therefore tend to spread hematogenously rather lymphatic spread[17].

Histology of sentinel lymphnode

Histology of SLNB is reported as follows:

- Negative

  No histological and immunochemical evidence of tumour in the lymph node[22].

- Macrometastasis

  Tumour deposits of >2 mm (pN1)[22]

- Micrometastasis
  Range in size from 0.2mm to ≤2 mm, or consist of >200 carcinoma cells in a single lymph node section (pN1mi) [22].

- Isolated cancer cells Isolated
  Isolated tumour cells are single cells or cell clusters each <0.2 mm in size and amounting to <200 carcinoma cells in one lymph node section [pN0 (i+)], regardless of method of detection[22].

Number of SLNBs

- Observation by Men Yi and colleagues showed that removing up to 5 SLNB is sufficient to identify metastatic carcinoma in >99% of patients. However there are several patient and tumour characteristics that influence the number of SLNBs that needs to be removed[23].

Histology of axilla

- As much as 50% of SLNB biopsies, SLNB is the only positive node in the axilla as was demonstrated by Jaka et al where 20 of 37 cases of SLNB had SLNB

as the only positive node[6, 7]. Thus many patients still undergo axillary lymph node clearance with no additional therapeutic or staging benefit[12, 15]. MSKCC (Memorial Sloan Kettering Cancer Center) nomogram with its criticism was developed in order to predict those patients with non-SLNB metastasis[16]. But this is not the purpose of this study.

SLNB technique

- Historically, the colorimetric detection of lymph nodes by the trypan blue (or equivalent) preceded the use of radioisotopes. For its rapid migration kinetics, the dye is injected in 1-4 ml intra-operatively either into or around the tumour, or superficially into the section of areolar tissue that correlates with the index quadrant, 5 to 10 minutes before surgery. Injection of the radio-isotope occurs between 2 and 24 h before surgery. Radioactive nanocolloid (e.g. Technetium-99m) is injected preoperatively, with the assistance of radiological imaging either around the tumour, or into the overlying skin. The advantage of vital dyes is their "real time" efficiency in guiding the gamma probe to the sentinel node. This reduces exploratory dissection and tissue plane disruption and leads to a surgical exploration much less aggressive than with isotope technique, as dissection is guided by hot and blue node. After the introduction of the isotope method, some teams have remained faithful to the colorimetric method while others are converted to the exclusive use of the radiocolloids; but the majority of teams use the combination of the two techniques. The reported technical success rates for SLNB are 69%-99% for radioisotope alone, 67%-93% for blue dye alone and 90%-100% for the combined use[22].
  In our institution the same method of identifying SLNB is used with addition of SPECT/CT to help further locate the node.

Histopathological analysis

- The histopathological analytical methods for excised SLNBs have been improved in order to increase staging accuracy and reduce false-negative rates. The relevant SLNB pathology must include multisection staining with haematoxylin and eosin. Immunohistochemical analysis with antibodies to cytokeratin is also commonly used for haematoxylin and eosin-negative cases and may facilitate the detection of small deposits, thereby enhancing the screening of SLNBs sections. The majority of SLNB cases can be classified as positive or negative based on the presence or absence of macrometastasis. The 2003 edition of the TNM classification uses 2.0 mm as the cut-off size that distinguishes between micro- and macrometastasis. The cut-off value for isolated tumour cells or so-called submicrometastasis is 0.2 mm[22].

In our hospital setting haematoxylin and eosin staining is used to evaluate the multisection of the SLNB, additional Immunohistochemical staining is rarely performed.

AIM AND OBJECTIVE OF THE STUDY

Primary aim

- To determine patient and tumour factors affecting involvement of axillary SLNB in breast cancer

Secondary aim

- The histological characteristics of positive SLNB
- Final histological status of the axilla if SLNB was positive

EXPECTED OUTCOME

My hypothesis

- Tumour size, site, grade, lymphovascular infiltration to affect lymphnode positivity
- Hormone receptors to have effect on the sentinel lymphnode

In Accordance with literature review

- Same patient characteristics as the rest of the world
- Size of tumour, lymphovascular infiltration and grade of tumor to affect sentinel lymphnode positivity

METHODOLOGY

- Study design
  Retrospective analytical study.
  The study will consist of retrospectively identified cases. Histology records, Meditech notes and files will be analysed retrospectively.

- Study location
  Universitas Academic Hospital, breast and endocrine unit.

- Study population
  Study will focus on all patients who had mastectomy/lumpectomy with sentinel lymphnode biopsy between 2007 and 2016 except those on the exclusion criteria. An estimated number of cases is roughly 60-70.

  Exclusion criteria.

  - SLNB in patients with DCIS
  - Males
  - Incomplete/untraceable notes

SNOMED search on the DISA and Labtrak system will be used to identify the cases. Patient numbers will be used to track Meditech and files of the patients for completion of the data sheet.

- Measurements
  - Demographic data
    Age and race will be captured on the data sheet to determine their effect on the histology of SLNB.
  - Tumour factors as described previously will be captured on the data sheet to determine their effect on the histology of SLNB.
  - Histology of the sentinel node will be captured on the data sheet to determine the most common type of lymph node metastasis.
  - The number of SLNBs removed will be captured on the data sheet to determine number of sentinel nodes affected.
  - Histology of the axilla
  - The final histology of the axilla will be captured on the data sheet to determine the numbers of axillae where SLNB was the only positive node.

- Methodological and measurement errors
  - Time
    Due to retrospective nature of the study, older records of patients might prove difficult to trace and therefore prolong the study
  - Sample
    Small sample size as most breast cancer patients present in advanced stages in our country and therefore small number of sentinel lymphnode biopsy are performed
  - Data
    Poor documentation of diagnosis, lost notes will affect the completeness of data and thus the number of patients in the study

- Pilot study
  The first ten cases will be used for the pilot study so as the data form can be tested and modified if needed. However, these cases will also be included in the main study.

DATA CAPTURE AND ANALYSIS

This will be undertaken by the researcher and Department of Biostatistics of the University of the Free-state. The researcher will enter the data into an Excel spreadsheet. Results will be summarized by frequencies and percentages (categorical variables) and means, standard deviations or percentiles (numerical variables). Associations will be investigated using contingency tables with appropriated hypothesis testing. Significant variables will be further analyzed in a multivariate regression analysis.

DURATION

The project is anticipated to run over two years to completion.

Ethics submission : February 2017

Data collection : April 2017 to April 2018

Data analysis : January 2019

Writing of article : June 2019

BUDGET

The budget for the entire study will be approximately R500 to R1000 for stationary. It will be funded by the Principal Investigator.

ETHICAL CONSIDERATIONS

The study protocol will be submitted to the Health Sciences Research Ethics Committee of the University of the Free State and Free State Department of Health.

Hospital number of the patient will be written on the data sheet to trace and to match patients results and note but will not be coded for so no association will be made between the patient and the information. Only the researchers will have access to the patient records.

The data file will be stored at the Department of Surgery and only be accessible to the researchers.

DISEMMINATION OF RESULTS

Results will be made available to all relevant departments; Surgery, Anatomical pathology, Ethics and Biostatistics.

IMPLEMENTATION OF RESULTS

This study will be used for the MMED dissertation to be handed in and marked as part of the curriculum for MMED. The study findings will determine the most patient and tumour factors affecting SLNB histology in our hospital settings. The results of the study will also be published.

APPENDIX F: Data sheet

E BIOPSY IN BREAST CANCER PATIENT AT UNIVERSITAS ACADEMIC HOSPITAL, BLOEMFONTEIN.

Hospital number — not code

Demographics

Age ____ years — 1 ____

Race — black 1 — white 2 — coloured 3 — other — 2 ____

Tumour factors

Size(cm) ____ — 4 ____

Site — 5 ____

- Upper right qaudrant ____ 1
- Lower right quadrant ____ 2
- upper left qaudrant ____ 3
- lower left quadrant ____ 4
- retro-areaolar ____ 5

Type of lesion — 6 ____

- Unifocal ____ 1
- Multifocal ____ 2

Histolgy of primary — 7 ____

- ductal ____ 1
- lobular ca ____ 2
- other ____ 3 _____

Histological grade — 8 ____

- Grade I ____ 1
- Grade II ____ 2
- Grade III ____ 3

Lymphovascular infiltration — 9 ____

Yes ____ 1 No ____ 2

Receptors

	positive	negitive	
ER	____ 1	____ 2	10 ____
PR	____ 1	____ 2	11 ____
HER	____ 1	____ 2	12 ____

Histology of the sentinel node — 13 ____

- Negative ____ 1
- Macrometastasis ____ 2
- Micrometastasis ____ 3
- Isolated cancer cells ____ 4

APPENDIX G: TURNITIN plagiarism report

Patient and tumour factors affecting histology of sentinel lymph node biopsy in breast cancer patients at Universit

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Appendix H: Instructions to Authors of the SAMJ

SAMJ Author Guidelines

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- Manuscripts must be written in UK English.
- The manuscript must be in Microsoft Word format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).
- Please make your article concise, even if it is below the word limit.
- Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
- Include sections on Acknowledgements, Conflict of Interest, Author Contributions and Funding sources. If none is applicable, please state 'none'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.
- Please be sure to insert proper symbols e.g. µ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.
- If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

SAMJ is a generalist medical journal, therefore for articles covering genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.

- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.

**NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'

- Use the latest approved gene or protein symbol as appropriate:

- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text .

Structured abstract

- This should be 250-400 words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
  - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
  - **Conclusion:** must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc)that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.

- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers
- Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.
- Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
- All illustrations (graphs, diagrams, charts, etc.) must be in PDF or jpeg form.
- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
- Scans/photos showing a specific feature e.g. *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings, and include units where necessary.
- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:

Each row of data must have its own proper row:

Do not: use separate columns for *n* and %:

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Combine into one column, *n* (%):

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Use <> symbols or numbers that don't overlap:

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must* ***not*** *be used.*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:
  - On the Crossref homepage, paste the article title into the 'Metadata search' box.

- Look for the correct, matching article in the list of results.
- Click Actions > Cite
- Alongside 'url =' copy the URL between { }.
- Provide as follows, e.g.: https://doi.org/10.7196/07294.937.98x

Some examples:

- *Journal references:* Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. http://dx.doi.org/10.1000/hgjr.182
- *Book references:* Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.
- *Chapter/section in a book:* Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.
- *Internet references:* World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).
- Legal references

  - Government Gazettes:

  National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.

  In this example, 17507 is the Gazette Number. This is followed by :1514 - this is the notice number in this Gazette.

  - Provincial Gazettes:

  Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

  - Acts:

  South Africa. National Health Act No. 61 of 2003.

  - Regulations to an Act:

  South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

  - Bills:

  South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.

  - Green/white papers:

  South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

  - Case law:

  Rex v Jopp and Another 1949 (4) SA 11 (N)

  Rex v Jopp and Another: Name of the parties concerned

  1949: Date of decision (or when the case was heard)

  (4): Volume number

  SA: SA Law Reports

  11: Page or section number

  (N): In this case Natal - where the case was heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.

  NOTE: no . after the v

- *Other references (e.g. reports) should follow the same format:* Author(s). Title. Publisher place: Publisher name, year; pages.
- Cited manuscripts that have been accepted but not yet published can be included as references followed by '(in press)'.
- Unpublished observations and personal communications in the text must **not** appear in the reference list. The full name of the source person must be provided for personal communications e.g. '...(Prof. Michael Jones, personal communication)'.