Abstract

Introduction

Knowledge about the oncologic status of pelvic lymph nodes forms an essential and integral part in the management of women with uterine cancer. Lymph node status is part of endometrial cancer staging and plays an important role in primary treatment and adjuvant treatment planning and prognosis in women with cervical cancer. Current practice in the management of uterine cancers involves systematic full pelvic lymphadenectomy, mainly to determine the oncological status of the nodes, as there is no high-quality evidence suggesting a therapeutic effect attributable to lymphadenectomy.

Imaging in the form of computed tomography (CT) scans and magnetic resonance (MRI) scan is not accurate to determine pelvic lymph node status in women with uterine cancer. Functional scans such as [18]Fluoro-deoxy-glucose positron emission/computed tomography (FDG-PET/CT) scan might provide better access in this setting.

Sentinel lymph node biopsy (SLNB) procedures, specifically the SLNB algorithm, have been proposed as a safe and accurate alternative procedure to full systematic lymphadenectomy in women with uterine cancers. It has also been proposed as a better alternative than complete omission of lymphadenectomy in women with presumed low risk early stage endometrial cancer. SLNB procedures might also be able to detect higher rates of lymph node metastases with the detection of micro metastases following pathological ultrastaging

The presence or absence of high risk human papilloma virus (hrHPV) DNA in sentinel lymph nodes of women with cervical cancer has also been suggested to be a useful adjunct to frozen section examination (FSE) in assisting with determination of the status of the non-sentinel nodes. Some data suggest the combination of negative FSE and absence of hrHPV accurately predict the absence of metastases. South African women have high prevalence of human immunodeficiency virus infection, tuberculosis (TB) and pelvic inflammatory disease (PID). All these

infections involve the lymphatic system. Data on SLNB procedures are form well-developed countries with different disease burdens and socioeconomic profiles, and there is no data from women living in low-resource settings.

Aims

This study aimed to determine the efficacy of and performance of FDG-PET/CT scan and SLNB and SLNB algorithm in accurately predicting the regional lymph node status of the pelvis in women with early stage cervical cancer and presumed early stage endometrial cancer. It also aimed to investigate the usefulness of HPV DNA testing of sentinel nodes in women with cervical cancer.

Population and setting

This was a prospective observational study performed in the Gynaecologic Oncology Unit at the Kalafong Provincial Tertiary Hospital and Steve Biko Academic Hospital.

Patients aged 18 years and older, with operable stages cervical cancer and presumed early stage endometrial cancer willing and able to provide informed consent were eligible for inclusion.

Materials and methods

Sentinel node mapping was done using methylene blue (MB) and indocyanine green (ICG) injected into the cervix after induction of anaesthesia at the time of primary surgery. ^{99}Technetium nanocolloid (^{99}Tc) was administered one day pre-operatively followed by lymphoscintigram. FDG-PET/CT scans were performed prior to surgery.

Following mapping and removal, FSE, HPV DNA typing, haematoxylin and eosin (H&E) examination with ultrastaging on H&E negative specimens were performed on the SLNs. All patients underwent systematic full pelvic lymphadenectomy and appropriate cancer surgery.

Results

One hundred patients were prospectively recruited to the study and results of 94 patients were available for analysis. SNL detection rate of the whole group was

60.6% with bilateral detection 29.2%. Twenty-four patients (25.5%) had pelvic metastases.

Sixty-five percent of women with cervical cancer in this study were HIV positive, and the SLN detection rate in this group was 65% with bilateral detection rate of 30%. The detection rate was significantly higher in women without nodal metastases, those with stage IA2 – IB2 disease, with tumour less than 2 cm and women with BMI less than 25 kg/m^2. HIV status, history of TB, PID and the presence of adhesions did not influence the SLN detection rate. The sentinel lymph node biopsy algorithm has a sensitivity of 100%, NPV of 100% and a false negative rate of 0% in this study. The SLNB procedure identified two women with only micro metastases (15.4%). These women would not have been identified with systematic lymphadenectomy and H&E examination.

Indocyanine green and the combination of methylene blue and ^{99}Technetium nanocolloid had significantly better sentinel node detection rates compared to methylene blue alone

FDG-PET/CT scan was performed in 28 women. The sensitivity, specificity, positive and negative predictive values of FDG-PET/CT scans to accurately predict nodal status, were 66.67%, 82%, 30.77% and 95.38% respectively. The false negative rate of FDG-PET/CT scans was 33.3%.

The sensitivity, specificity, PPV and NPV for FSE in this cohort was 66.67%, 100%, 100% and 96.05% respectively. The FNR for FSE was 23.1%.

Thirty-two patients with cervical cancer had tumour and SLN hrHPV DNA data. The sensitivity, specificity, PPV and NPV of sentinel lymph node HPV DNA to predict metastases was 50%, 69.6%, 30 and 84.2% respectively with a false negative rate of 42.8%.

Conclusions

Although the SLN detection rate was lower compared to the published literature, the SLNB algorithm performed excellently in this group of patients of which the majority were HIV-infected.

The SLNB procedure can be considered as a treatment option in selected cases in the management of women with early stage endometrial and cervical cancer.

PET/CT should not be used as part of the primary diagnosis and staging investigations in women with uterine cancer, and is recommended only in selected cases for initial staging of locally advanced cervical cancer being considered for radical chemoradiation therapy.

In this study, testing for the presence of hrHPV DNA in the sentinel lymph nodes was not useful as a predictor of pelvic lymph node status. The combination of negative FSE and negative hrHPV in the SLNs did not have a reliable negative predictive value for the absence of pelvic nodal metastases.

Key words

Sentinel lymph nodes, endometrial cancer, cervical cancer, PET/CT scan, Methylene blue, indocyanine green, sentinel lymph node algorithm, HPV types, pathological ultrastaging, frozen section examination.

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^{99m}Tc	^{99m}Technestium nanocolloid
AJCC	American Joint Committee on Cancer
ART	Anti-retroviral therapy
ARV	Anti-retrovirals
BMI	Body mass index
BSO	Bilateral salpingo oophorectomy
Ca-125	Cancer antigen 125
cCRT	Concurrent chemoradiation therapy
CI	Confidence interval
CT	Computed tomography
CXR	Chest X-ray
DFS	Disease free survival
DNA	Deoxyribonucleic acid
FBC	Full blood count
FDG-PET/CT	^{18}Fluoro-deoxy-glucose positron emission tomography/computed tomography
FIGO	Federation of Gynecology and Obstetrics
FNR	False negative rate
FSE	Frozen section examination
H&E	Haematoxylin and eosin
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
hrHPV	High-risk human papilloma virus
ICG	Indocyanine green
IHC	Immunohistochemistry
ITC	Isolated tumour cells
IUCC	International Union Against Cancer
IVP	Intravenous pyelogram
KPTH	Kalafong Provincial Tertiary Hospital
LFT	Liver function test
LND	Lymph node dissection

LVD	Low volume disease
LVD	Low volume disease
MB	Methylene blue
MM	Micro metastases
MRI	Magnetic resonance imaging
MTV	Metabolic tumour volume
NPV	Negative predictive value
OS	Overall survival
OSEM	Ordered subset expectation maximization
PBS	Phosphate buffered solution
PCR	Polymerase chain reaction
PET	Positron emission tomography
PPV	Positive predictive value
ROI	Regions of interest
RT	Radiotherapy
SBAH	Steve Biko Academic Hospital
SD	Standard deviation
SEM	Standard error of the mean
SLN	Sentinel lymph node
SLNA	Sentinel lymph node algorithm
SLNB	Sentinel lymph node biopsy
SLNs	Sentinel lymph nodes
SUV mean	Mean standardised uptake value
TAH	Total abdominal hysterectomy
TB	Tuberculosis
TLG	Total lesions glycolysis
TNM	Tumour-node-metastasis classification
U&E	Urea & electrolytes

Chapter 1

Overview

1. Introduction

Knowledge regarding lymph node status is essential in the management of women diagnosed with gynaecological cancers. In vulvar, endometrial and ovarian cancers, information about lymph node status is incorporated into the International Federation of Gynecology and Obstetrics (FIGO) staging of these cancers. Women with cervical cancer are staged clinically, and although lymph node status is not part of the staging, it has important prognostic and adjuvant therapeutic implications [1,2].

2. Cancer staging

Cancer staging is an important basic oncologic principle applicable to all cancers in human beings and essential for the optimal management of the disease. Cancer staging allows for treatment planning and enables healthcare providers to equip patients with information on the prognosis of the cancer they have been diagnosed with. Gynaecologic cancer staging is performed in the majority of cases using the rules and classification of malignant tumours of the female genital tract as adopted by FIGO.

2.1. Cervical cancer staging

The earliest efforts regarding the FIGO classification of cervical cancer dates back to 1928, and the first classification for cervical cancer was adopted in 1950, followed by uterine cancer in 1953. The International Union Against Cancer (IUCC) established a committee in 1954 to develop rules for the classification and clinical staging of malignant tumours. This committee proposed the tumour-node-metastasis (TNM) classification for cervical cancer in 1966. The American Joint Committee on Cancer (AJCC) accepted the FIGO stage grouping for gynaecological cancers in 1976 [3].

Cervical cancer staging is based mainly on clinical findings and is performed according to certain rules [4]. Physical examination of the patient, including general systemic and gynaecological examination consisting of vaginal and rectal inspection, provides information on the size and extent of the tumour. Special investigations include histology of the tumour obtained through punch biopsy, colposcopy directed biopsy, large loop excision of the transformation zone or cone biopsy. Cystoscopy, hysteroscopy and proctoscopy may also be utilised as part of the special investigations for staging purposes. Imaging includes ultrasound of the abdomen and pelvis, intravenous pyelogram (IVP) and chest X-ray (CXR) examination [5,6]. The 2009 staging system for cervical cancer is shown in Table 1 [1,6].

The fact that the FIGO staging of cervical cancer is based mainly on clinical findings with only limited use of imaging and special investigations contrary to surgically staged cancers such as endometrial cancer, can result in under staging of many patients. Clinical staging of cervical cancer has however shown a high correlation with surgical-pathological staging and with the use of more advanced imaging techniques [5,7,8].

The FIGO staging system for cervical cancer also excludes information of pelvic lymph node status, despite the significant impact it has on prognosis, as nodal status is besides disease stage the most important prognostic factor. The five-year survival of women with early stage disease and negative pelvic nodes after radical surgery ranges from 88% to 96% compared with 50% to 74% in women with early stage disease and nodal metastases [7,9,10]. The number of positive nodes seems to have an adverse effect on prognosis, with a higher number of positive nodes associated with a worse outcome [11].

Table 1: FIGO 2009 staging of cervical cancer

Stage	Definition
I	Tumour confined to the uterus regardless of corpus involvement
IA	Invasive carcinoma diagnosed microscopically regardless of lympho-vascular space involvement
IA1	Depth of invasion 3 mm or less; horizontal spread 7mm or less
IA2	Depth of invasion > 3mm and not more than 5 mm; horizontal spread 7mm or less
IB	Macroscopic lesion or microscopic lesion > than IA2
IB1	Tumour < 4 cm in largest diameter
IB2	Tumour ≥ 4 cm in largest diameter
II	Tumour extends beyond the cervix; pelvic sidewall and lower third of vagina not involved
IIA	Tumour extends to less than upper two thirds of vagina; no parametrial involvement
IIA1	Tumour ≤ 4 cm in largest diameter
IIA2	Tumour > 4 cm in largest diameter
IIB	Tumour with parametrial invasion
III	Tumour extends to pelvic sidewall and/or involves lower third of vagina and/or causes hydronephrosis or non-functioning kidney
IIIA	Tumour involves lower third of vagina, pelvic sidewall not involved
IIIB	Tumour extends to pelvic sidewall and/or causes hydronephrosis or non-functioning kidney
IV	Metastases to other organs
IVA	Invasion of bladder or rectal mucosa and/or tumour extends beyond true pelvis
IVB	Distant metastases

2.2. Endometrial cancer staging

The FIGO staging of endometrial cancer is, contrary to cervical cancer, based on surgical findings [5]. Pre-operative investigations following the histologically confirmed diagnosis, include systemic and gynaecological examinations, blood tests (FBC, U&E, LFT) and appropriate imaging such as CXR, ultrasound or magnetic resonance imaging (MRI). Tumour markers such as Ca-125 might be a useful marker for possible extra-uterine disease when values are raised [12-15]. The 2009 FIGO staging system for endometrial cancer is shown in Table 2 [1].

Table 2: FIGO 2009 staging of endometrial cancer

Stage	Definition
I	Tumour confined to the body of the uterus
IA	No or < 50% myometrial invasion
IB	≥ 50% myometrial invasion
II	Tumour confined to uterus with cervical stromal invasion
III	Local and/or regional spread
IIIA	Tumour invades serosa of uterus and/or adnexae
IIIB	Vaginal and or parametrial involvement
IIIC	Metastases to pelvic and/or parametrial involvement
IIIC1	Pelvic lymph node metastases
IIIC2	Para-aortic lymph node metastases with or without pelvic lymph node metastases
IV	Tumour invades bladder and/or bowel mucosa
IVA	Tumour invades bowel and/or bladder mucosa
IVB	Distant metastases including intra-abdominal structures and/or inguinal lymph nodes

3. Information on lymph node status

It is evident from the overview above that systematic regional lymphadenectomy is an essential part of the surgical management of most women with gynaecological cancers, as this is the most reliable method of obtaining information regarding the presence or absence of metastases in the regional lymph nodes. Lymphadenectomy is however not without morbidity and complications include lymph oedema, lymphocysts, vascular and neurologic injury, wound complications and bleeding [16-18]. In addition, the majority of women with early stage gynaecological cancers will not have lymph node metastases and will therefore not benefit from this procedure. Despite this fact, 10% to 15% of these women will have recurrent disease affecting the pelvis in up to 60% of cases [19].

The surgical management of vulva cancer is a good example of disease management that has evolved significantly during the past few decades, becoming much less radical with less procedure-related morbidity, but without affecting

oncological outcome. Traditionally, treatment of vulva cancer consisted of an en-block radical dissection of the vulva, bilateral inguinofemoral lymph nodes and excision of the bridging tissue between the vulva and the inguinal nodes. Treatment has become more individualised, consisting of wide local excision of the vulvar lesion with unilateral inguinofemoral lymph adenectomy in laterally situated tumours, and sentinel lymph node biopsy (SLNB) of the inguinofemoral nodes in small tumours [20].

Since Harry Reich performed the first laparoscopic assisted hysterectomy in 1989, minimally invasive surgery, associated with substantial benefits to women such as shorter hospital stay, less pain and quicker recovery times at the cost of longer operating times, has developed significantly [21]. This trend towards minimally invasive surgery has, to a certain extent, become the new standard in surgical management of women with early stage uterine cancers worldwide. Individualised cancer treatment is also increasing in relevance in modern-day management of gynaecologic malignancies of the uterus, yet further modifying the way these patients were traditionally treated.

Numerous publications since 1990 suggested the feasibility of minimally invasive surgical treatment of early stage endometrial and cervical cancer with similar survival outcomes to open surgery [22,23]. Oncological surgery is therefore moving towards less invasive surgery, associated with less morbidity, shorter hospital stay and quicker recovery in selected cases.

In the pursuit of finding the ever-crucial balance between treatment-related morbidity and optimal oncological outcomes, less morbid methods of reliably assessing regional lymph node status have been investigated. As already alluded to, sentinel lymph node biopsy in women with early stage unifocal squamous cell carcinoma of the vulva has become the standard of treatment in many institutions around the world, allowing for the safe omission of inguinofemoral lymph adenectomy in patients with sentinel lymph node biopsies negative for metastases [24]. Alternative options associated with less morbidity have also been investigated in the assessment of pelvic lymph nodes in women with uterine cancer.

4. Alternative assessment options of pelvic lymph nodes

4.1. Imaging

Clinical assessment of lymph nodes intra-operatively has a low sensitivity as approximately 40% of women with endometrial cancer and metastatic nodal disease have microscopic metastases, with nodes not clinically enlarged [25]. Pre-operative imaging with computed tomography (CT) scan and magnetic resonance imaging (MRI) also have limitations as these modalities require lymph nodes of 10 mm or more in size; also, no differentiation is possible between benign enlarged nodes and malignant nodes [26]. Positron emission tomography (PET) combined with CT scan (PET/CT) is a functional scan based on increased glucose metabolism by malignancies and can detect metastatic nodes up to 5 mm in size, but it also cannot reliably distinguish between malignancy and conditions such as tuberculosis (TB). PET/CT seems to be superior to CT and MRI in predicting pre-operative lymph node metastases [27].

4.2. Sentinel lymph node biopsy

The concept of the sentinel lymph node (SLN) was first described in 1960 in cancer of the parotid [28]. The philosophy of the sentinel node is that metastatic lymphatic spread of cancer occurs in an orderly or systematic fashion, first spreading to one node or group of nodes before involving nodes in the rest of the lymphatic chain. This principle allows for the ability to assess the status of the first node in the lymphatic chain known as the sentinel node, and use this information to predict the status of the rest of the lymphatic chain in that region without removing them (28, 29). Sentinel lymph node biopsy (SLNB) can therefore be used to determine local and regional lymph node involvement of cancers, by identifying a targeted sample, instead of performing a complete lymphadenectomy.

The SLNB has been used in the management of patients with penile cancer since 1977 [29], and has subsequently also been validated in patients treated for malignant melanoma and breast cancer [30,31].

SLNB has been investigated in gynaecological oncology [32] and it is now widely used in selected cases of women with early stage vulvar cancer [33]. Techniques for the detection of SLNs in endometrial and cervical cancer have also been described, with several publications suggesting the feasibility of an algorithm for SLNB in women with early stage cervical and endometrial cancer [34,35].

There is a clear trend towards less invasive surgical treatment of gynaecological malignancies in the quest to decrease morbidity associated with surgery and procedures that are not conveying significant benefit to the patient. At the same time, less invasive modalities with the main aim of avoiding morbidity should not be offered at the expense of disease free and overall survival. Individualisation of treatment to reduce therapy-associated morbidity is an important consideration in the surgical treatment of women with early stage cervical and endometrial cancer. SLN mapping and biopsy, and in particular the laparoscopic approach, is an important and potentially useful modality in cervical and endometrial cancer treatment. The relatively low rate of nodal metastasis in women with early stage cervical and endometrial cancer, necessitates investigating safe alternative and less invasive surgical treatment options to prevent morbidity associated with current standard surgical treatment options.

5. Research problem

There is very little to no data on the ability of imaging and SLNB procedures to predict the pelvic lymph node status in African or South African women with presumed early stage endometrial or cervical cancer. Findings on data published mainly from European and North American populations should be implemented only after validation in populations with significant differences in socio-economic and health status.

In addition to South Africa having one of the largest human immunodeficiency virus (HIV) infection rates worldwide, the population is also exposed to high rates of HIV associated infections such as pelvic inflammatory disease, tuberculosis (TB) and human papilloma virus (HPV) cervical infection. In 2012, approximately 12.2% of the South African population or 6.4 million persons were HIV-infected. The prevalence of

HIV infection is higher amongst females compared to males in all age groups (14.4% versus 9.9%), with the prevalence in Black African females of 31.6% [36]. In 2015, just more than one million people in South Africa were newly enrolled for HIV care, of which 11.7% or nearly 128 000 people were notified as TB cases [37]. The prevalence of high-risk HPV amongst the general female population is as high as 54.3% [38].

It is not known what the effects of these infections are on the lymphatic channels and the lymph nodes in these women. It is also evident that South African women face unique challenges, hence the contention that data from developed countries need to be verified before it can be applied in clinical practice in a country such as South Africa.

6. Aims and Objectives

6.1. Aims

6.1.1. To investigate the ability of PET-CT scan and the SLN algorithm to predict pelvic lymph node metastases in women with early stage endometrial cancer in a South African setting;

6.1.2. To investigate the ability of PET-CT scan, HPV DNA detection and the SLN algorithm to predict pelvic lymph node metastases in women with early stage cervical cancer in a South African setting.

6.2. Objectives

6.2.1. To establish the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the SLN algorithm in women with early stage cervical and endometrial cancer;

6.2.2. To establish the sensitivity, specificity, positive predictive value (PPV)

and negative predictive value (NPV) of PET-CT scan in women with early stage cervical and endometrial cancer;

6.2.3. To establish the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the combination of SLN algorithm and PET CT scan in women with early stage cervical and endometrial cancer;

6.2.4. To compare the detection rate of indocyanine green (ICG) versus blue dye versus ^{99m}Tc-Nanocolloid. Detection rates will be calculated on a patient and side-specific basis;

6.2.5. To investigate the presence and detection of hrHPV DNA in the primary cervical tumour and SLNs of women with cervical cancer;

6.2.6. To evaluate the ability of HPV DNA status and SLN histology to detect lymph node metastases;

6.2.7. To assess the ability of the combination of intra-operative frozen section examination (FSE) combined with HPV DNA detection in SLN to accurately predict regional lymph node status.

7. Materials and methods

7.1. Study population and setting

This was a prospective cohort study conducted at the Gynaecological Oncology Unit, Department Obstetrics and Gynaecology, University of Pretoria. The study was performed at Kalafong Provincial Tertiary Hospital (KPTH) and Steve Biko Academic Hospital (SBAH) during the period 1 April 2015 to 28 February 2017. The methods will be discussed in more detail in the relevant chapters.

Women with early stage cervical cancer (FIGO stage IA to IIA) and apparent early stage endometrial cancer (FIGO stage I and II) were eligible for recruitment

to the study. Recruitment was influenced by logistic considerations such as availability of methylene blue, appointments for lymphoscintigram and/or FDG PET/CT scan, which was performed on some women prior to surgical treatment.

Surgical treatment for cervical cancer patients consisted of laparoscopic or open radical hysterectomy and pelvic with or without para-aortic lymph adenectomy; for endometrial cancer patients laparoscopic or total abdominal hysterectomy, bilateral salpingo oophorectomy (BSO) and pelvic with or without para-aortic lymphadenectomy was performed. Laparoscopic or open SLNB procedure was performed on all women during these surgical procedures.

Sentinel lymph node detection was done using ^{99m}Tc-Nanocolloid and methylene blue labelling prior to surgery. The necessary equipment was made available on loan for a period of eight months during the study, and during this time indocyanine green (ICG) was also used in addition to methylene blue dye in a small number of patients. After identifying and removal of all SLNs, total pelvic lymphadenectomy was performed in addition to the appropriate type of hysterectomy with or without bilateral salpingo-oophorectomy. Para-aortic lymphadenectomy was performed at the discretion of the treating gynaecological oncologist. The SLN and the remainder of the pelvic lymph nodes were sent separately for histological examination.

Each sentinel lymph node was bisected into two halves. The one half was managed as a frozen section, while the other half was processed and stained using haematoxylin and eosin (H&E) as per normal routine. If after H&E examination the SLN was found to be negative for metastases, the rest of the node underwent ultrastaging.

In women with cervical cancer, HPV DNA was tested from the tumour as well as from swabs performed on the cut surface of the SLNs in those women where SLNs were detected and removed. A number of women in the study underwent pre-operative FDG-PET/CT scan to detect pelvic and para-aortic lymph node metastases.

7.2. Inclusion criteria

Patients aged 18 years and older, willing and able to provide informed consent, with any histological type FIGO stage IA1 with lymphovascular space invasion to stage IIA carcinoma of the cervix scheduled for primary surgical treatment, or diagnosed with any histological type endometrial carcinoma and after investigation appears to be stage I or II and who were scheduled for primary surgical treatment were eligible to partake in the study.

7.3. Exclusion criteria

Pregnant women, women with cervical cancer FIGO stage > IIA or women with endometrial cancer assumed to be stage III or IV, patients unfit for surgery, patients not willing or able to provide informed consent for the trail, and women with known allergies for ^{99m}Tc-Nanocolloid, contrast or blue dye were excluded from the study.

7.4. Data collection

The following data were collected:

Patient characteristics: age, ethnicity, BMI

Medical history: HIV status (CD4 count, viral load and treatment if applicable), history of TB, history of PID, history of STD (Chlamydia, gonorrhoea, genital warts), history of cone biopsy

Tumour characteristics: FIGO stage, lymphovascular space invasion (LVSI), size of tumour, histology

Surgical characteristics: grossly enlarged lymph nodes, adhesions (signs of previous PID), total blood loss during surgery

SLN characteristics: number of SLN found (hot and/blue), location of SLNs, unilateral/bilateral detection

Final histology characteristics: involvement SLN, involvement non-SLN, final histology cervix specimen final histology endometrial tumour, parametrial involvement, presence of grossly enlarged lymph nodes.

Adverse events: allergic reactions to technetium or blue dye

HPV DNA type of primary tumour and sentinel nodes

7.5. Statistical analysis

7.5.1. Sentinel lymph node biopsy

SLNs were considered positive if they contained macro metastases (tumour clusters larger than 2 mm), micro metastases (tumour clusters 0.2 - 2 mm in size), or isolated tumour cells (single tumour cells or tumour clusters smaller or equal to 0.2 mm in size).

7.5.2. Calculations

The detection rate was calculated as the number of patients with at least one detected pelvic SLN divided by the total number of patients who underwent SLN mapping. Diagnostic performance was calculated for hemi pelvises with at least one SLN harvested. Sensitivity was calculated as the proportion of true positives (patients with positive pelvic SLNs) among the patients with pelvic lymph-node metastases. NPV was calculated by dividing the number of true negatives (patients with negative pelvic SLNs) by the number of all patients without pelvic lymph-node metastases. Exact 95% confidence intervals (CI) for the proportions were calculated, and subgroup analysis was done using a two-sided χ^2 test or Fisher's exact test ($\alpha = 0.05$).

7.6. Sample size

A convenience sample size of 100 women was selected. A sample of at least 97 patients will estimate the 0.9 expected proportion of positive nodes (detection rate) with reference to the gold standard, with 95% confidence and an accuracy of 0.06. Detection rate, false negative rate and the negative predictive value (NPV) will be reported as percentages. A 95% confidence was determined for the detection rate.

7.7. Ethical considerations

Participation in the study was on a voluntary basis and all women who agreed to participate in the study provided informed consent.

Patients who declined participation were in no way disadvantaged and received standard treatment according to the guidelines of the Gynaecological Oncology Unit or as decided by the attending gynaecological oncology team.

Patients participating in the trial did not receive any financial or material benefits or rewards.

The University of Pretoria Faculty of Health Sciences Research Ethics Committee approved the study as well as amendments to the protocol (434/2014).

7.8. Definitions

The SLN detection rate was defined as the proportion of cases in which at least one SLN was identified among patients with attempted mapping.

Failed mapping refers to cases in which an SLN was not detected.

A true negative was defined as a negative SLN or algorithm in a patient with no nodal metastases on the side the SLN was detected.

A false- negative was a negative SLN or algorithm in a patient with nodal metastases.

A true-positive was defined as a positive SLN or algorithm in a patient with nodal metastases on the side the SLN was detected.

A false-positive was impossible by definition.

Sensitivity was calculated as the number of true positives divided by all patients with lymph node metastases.

The false-negative rate was the number of false-negatives divided by the number of patients with lymph node metastases.

Clinically, the false-negative rate refers to the detection of lymph node metastases in the completion LND when a SLN was excised and pathologically benign.

The negative predictive value was determined by dividing the number of true negatives by the number of patients with a negative test (SLN alone or algorithm).

8. Results

This section will provide the demographic data of the entire cohort, consisting of women with cervical cancer as well as women with endometrial cancer. The respective results of women with the two different types of cancer will be discussed in detail in Chapters 2 and 3 respectively.

One hundred women were recruited to the study between 1 April 2015 and 28 February 2017. Results for 94 women were available for interpretation. Seventy-eight women had cervical cancer and 22 women had endometrial cancer.

8.1. Demographic data

The demographic data is shown in Table 3.

Table 3: Demographic data

n = 100	Mean	SD	Range
Age	51.95	12.5	30 - 84
Parity	3.49	2.05	0 - 12
Gravidity	3.71	2.12	0 - 12
BMI	28.39	5.96	17.04 - 46.91

SD = Standard deviation;

8.2. HIV infection

All women were tested for HIV infection. Fifty women (50%) were HIV-infected. The mean CD4 count of this group was 423.8 cells/µl (SD = 226.34; SEM 32.33; range 14 - 950; 95% CI = 358.78 - 488.81). Five patients (5%) reported previous TB infection and 16 women (16%) reported previous PID.

8.3. Sentinel nodes

Sentinel lymph nodes were detected in 57 of 94 women for a SLN detection rate of 60.6%. The bilateral SLN detection rate was 29.2%. Twenty-nine women (30.8%) had clinically enlarged or palpable lymph nodes. The mean number of sentinel lymph nodes removed was 2.91 (SD = 1.80; SEM = 0.25; range = 1 - 90; 95% CI = 2.41 - 3.40). The mean pelvic lymph node count was 24.38 (SD = 9.99; SEM = 1.04; range = 1 - 57; 95% CI = 22.31 - 26.11). One patient with cervical cancer had extensive pelvic lymph node metastases and only one node was removed for histological confirmation. This patient did not undergo full planned surgical treatment. Para-aortic lymphadenectomy was performed in 13 women. The mean para-aortic lymph node count was 16.08 (SD = 7.85; SEM = 2.18; range = 7 - 28; 95% CI = 11.33 - 20.82).

8.4. Nodal metastases

Pelvic lymph node metastases were reported in 24 women (25.5%) and five women out of 13 (38.5%) had para-aortic lymph node metastases.

8.5. Sensitivity, specificity and detection rates

The sensitivity, specificity, negative and positive predictive values of the SLNB was 90%, 100%, 100% and 98.63% respectively.

The SLN detection rate for the first 40 patients was 62.5% and the detection rate of the second 60 patients was 56.6% (p = 0.5588). The bilateral detection rates were 37.5% and 23.33% respectively (p = 0.1280).

9. Discussion

The cohort of women with gynaecologic malignancies described in this dataset would usually be associated with post-menopausal women, but in this study, was found to be relatively young. The rate of HIV infection was surprisingly high at 50% with a mean BMI in the overweight category. The rate of 25% pelvic lymph node metastases in the group represented the higher end of what was expected in this cohort.

The SLN detection and bilateral SLN detection rates of 60.6% and 29.2% respectively were much lower than the published data in similar groups of women. The lower detection rate reported from this data for the entire cohort of women was not attributable to a learning curve, as there were no statistical differences in detection rates between the first 40 women and the last 60 women.

Issues influencing the SLNB and algorithm will be investigated in more detail in the remainder of this thesis.

Chapter 2 will discuss results of the patients treated for presumed early stage endometrial cancer.

The women treated for cervical cancer will be discussed in Chapter 3. This is a much larger cohort of women and provides a wide and in-depth range of comparative data of different subgroups within the cohort, examining different factors that may be involved in SLN detection.

The characteristics of FDG-PET/CT scan to accurately predict the regional nodes in the pelvises of women with endometrial and cervical cancer is investigated in Chapter 4.

In Chapter 5 the efficacy of HPV DNA detection in SLNs as an adjunctive test to predict the presence or absence of disease in the non-SLNs, is investigated.

In Chapter 6 we report the data of women who successfully mapped for SLNs, the distribution of these nodes and the performance of FSE and ultra-staging of SLNs.
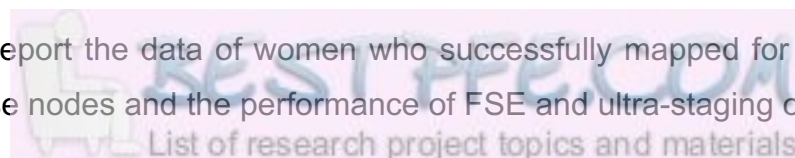
Chapter 7 summarises the findings and discusses suggestions for future research.

References

1. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet. 2009;105(2):103-4.

2. Delgado G, Bundy B, Zaino R, Sevin B-U, Creasman WT, Major F. Prospective surgical-pathological study of disease-free interval in patients with stage IB squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 1990;38(3):352-7.

3. Odicino F, Pecorelli S, Zigliani L, Creasman WT. History of the FIGO cancer staging system. Int J Gynecol Obstet. 2008;101(2):205-10.

4. Pecorelli S, Benedet JL, Creasman WT, Shepherd JH. FIGO staging of gynecologic cancer. 1994-1997 FIGO Committee on Gynecologic Oncology. International Federation of Gynecology and Obstetrics. Int J Gynaecol Obstet. 1999;64(1):5-10.

5. Benedet JL, Bender H, Jones H, 3rd, Ngan HY, Pecorelli S. FIGO staging classifications and clinical practice guidelines in the management of gynecologic cancers. FIGO Committee on Gynecologic Oncology. Int J Gynaecol Obstet. 2000;70(2):209-62.

6. Pecorelli S, Zigliani L, Odicino F. Revised FIGO staging for carcinoma of the cervix. Int J Gynaecol Obstet. 2009;105(2):107-8.

7. Quinn MA, Benedet JL, Odicino F, Maisonneuve P, Beller U, Creasman WT, et al. Carcinoma of the cervix uteri. FIGO 26th annual report on the results of treatment in gynecological cancer. Int J Gynaecol Obstet. 2006;95 Suppl 1:S43-103.

8. Tsunoda AT, Marnitz S, Soares Nunes J, Mattos de Cunha Andrade CE, Scapulatempo Neto C, Blohmer JU, et al. Incidence of histologically proven pelvic and para-aortic lymph node metastases and rate of upstaging in patients with locally advanced cervical cancer: results of a prospective randomized trial. Oncol. 2017;92(4):213-20.

9. Delgado G, Bundy B, Zaino R, Sevin BU, Creasman WT, Major F. Prospective surgical-pathological study of disease-free interval in patients with stage IB squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 1990;38(3):352-7.

10. Lee YN, Wang KL, Lin MH, Liu CH, Wang KG, Lan CC, et al. Radical hysterectomy with pelvic lymph node dissection for treatment of cervical cancer: a clinical review of 954 cases. Gynecol Oncol. 1989;32(2):135-42.

11. Tinga DJ, Timmer PR, Bouma J, Aalders JG. Prognostic significance of single versus multiple lymph node metastases in cervical carcinoma stage IB. Gynecol oncology. 1990;39(2):175-80.

12. Chung HH, Kim JW, Park NH, Song YS, Kang SB, Lee HP. Use of preoperative serum CA-125 levels for prediction of lymph node metastasis and prognosis in endometrial cancer. Acta Obstet Gynecol Scand. 2006;85(12):1501-5.

13. Hsieh CH, ChangChien CC, Lin H, Huang EY, Huang CC, Lan KC, et al. Can a preoperative CA 125 level be a criterion for full pelvic lymphadenectomy in surgical staging of endometrial cancer? Gynecol Oncology. 2002;86(1):28-33.

14. Kim HS, Park CY, Lee JM, Lee JK, Cho CH, Kim SM, et al. Evaluation of serum CA-125 levels for preoperative counseling in endometrioid endometrial cancer: a multi-center study. Gynecol Oncology. 2010;118(3):283-8.

15. Powell JL, Hill KA, Shiro BC, Diehl SJ, Gajewski WH. Preoperative serum CA-125 levels in treating endometrial cancer. J Reprod Med. 2005. 2005;50(8):585-90.

16. Franchi M, Ghezzi F, Riva C, Miglierina M, Buttarelli M, Bolis P. Postoperative complications after pelvic lymphadenectomy for the surgical staging of endometrial cancer. J Surg Oncol. 2001;78(4):232-7; discussion 237-40.

17. Matsuura Y, Kawagoe T, Toki N, Tanaka M, Kashimura M. Long-standing complications after treatment for cancer of the uterine cervix--clinical significance of medical examination at 5 years after treatment. Int J Gynecol Cancer. 2006;16(1):294-7.

18. Achouri A, Huchon C, Bats AS, Bensaid C, Nos C, Lecuru F. Complications of lymphadenectomy for gynecologic cancer. Eur J Surg Oncol. 2013;39(1):81-6.

19. Ho C-M, Chien T-Y, Huang S-h, Wu C-J, Shih B-Y, Chang S-C. Multivariate analysis of the prognostic factors and outcomes in early cervical cancer patients undergoing radical hysterectomy. Gynecol Oncol. 2004;93(2):458-64.

20. Hacker NF. Vulvar Cancer. In: Berek JS, Hacker NF, editors. Practical Gynecologic Oncology. Philadelphia, PA 19106 USA: Lippincott Williams & Wilkins; 2005. p. 549 - 7.

21. Reich H, De Caprio J, McGlyn F. Laparoscopic hysterectomy. J Gynecol Surg. 1989;5(2):213 - 6.

22. Bogani G, Cromi A, Uccella S, Serati M, Casarin J, Pinelli C, et al. Laparoscopic versus open abdominal management of cervical cancer: long-term results from a propensity-matched analysis. J Minim Invasive Gynecol. 2014;21(5):857-62.

23. Galaal K, Bryant A, Fisher AD, Al-Khaduri M, Kew F, Lopes AD. Laparoscopy versus laparotomy for the management of early stage endometrial cancer. Cochrane Database Syst Rev 2012;9. Art. No: CD006655. doi: 10.1002/14651858.CD006655.pub2.

24. Te Grootenhuis NC, van der Zee AG, van Doorn HC, van der Velden J, Vergote I, Zanagnolo V, et al. Sentinel nodes in vulvar cancer: long-term follow-up of the GROningen INternational Study on Sentinel nodes in Vulvar cancer (GROINSS-V) I. Gynecol Oncology. 2016;140(1):8-14.

25. Chuang L, Burke TW, Tornos C, Marino BD, Mitchell MF, Tortolero-Luna G, et al. Staging laparotomy for endometrial carcinoma: assessment of retroperitoneal lymph nodes. Gynecol Oncol. 1995;58(2):189-93.

26. Pelikan HM, Trum JW, Bakers FC, Beets-Tan RG, Smits LJ, Kruitwagen RF. Diagnostic accuracy of preoperative tests for lymph node status in endometrial cancer: a systematic review. Cancer Imaging. 2013;13(3):314-22.

27. Kitajima K, Murakami K, Yamasaki E, Fukasawa I, Inaba N, Kaji Y, et al. Accuracy of 18F-FDG PET/CT in detecting pelvic and paraaortic lymph node metastasis in patients with endometrial cancer. AJR Am J Roentgenol. 2008;190(6):1652-8.

28. Gould EA, Winship T, Philbin PH, Kerr HH. Observations on a "sentinel node" in cancer of the parotid. Cancer. 1960;13:77-8.

29. Cabanas RM. An approach for the treatment of penile carcinoma. Cancer. 1977;39(2):456-66.

30. Giuliano AE, Kirgan DM, Guenther JM, Morton DL. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. Ann Surg. 1994;220(3):391-8; discussion 8-401.

31. Morton DL, Wen DR, Wong JH, Economou JS, Cagle LA, Storm FK, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch Surg. 1992;127(4):392-9.

32. Levenback C, Burke TW, Gershenson DM, Morris M, Malpica A, Ross MI. Intraoperative lymphatic mapping for vulvar cancer. Obstet Gynecol. 1994;84(2):163-7.

33. Van der Zee AG, Oonk MH, De Hullu JA, Ansink AC, Vergote I, Verheijen RH, et al. Sentinel node dissection is safe in the treatment of early-stage vulvar cancer. J Clin Oncol. 2008;26(6):884-9.

34. Cormier B, Diaz JP, Shih K, Sampson RM, Sonoda Y, Park KJ, et al. Establishing a sentinel lymph node mapping algorithm for the treatment of early cervical cancer. Gynecol Oncol. 2011;122(2):275-80.

35. Barlin JN, Khoury-Collado F, Kim CH, Leitao MM, Jr., Chi DS, Sonoda Y, et al. The importance of applying a sentinel lymph node mapping algorithm in endometrial cancer staging: beyond removal of blue nodes. Gynecol Oncol. 2012;125(3):531-5.

36. Shisana O, Rehle T, Simbayi L, Zuma K, Jooste S, Zungu N, et al. South African national HIV prevalence, incidence and behaviour survey, 2012. Cape Town 2012. HSRC Press.

37. World Health Organization. Global tuberculosis report 2016. Switzerland: WHO; 2016. Available from: http://apps.who.int/iris/bitstream/10665/250441/1/9789241565394-eng.pdf?ua=1 [Accessed on 11 Oct 2017]

38. Richter K, Becker P, Horton A, Dreyer G. Age-specific prevalence of cervical human papillomavirus infection and cytological abnormalities in women in Gauteng Province, South Africa. South Afr Med J. 2013;103(5):313-7.

Chapter 2

Sentinel lymph node biopsy procedures in women with presumed early stage endometrial cancer

1. Introduction

Endometrial cancer is a common gynaecological cancer and in South African women it is the second most common cancer after cervical cancer. In countries with effective population based cervical cancer screening, endometrial cancer is the most common gynaecological cancer diagnosed and treated in gynaecologic oncology units [1]. The age standardised risk in South African women is estimated to be between 5.32 and 6.9/100 000 [2,3]. The estimated endometrial cancer lifetime risk of black South African females is 1:160, of Coloured women 1:125, while Asian and White women have lifetime risks of 1:76 and 1:130 respectively [4].

In developed countries roughly 70% of women with endometrial cancer are diagnosed with stage I disease, and the histological sub-type in more than 80% of cases are endometrioid adenocarcinoma [5]. Racial and population differences have been described [6,7]. Data from the University of Pretoria Gynaecologic Oncology Unit suggest local endometrial cancer figures are different from the developed world, with 62% of patients diagnosed with endometrioid histology and only 43% of women with FIGO stage I disease [8].

Surgery is the preferred treatment in women diagnosed with endometrial cancer. This involves hysterectomy, bilateral salpingo oophorectomy (BSO) and removal of pelvic and para-aortic lymph nodes. Most patients with high risk for recurrent disease, defined as endometrioid adenocarcinoma grade III and all other non-endometrioid histological sub-types, will undergo the abovementioned treatment. If para-aortic lymphadenectomy is performed it should be completed up to the level of the renal veins, as isolated positive para-aortic nodes between the aortic bifurcation and the inferior mesenteric artery is rare.

1.1. Lymphadenectomy in patients with presumed early stage endometrial carcinoma

In patients with presumed early stage low risk for recurrence disease, defined as endometrioid adenocarcinoma grade I and grade II, less than 50% myometrial invasion and tumour size less than 20 mm, lymphadenectomy can be omitted. The rationale is that if all three these parameters are present in the same patient, the risk for nodal metastases is less than 1% [9]. The caveat to consider is that the identification of this low risk group in the referenced study from the Mayo Clinic, was performed by means of very reliable intra-operative frozen section examination of the uterus, and lymphadenectomy was performed or omitted following information obtained from this frozen section examination. In most institutions frozen section examination is either not available or not as accurate and reliable as is the case at the Mayo Clinic who reported 89% accuracy of frozen section examination.

In the absence of intra-operative reliable and accurate frozen section examination, decisions on omission of lymphadenectomy is made on histological grade and pre-operative imaging with regard to tumour size and depth of myometrial invasion. This strategy however will result in missing the diagnosis of lymph node metastases in up to 11% of so-called low risk patients, and under staging of disease in up to 25% of cases [10-13]. In the absence of reliable intra-operative frozen section examination of the uterus, the diagnosis of low risk endometrial cancer becomes a retrospective diagnosis and theoretically means that all patients with endometrial cancer will require at least a full pelvic lymphadenectomy.

On the other hand, lymphadenectomy is associated with morbidity such as lymphocysts and lower limb oedema [14]. In addition, two prospective randomised trials and a Cochrane meta-analysis have failed to show survival benefit of lymphadenectomy in patients with endometrial cancer [15-17]. The low risk of lymph node metastases in FIGO stage I disease also implies that the vast majority of women will undergo this treatment unnecessary and without benefit if lymphadenectomy is performed as standard management in all patients with endometrial cancer.

The sentinel lymph node (SLN) algorithm has the potential to be a reasonable solution for the controversy regarding lymphadenectomy in women with possible low risk for recurrent endometrial cancer. It might also potentially provide a better alternative than complete omission of pelvic lymphadenectomy.

2. Literature overview

2.1. Sentinel lymph node biopsy and the sentinel lymph node algorithm

The idea of SLNs in women treated for endometrial cancer was first described by Burke et al in 1996 [18]. Isosulfan blue dye was injected into the fundus and posterior and anterior midline in the uteri of 15 women who underwent surgery for endometrial cancer. At least one SLN was detected in 67% of cases by means of this procedure.

In many populations more than 70% of women diagnosed with endometrial cancer will present with FIGO stage I disease. Pelvic lymph nodes will have metastases in 6% of women with FIGO stage IA moderately differentiated and 10% with poorly differentiated endometrioid adenocarcinomas respectively (5). These figures indicate that many women with presumed early stage endometrial cancer undergo lymphadenectomy without benefitting from the procedure.

There is no consensus on the role and place of lymphadenectomy in women treated for endometrial cancer. In addition, pelvic lymphadenectomy in these women has not been well-defined or standardised, and practices in this regard varies considerably, ranging from complete omission of lymph adenectomy in some cases based on pre-operative histological and imaging findings, to various practices of node sampling and systematic pelvic lymphadenectomy with or without para-aortic lymphadenectomy on all women. The extent of systematic lymphadenectomy varies from pelvic alone to pelvic plus para-aortic below the superior mesenteric artery or para-aortic up to the renal vessels.

The effect of para-aortic lymphadenectomy on survival in women with endometrial cancer remains unclear, with some data suggesting improved

survival [19]. Abu-Rustum et al published data on 1942 women treated for endometrial cancer and according to this prospectively maintained database only 1.6% of women with negative pelvic nodes will have positive para-aortic nodes. This low rate seems to be consistent for both low and high-grade histology-type tumours [20].

Ballester et al published the prospective multicentre SENTI-ENDO study in 2011 assessing SLNB in 113 women with FIGO stage I to II endometrial cancer [21]. Cervical injections with technetium the day before surgery and blue dye injected into the cervix pre-operatively after induction of anaesthesia were used and ultrastaging of negative SLNs was performed. Three patients had false negative SLNs giving a NPV of 97% (95% CI 91% - 99%) and a sensitivity of 84% (95% CI 62% - 95%). Using the hemi-pelvis as the unit of analysis, the NPV was 100% (95% CI 95% - 100%) and the sensitivity 100% (95% CI 63% - 100%). The long-term results of this trial were published in 2015 [22].

Levinson et al reviewed 19 studies on SLN dissection in women with endometrial cancer in 2013 [23]. Overall detection rates ranged from 62% to 100%, the false negative rate (FNR) ranged from 0% to 50%, and the NPV from 95% to 100%. No technique was superior with regard to the type of surgery modality, injection used, injection site, or pathology techniques.

Different injection sites for detection of SLNs in endometrial cancer have been investigated. These include the cervix, the fundus of the uterus as well as hysteroscopic peri-tumoural injections. Khoury-Collado et al published a literature review on lymphatic mapping in endometrial carcinoma in 2008 [24]. In seven studies using the subserosal myometrium of the uterine corpus as the injection site, SLN detection rates varied between 0% and 92%. Seven other studies employing the cervix as the injection site, reported detection rates varying between 84% and 100%, while in five studies reporting on the hysteroscopic peri-tumoural or endometrial injection, detection rates varied between 0% and 100%. The largest study in this review included 28 patients. In a prospective study of 80 women who had hysteroscopic injection of technetium followed by systematic pelvic and para-aortic lymph adenectomy, Solima et al observed a sensitivity of

26

90% (95% CI 55.5% - 95.5%) and an NPV of 98% (95% CI 89.4% - 100%) [25]. Other studies have also demonstrated less impressive figures at injection sites other than the cervix [26-28].

Barlin et al suggested an endometrial cancer SLN algorithm and determined its effectiveness in 498 patients with early stage endometrial cancer [29] (Figure 1). The algorithm is similar to the one used in cervical cancer, consisting of peritoneal washings, removal of all mapped SLNs with ultrastaging in nodes negative after H&E examination, removal of any suspicious enlarged nodes regardless of mapping, and side specific lymphadenectomy in the absence of SLN mapping on a hemi-pelvis. Para-aortic lymphadenectomy was performed at the discretion of the attending physician. Blue dye injected into the cervix was used as the only mapping method. At least one SLN was detected in 81% (n = 401) of women of which 96% mapped only to the pelvis and 0.5% had SLNs only in the para-aortic region; The FNR was 14.9%. Applying the endometrial cancer algorithm resulted in a sensitivity of 98.1%, a FNR of 1.9%, and an NPV of 99.8%.

According to results of a study from Raimond et al, SLN biopsy is able to detect lymphatic disease in three times more women compared to conventional standard lymphadenectomy [31]. In this retrospective multi-centre study involving 304 women, the SLN procedure detected disease in 16.2% versus 5.1% detected by lymphadenectomy. The difference was attributable to ultrastaging of negative SLNs following H&E examination.

Cormier et al published a systematic review on the SLN procedure in endometrial cancer. The review included seventeen studies with each more than 30 patients, totalling 1 572 patients, and overall detection rates ranged from 60% to 100%. In studies with more than 100 patients the overall detection rate was more than 80%. When the algorithm was applied retrospectively, the sensitivity was 95%, the negative predictive value was 99% and the false negative rate was 5% [32]. Lin et al published a systematic review and meta-analysis on 44 studies. The pooled overall detection rate was 83% with bilateral detection of 56% and the pooled sensitivity was 91% but the false negative rate was not reported [33]. A

systematic review and meta-analysis by Bodurtha Smith et al included 55 studies with 4 915 women and reported a detection rate of 81%, bilateral detection rate of 50% and sensitivity of 96%. The conclusion was that sentinel lymph node mapping may be considered an alternative standard of care in the staging of women with endometrial cancer [34]. Findings of the literature reviews and meta-analysis discussed here have also been confirmed by other reviews on this topic [35,35].

Peritoneal & serosal evaluation & washings

Retroperitoneal evaluation

Excision of all mapped SLN with ultrastaging

Any suspicious nodes must be removed regardless of mapping

If there is no mapping on a hemi-pelvis, a side-specific LND is performed

Paraaortic LND - at attending discretion
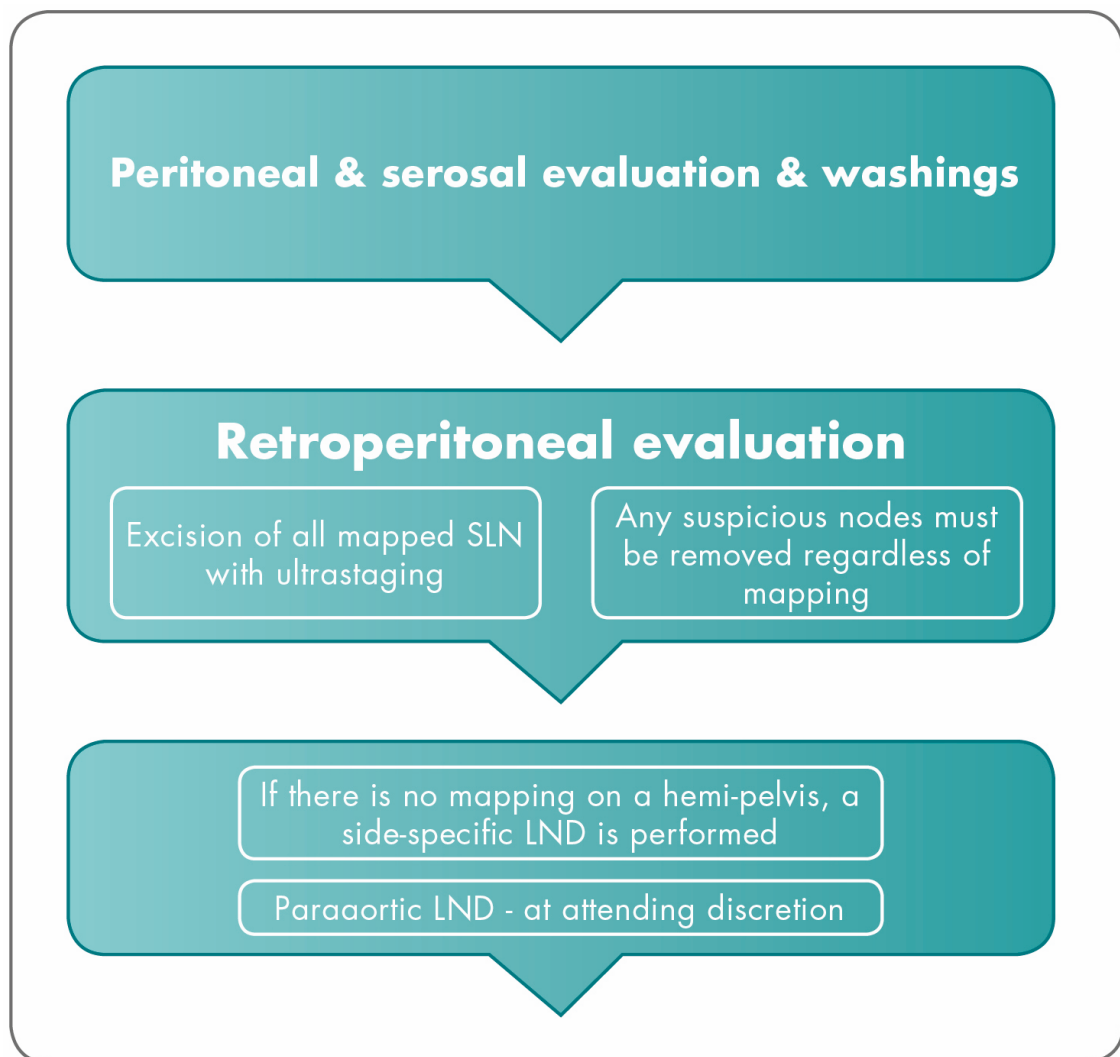
Figure 1: The sentinel lymph node algorithm for endometrial cancer [30]. (Reproduced with permission N Abu-Rustum)

Eighteen surgeons from ten centres participated in the FIRES multicentre prospective study where 385 patients with clinical FIGO stage I endometrial cancer were enrolled. Sentinel lymph node mapping with complete pelvic lymphadenectomy were performed in 340 women. The detection rate was 86%, sensitivity 97% and the negative predictive value was 99.6% [37]. Amant and Trum suggested in an editorial following the publication of the FIRES study, that the sentinel lymph node mapping procedure is the best compromise between complete versus no lymphadenectomy, and should be implemented in routine practice [38].

There is limited data on SLNB in women with high-risk endometrial cancer. Ehrisman et al performed SLN mapping prior to systematic lymphadenectomy in 36 women with high-risk endometrial cancer. Successful mapping was recorded in 83% and bilateral mapping in 56%. Metastatic nodes were identified in 25% of women [39]. Soliman et al prospectively performed SLNB in 123 women with high-risk endometrial cancer. The detection rate was 89% and the bilateral detection was 58%. They concluded that the SLN algorithm is a reasonable alternative to complete lymphadenectomy in high-risk presumed early stage endometrial cancer [40].

Over the past 15 years SLN biopsy in presumed early stage endometrial cancer has been gaining wider acceptance and may offer an alternative to complete pelvic lymphadenectomy in selected patients. Use of the surgical algorithm which integrates a side-specific evaluation, unilateral lymphadenectomy if no SLN is detected, ultrastaging and removal of clinical enlarged lymph nodes will increase the sensitivity and the NPV. It is reasonable to suggest that the sentinel lymph node algorithm might be a far safer oncological approach compared to not performing lymphadenectomy based on pre-operative histology and imaging information.

Sentinel lymph node mapping also provides the advantage of increased detection rate of nodal metastases since ultrastaging enables the detection of micro metastases and isolated tumour cells (ITC). In a study published by Holloway et al, in women who mapped, 30% had nodal metastases compared to findings from

pelvic lymphadenectomy where 14.7% had nodal metastases [41]. Other studies reported similar findings [42]. The effect of ITC on survival, the optimal adjuvant treatment and follow-up strategy in these patients are not clear yet [42,43].

The successful detection of SLNs involves, as with all surgical techniques, a learning curve, estimated to be around 30 cases [44]. The number of SLNs removed does not appear to influence the accuracy or FNR of the procedure [45].

The clinical outcome and risks associated with abandoning complete pelvic lymphadenectomy in cases with negative SLNs have not been studied in prospective trials.

A summary of publications on detection rates and accuracy of SLNB in endometrial cancer is provided in Table 1.

2.2. Tracers and injection sites for sentinel lymph node mapping

Published literature suggests that ICG has superior detection rates and bilateral detection rates as has been shown consistently in many studies, comparing ICG with blue dye, as well as literature reviews and meta-analysis. ICG alone is at least as accurate as the combination of blue dye and [99]Technetium [33,34,46-54].

Cervical injection of tracer seems to be the preferred site of injection [33,34,55,56]. Hysteroscopic injection into the fundus seems to increase the detection of para-aortic SLNs [32,56,57].

Multiple cervical injection techniques have been described. An array of injection sites, depths and volume of radio isotopic labelling with ^{99m}Tc-Nanocolloid, blue dye (Patent Blue®, Methylene Blue®, Isosulfan Blue® or Lymphazurin dye®) or a combination of the two have been used. None of the techniques resulted in a significantly better detection rate [23].

Table 1: Published literature on detection rates and accuracy of sentinel lymph node biopsy in endometrial cancer

First author	Year	Nr of Patients	Detection rate (%)	Sensitivity (%)	FNR (%)	NPV (%)
Soliman	2017	123	89%	95%	4.3%	98%
Rossi	2017	340	86%	97%	3%	99.6%
Ehrisman	2016	36	83%	NA	0	100%
Sawicki	2013	70	68 (97)	1/2 (50)	1/2 (50)	19/20 (95)
Torne	2013	67	55 (82)	12/13 (92)	1/13 (8)	42/43 (98)
How	2012	100	92 (92)	8/9 (89)	1/9 (11)	83/84 (99)
Buda	2012	35	32 (91)	3/3 (100)	0/3 (0)	22/22 (100)
Holloway	2012	35	35 (100)	9/10 (90)	1/10 (10)	25/26 (96)
Solima	2012	80	76 (95)	9/10 (90)	1/10 (10)	49/50 (98)
Barlin	2012	474	401 (85)	40/47 (85)	7/47 (15)	354/361 (98)
Ballester	2011	125	111 (89)	16/19 (84)	3/19 (16)	92/95 (97)
Mais	2010	34	21 (62)	3/3 (100)	0/3 (0)	18/18 (100)
Robova	2009	91	61 (67)	3/3 (100)	0/3 (0)	58/58 (100)
Bats	2008	43	30 (70)	8/8 (100)	0/8 (0)	22/22 (100)
Perrone	2008	40	27 (86)	6/6 (100)	0/6 (0)	21/21 (100)
Lopes	2007	40	31 (78)	5/6 (83)	1/6 (17)	25/26 (96)
Altgassen	2007	23	21 (91)	2/3 (66)	1/3 (33)	18/19 (95)
Li	2007	20	15 (75)	2/2 (100)	0/2 (0)	13/13 (100)
Delaloye	2007	60	49 (82)	8/8 (100)	0/8 (0)	41/41 (100)
Maccauro	2005	26	26 (100)	4/4 (100)	0/4 (0)	22/22 (100)
Niikura	2004	28	23 (82)	1/1 (100)	0/1 (0)	22/22 (100)
Holub	2004	25	21 (84)	2/2 (100)	0/2 (0)	19/19 (100)

FNR = False negative rate; NPV = Negative predictive value

The most commonly proposed technique consists of a 2 or 4-point peri-tumoural injection closest to the cervix-tumour interface with a 25-gauge spinal needle as shown in Figure 2. One-half of the volume should be injected deep into the stroma and the other half sub-mucosally with a total volume of 4 ml of blue dye and/or 0.1 - 0.5 mCi of ^{99m}Tc-Nanocolloid. Patients who have undergone a prior cone biopsy should be injected in the bed of the cone.

Injection of ^{99m}Tc-Nanocolloid may be performed the day prior to surgery or the morning of surgery. The blue dye should be injected in the operating theatre at the time of the examination under anaesthesia [58].

Data from the review published by Levinson suggest no difference in the detection rates between laparoscopic surgery and surgery performed via laparotomy in women with endometrial cancer [23].
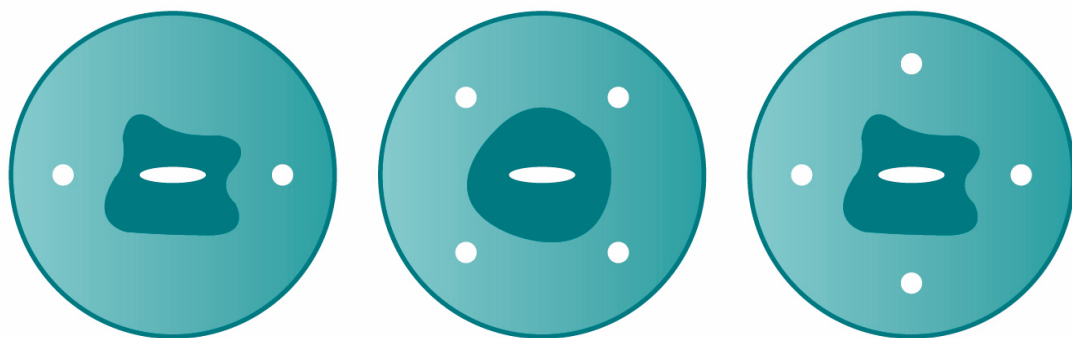
Figure 2: Options of sentinel lymph node cervical injection sites

2.3. Imaging of pelvic lymph nodes for detection of metastases

The identification of metastatic lymph nodes using CT scans and MRI has been based on nodal size, with a short axis diameter of more than 1 cm as the established criteria to diagnose cancer involvement. The widespread use of these two modalities is limited by their inability to detect metastases in normal sized nodes and the fact that they are unable to distinguish between malignant and reactive nodes. Functional imaging methods such as PET can establish

metabolic or functional parameters of tissue. Instead of using anatomical deviations to identify areas of abnormality, PET uses positron-emitting radiolabelled molecules to display molecular interactions of biological processes *in vivo*. The most commonly used radioisotope tracer is 18F-deoxy-glucose (FDG), a glucose analogue, which is preferentially taken up by and retained within malignant cells.

Recently, integrated PET/CT, in which a full-ring-detector clinical PET scanner and a multidetector helical CT scanner are combined, has enabled the acquisition of both metabolic and anatomic imaging data using one device in a single diagnostic session, providing precise anatomic localization of suspicious areas of increased FDG uptake and eliminating false-positive PET findings [59].

In 2008 Kitajima et al published the results of a study investigating the accuracy of FDG-PET/CT in 40 women with endometrial cancer FIGO stages I – III [60]. The overall patient based sensitivity was 50%, the specificity 86.7% and the accuracy was 77.5%.

Signorelli et al reported on the diagnostic accuracy of FDG-PET/CT in 37 women with high-risk early endometrial cancer in a prospective study [61]. The patient-based sensitivity, specificity, positive predictive value, negative predictive value and accuracy of FDG-PET/CT for detection of nodal disease were 77.8%, 100.0%, 100.0%, 93.1% and 94.4%, respectively.

A meta-analysis by Chang et al included seven studies involving 243 women on the ability of FDG-PET/CT to detect metastatic lymph nodes in endometrial cancer cases [62]. The overall pooled estimates for sensitivity and specificity of FDG-PET or FDG-PET/CT scans in the detection of pelvic and/or para - aortic metastases were 63.0% (95% CI, 48.7% - 75.7%) and 94.7% (95% CI, 90.4% - 97.4%), respectively. The positive likelihood ratio was 10.465 (95% CI, 5.646 - 19.396) and the negative likelihood ratio 0.399 (95% CI, 0.284 - 0.560). The overall diagnostic accuracy was 89.5%.

The conclusion from these and other studies is that FDG-PET/CT alone is not reliable enough to use as a single test to accurately predict lymph node metastases in women with early stage endometrial and cervical cancer [63].

3. Aims and objectives

3.1. Aims

3.1.1. To investigate the ability the SLN algorithm to predict pelvic lymph node metastases in women with early stage endometrial cancer in a South African setting.

3.2. Objectives

3.2.1. To establish the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the SLN algorithm in women with presumed early stage endometrial cancer

3.2.2. To establish the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of PET-CT scan in women with presumed early stage endometrial cancer

3.2.3. To compare the detection rate of ICG versus blue dye versus ^{99m}Tc-Nanocolloid. Detection rates will be calculated on a patient and side-specific basis

4. Materials and methods

4.1. Setting and study population

This was a prospective cohort study conducted at the Gynaecological Oncology Unit, Department Obstetrics and Gynaecology, University of Pretoria. The study

was performed at the Kalafong Provincial Tertiary Hospital (KPTH) and Steve Biko Academic Hospital (SBAH).

Women with apparent early stage endometrial cancer (FIGO stage I and II) were eligible for recruitment to the study. Depending on availability and logistic considerations, FDG-PET-CT scan were performed on some women prior to surgical treatment which consisted of laparoscopic or total abdominal hysterectomy, bilateral salpingo oophorectomy (BSO) and pelvic with or without para-aortic lymph adenectomy for women with endometrial cancer. Laparoscopic or open SLNB procedures were performed during these surgical procedures.

4.2. Inclusion criteria

All patients aged 18 years and older, willing and able to provide informed consent diagnosed with any histological type endometrial carcinoma and after investigation appears to be FIGO stage I or II and who were scheduled for primary surgical treatment were eligible for recruitment to the study.

4.3. Exclusion criteria

Women with endometrial cancer assumed to be FIGO stage III or IV, those who were regarded as unfit for surgery, patients not willing or able to provide informed consent for the trail, or patients with known allergy for ^{99m}Tc-Nanocolloid contrast, ICG or methylene blue were excluded from recruitment.

Recruitment was done taking into consideration the availability of tracers for injection, appointments for lymphoscintigram and or FDG-PET/CT scan.

4.4. Pre-operative evaluation

Women with presumed early stage endometrial cancer scheduled for surgery were investigated according to the standard protocol of the gynaecological oncology unit. Standard staging investigations included histology of the tumour, chest X-Rays, ultrasound of the pelvis and abdomen, vaginal ultrasound to assess depth of myometrial invasion and tumour size, full blood count, liver function tests, urea & electrolytes, Ca-125 level, syphilis test and HIV test. CD4

counts were assessed in HIV-positive women. Possible vaginal, paracervical and parametrial invasion were assessed by clinical examination.

4.5. Positron emission tomography/computed tomography

Some women scheduled for surgery underwent pre-operative FDG-PET/CT scan with the aim of assessing possible pelvic and para-aortic lymph node metastases. Women with contra-indications for FDG-PET/CT were not excluded from the rest of the study.

4.6. [99]Technetium and lymphoscintigraphy

A number of women scheduled for surgery had lymphoscintigraphy the day before surgery following intra-cervical injection with [99]Tc.

4.7. Intra-operative lymph node mapping

Sentinel lymph node detection was done using ^{99m}Tc-Nanocolloid, ICG and blue dye labelling (methylene blue) prior to surgery. ^{99m}Tc-Nanocolloid was injected in the cervix of women scheduled for surgery one day before the procedure, and blue dye and ICG were injected pre-operatively after induction of general anaesthesia. After identifying and removal of all SLNs, a total pelvic lymph node dissection was performed in addition to the appropriate type of hysterectomy with or without bilateral salpingo-oophorectomy. Para-aortic lymph adenectomy was performed at the discretion of the treating gynaecological oncologist.

The SLN and the remainder of the pelvic lymph nodes were sent separately for histological examination. Each sentinel lymph node was bisected into two halves. One half underwent frozen section examination, while the other half was processed and stained using H&E as per normal routine. If after H&E examination the SLN was found to be negative for metastases, the second half of the node was ultra-staged by performing additional level sections as well as immunohistochemistry staining.

After induction of general anaesthesia, a combined superficial (1 - 3 mm) and deep (1 - 2 cm) cervical injection (1 ml x 2 superficial and 1 ml x 2 deep for a total of 4 ml) of blue dye solution (Methylene Blue 1%) was injected at the 3 and 9

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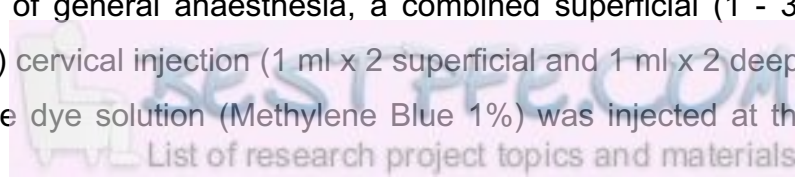
o'clock positions of intact-appearing epithelium of the uterine cervix. This was followed by injection of 4 ml of ICG in a similar fashion as described above in a smaller number of patients.

Intra-operatively the pelvic (and para-aortic) lymph nodes were examined for hot and/or blue lymph nodes. ICG stained nodes were identified using the Karl Storz near infrared fluorescent filter. The examination started before opening the retro-peritoneal space by tracking possible blue stained lymph nodes/vessels or green lime coloured ICG stained nodes using the described technology to show the green coloured nodes, and by examining for any hot nodes (hot nodes are defined as a radioactive count of more than 5 times the background count *in vivo* and more than 10 times *ex vivo*) using the laparoscopic or handheld gamma probe, through the intact peritoneum. Dissection commenced after mapping and recording any blue nodes, green nodes, vessels and/or hot nodes. Opening of the broad ligament and careful dissection of the broad ligament searching for a blue and or green stained lymph vessel or node was identified, tested with the gamma probe and removed. Blue and/or hot nodes identified were removed and send for histological examination separately with the position and radioactive count (if applicable) stated.

4.8. Surgical treatment

After removal of the SLNs, the remainder of the surgical procedure namely total abdominal hysterectomy, bilateral salpingo oophorectomy and pelvic lymph adenectomy for endometrial cancer were completed as per routine of the gynaecological oncology unit. Para-aortic lymphadenectomy was performed in the presence of macroscopic suspicious pelvic or para-aortic lymph nodes, at the discretion of the attending gynaecological oncologist and in patients with suspicious nodes pre-operatively identified on PET/CT.

Laparoscopic total hysterectomy and BSO with pelvic lymph adenectomy for endometrial cancer were performed at Kalafong Provincial Tertiary Hospital. Pelvic adhesions were recorded if present, for both open and laparoscopic procedures. The surgeon's impression of previous PID was noted. Complete pelvic lymphadenectomy was performed, as well as lymphadenectomy of any

other site containing at least one SLN (pre-sacral, common iliac and para-aortic basin). These lymph nodes were sent for histological examination as non-SLNs with stating the site of origin.

4.9. Histological evaluation

After intra-operative identification and removal of mapped SLNs each node was divided into two halves through the longitudinal axis. One half was sent for frozen section examination and the other half for routine H&E staining and ultrastaging if negative. All SLNs were labelled and couriered to the pathology laboratory immediately after removal and preservation to reach the laboratory within two hours after removal.

4.9.1. Frozen section examination

All SLNs collected underwent frozen section examination (FSE). For the purpose of the study and for logistical reasons, FSE were performed in the laboratory and not in the theatre. Specimens for FSE were placed in a container with a saline drenched swab and were not preserved in formalin.

The lymph node was submitted for H&E evaluation after frozen section procedure and routine processing.

4.9.2. Routine haematoxylin and eosin

All SLNs were marked and sent separately for histological evaluation. SLNs were divided in two parts. One part was examined using routine haematoxylin and eosin (H&E) staining. Ultrastaging was performed in all cases where the SLN was reported to be negative following FSE and routine H&E staining. Ultrastaging consisted of cutting two adjacent 5-μm sections from each paraffin block at two levels 50 μm apart. At each level, one side was stained with H&E and the other with immunohistochemistry (IHC) using the anti-cytokeratin AE1:AE3 (DAKO) for a total of five slides per block [11,64].

The presence of macro metastases, micro metastases and isolated tumour cells were recorded. Women with micro metastases and isolated tumour cells were regarded as having nodal metastases and were considered for

appropriate post-operative adjuvant treatment.

4.10.   Ethical considerations

Participation in the study was on a voluntary basis and all women who agreed to participate in the study provided informed consent.

Patients who declined participation were in no way disadvantaged and received standard treatment according to the guidelines of the Gynaecological Oncology Unit or as decided by the attending gynaecological oncology team. Patients participating in the trial did not receive any financial or material benefits or rewards.

The University of Pretoria Faculty of Health Sciences Research Ethics Committee approved the study and amendment to the protocol (434/2014)

5. Results

One hundred patients were recruited to the study, of which 22 patients were diagnosed with endometrial cancer.

5.1. Demographic data

The demographic data are shown in Table 2. All 22 patients were HIV negative. Five patients (22.7%) had previous surgery performed on them and five (22.7%) had diabetes mellitus.

5.2. Disease characteristics

The pre-operative histological sub-types are shown in Figure 3, and the post-operative histological sub-types in Figure 4. Post-operative histology displayed endometrioid histological sub-type present in eight women (36%), while six women (27%) had serous or papillary serous histology.

Five of ten patients (22.7%) with endometrioid histology were classified as FIGO grade III, three (13.6%) as FIGO grade II and two (9.1%) as FIGO grade I. Of the 22 patients, 17 (77.3%) had high-risk histological sub-types.

Table 2: Demographic data of women treated for presumed early stage endometrial cancer

n = 22	Mean	SD	Range
Age	68.05	8,168	54 - 85
Parity	4.4	2.73	0 - 12
Gravidity	4.6	2.55	0 - 12
BMI	31.69	7.038	17.04 - 46.91

SD = Standard deviation
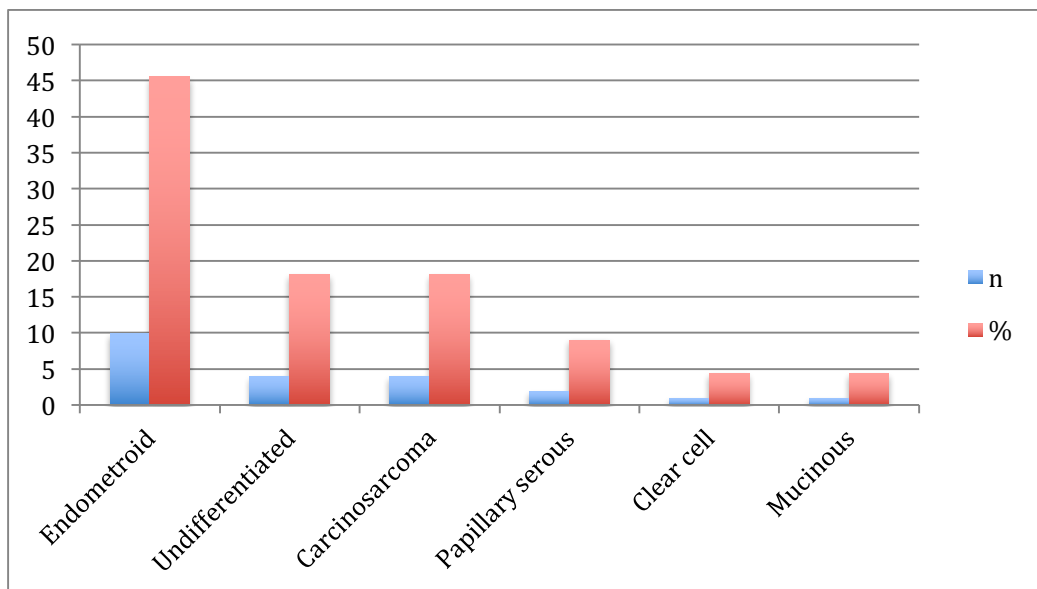
Figure 3: Pre-operative histology of women treated with presumed early stage endometrial cancer
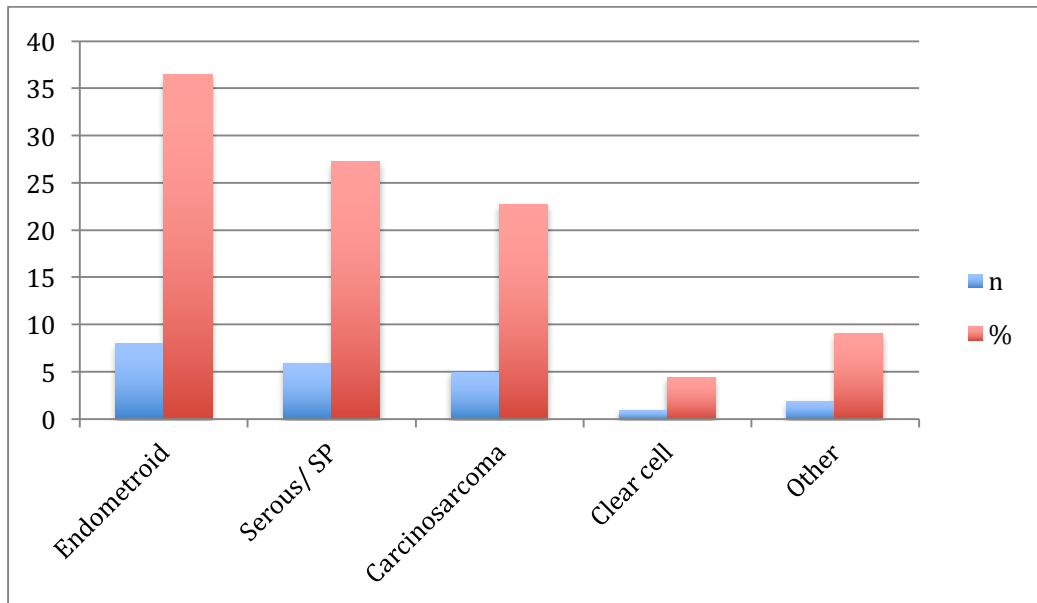
Figure 4: Post-operative histology of women treated with presumed early stage endometrial cancer

In ten patients (45.5%) the pre-operative histological subtype and grade were concordant with the post-operative histological subtype and grade. In three patients (13.6%) the pre- and post-operative histological subtypes were concordant, but they were discordant for histological grade. All three these cases were endometrioid histology, and in two cases the pre-operative grade changed from FIGO grade 3 to grade 2 and in one case from FIGO grade 1 to grade 2. In nine women (40.9%) the pre-operative histological subtype differed from the post-operative histological subtype as is shown Table 3.

The mean tumour diameter was 64.77 mm (range 20 - 95 mm; SD = 19.48; SEM = 4.15; 95% CI = 56.14 - 73.41. Twelve patients (54.5%) had FIGO stage I disease, and 6 women (27.3%) had FIGO stage III disease. The FIGO stage distribution is shown in Figure 5.

Ca-125 values were available in 18 women. The mean Ca-125 value was 18.61 (range 4 - 96; SD = 21.21; SEM = 5.00; 95% CI = 8.06 - 29.16). Of the ten women (45.5%) who had more than FIGO stage I disease, the Ca-125

value was elevated in one woman (10%) who had FIGO stage IIIC2 disease. Nine women with more than FIGO stage I disease had normal Ca-125 levels.

Table 3: Pre- and post-operative discordant histological subtypes of women treated for presumed early stage endometrial cancer

Study nr	Pre-operative		Post-operative		Treatment affected?
	Histology	Grade	Histology	Grade	
63	Carcinoma	3	Endometrioid	3	No
65	Endometrioid	3	Serous	3	No
68	Carcinoma	3	Leiomyosarcoma	3	No
70	Mucinous	3	Endometrioid	Unknown	Unsure
71	Carcinoma	3	Serous	3	No
72	Endometrioid	3	Endometrioid	2	Yes
73	Endometrioid	3	Endometrioid	2	Yes
74	Endometrioid	1	Endometrioid	2	No
78	Endometrioid	3	Carcinosarcoma	3	No
81	Endometrioid	3	Serous	3	No
83	Endometrioid	2	Serous	3	Yes
80	Undifferentiated		Serous	3	No
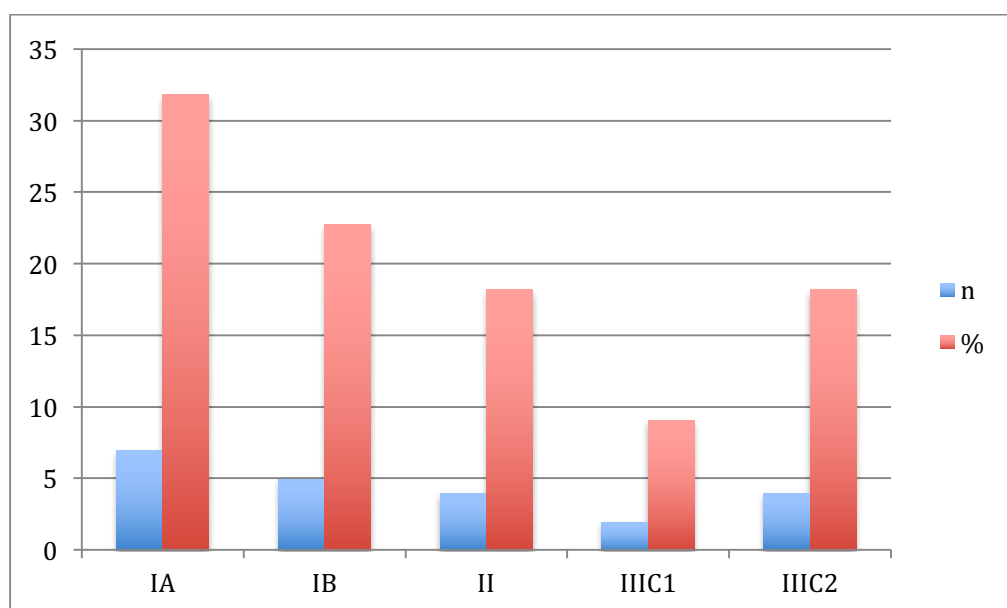
Figure 5: Stage distribution of women with endometrial cancer

Laparoscopic surgery was performed in three cases (13.6%) and 19 patients (86.4%) underwent open surgery. All 22 patients (100%) had full bilateral pelvic lymphadenectomy, while nine (40.9%) had para-aortic lymphadenectomy as well. Seventeen patients (77%) had high-risk histological sub-types and were eligible for para-aortic lymphadenectomy according to Unit guidelines. Para-aortic lymphadenectomy were performed in nine women (53%) in this group. The mean numbers of lymph nodes removed from the different regions are shown in Table 4.

Table 4: Mean number of lymph nodes removed in women with endometrial cancer

	Mean	SD	Range
Left pelvic n = 22	11.73	5.70	4 – 22
Right pelvic n = 22	11.27	4.32	5 – 21
Total pelvic n = 22	23	9.13	9 – 43
Para-aortic n = 9	17	8.03	7 – 28

SD = Standard deviation

5.3. Sentinel lymph node detection

Methylene blue (MB) was administered to all 22 patients with endometrial cancer. Seven patients (31.8%) also received intracervical administered ^{99m}Technetium nanocolloid tracer (^{99m}Tc) and lymphoscintigram examinations, while two patients (9.1%) were administered Indocyanine green (ICG) as well. One of the patients receiving ICG had methylene blue and ^{99}Technetium and one had methylene blue and ICG.

Sentinel lymph nodes were detected in ten cases (45.4%). Bilateral detection was achieved in four cases (18.2%). A total of 22 pelvic SLNs were removed, of which ten were located on the left side and twelve on the right side. The mean SLN

count was 2.2 (Range 1 - 3; SD = 0.79; SEM = 0.25; 95% CI 1.64 - 2.76). There were no para-aortic SLNs detected.

Nine SLNs (90%) were detected with MB. ICG successfully mapped in all three patients it was administered to, and detected one case that was not mapped using MB. ^{99m}Tc detected sentinel nodes in three patients (13.6%). Table 5 shows more information on how SLNs were detected.

Table 5: Detection of SLNs in women with endometrial cancer

Method	Number of SLNs detected
MB alone	9
^{99m}Tc alone	3
MB + ^{99m}Tc	2
ICG alone	3
MB + ICG	2

In three patients (13.6%) with metastatic nodes the sentinel nodes also showed metastatic disease, and in 11 patients (50.0%) with no lymph node metastases the sentinel lymph nodes were also negative. In no patient were the sentinel lymph node negative and the rest of the pelvic nodes positive for metastatic disease, indicating that there were no false negative sentinel lymph nodes.

5.4. Lymph node disease status

All 22 women with endometrial cancer underwent bilateral systematic pelvic lymphadenectomy. Seven women (31.8%) had metastatic disease in the pelvic nodes, of which two (9.1%) had unilateral metastatic disease. Seventeen women (77.3%) were candidates for systematic pelvic and para-aortic lymphadenectomy according to the Unit protocol. In this group, para-aortic lymphadenectomy up to the level of the renal veins was performed in nine women (52.9%), of which three (33.3%) had para-aortic lymph node metastases. No patient had isolated para-aortic lymph node metastases.

Seven women (31.8%) had grossly enlarged pelvic lymph nodes. Two of the nine women (22.2%) who underwent para-aortic lymphadenectomy had grossly enlarged lymph nodes. No sentinel nodes were detected in any of the patients with grossly enlarged lymph nodes. In seven women with grossly enlarged lymph nodes, three (42.9%) had metastatic disease in the lymph nodes. The histological findings of patients with grossly enlarged lymph nodes without histological evidence of metastatic disease is shown in Table 6. One of these patients was diagnosed with Kaposi sarcoma in the right pelvic lymph nodes.

Table 6: Histological findings of enlarged lymph nodes without metastases in women with endometrial cancer

Study number	Histology
72	Reactive follicular hyperplasia
74	Normal histological findings
75	Reactive changes
77	Reactive changes Kaposi sarcoma

5.5. [18]Fluoro-deoxy-glucose positron emission tomography / computed tomography scan

FDG-PET/CT scans were performed in four patients (18.2%), but none of these scans detected any suspicious or positive lymph nodes in any of the patients. One of the four patients had lymph node metastases that were not detected by FDG-PET/CT scan.

5.6. Sensitivity, specificity, positive and negative predicted values for pelvic sentinel lymph nodes

These calculations were made using hemi-pelvises. In 22 patients there were 44 hemi-pelvises of which 10 had metastatic disease and 34 had no metastatic disease. There were three true positive and 11 true negative SLNs. The sensitivity, specificity, positive and negative predicted values are shown in Table 7.

Table 7: Sensitivity, specificity, positive and negative predictive values for pelvic sentinel lymph nodes in women with endometrial cancer

	Value
Sensitivity	100%
Specificity	100%
Positive predictive value	100%
Negative predictive value	100%

CI = Confidence interval

5.7. Sensitivity, specificity, positive and negative predictive value for the sentinel lymph node algorithm

The sentinel lymph node algorithm would have resulted in systematic full pelvic lymphadenectomy in 30 out of 44 hemi-pelvises (68%). Full systematic pelvic lymphadenectomy would have been performed in 12 patients (54.5%), while six patients (27.3%) would have received unilateral full pelvic adenectomy. In four patients (18.2%) sentinel lymph node biopsy would have been sufficient and full pelvic lymphadenectomy could have been avoided.

These calculations were also performed for the algorithm and the information is shown in Table 8.

Table 8: Sensitivity, specificity, positive and negative predictive values for the pelvic sentinel lymph node algorithm in women with endometrial cancer

	Value
Sensitivity	100%
Specificity	100%
Positive predictive value	100%
Negative predictive value	100%

CI = Confidence interval

6. Discussion

The proportion of women with endometrial cancer in this study was relatively small. This reflects the burden of disease due to cervical cancer in South Africa as well as the Pretoria Gynaecologic Oncology Unit, which is a much larger problem compared to endometrial cancer with regard to absolute numbers. In developed countries cervical cancer is much less common and endometrial cancer more common, largely due to effective cervical cancer screening strategies and implemented screening programmes [65].

The mean age of this group corresponded well with data in published literature, as this is mainly a disease of post-menopausal women [65].

The significant discordance between pre-operative and post-operative histological diagnosis observed in this study in women with endometrial cancer is consistent with other data from our unit. Data from more than 100 patients treated over five years showed that pre- and post-operative histological sub-types were concordant in 61% of cases [8]. This issue limits decision making with regard to surgical treatment options, and does not allow omission of pelvic lymphadenectomy based on histological sub-type, with many institutions omitting lymphadenectomy in women with grade 1 or grade 2 histology sub-types.

The reasons why para-aortic lymphadenectomy was not performed on all patients where this was indicated were not investigated. The ultimate decision lies with the treating gynaecologists and is influenced by technical issues as well as patient co-morbidities.

The histological sub-type distribution is substantially different from international published data, where it is reported that up to 84% of women are diagnosed with endometrioid histological sub-type [5]. In this study it was only 36% of cases, while the five-year audit done in the Pretoria Gynaecologic Oncology Unit showed 62% of endometrial cancer patients had endometrioid sub-type histology [8].

Stage distribution of patients in this study also differed from that reported in the international literature, where it is reported about 71% of women are diagnosed as having FIGO stage I disease [5]. In this study around 55% of cases was having

stage I disease with stage III in as many as 27%. Data from our unit shows 43% of patients are diagnosed with FIGO stage I disease and 27% with FIGO stage III disease [8].

Other published data suggest significant differences in stage as well as histologic sub-type distribution in Black women [66]. A South African study published by Cronje et al showed more advanced disease and more high-risk histological sub-types in black South African women compared to their white counterparts [7].

Laparoscopic surgery has become the accepted surgical treatment of women with endometrial cancer. The Pretoria Gynaecologic Oncology Unit policy prescribes open surgery in women with high-risk histology sub-types as well as para-aortic lymphadenectomy in these women, and for this reason the majority of women underwent open surgery.

In this study 32% of women had pelvic lymph node metastases. This reflects the stage distribution and the high proportion of women with high-risk histological sub-types. The risk of nodal metastases depends on the depth of invasion as well as the histological sub-type of the endometrial cancer present in the patient [5,10]. No patient had isolated para-aortic nodes.

Enlarged lymph nodes did not reliably predict the presence of lymph node metastases, as only 43% of women with enlarged nodes had lymph node metastases. Other studies investigating this issue reported similar results. Not only are enlarged nodes unreliable, but metastases are also frequently present in nodes that appear to be macroscopically normal [67-69].

The detection rate of at least one sentinel lymph node in this group of women was much lower than what is reported in the international published literature. Sentinel nodes were detected in 45% of women and the bilateral detection rate was even lower at 18.2 %. The published detection rates for at least one sentinel lymph node is around 80% with bilateral detection rates of around 56% [32,33]. This group was not large enough to allow sub-group analysis.

In the small sample size, the sensitivity, specificity, positive and negative predictive values were 100% for all these parameters and the false negative rate was zero.

7. Conclusion

The small sample of women with endometrial cancer that was part of the 100 women recruited to this study represented a group of women with high-risk endometrial cancer with regard to stage distribution and histological sub-type.

Sentinel lymph node detection rate and bilateral detection rate was significantly lower than what is recorded in the international literature, but the reasons for this are unsure.

It is important to consider the different risk profile of black women diagnosed with endometrial carcinoma when applying sentinel lymph node procedures, as these procedures were mainly researched in women with a different stage and histological sub-type distribution of endometrial cancer. At least two published studies support the use of sentinel lymph node procedures in women with endometrial cancer with high-risk histology sub-types (39, 40). More research is necessary with regard to the effectiveness and reliability of sentinel lymph node procedures in African women with endometrial cancer, as this population have a different risk profile compared to women living in well-resourced countries.

The clinical value of the SLNB algorithm was not evaluated in this study, and therefore no findings about clinical outcomes can be made.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87-108.

2. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0 Cancer incidence and mortality worldwide: IARC CancerBase No. 11 [Internet] Lyon France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr. [Accessed on 11 Oct 2017]

3. The NIOH SA National Cancer Registry 2013: summary statistics of cancer diagnosed histologically in 2013. Available from: http://www.nioh.ac.za/assets/files/2013NCR.pdf [Accessed on 11 Oct 2017]

4. Herbst M. CANSA Fact sheet on the top ten cancers per population group South Africa 2017. Available from: http://www.cansa.org.za/files/2017/03/Fact-Sheet-Top-Ten-Cancers-per-Population-Group-in-SA-NCR-2012-web-Feb-2017.pdf [Accessed on 11 Oct 2017]

5. Creasman WT, Odicino F, Maisonneuve P, Quinn MA, Beller U, Benedet JL, et al. Carcinoma of the corpus uteri. FIGO 26th Annual report on the results of treatment in gynecological cancer. Int J Gynaecol Obstet. 2006;95 Suppl 1:S105-43.

6. Oliver KE, Enewold LR, Zhu K, Conrads TP, Rose GS, Maxwell GL, et al. Racial disparities in histopathologic characteristics of uterine cancer are present in older, not younger blacks in an equal-access environment. Gynecol Oncol. 2011;123(1):76-81.

7. Cronje HS, Fourie S, Doman MJ, Helms JB, Nel JT, Goedhals L. Racial differences in patients with adenocarcinoma of the endometrium. Int J Gynaecol Obstet. 1992;39(3):213-8.

8. Snyman LC, Barnard D, Dreyer G. Audit of endometrial cancer patients treated at the Gynaecology Oncology Unit, University of Pretoria. Int J Gynecol Cancer. 2014;24(4):1574.

9. Mariani A, Dowdy SC, Cliby WA, Gostout BS, Jones MB, Wilson TO, et al. Prospective assessment of lymphatic dissemination in endometrial cancer: a paradigm shift in surgical staging. Gynecol Oncol. 2008;109(1):11-8.

10. Milam MR, Java J, Walker JL, Metzinger DS, Parker LP, Coleman RL. Nodal metastasis risk in endometrioid endometrial cancer. Obstet Gynecol. 2012;119(2 Pt 1):286-92.

11. Kim CH, Khoury-Collado F, Barber EL, Soslow RA, Makker V, Leitao MM, Jr., et al. Sentinel lymph node mapping with pathologic ultrastaging: a valuable tool for assessing nodal metastasis in low-grade endometrial cancer with superficial myoinvasion. Gynecol Oncol. 2013;131(3):714-9.

12. Abu-Rustum NR, Khoury-Collado F, Pandit-Taskar N, Soslow RA, Dao F, Sonoda Y, et al. Sentinel lymph node mapping for grade 1 endometrial cancer: is it the answer to the surgical staging dilemma? Gynecol Oncol. 2009;113(2):163-9.

13. Bleu G, Arsene E, Merlot B, Kerdraon O, Bigot J, Boulanger L, et al. How to improve the preoperative staging of presumed early-stage endometrioid endometrial cancer? Eur J Gynaecol Oncol. 2015;36(6):698-702.

14. Lindqvist E, Wedin M, Fredrikson M, Kjolhede P. Lymphedema after treatment for endometrial cancer - a review of prevalence and risk factors. Eur J Obstet Gynecol Reprod Biol. 2017;211:112-21.

15. Kitchener H, Swart AM, Qian Q, Amos C, Parmar MK. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study. Lancet. 2009;373(9658):125-36.

16. Benedetti Panici P, Basile S, Maneschi F, Alberto Lissoni A, Signorelli M, Scambia G, et al. Systematic pelvic lymphadenectomy vs. no lymphadenectomy in early-stage endometrial carcinoma: randomized clinical trial. J Natl Cancer Inst. 2008;100(23):1707-16.

17. Frost JA, Webster KE, Bryant A, Morrison J. Lymphadenectomy for the management of endometrial cancer. Cochrane Database Syst Rev 2017, Issue 10. Art. No.: CD007585. doi: 10.1002/14651858.CD007585.pub4.

18. Burke TW, Levenback C, Tornos C, Morris M, Wharton JT, Gershenson DM. Intraabdominal lymphatic mapping to direct selective pelvic and paraaortic lymphadenectomy in women with high-risk endometrial cancer: results of a pilot study. Gynecol Oncol. 1996;62(2):169-73.

19. Todo Y, Kato H, Kaneuchi M, Watari H, Takeda M, Sakuragi N. Survival effect of para-aortic lymphadenectomy in endometrial cancer (SEPAL study): a retrospective cohort analysis. Lancet. 2010;375(9721):1165-72.

20. Abu-Rustum NR, Gomez JD, Alektiar KM, Soslow RA, Hensley ML, Leitao MM, Jr., et al. The incidence of isolated paraaortic nodal metastasis in surgically staged endometrial cancer patients with negative pelvic lymph nodes. Gynecol Oncol. 2009;115(2):236-8.

21. Ballester M, Dubernard G, Lecuru F, Heitz D, Mathevet P, Marret H, et al. Detection rate and diagnostic accuracy of sentinel-node biopsy in early stage endometrial cancer: a prospective multicentre study (SENTI-ENDO). Lancet Oncol. 2011;12(5):469-76.

22. Darai E, Dubernard G, Bats AS, Heitz D, Mathevet P, Marret H, et al. Sentinel node biopsy for the management of early stage endometrial cancer: long-term results of the SENTI-ENDO study. Gynecol Oncol. 2015;136(1):54-9.

23. Levinson KL, Escobar PF. Is sentinel lymph node dissection an appropriate standard of care for low-stage endometrial cancers? A review of the literature. Gynecol Obstet Invest. 2013;76(3):139-50.

24. Khoury-Collado F, Abu-Rustum NR. Lymphatic mapping in endometrial cancer: a literature review of current techniques and results. Int J Gynecol Cancer. 2008;18(6):1163-8.

25. Solima E, Martinelli F, Ditto A, Maccauro M, Carcangiu M, Mariani L, et al. Diagnostic accuracy of sentinel node in endometrial cancer by using hysteroscopic injection of radiolabeled tracer. Gynecol Oncol. 2012;126(3):419-23.

26. Robova H, Charvat M, Strnad P, Hrehorcak M, Taborska K, Skapa P, et al. Lymphatic mapping in endometrial cancer: comparison of hysteroscopic and subserosal injection and the distribution of sentinel lymph nodes. Int J Gynecol Cancer. 2009;19(3):391-4.

27. Perrone AM, Casadio P, Formelli G, Levorato M, Ghi T, Costa S, et al. Cervical and hysteroscopic injection for identification of sentinel lymph node in endometrial cancer. Gynecol Oncol. 2008;111(1):62-7.

28. Clement D, Bats AS, Ghazzar-Pierquet N, Le Frere Belda MA, Larousserie F, Nos C, et al. Sentinel lymph nodes in endometrial cancer: is hysteroscopic injection valid? Eur J Gynaecol Oncol. 2008;29(3):239-41.

29. Barlin JN, Khoury-Collado F, Kim CH, Leitao MM, Jr., Chi DS, Sonoda Y, et al. The importance of applying a sentinel lymph node mapping algorithm in endometrial cancer staging: beyond removal of blue nodes. Gynecol Oncol. 2012;125(3):531-5.

30. Abu-Rustum NR. Update on sentinel node mapping in uterine cancer: 10-year experience at Memorial Sloan-Kettering Cancer Center. J Obstet Gynaecol Res. 2014;40(2):327-34.

31. Raimond E, Ballester M, Hudry D, Bendifallah S, Darai E, Graesslin O, et al. Impact of sentinel lymph node biopsy on the therapeutic management of early-stage endometrial cancer: results of a retrospective multicenter study. Gynecol Oncol. 2014;133(3):506-11.

32. Cormier B, Rozenholc AT, Gotlieb W, Plante M, Giede C. Sentinel lymph node procedure in endometrial cancer: a systematic review and proposal for standardization of future research. Gynecol Oncol. 2015;138(2):478-85.

33. Lin H, Ding Z, Kota VG, Zhang X, Zhou J. Sentinel lymph node mapping in endometrial cancer: a systematic review and meta-analysis. Oncotarget. 2017;8(28):46601-10.

34. Bodurtha Smith AJ, Fader AN, Tanner EJ. Sentinel lymph node assessment in endometrial cancer: a systematic review and meta-analysis. Am J Obstet Gynecol. 2017;216(5):459-76.e10.

35. Tschernichovsky R, Diver EJ, Schorge JO, Goodman A. The role of lymphadenectomy versus sentinel lymph node biopsy in early-stage endometrial cancer: a review of the literature. Am J Clin Oncol. 2016;39(5):516-21.

36. Kadkhodayan S, Hasanzadeh M, Treglia G, Azad A, Yousefi Z, Zarifmahmoudi L, et al. Sentinel node biopsy for lymph nodal staging of uterine cervix cancer: a systematic review and meta-analysis of the pertinent literature. Eur J Surg Oncol. 2015;41(1):1-20.

37. Rossi EC, Kowalski LD, Scalici J, Cantrell L, Schuler K, Hanna RK, et al. A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): a multicentre, prospective, cohort study. Lancet Oncol. 2017;18(3):384-92.

38. Amant F, Trum H. Sentinel-lymph-node mapping in endometrial cancer: routine practice? Lancet Oncol. 2017;18(3):281-2.

39. Ehrisman J, Secord AA, Berchuck A, Lee PS, Di Santo N, Lopez-Acevedo M, et al. Performance of sentinel lymph node biopsy in high-risk endometrial cancer. Gynecol Oncol Rep. 2016;17:69-71.

40. Soliman PT, Westin SN, Dioun S, Sun CC, Euscher E, Munsell MF, et al. A prospective validation study of sentinel lymph node mapping for high-risk endometrial cancer. Gynecol Oncol. 2017;146(2):234-9.

41. Holloway RW, Gupta S, Stavitzski NM, Zhu X, Takimoto EL, Gubbi A, et al. Sentinel lymph node mapping with staging lymphadenectomy for patients with endometrial cancer increases the detection of metastasis. Gynecol Oncol. 2016;141(2):206-10.

42. Clinton LK, Kondo J, Carney ME, Tauchi-Nishi P, Terada K, Shimizu D. Low-volume lymph node metastases in endometrial carcinoma. Int J Gynecol Cancer. 2017 Jul;27(6):1165-70.

43. Plante M, Stanleigh J, Renaud MC, Sebastianelli A, Grondin K, Gregoire J. Isolated tumor cells identified by sentinel lymph node mapping in endometrial cancer: Does adjuvant treatment matter? Gynecol Oncol. 2017;146(2):240-6.

44. Khoury-Collado F, Glaser GE, Zivanovic O, Sonoda Y, Levine DA, Chi DS, et al. Improving sentinel lymph node detection rates in endometrial cancer: how many cases are needed? Gynecol Oncol. 2009;115(3):453-5.

45. Papadia A, Imboden S, Gasparri ML, Siegenthaler F, Fink A, Mueller MD. Endometrial and cervical cancer patients with multiple sentinel lymph nodes at laparoscopic ICG mapping: How many are enough? J Cancer Res Clin Oncol. 2016;142(8):1831-6.

46. Beavis AL, Salazar-Marioni S, Sinno AK, Stone RL, Fader AN, Santillan-Gomez A, et al. Sentinel lymph node detection rates using indocyanine green in women with early-stage cervical cancer. Gynecol Oncol. 2016;143(2):302-30 .

47. Buda A, Bussi B, Di Martino G, Di Lorenzo P, Palazzi S, Grassi T, et al. Sentinel lymph node mapping with near-infrared fluorescent imaging using indocyanine green: a new tool for laparoscopic platform in patients with endometrial and cervical cancer. J Minim Invasive Gynecol. 2016;23(2):265-9.

48. Buda A, Crivellaro C, Elisei F, Di Martino G, Guerra L, De Ponti E, et al. Impact of indocyanine green for sentinel lymph node mapping in early stage endometrial and cervical cancer: comparison with conventional radiotracer (99m)Tc and/or blue dye. Ann Surg Oncol. 2016;23(7):2183-91.

49. Buda A, Dell'Anna T, Vecchione F, Verri D, Di Martino G, Milani R. Near-infrared sentinel lymph node mapping with indocyanine green using the VITOM II ICG exoscope for open surgery for gynecologic malignancies. J Minim Invasive Gynecol. 2016;23(4):628-32.

50. Buda A, Di Martino G, Vecchione F, Bussi B, Dell'Anna T, Palazzi S, et al. Optimizing strategies for sentinel lymph node mapping in early-stage cervical and endometrial cancer: comparison of real-time fluorescence with indocyanine green and methylene blue. Int J Gynecol Cancer. 2015;25(8):1513-8.

51. Buda A, Papadia A, Zapardiel I, Vizza E, Ghezzi F, De Ponti E, et al. From conventional radiotracer Tc-99(m) with blue dye to indocyanine green fluorescence: a comparison of methods towards optimization of sentinel lymph node mapping in early stage cervical cancer for a laparoscopic approach. Ann Surg Oncol. 2016;23(9):2959-65.

52. Darin MC, Gomez-Hidalgo NR, Westin SN, Soliman PT, Escobar PF, Frumovitz M, et al. Role of indocyanine green in sentinel node mapping in gynecologic cancer: is fluorescence imaging the new standard? J Minim Invasive Gynecol. 2016;23(2):186-93.

53. How J, Gotlieb WH, Press JZ, Abitbol J, Pelmus M, Ferenczy A, et al. Comparing indocyanine green, technetium, and blue dye for sentinel lymph node mapping in endometrial cancer. Gynecol Oncol. 2015;137(3):436-42.

54. Ruscito I, Gasparri ML, Braicu EI, Bellati F, Raio L, Sehouli J, et al. Sentinel node mapping in cervical and endometrial cancer: indocyanine green versus other conventional dyes - a meta-analysis. Ann Surg Oncol. 2016;23(11):3749-56.

55. Khoury-Collado F, St Clair C, Abu-Rustum NR. Sentinel lymph node mapping in endometrial cancer: an update. Oncologist. 2016;21(4):461-6.

56. Holloway RW, Abu-Rustum NR, Backes FJ, Boggess JF, Gotlieb WH, Jeffrey Lowery W, et al. Sentinel lymph node mapping and staging in endometrial cancer: a Society of Gynecologic Oncology literature review with consensus recommendations. Gynecol Oncol. 2017;146(2):405-15.

57. Martinelli F, Ditto A, Bogani G, Signorelli M, Chiappa V, Lorusso D, et al. Laparoscopic sentinel node mapping in endometrial cancer after hysteroscopic injection of indocyanine green. J Minim Invasive Gynecol. 2017;24(1):89-93.

58. Abu-Rustum NR, Khoury-Collado F, Gemignani ML. Techniques of sentinel lymph node identification for early-stage cervical and uterine cancer. Gynecol Oncol. 2008;111(2 Suppl):S44-50.

59. Sironi S, Buda A, Picchio M, Perego P, Moreni R, Pellegrino A, et al. Lymph node metastasis in patients with clinical early-stage cervical cancer: detection with integrated FDG PET/CT. Radiology. 2006;238(1):272-9.

60. Kitajima K, Murakami K, Yamasaki E, Fukasawa I, Inaba N, Kaji Y, et al. Accuracy of 18F-FDG PET/CT in detecting pelvic and paraaortic lymph node metastasis in patients with endometrial cancer. AJR Am J Roentgenol. 2008;190(6):1652-8.

61. Signorelli M, Guerra L, Buda A, Picchio M, Mangili G, Dell'Anna T, et al. Role of the integrated FDG PET/CT in the surgical management of patients with high risk clinical early stage endometrial cancer: detection of pelvic nodal metastases. Gynecol Oncol. 2009;115(2):231-5.

62. Chang MC, Chen JH, Liang JA, Yang KT, Cheng KY, Kao CH. 18F-FDG PET or PET/CT for detection of metastatic lymph nodes in patients with endometrial cancer: a systematic review and meta-analysis. Eur J Radiol. 2012;81(11):3511-7.

63. Horowitz NS, Dehdashti F, Herzog TJ, Rader JS, Powell MA, Gibb RK, et al. Prospective evaluation of FDG-PET for detecting pelvic and para-aortic lymph node metastasis in uterine corpus cancer. Gynecol Oncol. 2004;95(3):546-51.

64. Kim CH, Soslow RA, Park KJ, Barber EL, Khoury-Collado F, Barlin JN, et al. Pathologic ultrastaging improves micrometastasis detection in sentinel lymph nodes during endometrial cancer staging. Int J Gynecol Cancer 2013;23(5):964-70.

65. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. Lancet. 2005;366(9484):491-505.

66. Babatunde OA, Adams SA, Eberth JM, Wirth MD, Choi SK, Hebert JR. Racial disparities in endometrial cancer mortality-to-incidence ratios among Blacks and Whites in South Carolina. Cancer Causes Control. 2016;27(4):503-11.

67. Reich O, Winter R, Pickel H, Tamussino K, Haas J, Petru E. Does the size of pelvic lymph nodes predict metastatic involvement in patients with endometrial cancer? Int J Gynecol Cancer. 1996;6(6):445-7.

68. Ayhan A, Tuncer ZS, Tuncer R, Yuce K, Kucukali T. Tumor status of lymph nodes in early endometrial cancer in relation to lymph node size. Eur J Obstet Gynecol Reprod Biol. 1995;60(1):61-3.

69. Boran N, Akdag D, Halici F, Tulunay G, Turan T, Bozok S, et al. A retrospective analysis of the diameter of metastatic lymph nodes in apparently early stage endometrial cancer. Tumori. 2008;94(5):681-5.

Chapter 3

Sentinel lymph nodes in women with early stage cervical cancer

1. Introduction

Cervical cancer is the second most common cancer in South African women, with an incidence of 31.5 per 100.000 women in 2012 [1]. Although the FIGO classification of cervical cancer does not include pelvic lymph node status, it is well recognised and accepted that lymph pelvic node metastasis is an important risk factor for recurrence and death in patients with cervical cancer [2,3]. Information about the presence of metastatic disease in the lymph nodes is therefore important insofar as it allows for planning of optimal treatment and adjuvant treatment strategies in patients with early stage disease. For the purpose of this study, early stage cervical cancer is defined as patients diagnosed and clinically staged as FIGO stages I to IIA.

Current primary treatment options for early stage cervical cancer consist of either surgery or chemo-radiation therapy. Both these options have reportedly similar survival rates [4,5]. If there were no metastatic spread to the lymph nodes through the lymphatic system, patients would probably benefit more from surgical treatment, considering the long-term complications associated with chemo-radiation therapy. In FIGO stage IB1 cervical cancer, the prevalence of lymph node involvement is approximately 15% [3,6]. This implies that up to 85% of these women do not benefit therapeutically or otherwise from lymphadenectomy, and the procedure could potentially be safely avoided if it was possible to obtain information with regard to the lymph node status in these patients without performing full pelvic lymphadenectomy.

Radical hysterectomy and complete pelvic with or without para-aortic lymph adenectomy is the current recommended surgical treatment for patients with early stage cervical cancer. Since the 1990s laparoscopic surgery has been shown to have similar survival outcomes with less morbidity [7,8]. Irrespective of the mode of surgery, pelvic lymphadenectomy is associated with prolonged duration of surgery,

increased blood loss, nerve injury, lymphocyst formation, vascular injury, and lower extremity lymph oedema [9].

Opinion is divided on the role of surgery in patients with bulky stage disease, which would include FIGO stages IB2 to IIA2. The prevalence of metastatic nodal disease in women with FIGO stage IB2 to IIA is about 60%, and therefore in many units these women are not offered surgical treatment, as a significant number of them will be candidates for adjuvant chemo-radiation following surgery, due to the presence of risk factors for recurrence. However, there is data suggesting some benefit of surgery in women with bulky early stage cervical cancer. Up to 30% of these women will benefit from surgery alone, and there might be improved survival outcomes following surgery combined with adjuvant therapy without significant increases in morbidity [8]. The role of sentinel lymph node biopsy (SLNB) in early stage bulky disease is still not all that clear, as it would appear that the sentinel lymph node (SLN) concept is most feasible and more reliable in smaller tumours with a diameter of less than 2 cm [10].

2. Sentinel lymph node literature overview

2.1. Sentinel lymph node detection

Echt et al was the first to suggest SLNB as an option to identify lymph node status in women with early stage cervical, endometrial and vulva cancer in 1999. The study investigated seven patients with endometrial cancer, 13 women with cervical cancer and 12 patients with vulvar carcinoma in this study. SLNs were detected in two of the thirteen cervical cancer cases. During that time SLNs were used in the treatment of melanoma and penile cancer, and this was the first published paper on SLNs in gynaecological cancer [11].

Altgassen et al assessed the diagnostic accuracy of SLN biopsy in all stages of cervical cancer [10]. This multicentre prospective cohort trial was conducted between 1998 and 2006, and included 507 patients for analysis. An overall SLN detection rate of 89.7% was reported, and an overall sensitivity of SLN of 77.4% (95% CI, 78.2% - 85.0%) with a negative predictive value (NPV) of 94.3% (95%

CI, 91.6% - 96.4%). Bilateral detection rate was found to be 42%. A significantly higher sensitivity (87.2%) was reported in patients where a bilateral SLN was found compared to a unilateral SLN. In a sub-group analysis, the detection rate (94%), sensitivity (90.9%) and NPV (99.1%) were significantly higher in women with tumour size ≤ 20 mm in diameter.

The SENTICOL study by Lécuru et al assessed the sensitivity and negative predictive value of laparoscopic SLN biopsy in cervical cancer FIGO stage IA1 with lymphovascular invasion up to stage IB1 [12]. This multicentre prospective longitudinal trial was conducted between 2005 and 2007, and included 139 patients with a diagnosis of early stage cervical cancer. They reported a SLN detection rate of 97.8%, and a bilateral detection rate of 76.5%. The sensitivity was 92.0% (95% CI, 74.0% - 99.0%), NPV of 98.2% (95% CI, 93.8% - 99.8%) and a false-negative rate (FNR) of 8.0%. In patients with a bilateral detection of SLNs the sensitivity was found to be 100% (95% CI, 96.5% - 100.0%), and a FNR of 0%.

Cormier et al reported a single centre cohort series of 122 cases from a prospective maintained database between 2003 and 2010. Patients with FIGO stage IA1 with lymphovascular invasion to FIGO stage IIA were included [13]. The SLN detection rate was determined to be 93.4%, with a bilateral detection rate of 74.6%. The overall sensitivity was found to be 87.5%, NPV of 96.8% and a FNR of 12.5%. Analysis of a side-specific diagnostic performance showed a sensitivity of 92.6%, NPV of 98.9 and a FNR of 7.4%.

A large multicentre retrospective cohort study of 645 cases by Cibula et al reported comparable results with the two more recent prospective studies of Cormier and Lécuru et al [14]. The cohort consisted of 645 FIGO IA - IIB cervical cancer cases. All patients had undergone SLN biopsy. A sensitivity of 91% (95%CI: 85% - 95%) for the whole cohort and an increased sensitivity of 97% (95%CI: 91% - 99%) in the subgroup with bilateral SLN detection was demonstrated. The bilateral detection rate was found to be 72% with a FNR of 1.3% in this group.

The lower sensitivity reported by Altgassen et al (77.4%) compared to Lécuru, Cormier and Cibula et al (92.0%, 87.5% and 91% respectively) is most likely representing the lack of histological ultra-staging of the SLN and a learning curve due to the fact that the Altgassen trial was a pioneer study that started recruiting in 1998. Another reason may be that Altgassen et al included all stages of cervical cancer. In more advanced stages, extensive invasion of lymph nodes will occur more often, possibly leading to a disruption of the lymphatic drainage system affecting the ability to identify the SLN.

Two systematic reviews from Selman et al published in 2008 and van de Lande et al published in 2007, prior to the publication of the four more recent large publications from Altgassen, Lécuru, Cormier, and Cibula et al, reported a similar detection rate of 95% - 96%, and a sensitivity of 89% - 90% [15,16]. Selman et al furthermore compared SLN biopsy with imaging modalities (MRI, CT and PET-CT) to determine lymph node status and found that SLN biopsy was superior to all imaging modalities [15].

Kadkhodayan et al published a review and meta-analysis of the pertinent literature in 2015 consisting of 67 studies. The pooled detection rate was 89% and the pooled sensitivity was 90% [17].

SLNB seems to be more accurate in smaller tumours and in patients with bilaterally detected SLNs [18-21]. Many authors are of the opinion that, because the cervix is a midline organ, lymphatic drainage involves both sides of the pelvis. It has also been shown that the SLN status on one side of the pelvis does not predict the presence or absence of metastasis on the contralateral side [13,16]. For this reason, the presence of the SLN should be evaluated side specifically, and SLN assessment should not only be patient specific [12-14,22,23].

SLNs that are used in isolation produce high false negative rates but this concern has been overcome by the introduction of the sentinel lymph node algorithm. Cormier et al published an algorithm for SLN mapping. This algorithm incorporates a side-specific evaluation and recommends lymphadenectomy on

the side(s) where no SLNs are detected, as well as removal of clinically enlarged lymph nodes. This strategy resulted in a sensitivity of 100% and NPV of 100% (Figure 1) [13]. The algorithm is shown in Figure 1.

Excision of all mapped SLNs
(submit for ultrastaging if negative on H&E)

Any suspicious nodes must be removed regardless of mapping

If there is no mapping on a hemi-pelvis, a side-specific LND is performed

Parametrectomy is performed en bloc with resection of the primary tumor
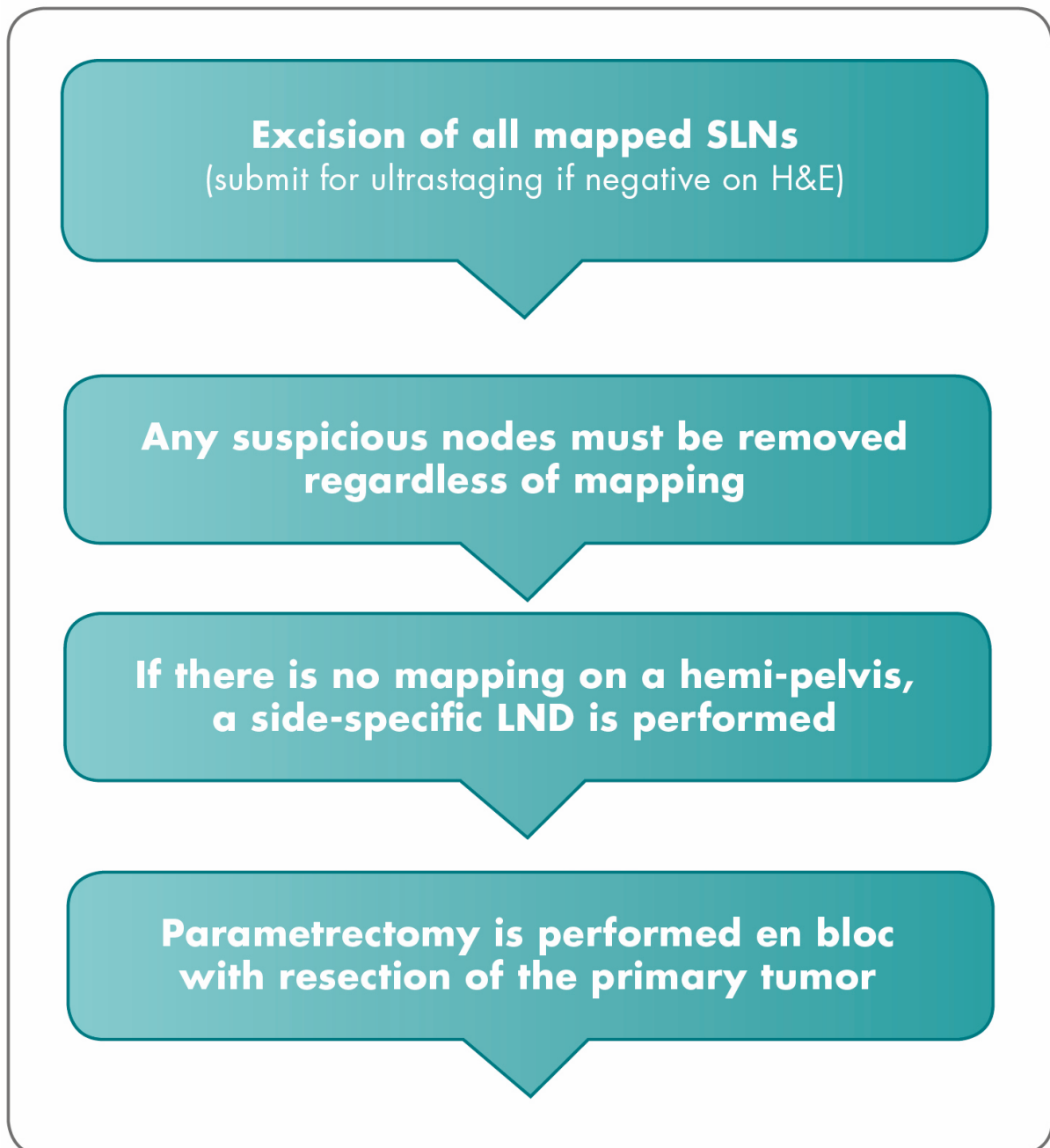
Figure 1: SLN algorithm for early cervical cancer [13]. (Reproduced with permission N Abu-Rustum)

Tax et al published a diagnostic review on the SLN procedure in early stage cervical cancer of 46 studies with 4 130 patients. In patients with tumours smaller than 40 mm and no pre-operative or intra-operative suspicious nodes with bilateral negative SLNs after ultrastaging, the FNR was 0.08% [24].

A summary of the published literature on sentinel lymph nodes in cervical cancer is shown in Table 1.

The requirement to remove clinically enlarged lymph nodes irrespective of the blue dye or ^{99m}Tc-Nanocolloid uptake, is based on the fact that the lymph drainage may be disrupted because of tumour burden in the metastatic lymph nodes. It is also possible that lymph nodes may be "clogged" with debris or lymph vessels may be disrupted following an inflammatory process caused by disease or surgery, and this can possibly alter bilateral drainage towards unilateral drainage. In addition, enlarged lymph nodes can be the result of other conditions such as infection or TB and histological confirmation of metastases is required. For this reason, a complete lymphadenectomy is mandatory on the side(s) where a SLN is not detected [25].

A study published by Zaal et al suggests improved survival in women with low volume early stage cervical cancer where positive SLNB is followed by pelvic lymph adenectomy [26], while a retrospective case matched study published by Gortzak-Uzan showed SLNB to have an increased sensitivity in the ability to detect lymph node metastases compared to full pelvic lymphadenectomy [27].

As with all surgical techniques there is a learning curve involved in the successful detection of SLNs. This is estimated to be around 30 cases [28].

Table 1: Summary of the published literature on sentinel lymph nodes in cervical cancer [18 - 21, 25, 31 -37]

First author	Nr of Patients	Year	Sensitivity (95%CI)	SLN Detection (95% CI)
Echt	13	1999	N/A	100(75-100)
O'Boyle	20	2000	75(19-99)	60(36-81)
Lantsch	14	2001	100(3-100)	93(66-100)
Malur	50	2001	100(40-100)	90(68-99)
Levenback	39	2002	88(47-100)	100(91-100)
Rhim	26	2002	83(36-100)	100(87-100)
Chung	26	2003	83(36-100)	100(87-100)
Hubalewska	37	2003	83(36-100)	97(86-100)
Lin	30	2003	100(69-100)	100(88-100)
Lambaudie	12	2003	67(9-99)	100(74-100)
Barranger	36	2004	100(63-100)	94(81-99)
Martinez	25	2004	100(29-100)	92 (74-99)
Pijpers	34	2004	92(62-100)	100(90-100)
Niikura	20	2004	100(16-100)	90(68-99)
Li Bin	28	2004	N/A	96(82-100)
Van Dam	25	2004	100(48-100)	84(64-95)
Malur	50	2004	N/A	76(53-92)
Marchiole	29	2004	38(9-76)	100(88-100)
Rob	183	2005	100(78-100)	96(90-99)
Roca	40	2005	100(40-100)	100(91-100)
Gil-Moreno	12	2005	N/A	100(74-100)
Silva	56	2005	82(57-96)	93(83-98)
Angioli	37	2005	100(54-100)	70(53-84)
Di Stefano	50	2005	90(55-100)	90(78-97)
Rob	183	2005	95(76-100)	80(71-87)
Fader	38	2008	83.3	92.1
Diaz-Feijoo	50	2008		100
Yamashita	58	2009		90
Pazin	50	2009	85	92
Cibula	44	2009		77
Darlin	105	2010	94(73-100)	90
Ogawa	82	2010	100	88
Diaz	81	2010	80	95
Roy	211	2011	87.9	96.9
Zhang	56	2014	90.9	87.5
Salvo	188	2017	96.4	90

CI = Confidence interval

2.2. Techniques of sentinel lymph node detection

Multiple cervical injection techniques have been described in the published literature on this topic. An array of injection sites, depths and volume of radio isotopic labelling with ^{99m}Tc-Nanocolloid, blue dye (Patent Blue®, Methylene Blue®, Isosulfan Blue® or Lymphazurin dye®) or a combination of the two have been used. None of the techniques have been shown to have a significantly better detection rate [29].

The most commonly proposed technique consists of a 2- or 4-point peri-tumoural injection closest to the cervix-tumour interface with a 25-gauge spinal needle as shown in Figure 2. One half of the volume should be injected deep into the stroma and the other half sub-mucosally, with a total volume of 4ml of blue dye and/or 0.1 - 0.5mCi of ^{99m}Tc-Nanocolloid. Patients who have undergone a prior cone biopsy should be injected in the bed of the cone.

Injection of ^{99m}Tc-Nanocolloid may be performed the day prior to surgery or the morning of surgery. The blue dye injection should be given in the operating theatre at the time of the examination under anaesthesia [30].

Figure 2 is a diagrammatic presentation of different cervical injection sites for SLN detection.
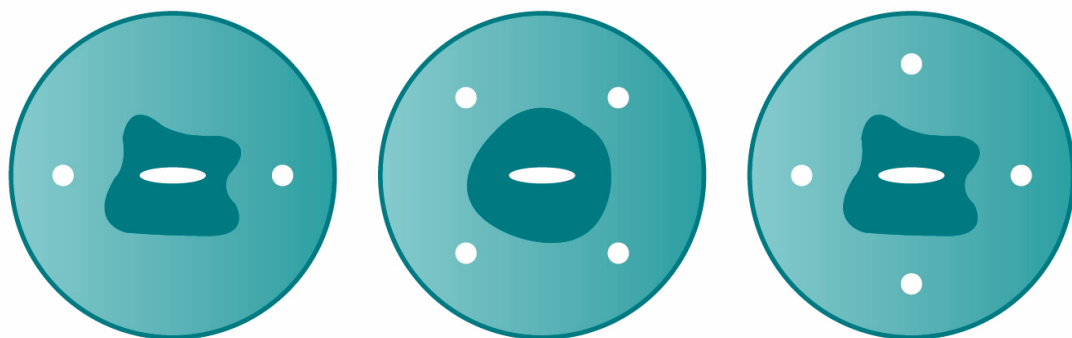
Figure 2: Options of cervical injection sites for sentinel lymph node detection

2.2.1. Laparotomy compared to laparoscopy

The SLN biopsy initially described by Echt et al in 1999 was performed through laparotomy [11]. The on going evolution of laparoscopic surgical techniques and the development of the laparoscopic gamma probe, prompted Dargent et al to propose the laparoscopic SLN biopsy technique in 2000 [38].

The concept of SLN biopsy contributes to the pursuit to decrease the surgical morbidity and post-operative complications by reducing the need for a complete pelvic lymphadenectomy. It is therefore rational to argue that the concept of SLN biopsy in cervical cancer fits well with minimally invasive surgical techniques such as laparoscopic surgery. Although more challenging and with a longer learning curve, the laparoscopic SLN technique has been found to be safe and feasible, with data from the review published by Levinson suggesting no difference in the detection rates between laparoscopic surgery and surgery performed via laparotomy in women with endometrial cancer [29].

2.2.2. Different mapping techniques

Different mapping techniques for locating the SLN have been reported. The most commonly used methods are radio isotopic labelling with 99mTc-Nanocolloid and blue dye, or a combination of the two. In penile, breast and vulvar cancer, radio isotopic labelling has been reported to be more sensitive compared to blue dye in identifying the SLN. However, in cervical cancer the complete lymphatic drainage route can be visualised by opening the broad ligament. This is further enhanced by the magnification involved in the use of laparoscopy, making it possible to identify and follow lymphatic vessels, which are blue labelled, until it reaches the SLN [13]. In penile, breast and vulvar cancer identification of the complete lymphatic tract towards the SLN is only possible following a large cutaneous incision.

In the published literature there is a trend favouring the combined technique in identifying the SLN, but nevertheless some uncertainty remains as to what

would be the preferred method in cervical cancer, especially if the procedure is performed by laparoscopy.

The multicentre pioneer study conducted by Altgassen et al in 2008 reported a significantly higher detection rate with the combined technique (^{99m}Tc-Nanocolloid and patent blue) versus ^{99m}Tc-Nanocolloid or patent blue solely [10]. More recent studies published by Lécuru et al and Cormier et al show a trend towards a superior detection rate using the combined technique of ^{99m}Tc-Nanocolloid labelling and patent blue dye, although the difference was not statistically significant [12,13]. Cormier et al found no significant difference in side specific diagnostic performance between Isosulfan blue dye and the combined technique [13].

The systematic review performed by van de Lande et al reported a significantly higher detection rate of 97% in the combined technique compared to 92% and 88% of technetium and blue dye respectively (16). The more recent systematic review of Selman et al reported a failure rate of 8.3% using blue dye alone compared to 4.4% for the combined technique, but this difference was not statistically significant [15]. Both these systematic reviews where performed prior to the publication of the three trials from Altgassen, Lécuru and Cormier et al, and do not correct for a side specific detection rate. In addition, both laparoscopy and laparotomy data were pooled in the systematic reviews.

Blue dye alone seems to be sufficient to detect sentinel nodes especially if nodes are remove 60 to 90 minutes after injection [39].

The published data on laparoscopic SLN detection in cervical cancer rates using different mapping techniques is summarised in Table 2.

Very little prospective data on SLN procedures in women with cervical cancer exist. Niikura et al reported on the outcomes of 35 women with FIGO stages IA1 to IIA1 of whom pelvic lymph adenectomy was omitted in 23 women who had negative bilateral SLNs [40]. The SLN group had no recurrences and an

incidence of 8.7% in lymph oedema compared to 42% in those who had systematic pelvic lymph adenectomy.

Table 2: Laparoscopic sentinel lymph node detection in cervical cancer rates using different mapping techniques

Publication	Blue dye mapping	Technetium mapping	Combined Blue and Technetium	Type of surgery	p-value
Devaja, 2012	91% (78/86)	96% (83/86)	98% (84/86)	LSC/LTO	
Lécuru, 2011	89.9% (125/139) BD 68.8% (86/125)	93.9% (123/131) BD 66.7% (82/123)	97.8% (136/139) BD 76.5% (104/136)	LSC	
Cormier, 2011	BD 73.1% (38/52)	-	BD 85.5% (53/62)	LSC (30%) LTO (70%)	0.112
Roy, 2011	92.8% (141/152) BD 69.1% (105/152)	96.9% (161/166) BD 86.7% (144/166)	99.1% (106/107) BD 90.6% 97/107)	LSC	
Altgassen, 2008	82.0% (160/195)	81.8% (45/55)	93.5% (318/340)	LSC (56%) LTO (44%)	< 0.001
Bats, 2007	83.3% (20/24) BD 45% (11/24)	75% (18/24) BD 50% (12/24)	87% (20/23) BD 78% (18/23)	LSC	
Rob, 2005	80% (80/100) BD 62% (62/100) LSC group: 90.5% (19/21)		96.4% (80/83) BD 90% (75/83) LSC group: 100% (18/18)	LSC (21%) LTO (79%)	Significant LSC group: non-significant
Plante, 2003	79% (23/29) BD 55% (16/29)		93% (27/29) BD 72% (21/29)	LSC	0.04 0.03
Lambaudie, 2003	90.9% (10/11) BD 63.6% (7/11)	90.9% (10/11) BD 63.6% (7/11)	100% (11/11) 100% (11/11)	LSC	
Malur, 2001	55.5% (5/9)	76.2% (16/21)	90.0% (18/21)	LSC (90%) LTO (10%)	

LSC = laparoscopy, LTO = laparotomy, BD = Bilateral detection rate

2.2.3. Pre-operative lymphoscintigraphy

The addition of a pre-operative lymphoscintigraphy (LSG) provides the surgeon with the additional value of localising SLN in abnormal anatomical locations. The systematic review by van de Lande et al demonstrated a high pooled SLN detection rate of 97% (95% CI, 95% - 98%) and high pooled sensitivity of 92% % (95% CI, 84% - 98%) with the combination of LSG and blue dye [16].

2.2.4. Other mapping techniques

More recently a novel mapping technique for cervical cancer SLN biopsy using indocyanine green (ICG) and near-infrared (NIR) fluorescence imaging has been described [41,42]. ICG is an agent that emits fluorescence that is generated by contact of ICG with plasma proteins. The fluorescence signal is captured by a near infrared laser and near infrared camera that transcribes the signal into a black and white image. Real-time laparoscopic images are then merged with the NIR fluorescence images (which are pseudo-coloured, lime green) and displayed on the screen when using the Novadaq® system [43]. When this technique is used with the system available from Karl Storz Laparoskope® the NIR fluorescence images reflects blue. The peri-tumoural cervical injection of ICG will subsequently lead to the identification of the SLN by the use of NIR fluorescence imaging.

Jewell et al demonstrated that NIR fluorescence imaging with ICG has a bilateral detection rate of 79% [41]. This technique has the potential to improve SLN detection rate in cervical cancer patients.

Since 2014 there has been several publications reporting on the high detection rates as well as the superior ability of ICG and NIR fluorescence imaging to detect SLNs compared to blue dye, and it is possible that this dye and technology will become the standard of care with regard to SLNs in uterine cancers [44-50].

Ruscito et al conducted a meta-analysis of 6 studies with 538 patients comparing ICG in cervical and endometrial cancer with other conventional dyes. ICG was found to be as effective in detecting SLNs as the combination of blue dye and ^{99}Tc [51]. A systematic review by Rocha et al included ten studies on ICG and infrared fluorescence of SLNs in endometrial and cervical cancer. Detection rates were reported ranging from 78% to 100% for cervical injection. The sensitivity and negative predictive value ranged from 50% to 100% and 88% to 100% respectively. The 10 included studies had only 422 patients who underwent laparoscopic surgery, robotic surgery as well as open surgery [52].

The current disadvantage of ICG and near infrared fluoroscopy technology is the fact that special camera equipment is required to enable visualisation of the fluorescence signal.

3. Aims and objectives

3.1. Aims

3.1.1. To investigate the ability of the SLN algorithm to predict pelvic lymph node metastases in women with early stage cervical cancer in a South African setting.

3.1.2. To compare the detection rate of ICG versus blue dye versus ^{99m}Tc-Nanocolloid, calculated on a patient and side-specific basis

3.1.3. To investigate the ability HPV DNA detection and the SLN algorithm to predict pelvic lymph node metastases in women with early stage cervical cancer in a South African setting.

3.2. Objectives

3.2.1. To establish the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the SLN algorithm in women with early stage cervical cancer;

3.2.2. To investigate the presence and detection of hrHPV DNA in the primary cervical tumour and SLNs of women with cervical cancer

3.2.3. To compare the detection rate of indocyanine green (ICG) versus blue dye versus ^{99m}Tc-Nanocolloid. Detection rates will be calculated on a patient and side-specific basis.

4. Materials and Methods

4.1. Setting and study population

A prospective cohort study was conducted at the Gynaecological Oncology Unit, Department Obstetrics and Gynaecology, University of Pretoria. The study was performed in Kalafong Provincial Tertiary Hospital (KPTH) and Steve Biko Academic Hospital (SBAH).

4.2. Inclusion criteria

All patients aged 18 years and older, willing and able to provide informed consent with any histological type FIGO stage IA1 with lymphovascular space invasion to FIGO stage IIA carcinoma of the cervix scheduled for primary surgical treatment.

4.3. Exclusion criteria

Pregnancy, women with cervical cancer FIGO stage > IIA, patients unfit for surgery, not willing or able to provide informed consent for the study and those with known allergies for ^{99m}Tc-Nanocolloid, contrast ICG or methylene blue.

4.4. Pre-operative evaluation

Prior to surgery all women with cervical cancer were assigned a clinical FIGO stage according to the standard protocol of the gynaecological oncology unit [56,57]. Standard staging investigations included histology of the tumour, chest X-Ray, ultrasound of the pelvis and abdomen, catheter specimen urine cytology [58], full blood count, liver function tests, urea & electrolytes, syphilis test and HIV test. The size of the tumour and possible paracervical and parametrial invasion were determined by clinical examination.

Cytobrush detection of hrHPV DNA was performed pre-operatively on the tumours of all women with cervical cancer.

4.5. [18]Fluoro-deoxy-glucose positron emission tomography/computed tomography scan

Based purely on availability of resources, some women scheduled for surgery underwent pre-operative FDG-PET/CT scan with the aim of assessing possible pelvic and para-aortic lymph node metastases. Women with contra-indications for FDG-PET/CT were not excluded from the rest of the study.

4.6. [99]Technetium and lymphoscintigraphy

Depending on availability and logistic issues, some women received [99]Technetium nanocolloid tracer injections.

4.7. Intra-operative lymph node mapping

After induction of general anaesthesia, a combined superficial (1 - 3 mm) and deep (1 - 2 cm) cervical injection (1 ml x 2 superficial and 1 ml x 2 deep for a total of 4 ml) of blue dye solution (Methylene Blue 1%) was injected at the 3 and 9 o'clock positions of intact-appearing epithelium of the uterine cervix using a 22-gauge spinal needle. This was followed by injection of 4 ml of ICG in a similar fashion as described above in the patients where this was used. Gynaecological oncologists in the unit were trained to perform the SLN technique.

Intra-operatively the pelvic and para-aortic lymph nodes were examined for hot and/or blue lymph nodes. ICG stained nodes were identified using the Karl Storz near infrared fluorescent filter. Examination started before opening the retroperitoneal space by looking for possible blue stained lymph nodes/vessels or blue coloured ICG stained nodes using the described technology. Hot nodes were defined as a radioactive count of more than 5 times the background count *in vivo* and more than 10 times *ex vivo* using the handheld gamma probe, through the intact peritoneum at open surgery and on the removed nodes in laparoscopic surgery as no laparoscopic gamma probe was available. After mapping and recording any blue nodes, green nodes, and/or hot nodes, dissection commenced. Opening of the right-sided broad ligament and careful dissection of the broad ligament until a blue and or green stained lymph vessel or node was identified, tested with the gamma probe, and removed. The same procedure was performed on the left side. Blue and/or hot nodes identified were sent for histological examination separately with the position and radioactive count (if applicable) stated. Any grossly enlarged lymph nodes encountered were considered to be SLN, and was removed separately.

4.8. Surgical treatment

Following removal of the SLNs, the remaining of the surgical procedure (type B, C1, C2 radical hysterectomy with pelvic lymph adenectomy) was completed as per the routine of the gynaecological oncology unit.

Para-aortic lymph adenectomy was performed using the following guidelines:

- Gynaecological Oncology Unit guidelines: in the presence of macroscopic suspicious pelvic lymph nodes;
- The discretion of the attending gynaecological oncologist;
- Patients with suspicious nodes pre-operatively identified on PET/CT where applicable

Laparoscopic radical hysterectomy and pelvic lymphadenectomy procedures were performed at Kalafong Provincial Tertiary Hospital. Patients were positioned in Lloyd Davis position, and following cleaning of the full abdomen, groin and vulvar region, laparoscopy was initiated by using an intra-umbilical direct primary

port entry or left subcostal (Palmer's point) Veress needle insertion, and insufflation of CO_2 until a pressure of 15 mmHg was obtained. Insertion of a 10mm intra-umbilical trocar followed, using a 10-mm laparoscopic 0° scope, and the abdominal cavity including the upper abdomen and liver was inspected. The patient was then positioned in Trendelenburg position. Additional insertion of 4 trocars (3 x 5mm and 1 x 10mm) was performed left and right 4 to 5 cm from the umbilicus at that level, and on the right and left McBurney's point respectively. The excised SLNs were placed in a bag made from a glove and removed through the 10-mm port placed at the McBurney's point on the right. A sterile disposable size 8 glove is used to make a bag. The fingers of the glove is removed with a scissors and the opening so created is closed with a Vicryl® tie.

Pelvic adhesions, if present, were recorded in both the open and laparoscopic procedures. The surgeon's impression of previous PID was noted and documented. Complete pelvic lymphadenectomy was performed, as well as lymphadenectomy of any other site containing at least one SLN (pre-sacral, common iliac and para-aortic basin). These lymph nodes were sent for histological examination as non-SLNs and stating the site of origin.

4.9. Histological evaluation

After intra-operative identification and removal of mapped SLNs, each node was divided into two halves through the longitudinal axis. One half was sent for frozen section examination and the other half for routine H&E staining and ultrastaging if negative. All SLNs were labelled and couriered to the pathology laboratory immediately after removal and preservation to reach the laboratory within two hours after removal.

4.10. HPV DNA analysis

Sample collection from the primary tumour was performed pre-operatively using a cytobrush to collect specimen sample directly from the tumour.

Intra-operatively identified SLNs were divided through the long axis. Sample collection was with a cytobrush from the central parts of both cut surfaces and was performed before fixation of the tissue. In cases where more than one

sentinel node were identified, separate brushes were used for each individual node.

The commercially available LINEAR ARRAY® HPV Genotyping Test (Roche Molecular Systems, Branchburg, NJ), a line-blot assay that individually identifies 37 HPV genotypes, was used to detect the HPV and cellular DNA in the sample. The pool of primers is designed to amplify HPV DNA from 18 high-risk/probable high-risk genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82), and 19 low/undetermined-risk types (6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84, IS39 and CP6108). The β-globin gene is amplified concurrently to assess cellular adequacy, extraction and amplification for each individual specimen.

4.11. Ethical considerations

Participation in the study was on a voluntary basis and all women agreeing to participate in the study signed an informed consent form. Consented patients who have initially agreed to participate, were allowed to withdraw consent should they wish to do so.

Patients who declined the option of participation were in no way disadvantaged and received standard treatment according to the guidelines of the Gynaecological Oncology Unit or as decided by the attending gynaecological oncology team. Patients participating in the trial did not receive any financial or material benefits or rewards.

The University of Pretoria Faculty of Health Sciences Research Ethics Committee approved the study as well as amendments to the protocol (434/2014).

5. Results

Of the 100 patients recruited to the study, 78 women were recruited to the cervical cancer arm of the study. Data of six women were excluded from analysis: one patient died after recruitment but before surgery, one hospital file was missing and four patients were upstaged. Of the patients who were upstaged, one patient was

diagnosed with lung metastases diagnosed on FDG-PET/CT scan, and three women were upstaged during examination under anaesthesia directly prior to undergoing surgery. Two of these women were upstaged to FIGO stage IIB and one to FIGO stage IIIB. Data on the remaining 72 women were available for analysis.

5.1. Demographic data

All women were tested for HIV infection and 47 women (65.3%) tested positive for HIV-infection. Thirteen women (18.1%) had previous surgery. Four patients (5.5%) provided history of previous tuberculosis infection (TB) of which three (4.2%) had pulmonary TB and one (1.4%) had abdominal TB. Thirteen patients (18%) were previously diagnosed with pelvic inflammatory disease (PID). The rest of the demographic data of this group is shown in Table 3.

Table 3: Demographic data for women treated with cervical cancer

n = 72	Mean	SD	Range
Age	47.21	9.09	32 – 77
Parity	3.10	1.63	0 – 8
Gravidity	3.28	1.69	0 – 8
BMI	27.51	5.41	18.37 – 43.03

SD = Standard deviation

5.2. Disease characteristics

Forty-eight women (66.7%) were FIGO stage IB1. The FIGO stage distribution of the cervical cancer patients is shown in Figure 3.

Sixty-two women (86.1%) had squamous cell carcinoma. The histological type distribution is shown in Figure 4.
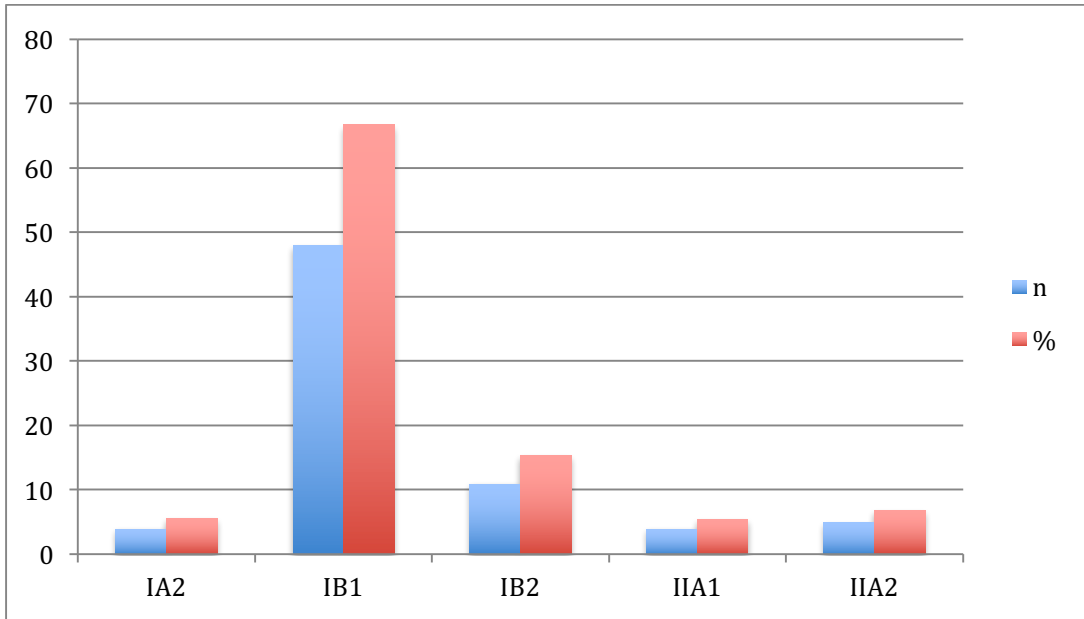
Figure 3: FIGO cervical cancer stage distribution in women treated for cervical cancer
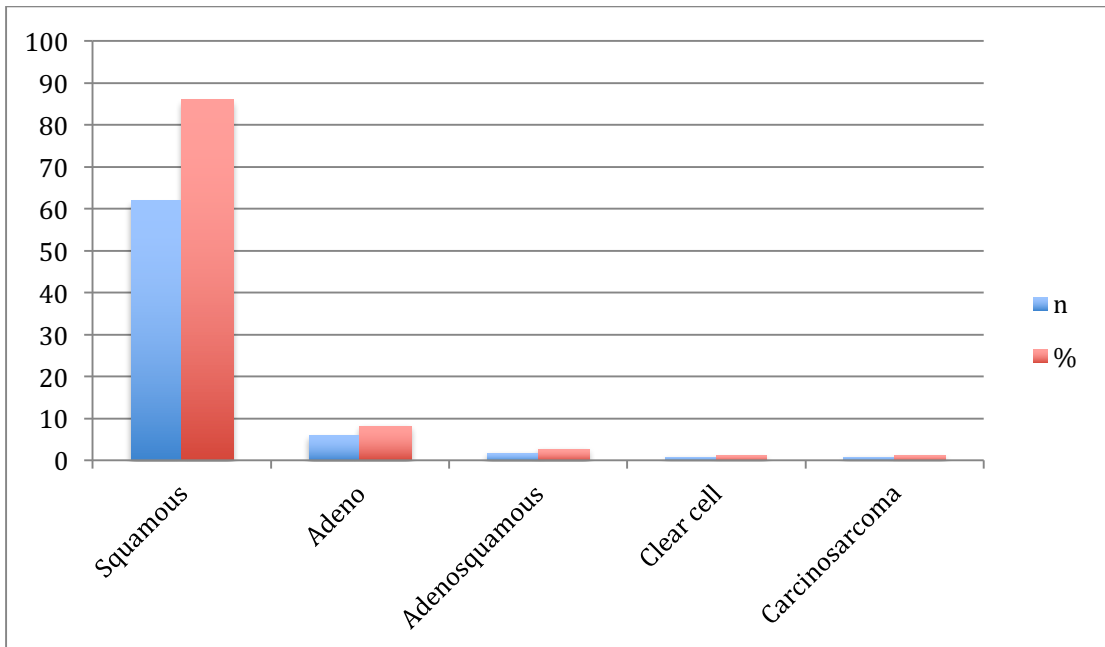
Figure 4: Cervical cancer histology distribution

5.3. Surgical access and type of surgery

Seventy-one patients (98.6%) underwent radical hysterectomy procedures and pelvic lymphadenectomy procedures. Laparoscopic radical hysterectomy procedures with pelvic lymphadenectomy were completed in twenty-one patients (29.2%). In another nine patients (12.5%), the laparoscopic approach was started but converted to open radical hysterectomy, and 41 patients (56.9%) had planned open hysterectomy from the start. The reasons for conversion from laparoscopy to open procedures are shown in Table 4.

Table 4: Reasons for conversion from laparoscopy to open procedure in women treated for cervical cancer

Reason	n
Excessive bleeding	2
Dense adhesions	2
Extensive nodal disease	1
Obesity and inability to tolerate Trendelenburg	1
Ureteric injury	1
Equipment failure	1
Enlarged uterus	1

Type C2 radical hysterectomy and pelvic lymphadenectomy were performed in 44 women (62%). The type distribution of radical hysterectomy procedures performed is shown in Figure 5.
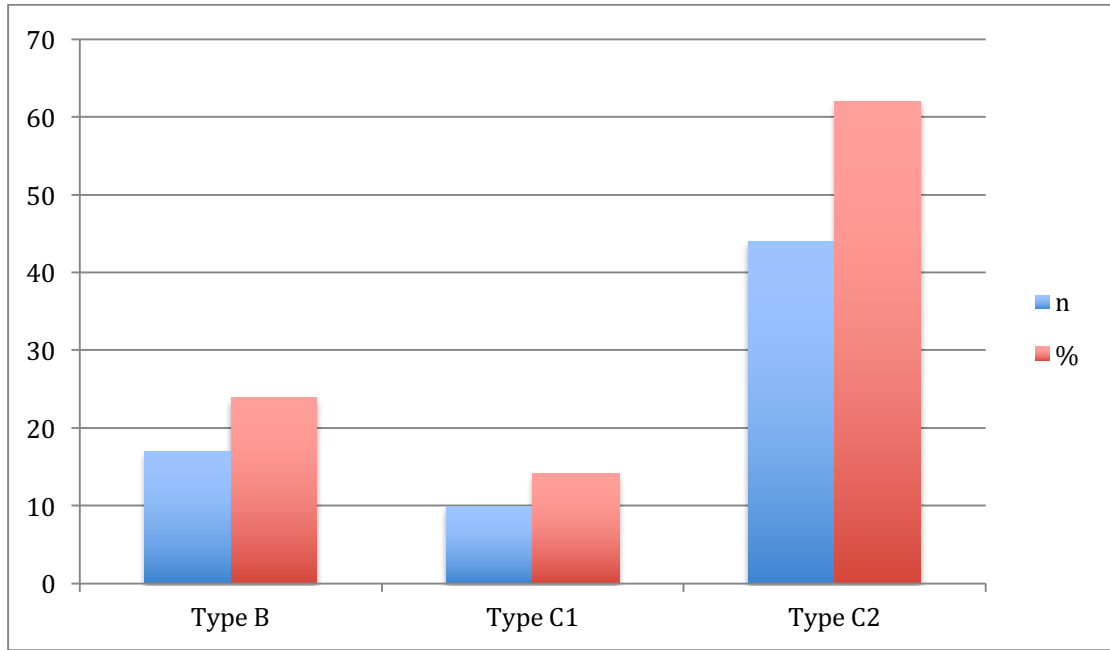
Figure 5: Type distribution of radical hysterectomy procedures performed in women treated for cervical cancer

5.4. Operative complications

Six patients (8.3%) had intra-operative complications. In the laparoscopy group there was one bladder injury, one ureteric injury and one anaesthetic-related hypotensive episode, while in the open surgery group there was one bladder injury, one ureteric injury and one vascular injury. The details of the 12 post-operative complications (16.7%) reported in nine women (12.5%) are shown in Table 5.

There were no complications reported as a result of the sentinel lymph node procedure itself. No adverse drug reactions were reported.

Table 5: Details of post-operative complications in women treated for cervical cancer

Case number	Complication	Type of surgery
3	Sepsis (demised 45 days post-op)	Laparoscopy
6	Pulmonary embolus	Open
6	Uretero-vaginal fistula	Open
8	Vesico-vaginal fistula	Open
8	Wound sepsis	Open
9	Sepsis	Laparoscopy
9	Uretero-vaginal fistula	Laparoscopy
13	Urinary retention	Open
18	Vault abscess	Laparoscopy
20	Wound sepsis	Open
21	Vesico-vaginal fistula	Open
101	Wound sepsis	Open

Tumour diameter and total pelvic lymph node count were significantly larger and higher in the open group, and there were significantly less adhesions in the laparoscopy group. Although the SLN detection rate was higher in the laparoscopy group, this difference did not reach statistical significance. The remainder of the comparative data between the open and laparoscopic surgery groups is presented in Table 6.

5.5. HIV status

There were 25 HIV negative and 47 HIV positive women treated for cervical cancer. Forty-three of the 47 HIV positive women (91.5%) were using anti-retroviral therapy, of which 28 women (80.8%) have been using it for more than six months. The mean CD4 count in HIV positive patients were 434.60 cells/µl (range 43 - 950; SD = 222.13; SEM = 33.11) and the mean viral load was 24 916.29 copies/ml (range undetectable - 666 811; SD = 119 540.02; SEM = 21 470.02). HIV infected women were significantly younger, had lower BMI, and statistically fewer women in this group had tumours ≥ 2 cm compared to HIV negative women. The rest of the comparative data is shown in Table 7.

Table 6: Comparative data between open and laparoscopic procedures in women treated for cervical cancer

	Open procedure n = 50		Laparoscopy n = 21		p-value
	Mean	**SD**	**Mean**	**SD**	
Age (years)	46.96	8.46	48.29	10.57	0.5768
Parity	3.16	1.65	2.9	1.64	0.5458
Gravidity	3.31	1.71	3.14	1.68	0.7020
BMI (kg/m^2)	27.95	4.96	25.74	5.27	0.0970
Tumour diameter (mm)	28.58	26.06	13.44	15.96	0.0160
Pelvic node count	27.10	10.10	20.71	8.26	0.0126
	n	**%**	**n**	**%**	
HIV infected	35	70	12	57.1	0.2978
Previous TB	1	2	4	19	0.0111
Previous PID	9	18	4	19	0.9213
Previous surgery	10	20	2	9.5	0.2846
Tumour ≥2 cm	28	56	7	33.3	0.0830
Enlarged nodes	17	34	5	23.8	0.3996
Nodal metastases	15	30	2	9.5	0.0666
Adhesions	13	26	0	0	0.0103
SLN detection rate	29	58	16	76	0.1538
Bilateral SLN detection	14	28	7	33.3	0.6574
Intra-op complications	5	10	3	14.3	0.6036
Post-op complications	8	16	5	23.8	0.4412

The FIGO cervical cancer stage distribution of HIV negative and HIV positive women is shown in Table 8. There were no statistically significant differences in stage distribution between the two groups.

Table 7: Comparative data HIV negative and HIV positive women treated for cervical cancer

	HIV negative n = 25		HIV positive n = 47		p-value
	Mean	**SD**	**Mean**	**SD**	
Age (years)	52.84	9.99	44.21	7.01	< 0.0001
Parity	3.33	1.61	2.98	1.65	0.3905
Gravidity	3.33	1.61	3.13	1.73	0.6340
BMI (kg/m^2)	29.25	5.63	26.59	5.11	0.0462
Tumour diameter (mm)	28.22	24.60	21.71	24.23	0.2840
Macro SLN count	2.07	1.03	2.13	1.34	0.8459
Histology SLN count	2.80	1.52	3.00	2.08	0.6730
Pelvic node count	22.56	9.80	26.07	10.41	0.1691
	n	**%**	**n**	**%**	
Previous TB	0	0	5	10.6	0.0938
Previous PID	3	12	10	21.3	0.3322
Previous surgery	6	24	7	14.9	0.3426
Tumour ≥2 cm	15	60	21	44.7	< 0.0001
Enlarged nodes	8	32	15	31.9	0.9931
Nodal metastases	6	24	12	25	0.9258
SLN detection rate	15	60	32	68.1	0.4949
Bilateral SLN detection	7	28	14	29.8	0.8738
Intra-op complications	3	12	5	10.6	0.8580
Post-op complications	5	20	8	17	0.7543

SD = Standard deviation

Table 8: Stage distribution HIV negative and HIV positive women treated for cervical cancer

FIGO stage	**HIV negative n = 25**		**HIV positive n = 47**		**p-value**
	n	**%**	**n**	**%**	
IA2	1	4	1	2.1	0.6417
IB1	14	56	34	72.3	0.1655
IB2	4	16	7	14.9	0.9024
IIA1	2	8	2	4.3	0.5180
IIA2	4	16	3	6,4	0.1939

5.6. Enlarged lymph nodes

Macroscopically enlarged pelvic lymph nodes were present in 23 patients (31.9%) and enlarged para-aortic nodes in 4 women (5.5%). Of the 23 patients with enlarged lymph nodes, 12 women (52.2%) had lymph node metastases. The macroscopically enlarged pelvic lymph nodes involved 37 hemi-pelvises. When assessing patients with enlarged lymph nodes where any enlarged lymph node was regarded as a true positive if there were pelvic nodal metastases on any side, or false positive if there were no metastases, the sensitivity, specificity and positive predicative values were 100%, 0% and 52.17% respectively. If the macroscopic appearance of pelvic nodes was used where enlarged lymph nodes were regarded as positive and non-enlarged lymph nodes as negative for predicting nodal metastases, the sensitivity, specificity, positive and negative predicted values were 66.7%, 79.3%, 52.2% and 87.5% respectively. Calculated per hemi-pelvis, the sensitivity, specificity, positive and negative predictive values of grossly enlarged lymph nodes were 83.3%, 32.1%, 44.1% and 75% respectively for pelvic nodal metastases.

5.7. Sentinel lymph node detection

SLNs were detected in 47 patients, giving a detection rate of 65.3%. Bilateral SLN detection was achieved in 22 patients for a bilateral detection rate of 30.5% in patients with cervical cancer. The macroscopic total pelvic SLN count was 98, of which 48 were located on the left side and 50 on the right side, with a mean SLN count of 2.09 (range 1 - 6; SD = 1.21). The histologically confirmed SLN count was 135 nodes of which 62 was on the left side and 73 on the right side with a mean count of 3.0 (range 1 - 9; SD = 1.92).

In 66 hemi-pelvises, there were six true positives, 59 true negatives and one false negative SLN. Per definition, false positive SLNs do not exist.

The sensitivity, specificity, negative and positive predictive values of SLN biopsy in women with cervical cancer was 85.7%, 100%, 100% and 98.33% respectively. The false negative rate was 14.3%.

5.8. Methods of sentinel lymph node detection

Methylene blue (MB) was administered to all 72 patients with cervical cancer. Thirty-six patients (50%) also received intracervical administered ^{99m}Technetium nanocolloid tracer (^{99m}Tc) with lymphoscintigram examinations, while 8 patients (11.1%) were administered Indocyanine green (ICG) as well. Thirty-six patients (50%) were administered MB and ^{99m}Tc. Three patients (4.2%) received MB and ICG, and five patients (6.9%) were administered MB, ^{99m}Tc and ICG.

In the 72 women who received MB, SLNs were detected with MB in 41 patients (56.9%). In the 36 women who received MB and ^{99m}Tc, SLNs were detected by ^{99m}Tc in 25 patients (69.4%). SLNs were detected with ICG in seven patients (87.5%). The combination of MB and ^{99m}Tc detected SLNs in 33 women (91.7%).

In the 33 women where MB was the only detection method used, SLNs were detected in 13 women (39.4%) with bilateral detection in four women (12.1%). The comparative detection rate data is shown in Table 9.

Table 9: Comparative sentinel lymph node detection rates between MB, ^{99m}Tc and ICG

MB only		^{99}Tc only		MB +^{99m}Tc		ICG only		p value
Detect rate%	n	Detect rate%	n	Detect rate%	n	Detect rate%	n	
56.9	72	69.4	36					0.2113
39.4	33			91.7	36			< 0.0001
		69.4	36	91.7	36			0.0176
56.9	72					87.5	8	0.0959
		69.4	36			87.5	8	0.3042
				91.7	36	87.5	8	0.7114

5.9. Nodal status

Seventy-one patients (98.6%) underwent full systematic pelvic lymphadenectomy. One patient (1.4%) had morbidly adherent lymph node metastases that were assessed as not safely removable, and these were only biopsied. No hysterectomy was performed in this patient.

Eighteen patients (25%) had pelvic lymph node metastases. According to unit protocol all patients with enlarged or suspected pelvic nodes should undergo para-aortic lymphadenectomy as well. Four of the 23 patients (17.4%) with enlarged nodes had para-aortic lymphadenectomy, two (50%) of which had metastatic disease in the para-aortic nodes.

The mean pelvic lymph node counts are shown in Table 10.

Table 10: Pelvic lymph node counts in women treated for cervical cancer

n = 69	n	Mean	SD	SEM	Range	95% CI
Left pelvic	69	12.49	5.52	0.66	2 - 33	11.17 - 13.82
Right pelvic	69	12.68	5.74	0.69	4 - 30	11.30 - 14.06
Total pelvic	69	25.16	9.92	1.19	6 - 57	22.78 - 27.54
Para-aortic	4	14.00	8.12	4.06	7 - 22	1.07 - 26.93

SD = Standard deviation; SEM = Standard error of the mean; CI = Confidence interval

Patients with nodal metastases had significantly larger tumours and more women had tumours ≥ 2 cm and grossly enlarged lymph nodes. The SLN detection rate in women with no metastases was 72.2% compared to 44.4% in women with metastases. This difference was statistically significant.

Comparative data of women with and without pelvic lymph node metastases are shown in Table 11.

Statistically more women without nodal metastases had FIGO stage IB1 disease. The stage distribution of node negative and node positive patients is shown in Table 12.

Table 11: Comparison of lymph node negative and lymph node positive cervical cancer patients

	Node negative n = 54		Node positive n = 18		p-value
	Mean	SD	Mean	SD	
Age (years)	47.91	9.36	44.68	8.01	0.1845
Parity	3.11	1.75	3.00	1.15	0.7999
Gravidity	3.30	1.82	3.16	1.17	0.7558
BMI (kg/m^2)	27.56	5.33	27.50	5.75	0.9671
Tumour diameter (mm)	23.26	21.51	40.88	26.66	0.0087
Pelvic node count	24.44	10.05	26.16	11.16	0.5374
Mean SLN count	3.14	2.04	2.44	1.24	0.3326
	n	**%**	**n**	**%**	
HIV infected	35	64.8	12	66.7	0.8842
Previous TB	5	9.3	0	0	0.1828
Previous PID	13	24.1	0	0	0.0233
Previous surgery	8	14.8	5	27.8	0.2175
Adhesions	10	18.5	6	33.3	0.1938
Tumour ≥ 2 cm	22	40.7	15	83.3	0.0019
Enlarged nodes	11	20.4	12	66.7	0.0003
SLN detection rate	39	72.2	8	44.4	0.0332
Bilateral SLN detection	19	35.2	3	16.6	0.1407
Intra-op complications	7	13.0	0	0	0.1098
Post-op complications	9	16.7	4	22.2	0.6020

Table 12: Stage distribution node negative compared to node positive cervical cancer patients

FIGO Stage	Nodes negative n = 54		Nodes positive n = 18		p-value
	n	%	n	%	
IA2	2	100	0	0	0.0833
IB1	40	83.3	8	16.7	<000.1
IB2	7	63.6	4	36.4	0.2126
IIA1	2	50.0	2	50.0	1.000
IIA2	3	42.9	4	57.1	0.6087

5.10. Tumour size

The mean tumour diameter from the histological examination was 23.97 mm (range 1 - 110 mm; SD = 24.38; SEM = 2.93; 95% CI = 8.12 - 29.83). In 37 women (51.4%) the tumour diameter was ≥ 2 cm.

There were statistically significantly less women with TB and PID and more with previous surgery in the group with tumour diameter ≥ 2 cm, while the smaller tumour diameter group had significantly less nodal metastases. The SLN detection rate was significantly better at 77.1% compared to 54% in the ≥ 2cm group. Comparative data between women with tumour size ≥ 2 cm and those with tumour size < 2cm is depicted in Table 13.

5.11. Body mass index

The mean BMI of patients with cervical cancer was 27.51 kg/m^2. In 27 women (37.5%) the BMI was < 25 kg/m^2. Only one woman (1.4%) was underweight, with a BMI of 18.37 kg/m^2. The BMI was ≥ 25 kg/m^2 in 45 women (62.5%) and in 23 women (31.9%) it was ≥ 30 kg/m^2.

Women with BMI ≥ 25 kg/m^2 were significantly older than women with BMI < 25 kg/m^2. More women in the normal BMI group were HIV infected, although this comparison did not reach statistical significance. The macroscopically detected number of SLNs in the women with BMI ≥ 25 kg/m^2 was significantly lower than what was histologically detected. This same comparison was not significantly different for the normal BMI group.

SLN detection was 77.7% in women with normal BMI compared to 57.8% in women with BMI ≥ 25 kg/m^2 (p = 0.0865). The comparative data between the two groups are shown in Table 14.

The BMI in 23 women (31.9%) were ≥30 kg/m^2. The mean BMI in this group was 33.74 (range 30.06 – 43.03; SD = 3.69; SEM = 0.77; 95% CI = 32.15 - 35.34). SLNs were detected in 10 patients for a detection rate of 43.5%, and bilateral detection in 5 patients for a bilateral detection rate of 21.7%.

The SLN detection rate in women with BMI < 25 kg/m^2 of 77.7% is significantly better compared to the detection rate of 43.5% in women with BMI ≥ 30 kg/m^2 (p = 0.0140).

Table 13: Comparative data between patients with tumour ≥ 2 cm and patients with tumour < 2 cm

	Tumour < 2cm n = 35		Tumour ≥ 2 cm n = 37		p-value
	Mean	SD	Mean	SD	
Age (years)	48.46	10.65	46.03	7.27	0.2598
Parity	3.00	1.74	3.19	1.54	0.6271
Gravidity	3.21	1.74	3.35	1.67	0.7305
BMI (kg/m^2)	27.18	5.18	27.23	5.68	0.6568
Tumour diameter (mm)	6.64	5.5	42.71	20.63	<0.0001
Macro SLN count	2.3	1.41	1.80	0.83	0.1647
Histology SLN count	3.15	2.07	2.79	1.72	0.5401
Pelvic node count	24.59	9.90	25.03	10.73	0.8592
	n	%	n	%	
HIV positive	25	71.4	22	59.5	0.3083
Previous TB	4	11.4	0	0	0.0359
Previous PID	10	28.6	3	8.1	0.0243
Previous surgery	3	8.6	10	27.0	0.0439
Adhesions	7	20.0	9	24.3	0.6845
Enlarged nodes	10	28.6	13	37.1	0.4464
Nodal metastases	3	8.6	15	42.9	0.0010
SLN detection rate	27	77.1	20	54.0	0.0411
Bilateral SLN detection	13	37.1	9	25.7	0.3003
Intra-op complications	4	11.4	4	10.8	0.9359
Post-op complications	7	20.0	5	13.5	0.4625

Table 14: Comparative data between women with BMI ≥ 25 kg/m^2 and those with BMI < 25 kg/m^2

	BMI < 25 kg/m^2 n = 27		BMI ≥ 25 kg/m^2 n = 45		p-value
	Mean	**SD**	**Mean**	**SD**	
Age (years)	44.41	9.56	48.89	8.46	0.0420
Parity	3.04	1.83	3.14	1.52	0.8042
Gravidity	3.19	1.86	3.34	1.60	0.7197
BMI (kg/m^2)	22.32	2.06	30.63	4.27	< 0.0001
Tumour diameter (mm)	28.49	26.83	27.75	22.59	0.9096
Macro SLN count	2.14	1.15	2.04	1.28	0.7819
Histology SLN count	2.85	1.81	3.08	2.06	0.6967
Pelvic node count	25.46	11.48	24.43	9.59	0.6880
	n	**%**	**n**	**%**	
HIV positive	21	77.8	26	57.8	0.0865
Previous TB	3	11.1	2	4.4	0.2813
Previous PID	5	18.5	8	17.8	0.9408
Previous surgery	4	14.8	9	20.0	0.5813
Adhesions	7	25.9	9	20.0	0.5626
Enlarged nodes	6	22.2	17	37.8	0.1723
Tumour ≥ 2 cm	14	51.8	23	51.1	0.9544
Nodal metastases	8	29.6	18	40.0	0.3771
SLN detection rate	27	77.7	26	57.8	0.0882
Bilateral SLN detection	11	40.7	11	24.4	0.1487
Intra-op complications	2	7.4	6	13.3	0.4434
Post-op complications	5	18.5	8	17.8	0.9408

SD = Standard deviation

5.12. FIGO stage

Fifty-one women (70.8%) had early-stage disease defined as FIGO stage IA2 - IB1, while 21 women (29.2%) had locally advanced disease defined as FIGO stage IB2 - IIA2. Women diagnosed with locally advanced disease were significantly older compared to women diagnosed with early stage cervical cancer. Women with locally advanced disease also had significantly larger

tumours, while proportionally more had enlarged nodes and nodal metastases. The SLN detection rate was significantly better in women with FIGO stage IA2 - IB1 early-stage disease compared to women with locally advanced disease. The comparative data for the two groups are shown in Table 15.

Table 15: Comparative data early stage disease compared to locally advanced disease in women treated for cervical cancer

	FIGO Stage IA - IB1 n = 51		FIGO Stage IB2 - IIA2 n = 21		p-value
	Mean	**SD**	**Mean**	**SD**	
Age (years)	48.82	9.51	43.29	6.69	0.0179
Parity	3.14	1.71	3.00	1.45	0.7435
Gravidity	3.32	1.74	3.19	1.60	0.7697
BMI (kg/m^2)	27.42	5.29	27.73	5.82	0.8269
Tumour diameter (mm)	14.85	12.61	53.75	20.08	< 0.0001
Macro SLN count	2.11	1.31	2.00	0.71	0.8097
Histology SLN count	3.11	2.05	2.56	1.24	0.4475
Pelvic node count	24.37	9.01	27.10	11.90	0.3032
	n	**%**	**n**	**%**	
HIV positive	36	70.6	11	52.4	0.1431
Previous TB	4	7.8	0	0	0.1910
Previous PID	11	52.4	2	9.5	0.0008
Previous surgery	8	15.7	5	23.8	0.4200
Adhesions	12	23.5	4	19.1	0.6852
Enlarged nodes	10	19.6	10	47.6	0.0166
Tumour ≥ 2 cm	16	31.4	21	100	< 0.0001
Nodal metastases	8	15.7	10	47.6	0.0048
SLN detection rate	36	74.5	9	42.9	0.0110
Bilateral SLN detection	16	31.4	6	28.6	0.8160
Intra-op complications	6	11.8	1	4.8	0.3663
Post-op complications	8	15.7	5	23.8	0.4200

SD = Standard deviation

5.13. History of tuberculosis, pelvic inflammatory disease and the presence of pelvic adhesions

Twenty-seven women (37.5%) had a history of TB and/or PID and/or the presence of adhesions at the time of surgery, compared to 45 women (62.5%) who did not. The SLN detection rate in the TB/PID/adhesion group was 63.0% compared to 66.7% in the comparative group (p = 0.7512).

5.14. Bilharzia and tuberculosis

One patient had bilharzia ova reported in the lymph nodes and in another patient the presence of tuberculosis was reported. The patient with tuberculosis in her nodes had micro metastases in her SLN and the non-SLNs were negative for metastases.

5.15. The sentinel lymph node biopsy algorithm

According to the algorithm, patients without mapping receive full pelvic lymphadenectomy, and all enlarged or suspicious nodes are also removed. In this cohort the SLN biopsy had a FNR of 14.3% because of a false negative SLN biopsy in one patient. This patient had enlarged nodes on the side of lymph node metastases, and if the algorithm were applied, the false negatives would have been zero with a false negative rate of 0%.

The sensitivity, specificity, negative and positive predictive values of SLNB algorithm in women with cervical cancer was 100%, 100%, 100% and 100% respectively with a false negative rate of 0%.

6. Discussion

6.1. HIV prevalence

HIV prevalence in this group of women was unexpectedly high, as the study design and recruitment were not designed to ultimately have more HIV infected women compared to HIV negative women. Previous results from the same unit recorded a consistent HIV infection prevalence of 26% of women amongst all FIGO stages over a number of years [58]. The fact that HIV infected women

recruited to this study were significantly younger than the HIV negative group, was also consistent with previously published data, including data from the Pretoria Unit [58,59]. HIV-infected women were 8 years younger than HIV negative women, while HIV negative women had significantly higher BMI compared to HIV positive women. Although there were no significant differences with regard to mean tumour diameter between the two groups, significantly fewer women in the HIV infected group had tumours measuring ≥ 2 cm, despite the similar FIGO stage distribution between the two groups. This is probably a reflection of screening practices in HIV infected women receiving ARV treatment. HIV positive women tended to have more previous episodes of tuberculosis infection although due to small numbers this difference did not reach statistical significance.

The almost similar proportion of HIV positive and HIV negative women with enlarged lymph nodes as well as with nodal metastases were somewhat unexpected, as higher rates of both in the HIV infected group were anticipated. The similarity in SLN detection rates between the two groups was an important finding, as there is no published data on the detection rate of SLNs in HIV infected women.

6.2. Body mass index

The mean BMI of this group was ≥ 25 kg/m^2, which reflects the current obesity phenomenon experienced worldwide as well as in South Africa. It is reported that in sub-Saharan Africa, South African women have the highest prevalence of obesity, with 42% of women being obese [60]. The mean BMI-value in the HIV negative group was statistically more elevated compared to HIV positive women. This finding reflects the known higher prevalence of obesity in HIV–infected women on ARV treatment, as the vast majority of these women were on ARV treatment for more than six months [61]. In the current study, women with BMI ≥ 25 kg/m^2 were significantly older compared to women with BMI of < 25 kg/m^2.

The SLN detection rate in women with BMI < 25 kg/m^2 tended to be higher at 77.7% compared to 57.8% in women with BMI ≥ 25 kg/m^2, although this

difference did not reach statistical significance (p = 0.0882). In the group of women with BMI ≥ 25 kg/m^2, the SLN detection rate was significantly lower at 43.5% when compared to women in the BMI < 25 kg/m^2 group (p = 0.0140). BMI is therefore a significant factor in the detection rate of SLNs. This finding is consistent with similar findings in the published literature. Salvo et al reported significantly lower detection rate for bilateral SLNs in women with BMI >30 kg/m^2 in a study of 181 women with early stage cervical cancer [37].

6.3. Disease characteristics

The disease characteristics found in the group of women with cervical cancer are as expected, with the vast majority of women having squamous cell carcinoma followed by adenocarcinoma as the second most common. The majority of women had FIGO stage IB1 disease.

6.4. Surgical access

Most women underwent open surgery. The trial was conducted midst the course of normal clinical service delivery to patients as provided by the gynaecologic oncology team and fellows in training at the two sites, with one site and one person performing laparoscopic radical hysterectomy surgery. The group of women who underwent laparoscopic surgery had significantly smaller tumours and were less likely to have nodal metastases, a result of patient selection and current Unit protocol limiting laparoscopic radical hysterectomy to patients with FIGO stage IB1 disease. This selection bias resulted in the detection rate of 18% higher in the laparoscopy group. The difference did not reach statistical significance, most likely attributable to the small sample size. Statistically significant difference in lymph node counts between the two groups favouring open surgery is due to the fact that the common iliac nodes were not routinely removed in all of the laparoscopy cases. Common iliac nodes at laparoscopy were removed only in cases with suspicious pelvic nodes, whereas common iliac nodes are routinely removed in all open surgery cases regardless of the macroscopic status of the pelvic nodes. An underlying selection bias existed in that non- obese patients considered to be "ideal for laparoscopic surgery" were more easily selected to undergo laparoscopic surgery.

6.5. Enlarged lymph nodes

Almost 32% of women had clinically enlarged lymph nodes. HIV status did not show an effect on the macroscopic nodal status, with almost identical proportions of women identified with enlarged nodes in both groups. This was unexpected as one would have anticipated a higher proportion of women to present with clinically enlarged nodes in the HIV infected group of women.

The macroscopic appearance of lymph nodes in women with cervical cancer is not a good predictor of the presence of pelvic nodal metastatic disease with 52% of women with macroscopic enlarged nodes having metastatic disease in these enlarged nodes. The sensitivity, specificity, PPV and NPV of enlarged lymph nodes of 83.3%, 32.1%, 44.1% and 75% respectively were not as good as what is reported for the SLN algorithm. Macroscopic assessment based on lymph node size alone is not accurate for clinical decision making of women treated for cervical cancer.

The reasons why para-aortic lymphadenectomy was not performed on all patients where this was indicated were not investigated, as the final decision whether or not para-aortic lymphadenectomy is performed, lies with the treating gynaecologist.

6.6. Detection of sentinel lymph nodes

6.6.1. Detection rates

The overall SLN detection rate of 65% in this study population is much lower than the detection rates published in the international literature.

6.6.2. Detection methods

The detection rate of the combination of MB and ^{99m}Tc were statistically significantly better compared to MB alone or ^{99m}Tc alone. There was no statistically significant difference between MB + [99m]Tc compared to ICG alone. ICG performed better than MB alone, although the difference did not reach statistical significance. The sample size of ICG was very small.

Detection patterns displaying the combination of MB and ^{99m}Tc to be better than either on its own, and ICG having detection rates as good as the combination of MB and ^{99m}Tc are consistent with the published literature on this issue [16,22,44,46,51,52,62,63].

6.7. Factors influencing detection rate of sentinel lymph nodes

6.7.1. Body mass index

The influence of BMI has already been discussed, with a better detection rate in patients with normal BMI compared to those with being overweight and obese.

6.7.2. Pelvic lymph node status

The rate of nodal metastases of 25% was slightly higher than reported in other studies where the node positive rates vary from 16 – 21% [14,19,22,36,39].

As expected, women with nodal metastases had significantly larger tumours and more women had tumours ≥ 2 cm and grossly enlarged lymph nodes. The SLN detection rate in women without metastases was significantly higher at 72.2% compared to 44.4% in women with metastases, also consistent with published literature. In a study published by Plante et al, the detection rate in women with normal nodes was 75% and 56% in those with macroscopic affected nodes [22].

The better detection rate in node negative women with early stage cervical cancer is a reassuring finding, as this might result in less unnecessary pelvic lymphadenectomy procedures in the appropriate group of patients if the sentinel lymph node algorithm is followed correctly.

Although HIV status did not influence the rate of pelvic lymph node metastases, there were statistically more women with nodal metastases in the group without a history of previous PID, compared to those who had a history

of previous PID. This is in all likelihood not a causal association. No published literature addressing this issue could be found on PubMed.

6.7.3. Tumour size

Tumour size was, as expected, significantly associated with less nodal metastases. SLN detection was statistically significantly better at 77.1% compared to 54% in those with tumour size ≥ 2 cm.

In the group of women with tumour size < 2 cm, significantly more women had a history of previous TB and previous PID, while the group with tumour size ≥ 2 cm reported significantly more previous surgery. As expected, significantly more women in the ≥ 2cm tumour size group had nodal metastases compared to the < 2 cm size tumour group.

Published literature on this issue also showed a correlation between tumour size and detection rate, with significantly better detection rates when tumour size was less than 2 cm [19]. When 4 cm tumour size was used as a measure, there was also a significant difference in detection rates [39]. Smaller tumours were associated with better detection rates of SLNs [65].

6.7.4. FIGO stage

Women with FIGO stage IA – IB1 were significantly older at 48.8 years compared to women with FIGO stages IB2 – IIA2 who had a mean age of 43.3 years. Significantly more women in the FIGO stage IA – IB1 group had a history of previous PID.

The SLN detection rate was significantly higher (74.5%) in the FIGO stage IA – IB1 group compared to the IB2 – IIA2 group (42.9%). This correlated well with the findings of better detection in smaller tumours as well as in women with no nodal metastases.

7. Conclusion

The SLN algorithm is a feasible option to gain knowledge with regard to the oncological status of the pelvic lymph nodes in South African women with early stage cervical cancer. Although the detection rate was generally lower than in reported literature, selected sub-groups of women showed comparable detection rates to those reported elsewhere.

Factors influencing sentinel lymph node detection rates were pelvic lymph node status, tumour size, FIGO stage and BMI. Women with smaller tumours, normal BMI and lower FIGO stage disease had better detection rates and these findings were statistically significant.

The procedure had a high sensitivity and the FNR for this cohort of women when using the algorithm was zero.

There is still a lack of prospective data on the use of SLN procedures in women with early stage cervical cancer and it is unsure what the effect on disease free and overall survival will be should this concept be implemented in routine care. With the current knowledge it would be reasonable to suggest the use of the SLN algorithm as part of individualised care of selected women.

The results reported here is the first set of results with regard to a SLN procedure in South African women who are subjected to high prevalence of HIV, tuberculosis and pelvic infections. This is also the first report of SLNs performed in a substantial group of HIV infected women.

References

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0 Cancer incidence and mortality worldwide: IARC CancerBase No. 11 [Internet] Lyon France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr. [Accessed on 11 Oct 2017]

2. Sevin BU, Lu Y, Bloch DA, Nadji M, Koechli OR, Averette HE. Surgically defined prognostic parameters in patients with early cervical carcinoma. A multivariate survival tree analysis. Cancer. 1996;78(7):1438-46.

3. Delgado G, Bundy BN, Fowler WC, Jr., Stehman FB, Sevin B, Creasman WT, et al. A prospective surgical pathological study of stage I squamous carcinoma of the cervix: a Gynecologic Oncology Group Study. Gynecol Oncol. 1989;35(3):314-20.

4. Undurraga M, Loubeyre P, Dubuisson JB, Schneider D, Petignat P. Early-stage cervical cancer: is surgery better than radiotherapy? Expert Rev Anticancer Ther. 2010;10(3):451-60

5. Waggoner SE. Cervical cancer. Lancet. 2003;361(9376):2217-25.

6. Look KY, Brunetto VL, Clarke-Pearson DL, Averette HE, Major FJ, Alvarez RD, et al. An analysis of cell type in patients with surgically staged stage IB carcinoma of the cervix: a Gynecologic Oncology Group study. Gynecol Oncol. 1996;63(3):304-11.

7. Bogani G, Cromi A, Uccella S, Serati M, Casarin J, Pinelli C, et al. Laparoscopic versus open abdominal management of cervical cancer: long-term results from a propensity-matched analysis. J Minim Invasive Gynecol. 2014. 21(5):857-62.

8. Park JY, Kim DY, Kim JH, Kim YM, Kim YT, Kim YS, et al. Comparison of outcomes between radical hysterectomy followed by tailored adjuvant therapy versus primary chemoradiation therapy in IB2 and IIA2 cervical cancer. J Gynecol Oncol. 2012;23(4):226-34.

9. Matsuura Y, Kawagoe T, Toki N, Tanaka M, Kashimura M. Long-standing complications after treatment for cancer of the uterine cervix--clinical significance of medical examination at 5 years after treatment. Int J Gynecol Cancer.2006;16(1):294-7.

10. Altgassen C, Hertel H, Brandstadt A, Kohler C, Durst M, Schneider A. Multicenter validation study of the sentinel lymph node concept in cervical cancer: AGO Study Group. J Clin Oncol. 2008;26(18):2943-51.

11. Echt ML, Finan MA, Hoffman MS, Kline RC, Roberts WS, Fiorica JV. Detection of sentinel lymph nodes with lymphazurin in cervical, uterine, and vulvar malignancies. South Med J. 1999;92(2):204-8.

12. Lecuru F, Mathevet P, Querleu D, Leblanc E, Morice P, Darai E, et al. Bilateral negative sentinel nodes accurately predict absence of lymph node metastasis in early cervical cancer: results of the SENTICOL study. J Clin Oncol.2011;29(13):1686-91.

13. Cormier B, Diaz JP, Shih K, Sampson RM, Sonoda Y, Park KJ, et al. Establishing a sentinel lymph node mapping algorithm for the treatment of early cervical cancer. Gynecol Oncol. 2011;122(2):275-80.

14. Cibula D, Abu-Rustum NR, Dusek L, Slama J, Zikan M, Zaal A, et al. Bilateral ultrastaging of sentinel lymph node in cervical cancer: lowering the false-negative rate and improving the detection of micrometastasis. Gynecol Oncol.2012;127(3):462-6.

15. Selman TJ, Mann C, Zamora J, Appleyard TL, Khan K. Diagnostic accuracy of tests for lymph node status in primary cervical cancer: a systematic review and meta-analysis. CMAJ. 2008;178(7):855-62.

16. van de Lande J, Torrenga B, Raijmakers PG, Hoekstra OS, van Baal MW, Brolmann HA, et al. Sentinel lymph node detection in early stage uterine cervix carcinoma: a systematic review. Gynecol Oncol. 2007;106(3):604-13.

17. Kadkhodayan S, Hasanzadeh M, Treglia G, Azad A, Yousefi Z, Zarifmahmoudi L, et al. Sentinel node biopsy for lymph nodal staging of uterine cervix cancer: a systematic review and meta-analysis of the pertinent literature. Eur J Surg Oncol. 2015;41(1):1-20.

18. Fader AN, Edwards RP, Cost M, Kanbour-Shakir A, Kelley JL, Schwartz B, et al. Sentinel lymph node biopsy in early-stage cervical cancer: utility of intraoperative versus postoperative assessment. Gynecol Oncol. 2008;111(1):13-7.

19. Yamashita T, Katayama H, Kato Y, Nishiwaki K, Hayashi H, Miyokawa N, et al. Management of pelvic lymph nodes by sentinel node navigation surgery in the treatment of invasive cervical cancer. Int J Gynecol Cancer. 2009;19(6):1113-8.

20. Darlin L, Persson J, Bossmar T, Lindahl B, Kannisto P, Masback A, et al. The sentinel node concept in early cervical cancer performs well in tumors smaller than 2 cm. Gynecol Oncol. 2010;117(2):266-9.

21. Ogawa S, Kobayashi H, Amada S, Yahata H, Sonoda K, Abe K, et al. Sentinel node detection with (99m)Tc phytate alone is satisfactory for cervical cancer patients undergoing radical hysterectomy and pelvic lymphadenectomy. Int J Clin Oncol. 2010;15(1):52-8.

22. Plante M, Renaud MC, Tetu B, Harel F, Roy M. Laparoscopic sentinel node mapping in early-stage cervical cancer. Gynecol Oncol. 2003;91(3):494-503.

23. Hauspy J, Beiner M, Harley I, Ehrlich L, Rasty G, Covens A. Sentinel lymph nodes in early stage cervical cancer. Gynecol Oncol. 2007;105(2):285-90.

24. Tax C, Rovers MM, de Graaf C, Zusterzeel PL, Bekkers RL. The sentinel node procedure in early stage cervical cancer, taking the next step; a diagnostic review. Gynecol Oncol. 2015;139(3):559-67.

25. Eichner E, Goldberg I, Bove ER. In vivo studies with direct sky blue of the lymphatic drainage of the internal genitals of women. Am J Obstet Gynecol. 1954;67(6):1277-87.

26. Zaal A, Zweemer RP, Zikan M, Dusek L, Querleu D, Lecuru F, et al. Pelvic lymphadenectomy improves survival in patients with cervical cancer with low-volume disease in the sentinel node: a retrospective multicenter cohort study. Int J Gynecol Cancer. 2014;24(2):303-11.

27. Gortzak-Uzan L, Jimenez W, Nofech-Mozes S, Ismiil N, Khalifa MA, Dube V, et al. Sentinel lymph node biopsy vs. pelvic lymphadenectomy in early stage cervical cancer: is it time to change the gold standard? Gynecol Oncol. 2010;116(1):28-32.

28. Khoury-Collado F, Glaser GE, Zivanovic O, Sonoda Y, Levine DA, Chi DS, et al. Improving sentinel lymph node detection rates in endometrial cancer: how many cases are needed? Gynecol Oncol. 2009;115(3):453-5.

29. Levinson KL, Escobar PF. Is sentinel lymph node dissection an appropriate standard of care for low-stage endometrial cancers? A review of the literature. Gynecol Obstet Invest. 2013;76(3):139-50.

30. Abu-Rustum NR, Khoury-Collado F, Gemignani ML. Techniques of sentinel lymph node identification for early-stage cervical and uterine cancer. Gynecol Oncol. 2008;111(2 Suppl):S44-50.

31. Diaz-Feijoo B, Gil-Moreno A, Perez-Benavente MA, Morchon S, Martinez-Palones JM, Xercavins J. Sentinel lymph node identification and radical hysterectomy with lymphadenectomy in early stage cervical cancer: laparoscopy versus laparotomy. J Minim Invasive Gynecol.2008;15(5):531-7.

32. Pazin V, Dragojevic S, Mikovic Z, Dukic M, Rakic S, Marinkovic J, et al. The value of sentinel lymphadenectomy in radical operative treatment of cervical cancer. Vojnosanit Pregl. 2009;66(7):539-43.

33. Cibula D, Kuzel D, Slama J, Fischerova D, Dundr P, Freitag P, et al. Sentinel node (SLN) biopsy in the management of locally advanced cervical cancer. Gynecol Oncol. 2009;115(1):46-50.

34. Diaz JP, Gemignani ML, Pandit-Taskar N, Park KJ, Murray MP, Chi DS, et al. Sentinel lymph node biopsy in the management of early-stage cervical carcinoma. Gynecol Oncol. 2011;120(3):347-52.

35. Roy M, Bouchard-Fortier G, Popa I, Gregoire J, Renaud MC, Tetu B, et al. Value of sentinel node mapping in cancer of the cervix. Gynecol Oncol. 2011;122(2):269-74.

36. Zhang Z, Chang Q. Clinical analysis of sentinel lymph node identification in patients with cervical cancer. Eur J Gynaecol Oncol. 2014;35(1):26-31.

37. Salvo G, Ramirez PT, Levenback CF, Munsell MF, Euscher ED, Soliman PT, et al. Sensitivity and negative predictive value for sentinel lymph node biopsy in women with early-stage cervical cancer. Gynecol Oncol. 2017;145(1):96-101.

38. Dargent D, Martin X, Mathevet P. Laparoscopic assessment of the sentinel lymph node in early stage cervical cancer. Gynecol Oncol. 2000;79(3):411-5.

39. Yuan SH, Xiong Y, Wei M, Yan XJ, Zhang HZ, Zeng YX, et al. Sentinel lymph node detection using methylene blue in patients with early stage cervical cancer. Gynecol Oncol. 2007;106(1):147-52.

40. Niikura H, Okamoto S, Otsuki T, Yoshinaga K, Utsunomiya H, Nagase S, et al. Prospective study of sentinel lymph node biopsy without further pelvic lymphadenectomy in patients with sentinel lymph node-negative cervical cancer. Int J Gynecol Cancer. 2012;22(7):1244-50.

41. Jewell EL, Huang JJ, Abu-Rustum NR, Gardner GJ, Brown CL, Sonoda Y, et al. Detection of sentinel lymph nodes in minimally invasive surgery using indocyanine green and near-infrared fluorescence imaging for uterine and cervical malignancies. Gynecol Oncol. 2014;133(2):274-7.

42. van der Vorst JR, Hutteman M, Gaarenstroom KN, Peters AA, Mieog JS, Schaafsma BE, et al Optimization of near-infrared fluorescent sentinel lymph node mapping in cervical cancer patients. Int J Gynecol Cancer. 2011;21(8):1472-8.

43. Schaafsma BE, van der Vorst JR, Gaarenstroom KN, Peters AA, Verbeek FP, de Kroon CD, et al. Randomized comparison of near-infrared fluorescence lymphatic tracers for sentinel lymph node mapping of cervical cancer. Gynecol Oncol. 2012;127(1):126-30.

44. Beavis AL, Salazar-Marioni S, Sinno AK, Stone RL, Fader AN, Santillan-Gomez A, et al. Sentinel lymph node detection rates using indocyanine green in women with early-stage cervical cancer. Gynecol Oncol. 2016. 143(2):302-6.

45. Buda A, Bussi B, Di Martino G, Di Lorenzo P, Palazzi S, Grassi T, et al. Sentinel lymph node mapping with near-infrared fluorescent imaging using indocyanine green: a new tool for laparoscopic platform in patients with endometrial and cervical cancer. J Minim Invasive Gynecol.2016;23(2):265-9.

46. Buda A, Crivellaro C, Elisei F, Di Martino G, Guerra L, De Ponti E, et al. Impact of indocyanine green for sentinel lymph node mapping in early stage endometrial and cervical cancer: comparison with conventional radiotracer (99m)tc and/or blue dye. Ann Surg Oncol. 2016;23(7):2183-91.

47. Darin MC, Gomez-Hidalgo NR, Westin SN, Soliman PT, Escobar PF, Frumovitz M, et al. Role of indocyanine green in sentinel node mapping in gynecologic cancer: is fluorescence imaging the new standard? J Minim Invasive Gynecol.2016;23(2):186-93.

48. Handgraaf HJ, Verbeek FP, Tummers QR, Boogerd LS, van de Velde CJ, Vahrmeijer AL, et al. Real-time near-infrared fluorescence guided surgery in gynecologic oncology: a review of the current state of the art. Gynecol Oncol. 2014;135(3):606-13.

49. Holloway RW, Abu-Rustum NR, Backes FJ, Boggess JF, Gotlieb WH, Jeffrey Lowery W, et al. Sentinel lymph node mapping and staging in endometrial cancer: a Society of Gynecologic Oncology literature review with consensus recommendations. Gynecologic oncology. 2017; 146(2):405-15.

50. Diab Y. Sentinel lymph nodes mapping in cervical cancer a comprehensive review. Int J Gynecol Cancer.2017;27(1):154-8.

51. Ruscito I, Gasparri ML, Braicu EI, Bellati F, Raio L, Sehouli J, et al. Sentinel node mapping in cervical and endometrial cancer: indocyanine green versus other conventional dyes-a meta-analysis. Ann SurgOncol. 2016;23(11):3749-56.

52. Rocha A, Dominguez AM, Lecuru F, Bourdel N. Indocyanine green and infrared fluorescence in detection of sentinel lymph nodes in endometrial and cervical cancer staging - a systematic review. Eur J Obstet Gynecol Reprod Biol.2016;206:213-9.

53. Cibula D, Abu-Rustum NR. Pelvic lymphadenectomy in cervical cancer--surgical anatomy and proposal for a new classification system. Gynecol Oncol. 2010;116(1):33-7.

54. Cibula D, Abu-Rustum NR, Benedetti-Panici P, Kohler C, Raspagliesi F, Querleu D, et al. New classification system of radical hysterectomy: emphasis on a three-dimensional anatomic template for parametrial resection. Gynecol Oncol. 2011;122(2):264-8.

55. Querleu D, Morrow CP. Classification of radical hysterectomy. Lancet Oncol. 2008;9(3):297-303.

56. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet. 2009;105(2):103-4.

57. FIGO staging classifications and clinical practice gudelines in the management of gynecologic cancers. Int J Gynaecol Obstet. 2000;70(2):209-62.

58. Snyman LC, Zondagh BA, Dreyer G, Lindeque BG, Louw M. Urine cytology as a screening test for bladder infiltration in cervical cancer. Int J Gynaecol Obstet. 2006;16(4):1587-90.

59. Mangena M, Snyman LC, Dreyer G, Bassa S, Becker P. The impact of HIV on women receiving radiation for cervical cancer. South Afr J Gynaecol Oncol. 2015;7(2):44-51.

60. Gradidge PJ, Crowther NJ. Review: Metabolic syndrome in black South African women. Ethn Dis. 2017;27(2):189-200.

61. Freitas P, Carvalho D, Santos AC, Matos MJ, Madureira AJ, Marques R, et al. Prevalence of obesity and its relationship to clinical lipodystrophy in HIV-infected adults on anti-retroviral therapy. J Endocrinol Invest. 2012;35(11):964-70.

62. Buda A, Papadia A, Zapardiel I, Vizza E, Ghezzi F, De Ponti E, et al. From conventional radiotracer tc-99(m) with blue dye to indocyanine green fluorescence: a comparison of methods towards optimization of sentinel lymph node mapping in early stage cervical cancer for a laparoscopic approach. Ann Surg Oncol. 2016;23(9):2959-65.

63. Plante M, Touhami O, Trinh XB, Renaud MC, Sebastianelli A, Grondin K, et al. Sentinel node mapping with indocyanine green and endoscopic near-infrared fluorescence imaging in endometrial cancer. A pilot study and review of the literature. Gynecol Oncol. 2015;137(3):443-7.

64. Devaja O, Mehra G, Coutts M, Montalto SA, Donaldson J, Kodampur M, et al. A prospective single-center study of sentinel lymph node detection in cervical carcinoma: is there a place in clinical practice? Int J Gynecol Cancer.2012;22(6):1044-9.

65. Zarganis P, Kondi-Pafiti A, Arapantoni-Dadioti P, Trivizaki E, Velentzas K, Vorgias G, et al. The sentinel node in cervical cancer patients: role of tumor size and invasion of lymphatic vascular space. In Vivo. 2009;23(3):469-73.

Chapter 4

The ability of [18]Fluoro-deoxy-glucose positron emission tomography/computed tomography scan to accurately predict pelvic nodal status in women with uterine cancer

1. Introduction

The need to have accurate knowledge regarding the pelvic and where necessary para-aortic lymph node status has been discussed in depth in Chapter one of this thesis. In essence, the gold standard method of obtaining this information in women with uterine cancer is histological examination of all the removed nodes following systematic pelvic and where indicated para-aortic lymphadenectomy.

The sentinel lymph node concept in uterine cancer has been proposed as a feasible alternative to systematic pelvic lymphadenectomy in patients treated for presumed early stage endometrial cancer and early stage cervical cancer. This concept is based on the notion that lymph node metastases from cervical or endometrial cancer first involves one or two pelvic nodes known as the sentinel nodes on each side before metastasising to other nodes in the region. The status of the sentinel nodes is believed to reflect the status of the rest of the nodes on that specific side with a very high degree of accuracy.

1.1. The role of imaging in predicting lymph node status in patients with uterine malignancies

1.1.1. Ultrasound, magnetic resonance imaging and computed tomography scan

Imaging plays an important role in obstetrics & gynaecology, with ultrasound investigations the main modality used by all gynaecologists, especially in the practice of obstetrics. Ultrasound in gynaecology has also evolved rapidly with the availability of high quality ultrasound equipment and trans-vaginal probes

allowing access close to the anatomy that is being imaged. The use of ultrasound in detecting or assessing lymph nodes is to a large extent inadequate, especially with regard to deeply located nodes [1]. The data on the ability of ultrasound to reliably detect lymph node metastases are limited, with a reported sensitivity ranging from 38 - 43%. Ultrasound, like other imaging modalities depends on lymph node size to detect metastases and will not detect disease in lymph nodes that are not enlarged [2,3].

Lymph nodes can be involved in a variety of pathologies, including infectious and neoplastic conditions. The identification of metastatic lymph nodes using computed tomography (CT) scans and magnetic resonance imaging (MRI) is mainly based on assessment of nodal size. In normal sized nodes the short axis diameter usually does not exceed 1 cm. A short axis diameter of more than 1 cm is usually regarded as enlarged and in the context of cancer staging will be regarded as indicative of nodal metastases. The widespread use of CT scans and MRI is reduced by their inability to detect metastases in normal sized nodes, and their inability to accurately distinguish on morphological features between malignant and reactive or benign enlarged nodes [1]. Benedetti et al reported the diameter of nodal metastases in cervical cancer patients was less than 10 mm in more than 80% of positive nodes [4].

Supplementary imaging modalities such as CT scan and MRI are important special investigations available to gynaecologic oncologists to assess extend and possible spread of disease in women with gynaecologic cancer. The sensitivity of CT scan to detect nodal metastasis in endometrial cancer ranges from 30 - 52% with the specificity ranging from 93 to 98% [5]. A meta-analysis on the ability of diffusion weighted MRI to detect metastatic pelvic nodal disease in women with cervical cancer published by Shen et al, showed pooled sensitivity and specificity of 86% and 84% respectively [6]. The ability to detect nodal disease on CT scan and MRI seems to be comparable [7].

1.1.2. [18]Fluoro-deoxy-glucose positron emission tomography/computed tomography scan

Functional imaging methods such as positron emission tomography (PET) can establish the metabolic or functional parameters of tissue. Instead of using anatomical deviations such as size to identify areas of abnormality, PET uses positron-emitting radiolabeled molecules to display molecular interactions of biological processes *in vivo*. The most commonly used radioisotope tracer is [18]Fluoro-deoxy-glucose (FDG), a glucose analog which is preferentially taken up by and retained within malignant cells.

Since the 2000s, integrated PET/CT, in which a full-ring-detector clinical PET scanner and a multidetector helical CT scanner are combined, has enabled the acquisition of both metabolic and anatomic imaging data using one device in a single diagnostic session, and this provides precise anatomic localization of suspicious areas of increased FDG uptake and eliminates false-positive PET findings [8].

Sironi et al in 2006 prospectively evaluated the accuracy of FDG-PET/CT in 47 women with early stage cervical cancer. Results showed an overall patient based sensitivity of 73% (95% CI 48 - 89.1), specificity 97% (84.3 - 99.4), positive predictive value (PPV) of 92%, and NPV of 89%. The accuracy of PET/CT was 89% and four patients (8.5%) had false negative findings. Loft et al reported results of 27 women who had FDG-PET/CT scan before undergoing radical hysterectomy for early stage cervical cancer [9]. The sensitivity of FDG-PET/CT was 75%, the specificity 96%, the PPV 75% and the NPV 96%. The false negative rate was 4% [8].

Signorelli et al published the largest study to date that evaluated FDG-PET/CT scans in 159 women treated surgically for early stage cervical cancer (FIGO stage I - IIA). Twenty-eight women (18%) in the study population had nodal metastases. FDG-PET/CT correctly identified pelvic lymph node lesions in 9 out of 28 women, with a sensitivity of 32.1% (95% CI 14.8 - 49.4%). The PPV was 69.2% (95% CI 44.1 - 94.3%). The absence of metastatic pelvic lymph nodes was correctly diagnosed in 127 of 131 patients, resulting in a

specificity of 96.9% (95% CI 94.0 - 99.8%) with a NPV of 86.9% (95% CI 81.5 - 92.4%). The FNR was not reported [10].

Selman et al published a systematic review and meta-analysis of 72 studies involving 5 042 women on the diagnostic accuracy of tests for lymph node status in primary cervical cancer. Sentinel lymph node biopsy had a sensitivity of 91.4% (95% CI 87.1 - 94.6), specificity of 100% (99.6 - 100) with a pooled positive likelihood ratio of 40.8 (24.6 - 67.6) and a pooled negative likelihood ration of 0.18 (0.14 - 0.24), while the respective values for FDG-PET/CT were 74.7 (63.3 - 84.0), 97.6 (95.4 - 98.9), 15.3 (7.9 - 29.6) and 0.27 (0.11 - 0.66) respectively [7].

Kitajima et al investigated the accuracy of FDG-PET/CT in 40 women with endometrial cancer FIGO stages I - III. The overall patient based sensitivity was 50%, the specificity 86.7% and the accuracy was 77.5% [11].

Signorelli et al calculated the diagnostic accuracy of FDG-PET/CT in 37 women with high-risk early endometrial cancer in a prospective study. Patient-based sensitivity, specificity, positive predictive value, negative predictive value and accuracy of FDG-PET/CT for detection of nodal disease were 77.8%, 100.0%, 100.0%, 93.1% and 94.4%, respectively [12].

Chang et al published a meta-analysis including seven studies involving 243 women on the ability of FDG-PET/CT to detect metastatic lymph nodes in women with endometrial cancer. The overall pooled estimates for sensitivity and specificity of FDG-PET or FDG-PET/CT scans in the detection of pelvic and/or para - aortic metastases were 63.0% (95% CI, 48.7 - 75.7%) and 94.7% (95% CI, 90.4 - 97.4%), respectively. The positive likelihood ratio was 10.465 (95% CI, 5.64 - 19.39) and the negative likelihood ratio 0.399 (95% CI, 0.28 - 0.56). The overall diagnostic accuracy was 89.5% [13].

The conclusion from these and other studies is that FDG-PET/CT alone is not reliable enough to use as a test to accurately predict lymph node metastases in women with early stage endometrial and cervical cancer [14-16].

A summary of the literature with regard to the ability of FDG-PET and FDG-PET/CT to detect nodal metastases is given in Table 1.

Table 1: Literature review of diagnostic accuracy of FDG-PET or FDG-PET/CT scan in detecting nodal metastases [10].

Author	No of patients	Stage	Imaging	% Nodes Positive	Sens	Spec	PPV	NPV
Belhocine	18	IA-IVA	PET	56	20	63	40	38
Williams	18	NR	PET	44	25	77	26	76
Reinhardt	35	IB1-IIB	PET	31	91	100	100	96
Park	36	IB1-IIA	PET	39	43	100	100	73
Wright	59	IA2-IB1-IIA	PET	32	53	90	71	80
Chou	60	IA2-IIA	PET	17	10	94	25	84
Yu	16	IB1-IB2	PET/CT	6	NA	NA	NA	94
Amit	16	NR	PET/CT	25	NA	92	NA	73
Choi	22	IB-IVA	PET/CT	59	77	56	71	63
Loft	27	IB1-IIA	PET/CT	15	75	96	75	96
Sironi	47	IA2-IB2	PET/CT	32	73	97	92	89
Ramirez	60	IB2-IIIB	PET/CT	23	36	96	71	83
Goyal	80	IB1-IIA	PET/CT	30	58	93	78	84
Chung	83	IB1-IIB	PET/CT	33	29	84	47	70
Signorelli	159	IB1-IIA	PET/CT	18	32	97	69	87

Sens = Sensitivity; Spec = Specificity; PPV = Positive predictive value; NPV = Negative predictive value

Chung et al compared the ability of MRI and PET/CT to pre-operatively detect nodal metastases in cervical cancer patients. This retrospective study on 83 women, of which 28 (33.7%) had nodal spread, concluded that MRI was significantly better than PET/CT to detect nodal metastases [17].

Inflammatory and infectious conditions can result in false positive lymph node findings in patients investigated with FDG-PET examinations. Patients with HIV infection as well as those with tuberculosis (TB) infection will have increased FDG uptake in involved lymph nodes. In cancer patients who have

HIV infection or TB, or both, FDG-PET is not a reliable test for assessing metastatic lymph node disease [18].

2. Rationale for the study

The data referred to and discussed above is mainly from Europe and North America. Very limited to zero data investigating the effectiveness of FDG-PET/CT scans in accurately predicting the pelvic lymph node status in South African women exist. It is well known that the prevalence of human immunodeficiency virus (HIV) and human papilloma virus (HPV) infections, TB and pelvic inflammatory disease (PID) is significantly high in South African women [19-21]. In addition, it remains a priority to find ways of safely avoiding or limiting the use of unnecessary invasive procedures in women who are at risk of the surgical morbidity described in patients living in a low socio-economic environment.

3. Aim and Objectives

3.1. Aim

3.1.1. To investigate the ability of FDG-PET/CT scans to predict pelvic lymph node metastases in women with early stage endometrial and cervical cancer in a South African setting

3.2. Objectives

3.2.1. To establish the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of FDG-PET/CT scan in women with early stage cervical and endometrial cancer

3.2.2. To determine the SLN detection rate in women who had FDG-PET/CT scans and lymphoscintigram examinations

4. Materials and methods

4.1. Study population and setting

A prospective cohort study was conducted at the Gynaecological Oncology Unit, Department Obstetrics and Gynaecology, University of Pretoria. The study was performed at Kalafong Provincial Tertiary Hospital (KPTH) and Steve Biko Academic Hospital (SBAH).

Women with early stage cervical cancer (FIGO stage IA to IIA) and apparent early stage endometrial cancer (FIGO stage I and II) were eligible for recruitment to the study. Recruitment was influenced by logistic considerations such as availability of methylene blue, appointments for lymphoscintigram and or FDG-PET/CT scan, which was performed on some women prior to surgical treatment.

Surgical treatment consisted of laparoscopic or open radical hysterectomy and pelvic with or without para-aortic lymph adenectomy for cervical cancer, and laparoscopic or total abdominal hysterectomy, bilateral salpingo oophorectomy (BSO) and pelvic with or without para-aortic lymph adenectomy for women with endometrial cancer. Laparoscopic or open SLNB procedure was performed on all women during these surgical procedures.

Sentinel lymph node detection was done using ^{99m}Tc-Nanocolloid and methylene blue labelling prior to surgery. In some patients indocyanine green (ICG) was also used in addition to methylene blue dye. After identifying and removal of all SLNs, total pelvic lymphadenectomy was performed in addition to the appropriate type of hysterectomy with or without bilateral salpingo-oophorectomy. Para-aortic lymphadenectomy was performed at the discretion of the treating gynaecological oncologist.

The SLN and the remainder of the pelvic lymph nodes were sent separately for histological examination. Each sentinel lymph node was bisected into two halves. The one half was managed as a frozen section, while the other half was processed and stained using haematoxylin and eosin (H&E) as per normal routine. If after H&E examination the SLN was found to be negative for

metastases, the rest of the node underwent ultrastaging.

4.2. Imaging

4.2.1. [18]Fluoro-deoxy-glucose positron emission tomography/computed tomography

Based on the availability of resources, some women scheduled for surgery also underwent pre-operative FDG-PET/CT scan with the aim of assessing possible pelvic and para-aortic lymph node metastases. Women with contra-indications for FDG-PET/CT were not excluded from the rest of the study. FDG-PET/CT was performed on a Siemens Biograph 40 PET/CT scanner following standard preparation of the Nuclear Medicine Department at Steve Biko Academic Hospital.

Preparation consisted of a four-hour fast and avoidance of strenuous exercise on the day prior to the study. Blood glucose was obtained with a portable capillary glucometer (cut-off for inclusion was 140 mg/dl). Patients received a dose of FDG based on their body weight using the formula {(body weight/10) +1} *37 MBq.

Patients were covered with a blanket to keep them warm and prevent brown fat uptake. Visual and auditory stimuli were avoided for at least 45 minutes to prevent uptake of the radiotracer at physiological sites excited by these stimuli, which could result in artefacts. During this phase, patients were instructed to drink 1 litre of contrast material (barium diluted in water). Images were acquired in a three-dimensional mode with a 3-minute emission scan for each of 9 bed positions (Matrix size 512 x 512) from the skull base to the pelvis. Patients were asked not to move while on the scanning table.

For CT imaging, contrast enhancement was achieved by intravenous administration of 100 ml of non-ionic contrast material (Ultravist) at a rate of 2 ml/s. Images were reconstructed with and without attenuation correction (CT based) using ordered subset expectation maximization (OSEM) to yield axial, sagittal and coronal slices.

4.2.2. Image analysis & interpretation

Findings were evaluated and reported by two independent physicians who had access to the patient history and examination, but were blinded to the morphological and histology results. Agreement was reached by consensus.

4.2.3. Qualitative Analyses

Any areas of abnormal tracer accumulation were documented and graded visually, with the mediastinal blood pool and liver serving as the internal reference points, as follows:

Lesions in the thorax:

0 = Less intense than the mediastinal blood pool

1 = Equal in intensity to the mediastinal blood pool

2 = More intense than the mediastinal blood pool

Lesions in the abdomen:

0 = Less intense than the liver

1 = Equal in intensity to the liver

2 = More intense than the liver

4.2.4. Semi-quantitative Analyses

PET/CT parameters; maximum standardised uptake value (SUV max), mean standardised uptake value (SUV mean), metabolic tumour volume (MTV), total lesion glycolysis (TLG), and uptake pattern were recorded.

Regions of interest (ROIs) were placed manually over areas of abnormal ^{18}F-FDG accumulation and SUVmax was determined for all areas of pathological uptake noted on ^{18}F-FDG. ROI was created manually by the interpreting nuclear physician. The value obtained from this ROI reflects the maximum intensity obtained from a pixel within the selected ROI. This value was used to quantify areas of abnormal tracer accumulation and was expressed as the SUVmax.

SUV was calculated as radiotracer activity x weight of the patient/injected dose, which characterised the relative concentration of the radiotracer in the

lesion of interest. The SUV for the pixel with the greatest uptake in the lesion (maximum pixel SUV) was used.

MTV was defined as all tumour voxels exceeding a cut-off SUV of 2.5 and TLG was defined as total lesion glycolysis: MTV multiplied by its mean SUV.

The corresponding CT image was used as an anatomical landmark. In tumour lesions, which extended over several slices in the cranio-caudal direction, the ROI was placed over the mid-portion of the lesion where the maximal SUV was measured

4.2.5. Image acquisition

A dynamic acquisition was acquired between 30 - 60 seconds per frame over 20 - 30 minutes starting immediately after injection. All planar images were acquired no later than one hour post tracer injection.

4.2.6. CT acquisition

CT was performed with the SPECT A low dose CT (140 kV, 2.5 mA). It was acquired over the 40 cm of the field of view of the SPECT study. This was performed after the SPECT study which itself was performed immediately after identifying the sentinel lymph node on planar images. Images were registered and fused using fusion software provided on the Xeleris workstation.

Analysis included interpretation of the planar images and SPECT images by two experienced nuclear physicians.

A pre-operative planar lymphoscintigram was taken 20 - 30 min after injection to locate the nodes. Gamma probes, such as the Europrobe or C-Trak laparoscopic SLN probe or a handheld open procedure SLN probe was used intra-operatively to detect the presence of any "hot" nodes.

4.3. Ethical considerations

Participation in the study was on a voluntary basis and all women who agreed to participate in the study provided informed consent. Patients who declined participation were in no way disadvantaged and received standard treatment according to the guidelines of the Gynaecological Oncology Unit or as decided by the attending gynaecological oncology team. Patients participating in the trial did not receive any financial or material benefits or rewards.

The University of Pretoria Faculty of Health Sciences Research Ethics Committee approved the study (434/2014).

5. Results

FDG-PET/CT scans were performed on thirty-two women. Data of four women were excluded from the analysis; three women were upstaged, and one demised prior to planned surgery. Results of 28 women were available for analysis. Twenty-four of the 28 women (85.7%) had early stage cervical cancer, and four women were diagnosed with presumed early stage endometrial cancer.

5.1. Demographic data

The mean age of the women in this group was 52.1 years. The mean age of the four women with endometrial cancer was 76 years (SD = 9.56; 62 - 83 years; 95% CI 60.79 - 91.21).

Five women (17.8%) had previous surgery. Two patients (7.1%) provided a history of previous tuberculosis infection. Four patients (14.2%) were previously diagnosed with pelvic inflammatory disease (PID), and five women (17.9%) underwent previous surgery. The rest of the demographic data of this group is shown in Table 2.

Table 2: Demographic data

n = 24	Mean	SD	Range
Age	52.11	14.59	33 – 83
Parity	3.57	2.35	1 – 12
Gravidity	3.71	2.34	1 – 12
BMI	26.46	4.61	18.59 – 35.44

SD = Standard deviation

5.2. Disease characteristics

The FIGO stage distribution of the 24 patients in this group who had cervical cancer is shown in Figure 1.
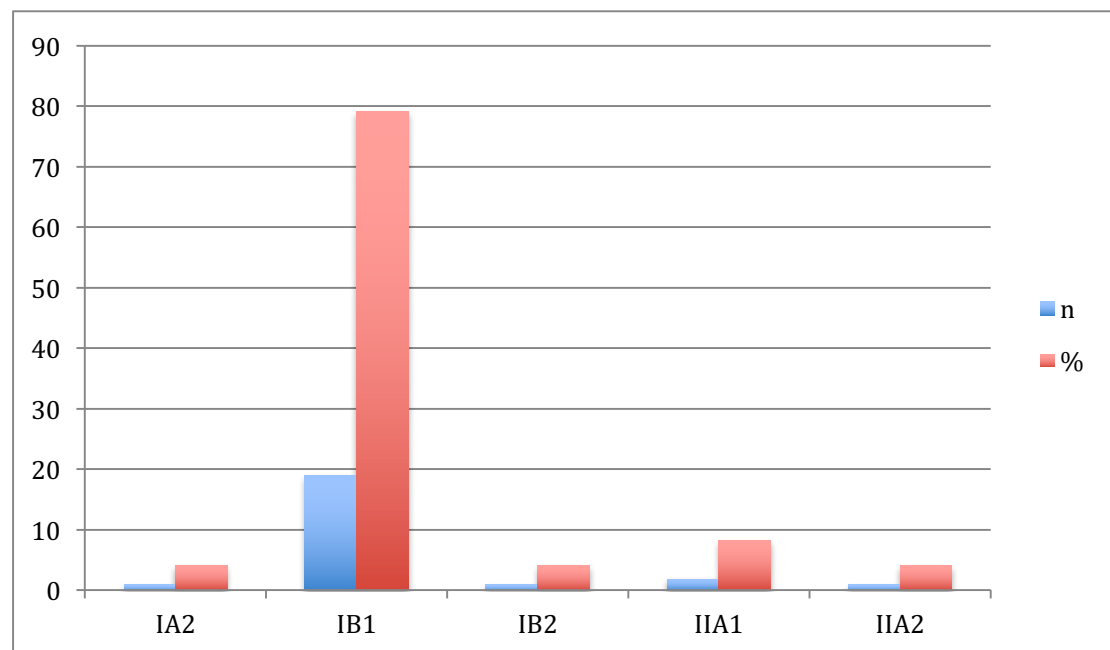
Figure 1: FIGO cervical cancer stage distribution

Twenty-three women (95.8%) had squamous cell carcinoma, and one woman (4.2%) had adenocarcinoma. Nine of the 24 cervical cancer patients (37.5%), had tumours > 2cm.

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Two of the four women with endometrial cancer in this group were FIGO stage IA, one had FIGO stage IB and one was FIGO stage II. Two patients had endometrioid adenocarcinoma, and two patients had serous adenocarcinoma on final histology.

5.3. Surgical access and type of surgery

Laparoscopic radical hysterectomy with pelvic lymphadenectomy was completed in 15 of the 24 cervical cancer patients (62.5%) and total laparoscopic hysterectomy and pelvic lymphadenectomy in two of the four women with endometrial cancer (50.0%). In two of the 24 cervical cancer patients (8.3%), the laparoscopic approach was started but converted to open radical hysterectomy, because of an enlarged uterus in one patient and extensive nodal disease in the other patient. Seven of the 24 cervical cancer patients (29.2%) had open radical hysterectomy with pelvic lymphadenectomy and two of the four women with endometrial cancer (50.0%) underwent open hysterectomy, BSO and pelvic lymphadenectomy.

5.4. HIV status

All women were tested for HIV infection and 14 women (50.0%) tested positive for HIV-infection. All of them were in the cervical cancer group, where 58.3% were HIV positive. All HIV-infected women were using anti-retroviral therapy. Ten women (71.4%) were using ARV therapy for more than six months at the time of treatment for cervical cancer. The mean CD4 count in HIV-positive patients were 448.15 cells/µl (range 189 - 784; SD = 204.79; SEM = 56.80, 95% CI = 324.40 - 571.91). HIV-infected women were significantly younger compared to HIV-negative women. The rest of the comparative data is shown in Table 3.

5.5. Sentinel lymph nodes

All patients in this cohort received MB for sentinel node detection, and 26 patients (92.9%) also received [99]Technetium nanocolloid (^{99m}Tc) with lymphoscintigram. ICG was used in five women (17.8%).

SLNs were detected in 24 patients, representing a detection rate of 85.7%. Bilateral SLN detection was achieved in 12 patients for a bilateral detection rate

of 42.9%. The mean macroscopic pelvic SLN count was 1.96 (range = 1 - 4; SD = 1.00; SEM = 0.20; 95% CI = 1.54 - 2.38). The histologically confirmed mean SLN count was 2.96 (range 1 - 7; SD = 1.85; SEM = 0.38; 95% CI = 2.18 - 3.74). Four patients (14.3%) had metastases in the sentinel lymph nodes.

Table 3: Comparative data of HIV-negative compared to HIV-positive women

	HIV-negative n = 14		HIV-positive n = 14		p-value
	Mean	SD	Mean	SD	
Age (years) (all)	62.00	13.79	42.21	6.40	< 0.0001
Age (ca cervix only)	56.40	11.06	42.21	6.40	0.0006
Parity	4.79	2.72	2.36	0.93	0.0039
Gravidity	5.07	2.56	2.36	0.93	0.0010
BMI (kg/m^2)	27.53	4.82	25.29	4.31	0.2063
Tumour diameter (mm)	22.20	21.94	17.14	15.61	0.5149
Macro SLN count	1.77	0.93	2.09	1.04	0.4346
Histology SLN count	2.54	1.61	3.36	2.01	0.2789
Pelvic node count	22.14	8.50	24.50	7.83	0.4517
	n	%	n	%	
Previous TB	0	0	2	14.28	0.1496
Previous PID	2	14.28	2	14.28	1.0000
Previous surgery	4	28.57	1	7.14	0.1460
Tumour ≥2 cm	5	35.71	4	28.57	0.6912
Enlarged nodes	1	7.14	4	28.57	0.1460
Nodal metastases	2	14.28	4	28.57	0.3655
SLN detection rate	13	92.86	11	78.57	0.2887
Bilat SLN detection	6	42.86	6	42.86	1.000

SD = Standard deviation

5.6. Pelvic lymph nodes

Six patients (21.4%) had pelvic lymph node metastases. The mean pelvic lymph node count was 23.32 (range = 9 - 37; SD = 8.11; SEM = 1.53; 95% CI = 20.18 - 26.47).

One patient had tuberculosis reported in the pelvic nodes after histological examination. This patient had micro metastases on histology of her SLN and the non-SLNs were negative for metastases. The FDG-PET/CT was a true positive on the one side and true negative on the other side.

5.7. Sensitivity, specificity, positive and negative predictive values

Calculated per hemi-pelvis for the whole group, there were four true positives, 41 true negatives, two false negatives and nine false positives.

The sensitivity, specificity, positive and negative predictive values of FDG-PET/CT scans to accurately predict nodal status, were 66.7%, 82%, 30.8% and 95.4% respectively. The false negative rate of FDG-PET/CT scans for the entire cohort was 33.3%.

6. Discussion

This cohort of patients was selected based on the availability of resources to perform FDG-PET/CT scans and lymphoscintigraphy on them.

It was possible to perform FDG-PET/CT scans in only 32 women (32%) from the entire cohort over a period of twenty months, which highlights the pressure on scarce commodities in healthcare such as FDG-PET/CT scan. Logistic factors such as time slots and other related factors limit the availability to perform these investigations on all patients intended to be investigated.

The demographic data of this group of women compares well with that of the entire group reported on in Chapter one.

The phenomenon that HIV-infection results in cervical cancer being diagnosed at a significantly younger age is once again confirmed in this dataset. The HIV-infected women in this group were 14 years younger than their HIV-positive counterparts, a finding that is consistent with published reports from elsewhere and from the Pretoria Gynaecologic Oncology Unit. In addition, parity and gravidity were also significantly lower in HIV-positive women. The rest of the comparative data between HIV-positive and HIV-negative women did not reach statistical significance, a finding that might be due to antiretroviral treatment.

The stage distribution of the 24 women in this group who had cervical cancer was different compared to that of the women reported on in Chapter three, with a much lower proportion of women with FIGO stage IB2 disease of 4% in this cohort compared to 15% in the Chapter 3-cohort. In this cohort, 79% of women had FIGO stage IB1 disease compared to 68% in the cohort reported on in chapter three. The p values of 0.25 and 0.15 respectively for the comparison between FIGO stages IB1 and IB2 did not reach statistical significance.

The SLN detection rate of 85.7% in this cohort was statistically significantly better compared to the 60.6% of the entire cohort reported on in chapter one (p = 0.0141). This is most likely due to the fact that MB with ^{99m}Tc was used to detect SLNs in nearly 93% of women in this cohort, compared to 43% in the entire cohort. It has been reported that the combination of blue dye and ^{99m}Tc resulted in higher detection rates compared to either agent used alone [22,23]. In addition to this, more women in this cohort had stage IB1 disease.

The sensitivity, specificity, PPV and NPV for SLNs in endometrial cancer, cervical cancer and for FDG-PET/CT scan is shown in Table 4.

Table 4: Comparison of sensitivity, specificity, PPV and NPV of SLNB in endometrial and cervical cancer with FDG-PET/CT scan in endometrial and cervical cancer

	SLNB Endometrial cancer	SLNB Cervical cancer	FDG-PET/CT
Sensitivity	100	85	66.67
Specificity	100	100	82.00
PPV	100	100	30.77
NPV	100	98.33	95.35
FNR	0	14.3	33.3

PPV= Positive predictive value; NPV = Negative predictive value; FNR = False negative rate

The most important marker of interest is the FNR, which should preferably be as close to zero as possible. The FNR of FDG-PET/CT is 33.3% and of SLNB, without applying the algorithm, is 14.3%. The relatively high false negative rates of both the SLNB in cervical cancer patients in this study and [18]FDG-PET/CT scan, renders these as stand-alone tests insufficient to be applied to a high-stakes decision such as the oncological status of the rest of the regional lymph nodes in women investigated for presumed early stage endometrial and cervical cancer. The FNR of the SLN algorithm in women with cervical cancer in this study is zero, which is consistent with the published literature suggesting a very low FNR for the SLN algorithm [24].

The sensitivity, specificity, PPV and NPV of FDG-PET/CT scan performed on South African women with high prevalence of HIV, TB and PID is comparable to that published in the international literature [19-21]. This is the first study reporting on the sentinel lymph node procedures in patients who are HIV-infected.

7. Conclusions

The 28 women in this cohort demonstrated a SLN detection rate very much comparable to the best internationally published data. This is mainly attributable to the use of two detection modes in 26 of the 28 women, as well as the fact that they mostly had FIGO stage IB1 disease.

The sensitivity, specificity, PPV, NPV and FNR of FDG-PET/CT scan compares less favourably to that of SLN biopsy and the SLN algorithm. The high FNR of FDG-PET/CT limits its use to accurately predict the status of the rest of the pelvic lymph nodes in early stage endometrial and cervical cancer and support the Radiology Society of South Africa PET/CT guidelines. According to these guidelines PET/CT should not be used as part of the diagnosis and staging investigations, and is only recommended in selected cases for initial staging of locally advanced cervical cancer being considered for radical chemoradiation therapy [25].

The results presented in this chapter confirms the conclusion from several other studies suggesting that FDG-PET/CT alone is not reliable enough to use as a stand-alone test to accurately predict lymph node metastases in women with early stage endometrial and cervical cancer [14-16].

In addition, these results confirm the pressure on existing infrastructure in low-resource settings for special investigations such as FDG PET/CT scans. It is imperative for healthcare workers in resource-poor settings to appropriately prioritise patients undergoing special investigations.

References

1. Luciani A, Itti E, Rahmouni A, Meignan M, Clement O. Lymph node imaging: basic principles. Eur J Radiol. 2006;58(3):338-44.

2. Fischerova D, Cibula D. Ultrasound in gynecological cancer: is it time for re-evaluation of its uses? Curr Oncol Rep. 2015;17(6):28.

3. Palsdottir K, Fischerova D, Franchi D, Testa A, Di Legge A, Epstein E. Preoperative prediction of lymph node metastasis and deep stromal invasion in women with invasive cervical cancer: prospective multicenter study using 2D and 3D ultrasound. Ultrasound Obstet Gynecol. 2015;45(4):470-5.

4. Benedetti-Panici P, Maneschi F, Scambia G, Greggi S, Cutillo G, D'Andrea G, et al. Lymphatic spread of cervical cancer: an anatomical and pathological study based on 225 radical hysterectomies with systematic pelvic and aortic lymphadenectomy. Gynecol Oncol. 1996;62(1):19-24.

5. Lakhman Y, Katz SS, Goldman DA, Yakar D, Vargas HA, Sosa RE, et al. Diagnostic performance of computed tomography for preoperative staging of patients with non-endometrioid carcinomas of the uterine corpus. Ann Surg Oncol. 2016;23(4):1271-8.

6. Shen G, Zhou H, Jia Z, Deng H. Diagnostic performance of diffusion-weighted MRI for detection of pelvic metastatic lymph nodes in patients with cervical cancer: a systematic review and meta-analysis. Br J Radiol. 2015;88(1052):20150063.

7. Selman TJ, Mann C, Zamora J, Appleyard TL, Khan K. Diagnostic accuracy of tests for lymph node status in primary cervical cancer: a systematic review and meta-analysis. CMAJ. 2008;178(7):855-62.

8. Sironi S, Buda A, Picchio M, Perego P, Moreni R, Pellegrino A, et al. Lymph node metastasis in patients with clinical early-stage cervical cancer: detection with integrated FDG PET/CT. Radiology. 2006;238(1):272-9.

9. Loft A, Berthelsen AK, Roed H, Ottosen C, Lundvall L, Knudsen J, et al. The diagnostic value of PET/CT scanning in patients with cervical cancer: a prospective study. Gynecol Oncol. 2007;106(1):29-34.

10. Signorelli M, Guerra L, Montanelli L, Crivellaro C, Buda A, Dell'Anna T, et al. Preoperative staging of cervical cancer: is 18-FDG-PET/CT really effective in patients with early stage disease? Gynecol Oncol. 2011;123(2):236-40.

11. Kitajima K, Murakami K, Yamasaki E, Fukasawa I, Inaba N, Kaji Y, et al. Accuracy of 18F-FDG PET/CT in detecting pelvic and paraaortic lymph node metastasis in patients with endometrial cancer. AJR Am J Roentgenol. 2008;190(6):1652-8.

12. Signorelli M, Guerra L, Buda A, Picchio M, Mangili G, Dell'Anna T, et al. Role of the integrated FDG PET/CT in the surgical management of patients with high risk clinical early stage endometrial cancer: detection of pelvic nodal metastases. Gynecol Oncol. 2009;115(2):231-5.

13. Chang MC, Chen JH, Liang JA, Yang KT, Cheng KY, Kao CH. 18F-FDG PET or PET/CT for detection of metastatic lymph nodes in patients with endometrial cancer: a systematic review and meta-analysis. Eur J Radiol. 2012;81(11):3511-7.

14. Horowitz NS, Dehdashti F, Herzog TJ, Rader JS, Powell MA, Gibb RK, et al. Prospective evaluation of FDG-PET for detecting pelvic and para-aortic lymph node metastasis in uterine corpus cancer. Gynecol Oncol. 2004;95(3):546-51.

15. Bentivegna E, Uzan C, Gouy S, Leboulleux S, Duvillard P, Lumbroso J, et al. Correlation between [18f]fluorodeoxyglucose positron-emission tomography scan and histology of pelvic nodes in early-stage cervical cancer. Anticancer Res. 2010;30(3):1029-32.

16. Signorelli M, Crivellaro C, Buda A, Guerra L, Fruscio R, Elisei F, et al. Staging of high-risk endometrial cancer with PET/CT and sentinel lymph node mapping. Clin Nucl Med. 2015;40(10):780-5.

17. Chung HH, Kang KW, Cho JY, Kim JW, Park NH, Song YS, et al. Role of magnetic resonance imaging and positron emission tomography/computed tomography in preoperative lymph node detection of uterine cervical cancer. Am J Obstet Gynecol. 2010;203(2):156.e1-5.

18. Sathekge M, Maes A, Kgomo M, Pottel H, Stolz A, Van De Wiele C. FDG uptake in lymph-nodes of HIV+ and tuberculosis patients: implications for cancer staging. Q J Nucl Med Mol Imaging. 2010;54(6):698-703.

19. World Health Organization. Global tuberculosis report 2016. Switzerland: WHO; 2016. Available from: http://apps.who.int/iris/bitstream/10665/250441/1/9789241565394-eng.pdf?ua=1 [Accessed on 11 Oct 2017]

20. Richter K, Becker P, Horton A, Dreyer G. Age-specific prevalence of cervical human papillomavirus infection and cytological abnormalities in women in Gauteng Province, South Africa. South Afr Med J. 2013;103(5):313-7.

21. Shisana O, Rehle T, Simbayi L, Zuma K, Jooste S, Zungu N, et al. South African national HIV prevalence, incidence and behaviour survey, 2012. Cape Town 2012. HSRC Press.

22. Jewell EL, Huang JJ, Abu-Rustum NR, Gardner GJ, Brown CL, Sonoda Y, et al. Detection of sentinel lymph nodes in minimally invasive surgery using indocyanine green and near-infrared fluorescence imaging for uterine and cervical malignancies. Gynecol Oncol. 2014;133(2):274-7.

23. vvan der Vorst JR, Hutteman M, Gaarenstroom KN, Peters AA, Mieog JS, Schaafsma BE, et al. Optimization of near-infrared fluorescent sentinel lymph node mapping in cervical cancer patients. Int J Gynecol Cancer. 2011;21(8):1472-8.

24. Cormier B, Diaz JP, Shih K, Sampson RM, Sonoda Y, Park KJ, et al. Establishing a sentinel lymph node mapping algorithm for the treatment of early cervical cancer. Gynecol Oncol. 2011;122(2):275-80.

25. Radiological Society of South Africa PET-CT Referral Guidelines Version 2 2013. Available from: http://rssa.co.za/downloads/doc_download/1762-rssa-pet-ct-guidelines-version-2-august-2013 [Accessed on 11 Oct 2017]

Chapter 5

High-risk human papilloma virus DNA in sentinel lymph nodes in women with cervical cancer

1. Introduction

Cancer of the uterine cervix is caused by persistent infection of the cervix with high risk human papilloma virus types (hrHPV) [1]. Fifteen types of hrHPV have been described, of which HPV types 16 and 18 are responsible for the majority of cases with cervical cancer worldwide as well as in Africa and southern Africa [2].

Part of the surgical treatment of patients with early stage cervical cancer is systematic pelvic lymphadenectomy. This procedure is performed to obtain reliable information on the oncologic status of the pelvic nodes, to assist in the determination of prognosis and planning of adjuvant treatment, as lymph node status is not part of the FIGO staging classification for cervical cancer. There is no good quality data suggesting any therapeutic benefit of lymphadenectomy in patients with cervical cancer, and therefore the procedure will be performed without benefit to patients in up to 80% of cases of early stage cervical cancer [3].

Imaging and clinical palpation are not reliable methods of pre- or intra-operative lymph node assessment for possible metastases [4,5]. The sentinel lymph node algorithm has been proposed as a reliable alternative to systematic lymphadenectomy in these patients.

A few publications in the recent literature have suggested the presence or absence of high-risk HPV types in the sentinel lymph nodes of women with cervical cancer may further assist in the assessment of prognosis and the oncological status of pelvic lymph nodes in women treated for cervical cancer.

2. Literature review

2.1. Human papilloma virus in non-sentinel lymph nodes

Lancaster et al were the first to report on the presence of HPV DNA in pelvic and para-aortic lymph nodes in 13 women with cervical cancer [6]. There were 13 nodes of which six were metastatic nodes. All six these nodes were positive for HPV DNA, while only one non-metastatic lymph node tested positive. Results from a study published by Kobayashi et al suggest the presence of hrHPV DNA in histologically metastatic free nodes is associated with recurrent disease [7].

Several publications with different populations and study methodologies have reported conflicting data regarding the prognostic value of the presence of HPV in pelvic lymph nodes, with most of the smaller studies not suggesting a correlation [8-11], while most of the larger studies have shown some correlation between the presence of HPV and adverse prognosis [7,12-18].

2.2. Human papilloma virus in sentinel lymph nodes

Three studies investigating HPV status in sentinel lymph nodes of women with cervical cancer have been published.

In a study involving 57 women with FIGO stage I-II cervical cancer Lee et al investigated frozen section histology and HPV DNA in 79 sentinel nodes [19]. HPV typing was performed on tissue and the sub-types identified were not reported. Ten women with metastases were identified on frozen section, while histopathology identified 11 women. HPV DNA was detected in the primary cervical cancer lesion of 55 women (96.5%) and in the sentinel nodes of 44 women (80%). HPV DNA was present in 10 of the 11 women with nodal metastases as well as in all five women with recurrences. The combination of frozen section and HPV had a 100% NPV in predicting non-metastatic disease and recurrence free survival.

Coutant et al performed polymerase chain reaction (PCR) in the primary tumour and sentinel nodes by E6-specific PCR for HPV DNA (HPV 16, 18 and 31) in the primary tumour and sentinel nodes of 59 patients with FIGO stage I - II cervical

cancer [20]. Histological examination of the SLN was performed by H&E stain and ultrastaging of negative H&E nodes. Fifteen women had at least one metastatic SLN. Twenty-nine of 51 tumours tested were positive for HPV DNA. Ten out of 20 metastatic SLNs in 7 out of 15 women were positive for HPV DNA, while five out of 90 non-metastatic nodes in five out of 44 women tested positive for HPV DNA. This study found no relation between HPV DNA status of SLNs and the risk of recurrence [20].

Slama et al published a study involving 49 women with FIGO stage IA2 - IIB cervical cancer [21]. A cytobrush was used for detection of HPV DNA on the tumour and the cut surfaces of both halves of the SLN. Histopathology on the SLN was by means of H&E and ultrastaging. They detected hrHPV DNA in the primary cervical tumours of 45 out of 48 women (91.8%) and in the SLNs of 21 women (49.9%). Six women (12.2%) had regional metastatic disease and they were all positive for hrHPV DNA, while in 15 patients (30.6%) without metastatic nodal disease SLNs were positive for hrHPV DNA. The follow-up period in this study was not sufficient to investigate correlation with disease free or overall survival [21].

3. Rationale for the study

Data on the potential value of hrHPV DNA in SLNs is limited and conflicting, and this topic requires further investigation. An important aspect to investigate is whether HPV DNA testing will have the ability to improve the accuracy of FSE as is reported by Lee et al [19,22].

4. Aims and Objectives

4.1. To investigate the presence and detection of hrHPV DNA in the primary cervical tumour and SLNs of women with cervical cancer.

4.2. To evaluate the ability of HPV DNA status and SLN histology to detect lymph node metastases

4.3. To assess the ability of the combination of intra-operative FSE combined with HPV DNA detection in SLN to accurately predict regional lymph node status

5. Materials and methods

5.1. Study design and setting

This was a prospective cohort study conducted at the Gynaecological Oncology Unit, Department Obstetrics and Gynaecology, University of Pretoria. The study was performed at Kalafong Provincial Tertiary Hospital (KPTH) and Steve Biko Academic Hospital (SBAH).

5.2. Study population

Women with early stage cervical cancer (FIGO stage IA to IIA) were eligible for recruitment to the study. This cohort consists of women with early stage cervical cancer who underwent surgical treatment, and in whom sentinel nodes were detected and removed.

5.3. Surgical treatment and sentinel lymph node detection

Surgical treatment consisted of laparoscopic or open radical hysterectomy and pelvic with or without para-aortic lymph adenectomy. Laparoscopic or open sentinel lymph node biopsy (SLNB) procedure was performed on all women during these surgical procedures.

Sentinel lymph node detection was done using ^{99m}Tc-Nanocolloid and methylene blue labelling prior to surgery. In some patients indocyanine green (ICG) was also used in addition to methylene blue dye. After identifying and removal of all SLNs, total pelvic lymphadenectomy was performed in addition to the appropriate type of hysterectomy with or without bilateral salpingo-oophorectomy. Para-aortic lymphadenectomy was performed at the discretion of the treating gynaecological oncologist.

5.4. Removal of sentinel nodes

The SLN and the remainder of the pelvic lymph nodes were sent separately for

histological examination. Each sentinel lymph node was bisected into two halves. The one half was managed as a frozen section, while the other half was processed and stained using haematoxylin and eosin (H&E) as per normal routine. If after H&E examination the SLN was found to be negative for metastases, the rest of the node underwent ultrastaging.

5.5. Human papilloma virus DNA analysis

5.5.1. Sample collection

A cytobrush was used to collect cells directly from the primary tumour pre-operatively.

Intra-operatively identified SLNs were divided through the long axis. Sample collection was with a cytobrush from the central parts of both cut surfaces and was performed before fixation of the tissue. In cases where more than one sentinel node was identified, separate brushes were used for each individual node. All cytobrush tips were placed in the same container with PreservCyt® transport medium/phosphate buffered saline (PBS). The tip of each cytobrush was submerged into the container filled with PreservCyt® transport medium/phosphate buffered saline immediately after collection. All material was released from the cytobrush by vortexing in the lab before processing.

Cytobrush collection as described above was deemed the most feasible option. The primary aim of the study was to have the tissue examined histologically and there was not enough tissue available to perform DNA testing on tissue

5.5.2. HPV Genotyping

In the laboratory specimens were centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the cell pellets were stored at -70°C until testing could be performed in batches.

HPV and cellular DNA were released by either manual extraction (as described by the manufacturer as per package insert) or by using the DNA

Isolation Kit (Roche Molecular Systems, Branchburg, NJ), an automated extraction on the MagNAPure instrument.

The commercially available LINEAR ARRAY® HPV Genotyping Test (Roche Molecular Systems, Branchburg, NJ), a line-blot assay that individually identifies 37 HPV genotypes, was used to detect the HPV and cellular DNA in the sample. The pool of primers is designed to amplify HPV DNA from 18 high-risk/probable high-risk genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82), and 19 low/undetermined-risk types (6, 11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84, IS39 and CP6108). The β-globin gene is amplified concurrently to assess cellular adequacy, extraction and amplification for each individual specimen.

5.6. Data collection

The following data were collected:

Patient characteristics: age, ethnicity, BMI

Medical history: HIV status (CD4 count, viral load and treatment if applicable), history of TB, history of PID, history of STD (Chlamydia, gonorrhoea, genital warts), history of cone biopsy

Tumour characteristics: FIGO stage, lymphovascular space invasion (LVSI), size of tumour, histology

Surgical characteristics: grossly enlarged lymph nodes, adhesions (signs of previous PID)

SLN characteristics: number of SLN found (hot and/blue), location of SLNs, unilateral/bilateral detection

Final histology characteristics: involvement SLN, involvement non-SLN, final histology cervix specimen final histology endometrial tumour, parametrial involvement, involvement of grossly enlarged lymph nodes

Adverse events: allergic reactions to technetium or blue dye

HPV DNA type of primary tumour and sentinel nodes

5.7. Inclusion criteria

Patients aged 18 years and older, willing and able to provide informed consent with any histological type FIGO stage IA1 with lymphovascular space invasion to FIGO stage IIA carcinoma of the cervix scheduled for primary surgical treatment, or diagnosed with any histological type endometrial carcinoma and after investigation appears to be FIGO stage I or II and who were scheduled for primary surgical treatment.

5.8. Exclusion criteria

Pregnancy, women with cervical cancer FIGO stage > IIA or women with endometrial cancer assumed to be stage III or I patients unfit for surgery, patients not willing or able to provide informed consent for the trail, and women with known allergy for ^{99m}Tc-Nanocolloid, contrast or blue dye.

5.9. Ethical considerations

Participation in the study was on a voluntary basis and all women who agreed to participate in the study provided informed consent. Patients who declined participation were in no way disadvantaged and received standard treatment according to the guidelines of the Gynaecological Oncology Unit or as decided by the attending gynaecological oncology team. Patients participating in the trial did not receive any financial or material benefits or rewards.

The University of Pretoria Faculty of Health Sciences Research Ethics Committee approved the study (434/2014).

6. Results

6.1. HPV tumour data

6.1.1. Demographic data

HPV typing was performed on 42 patients with cervical cancer. Interpretable HPV DNA test results were available for both the primary tumour and the sentinel lymph nodes in 29 patients.

The mean age of the 42 patients with tumour HPV data was 46.79 years and 28 women (65.12%) were HIV-positive. The mean CD4 count was 423.43 (SD = 248.10; SEM = 46.89; range: 43 - 950). Four women (9.52%) previously had TB of which one had abdominal TB, and seven women (16.67%) previously had PID. The rest of the demographic data is shown in Table 1.

Table 1: Clinical and demographic data women with HPV tumour results

n = 42	Mean	SD	Range
Age	46.79	9.11	32 - 77
Parity	3.24	1.59	1 - 8
Gravidity	3.40	1.68	1 - 8
BMI	26.78	5.40	18.37 - 40.90

SD = Standard deviation

6.1.2. Disease characteristics

In 15 women (35.7%) the cervical tumour size was ≥ 2cm. Thirty-one women (73.8%) in this group had FIGO stage IB1 disease. The stage distribution of cervical cancer in this group is shown in Figure 1.
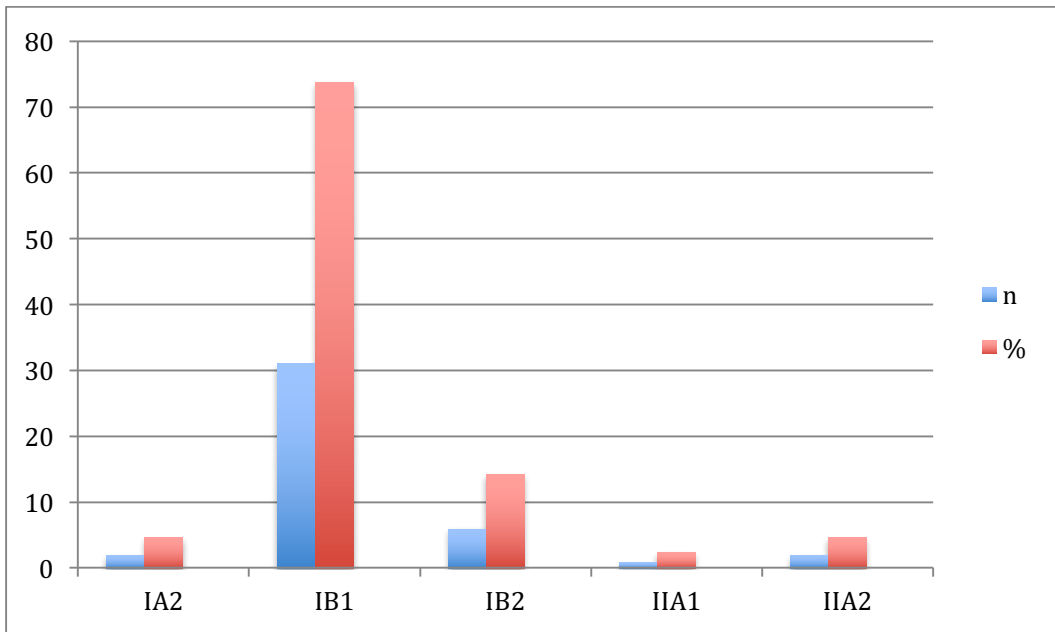
Figure 1: Cervical cancer stage distribution of women with HPV tumour results
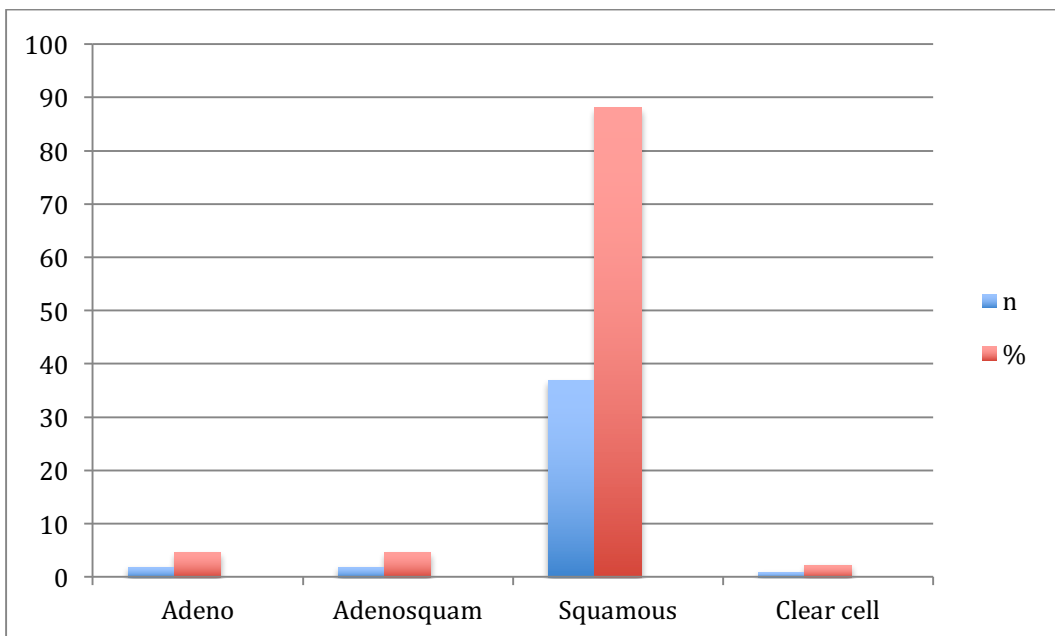
Figure 2: Cervical cancer histopathological distribution of women with tumour HPV results

The most common histological sub-type was squamous cell carcinoma, found in 37 women (88.10%). The rest of the histological sub-types are shown in Figure 2.

6.1.3. Tumour HPV types

HPV 16 was the most common hrHPV type identified from cervical cancer tumours in this group of women. The hrHPV type distribution from these tumours is shown in Figure 3 and the comparison between HIV positive and negative women is shown in Figure 4

6.2. Tumour with SLN data

6.2.1. Demographic data

Tumour and SLN HPV results were available for 29 women, of whom 20 patients (68.9%) were HIV-infected and all were using ART; 17 of the 20 HIV infected women were on ART for more than six months. Three women (10.3%) previously had TB and seven (24.1%) previously had PID. The rest of the demographic data of these patients are shown in Table 2.
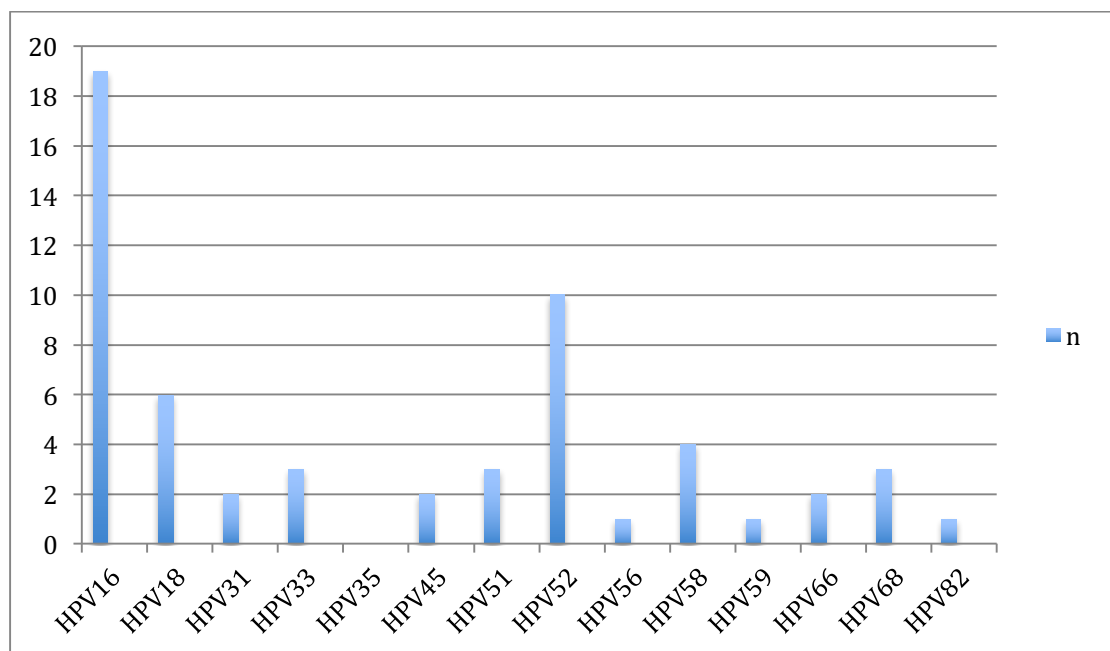
Figure 3: hrHPV type distribution of the group of women with HPV tumour results
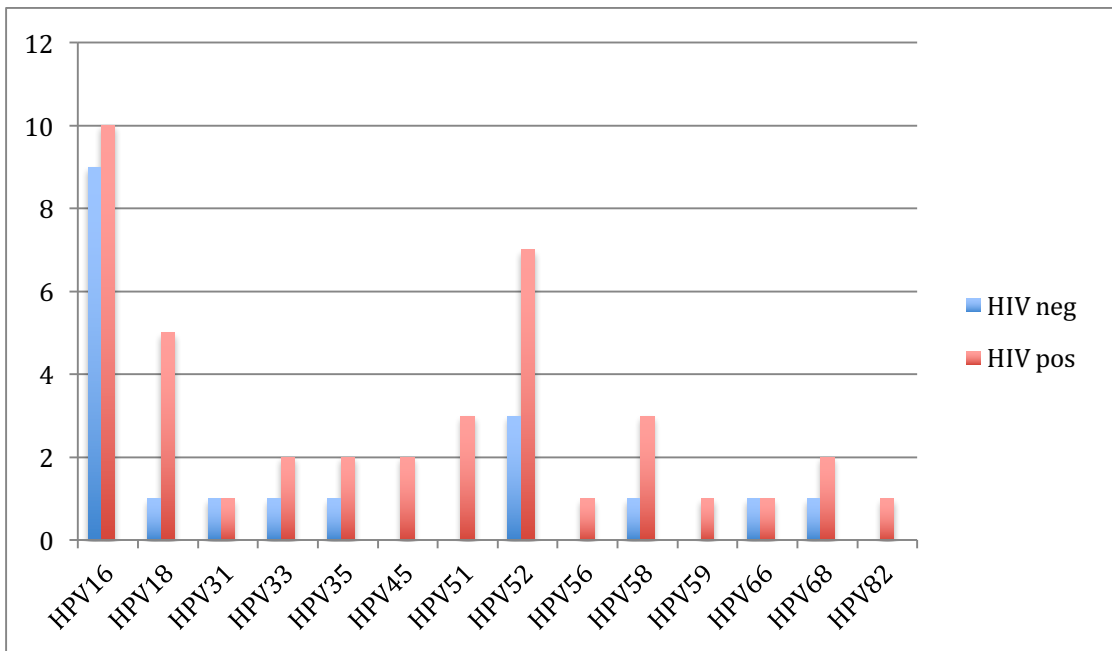
Figure 4: hrHPV tumour type distribution in HIV-positive and HIV-negative women

Table 2: Demographic data for women with HPV tumour and SLN data

n = 29	**Mean**	**SEM**	**Range**
Age	45.86	1.83	32 - 77
Parity	3.03	0.27	1 - 7
Gravidity	3.10	0.26	1 - 7
BMI	25.90	0.95	18.37 - 40.90

SD = Standard deviation

6.2.2. Disease characteristics

The stage distribution of this group is shown in Figure 5. Most patients were FIGO stage IB1

The most common histological sub-type was squamous cell carcinoma in 26 women (89.6%). The rest of this data is shown in Figure 6.

Ninety-one SLNS were removed for a mean SLN count of 3.14 (SD = 1.64; 95% CI = 2.51 - 3.76)
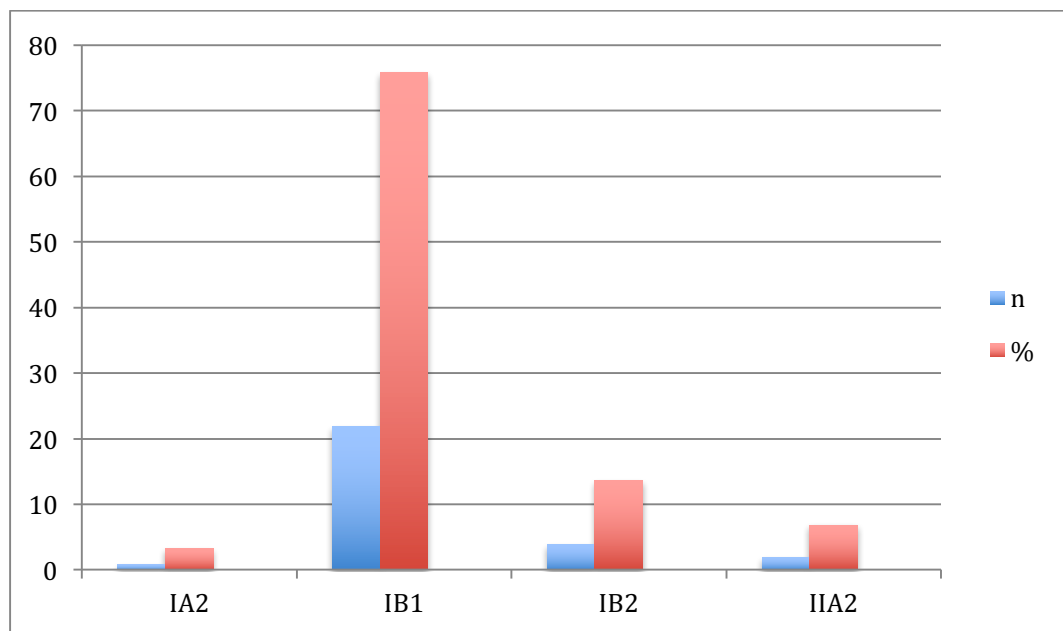
Figure 5: Cervical cancer stage distribution of women with tumour and SLN data
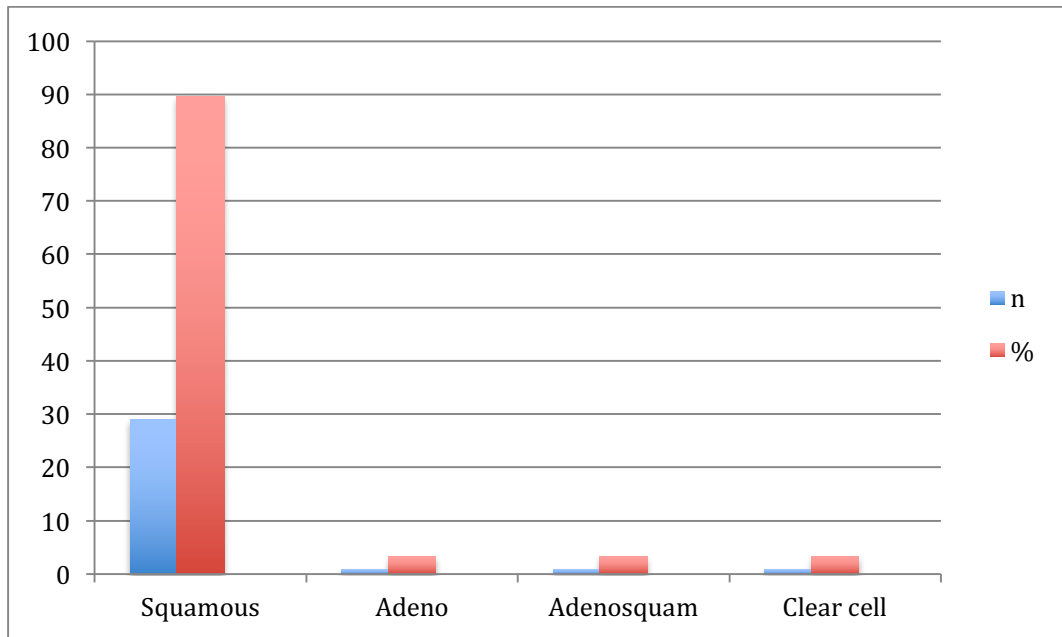
Figure 6: Cervical cancer histopathological distribution of women with tumour and SLN HPV data

6.2.3. HPV types in cervical lesions

In five patients (17.2%) hrHPV types were not associated with cervical cancer lesions. Three of the five tested negative for any HPV and in two patients non-high-risk types were associated (types 84 and 62 respectively). In the three patients without any identified hrHPV types associated with their cervical tumours, one patient had five HPV types identified in the SLN, of which three were hrHPV types. High-risk HPV types were identified in the cervical cancer lesions of 24 patients (82.76%). Of the 24 patients with hrHPV types identified, 14 (58.3%) had one hrHPV type, 6 (25%) had two types, one (4.1%) had three types and three (12.5%) had four types. In one woman, 12 different HPV types were identified of which four were hrHPV types. No HPV types were identified in the SLN of this patient.

The most common HPV type identified in these lesions was HPV 16 followed by HPV 52. Figure 7 shows the distribution of hrHPV in the cervical tumours of this group of women. HPV 16 was the most common virus isolated, followed by HPV 52 and HPV 18.
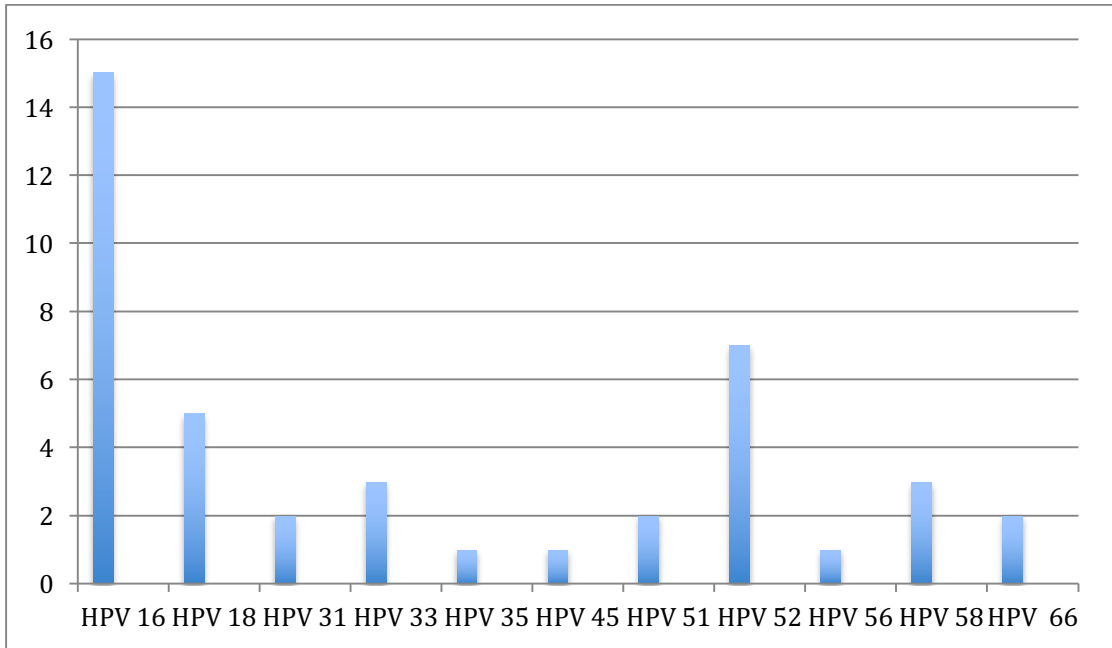
Figure 7: High-risk HPV types isolated from cervical cancer tumours

6.2.4. HPV types in SLNs

HPV types were identified in the SLNs of 11 of the 29 patients (37.9%), of which ten patients had hrHPV in the SLNs and one patient had non-hrHPV (HPV 62). In eight women (27.5%) one type hrHPV was identified, while one woman (3.4%) had two hrHPV types and one woman (3.4%) had three types isolated from the SLNs. The most common hrHPV type isolated in SLNs was HPV 16. The distribution of hrHPV types isolated from SLNs is shown in Figure 8, and the combined results are shown in Figure 9.

140
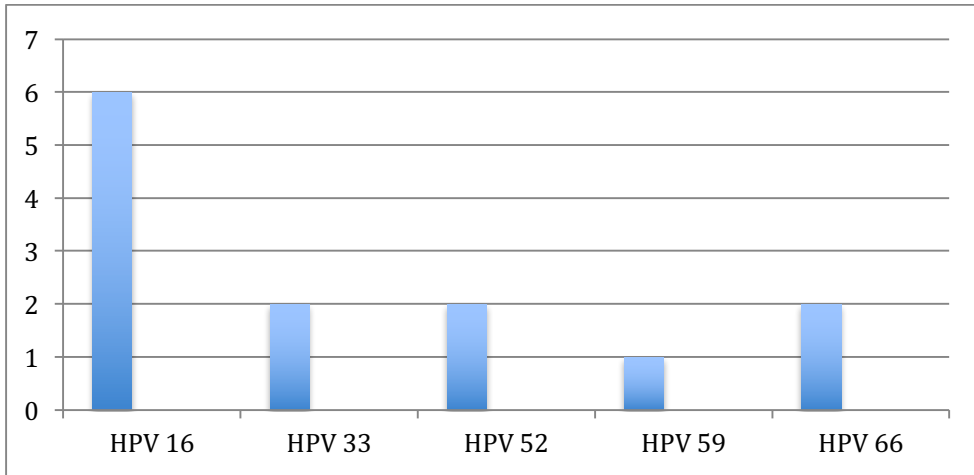
Figure 8: High-risk HPV types isolated from sentinel lymph nodes
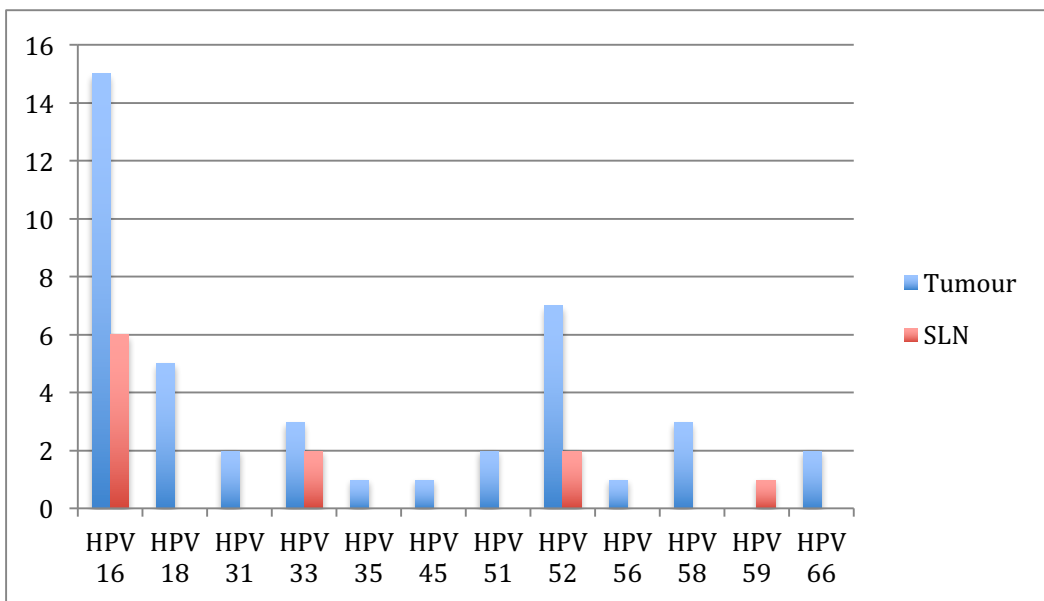
Figure 9: High-risk HPV types for both tumours and sentinel lymph nodes

6.2.5. Lymph node metastases

Lymph node metastases were found in seven of the 29 women (24.1%) in the group. In four of these patients no HPV types were isolated in the SLNs. In the other three women, HPV 16 were isolated in the SLNs of two patients and HPV 33, 66, 61 and 84 were identified in the SLN of the third patient.

High-risk HPV types were identified in seven patients (31.8%) without lymph node metastases. In six of the seven women a single type was identified and in one woman five different types were identified of which three were hrHPV types.

6.2.6. Predictive values of HPV in SLNs for metastatic nodal disease

For to purpose of this assessment the presence of hrHPV was assumed to be an indicator of metastatic nodal disease and the absence of hrHPV was an indicator for the absence of metastatic nodal disease. Using this hypothesis there were three true positives, 16 true negatives, seven false positives and three false negatives.

The sensitivity, specificity, positive and negative predictive values are shown in Table 3.

Table 3: Sensitivity, specificity, positive and negative predictive values

	Value	95% CI
Sensitivity	50%	11.81 - 88.19%
Specificity	69.6%	47.08 - 86.79%
Positive predictive value	30%	14.49 - 54.08%
Negative predictive value	84.2%	69.62 - 92.54%

CI = Confidence interval

The false negative rate for hrHPV is 42.8%.

Frozen section of sentinel lymph nodes showed metastatic disease in one case out of four true positive SLNs. The sensitivity, specificity PPV and NPV for FSE in this group of women were 20%, 100%, 100% and 90.91% respectively, while the FNR for FSE was 57.14%.

Seven patients in this group had pelvic lymph node metastases. In three of these (42.9%) both the FSE and hrHPV DNA were negative.

7. Discussion

7.1. HPV distribution in tumours

HPV 16 was the most frequently encountered type identified in cervical tumours of this cohort, followed by HPV 52 and then HPV 18 in the third place. This finding differs from the findings published by Denny et al, where HPV 18 was much more common than HPV 52 [2]. The difference in HPV type distribution might be as a result of sample collection, however in the study published by Slama et al, sample collection was performed using a cytobrush as well and this did not result in a different HPV type distribution [21]. The very high prevalence of 65% HIV infection in this cohort might be a more likely explanation for this finding.

7.2. HPV distribution in sentinel lymph nodes

The ability of intra-operative frozen section examination of SLNs to accurately predict lymph node status is limited with a false negative rate of 8% - 32% [19]. Additional markers such as hrHPV DNA typing have low sensitivity, specificity, PPV and NPV, with a very high false negative rate when used as a stand-alone test.

In addition, the combination of negative hrHPV in the SLN and negative frozen section examination was shown to accurately predict the absence of metastatic disease in the pelvic lymph nodes. High risk HPV DNA does not seem to be a

useful tool to compensate for the high false negative rate of FSE as suggested by Noventa et al [22].

The findings in this study differ from the published data on this topic. The presence of hrHPV had a 100% PPV in the study published by Slama et al [21]. In the study published by Lee et al, the combination of absent hrHPV DNA and negative FSE had a 100% NPV for the absence of metastatic lymph node disease (19). Coutant et al showed a correlation between the presence of hrHPV in SLNs and metastatic disease [20]. In all three these studies different HPV tests were performed and this might also impact on results. Lee et al used the Mygene® test, Coutant used an E6 specific PCR and Slama used the Roche Amplicor® assay [19-21].

The finding of single HPV type in the tumour with multiple types has not been reported previously. In the study published by Slama et al, one patient had two HPV types in the tumour and four types in the nodes, but no patient with one type in the tumour had multiple types in the SLNs [21].

It is possible that the HIV prevalence in this group of 69% impacted significantly on these results. HIV infected women usually have multiple HPV types and HIV also alters the relative carcinogenicity of HPV types [23]. Data on the effect of anti-retroviral therapy on HPV detection of the cervix is limited, but it does seem to reduce HPV infection [24]. The effect of ART on HPV has not been studied on HPV infection or detection in lymph nodes.

This study aimed to assess methods of reliably predicting the status of pelvic nodes and was not designed to assess the prognostic implications of detecting hrHPV DNA in patients with cervical cancer. Some studies have shown the presence of hrHPV DNA in the pelvic nodes to be associated with a poor prognosis and a risk for recurrent disease.

8. Conclusion

In this study, testing for the presence of hrHPV DNA in the sentinel lymph nodes was not useful as a predictor of pelvic lymph node status. The presence of hrHPV in SLNs did not accurately identify patients with lymph node metastases, while the absence of hrHPV in SLNs did not accurately predict the absence of lymph node metastases.

The combination of negative FSE and negative hrHPV in the SLNs did not have a reliable negative predictive value for the absence of pelvic nodal metastases.

References

1. Bosch FX, de Sanjosé S. Chapter 1: Human papillomavirus and cervical cancer—burden and assessment of causality. J Natl Cancer Inst Monogr. 2003;(31):3-13.

2. Denny L, Adewole I, Anorlu R, Dreyer G, Moodley M, Smith T, et al. Human papillomavirus prevalence and type distribution in invasive cervical cancer in sub-Saharan Africa. Int J Cancer.2014;134(6):1389-98.

3. Ho C-M, Chien T-Y, Huang S-h, Wu C-J, Shih B-Y, Chang S-C. Multivariate analysis of the prognostic factors and outcomes in early cervical cancer patients undergoing radical hysterectomy. Gynecol Oncol. 2004;93(2):458-64.

4. Chuang L, Burke TW, Tornos C, Marino BD, Mitchell MF, Tortolero-Luna G, et al. Staging laparotomy for endometrial carcinoma: assessment of retroperitoneal lymph nodes. Gynecol Oncol. 1995;58(2):189-93.

5. Kitajima K, Murakami K, Yamasaki E, Fukasawa I, Inaba N, Kaji Y, et al. Accuracy of 18F-FDG PET/CT in detecting pelvic and paraaortic lymph node metastasis in patients with endometrial cancer. AJR Am J Roentgenol.2008;190(6):1652-8.

6. Lancaster WD, Castellano C, Santos C, Delgado G, Kurman RJ, Jenson AB. Human papillomavirus deoxyribonucleic acid in cervical carcinoma from primary and metastatic sites. Am J Obstet Gynecol. 1986;154(1):115-9.

7. Kobayashi Y, Yoshinouchi M, Tianqi G, Nakamura K, Hongo A, Kamimura S, et al. Presence of human papilloma virus DNA in pelvic lymph nodes can predict unexpected recurrence of cervical cancer in patients with histologically negative lymph nodes. Clin Cancer Res. 1998;4(4):979-83.

8. Baay MF, Koudstaal J, Hollema H, Duk JM, Burger MP, Quint WG, et al. Detection of HPV-16 DNA by PCR in histologically cancer free lymph nodes from patients with cervical cancer. J Clin Pathol. 1997;50(11):960-1.

9. Chan PK, Yu MM, Cheung TH, To KF, Lo KW, Cheung JL, et al. Detection and quantitation of human papillomavirus DNA in primary tumour and lymph nodes of patients with early stage cervical carcinoma. J Clin Virol. 2005;33(3):201-5.

10. Fuchs PG, Girardi F, Pfister H. Human papillomavirus 16 DNA in cervical cancers and in lymph nodes of cervical cancer patients: a diagnostic marker for early metastases? Int J Cancer. 1989;43(1):41-4.

11. Füle T, Csapó Z, Máthé M, Tátrai P, László V, Papp Z, et al. Prognostic significance of high-risk HPV status in advanced cervical cancers and pelvic lymph nodes. Gynecol Oncol. 2006;100(3):570-8.

12. Hernadi Z, Szarka K, Sapy T, Krasznai Z, Veress G, Poka R. The prognostic significance of HPV-16 genome status of the lymph nodes, the integration status and p53 genotype in HPV-16 positive cervical cancer: a long term follow up. BJOG. 2003;110(2):205-9.

13. Lukaszuk K, Liss J, Gulczynski J, Nowaczyk M, Nakonieczny M, Piatkowski M, et al. Predictive value of HPV DNA in lymph nodes in surgically treated cervical carcinoma patients - a prospective study. Gynecol Oncol. 2007;104(3):721-6.

14. Pilch H, Gunzel S, Schaffer U, Tanner B, Brockerhoff P, Maeurer M, et al. Human papillomavirus (HPV) DNA in primary cervical cancer and in cancer free pelvic lymph nodes--correlation with clinico-pathological parameters and prognostic significance. Zentralbl Gynakol. 2001;123(2):91-101.

15. Rolla M, Berretta R, Patrelli TS, Merisio C, Gramellini D, Fadda GM, et al. A perspective study on correlation between HPV DNA and lymph nodes in surgically treated cervical carcinoma patients. Preliminary data. Eur J Gynaecol Oncol. 2009;30(5):557-61.

16. Zhang F, Liu D, Lin B, Hao Y, Zhou D, Qi Y, et al. Expression of high-risk HPV DNA and CK19 in pelvic lymph nodes in stage Ia-IIa cervical cancer and their clinical value. Oncol Rep. 2012;27(6):1801-6.

17. Kedzia H, Gozdzicka-Jozefiak A, Kedzia W, Poreba E. The value of the presence of HPV16 in pelvic lymph nodes of cervical cancer patients. Eur J Gynaecol Oncol. 1995;16(3):199-202.

18. Durst M, Hoyer H, Altgassen C, Greinke C, Hafner N, Fishta A, et al. Prognostic value of HPV-mRNA in sentinel lymph nodes of cervical cancer patients with pN0-status. Oncotarget. 2015;6(26):23015-25.

19. Lee YS, Rhim CC, Lee HN, Lee KH, Park JS, Namkoong SE. HPV status in sentinel nodes might be a prognostic factor in cervical cancer. Gynecol Oncol. 2007;105(2):351-7.

20. Coutant C, Barranger E, Cortez A, Dabit D, Uzan S, Bernaudin JF, et al. Frequency and prognostic significance of HPV DNA in sentinel lymph nodes of patients with cervical cancer. Ann Oncol. 2007;18(9):1513-7.

21. Slama J, Drazdakova M, Dundr P, Fischerova D, Zikan M, Pinkavova I, et al. High-risk human papillomavirus DNA in the primary tumor, sentinel, and nonsentinel pelvic lymph nodes in patients with early-stage cervical cancer: a correlation with histopathology. Int J Gynecol Cancer.2009;19(4):703-7.

22. Noventa M, Ancona E, Saccardi C, Litta P, D'Antona D, Nardelli GB, et al. Could HPV-DNA test solve the dilemma about sentinel node frozen section accuracy in early stage cervical cancer? Hypothesis and rationale. Cancer Invest. 2014;32(5):206-7.

23. Clifford GM, de Vuyst H, Tenet V, Plummer M, Tully S, Franceschi S. Effect of HIV infection on human papillomavirus types causing invasive cervical cancer in Africa. J Acquir Immune Defic Syndr. 2016;73(3):332-9.

24. Zeier MD, Botha MH, Engelbrecht S, Machekano RN, Jacobs GB, Isaacs S, et al. Combination antiretroviral therapy reduces the detection risk of cervical human papilloma virus infection in women living with HIV. AIDS. 2015;29(1):59-66.

Chapter 6

Analysis of the data of women in whom sentinel nodes were detected and the role of different histological examinations of sentinel lymph nodes

1. Introduction

The standard surgical treatment for women with early stage cervical cancer is radical hysterectomy with pelvic lymphadenectomy. Patients with presumed early stage endometrial cancer are also treated surgically, and lymph node status is part of the FIGO staging, which means theoretically the vast majority of these patients require at least systematic pelvic lymphadenectomy.

The histologic examination of removed lymph nodes involves paraffin embedding and haematoxylin and eosin (H&E) staining of all the nodes harvested for histologic examination.

The sentinel lymph node biopsy procedure has the added advantage of frozen section examination (FSE) in patients treated for cervical cancer. In cases where the FSE reveals the presence of metastases the option of abandoning surgery in favour of primary radiation therapy (RT) or concurrent chemoradiation therapy (cCRT) can be implemented. Primary surgical treatment and primary cCRT has the same overall survival (OS) and disease-free survival (DFS) [1]. In patients with cervical cancer treated surgically, those with lymph node metastases will receive adjuvant CRT. From the available data it seems that the combination of surgical treatment and adjuvant CRT does not translate into superior survival, but it does increase morbidity [2]. FSE has less of a role in patients treated with presumed early stage endometrial cancer where the primary treatment remains surgical regardless of lymph node status.

Ultrastaging is an important prerequisite for SLNB procedure, and all SLNs negative on H&E examination should undergo ultrastaging.

This chapter investigates the contributions of FSE and ultrastaging in the SLNB procedure, and will also discuss and examine the differences between women who had successful SLN mapping compared to those who failed SLN mapping.

2. Histological evaluation of the SLN

2.1. Intra-operative assessment

The SLNB technique can be combined with intra-operative histological examination (frozen section and/or imprint cytology) to identify cervical cancer patients amenable to radical hysterectomy without lymph adenectomy, or those with a positive SLN, which might benefit from primary RT or cCRT.

Intra-operative examination of the SLN in early cervical cancer has its limitations and published results in the scientific literature vary widely. The main limitation of FSE is its inability to identify low volume metastatic disease such as micro metastases and isolated tumour cells (ITC).

A sensitivity of 22.7% was reported in 102 cases of intra-operative frozen section and imprinting cytology collected during the multicentre prospective SENTICOL trial by Bats et al [3].

Martinez et al reported the findings of intra-operative frozen section performed in 94 patients with FIGO stage IA1 to IB1 cervical cancer [4]. Eleven patients had metastatic sentinel nodes of which eight were macro metastases, two were micro metastases and one had ITC. Intra-operative FSE identified all eight cases with macro metastases, and the sensitivity of FSE was 64.3% with an NPV of 98% for all types of lymph node involvement.

The biggest study on the ability of frozen section to predict regional lymph node status was published by Slama et al, who reported the results of 225 patients with

FIGO stage I to IIB cervical cancer [5]. Frozen section identified 41 out of 73 cases with metastatic SLNs. Of the 73 cases, 44 had macro metastases and FSE identified 39 (88.6%). When ITC was excluded, the sensitivity of FSE was 63%, specificity was 100% and the NPV 91.

How et al reported on the SLN and FSE in 100 endometrial cancer patients [6]. In this series, from nine women with metastatic nodes in whom SLNs were detected, the SLN FSE correctly identified eight women, yielding a sensitivity of 89% (95% CI 50 - 99) with a NPV of 99%. Mosgaard et al reported similar results for FSE in SLN missing no metastatic disease in a smaller study of 32 patients [7].

In summary, the limitation of intra-operative FSE is its inability to reliably detect SLNs with MM. It has a role to play insofar as it has the ability to reliably detect macro metastases, allowing decision making in patients with early stage cervical cancer planned to undergo surgical treatment.

2.2 Pathologic ultrastaging

Ultrastaging refers to the systematic analysis of lymph nodes using serial sectioning and immunohistochemistry (IHC). This technique is more sensitive in the detection of lymph node metastases compared to routine H&E staining. Although it is time consuming and costly and therefore not routinely used in examining lymph nodes obtained from systematic pelvic and or para-aortic lymphadenectomy, pathologic ultrastaging forms an inherent part of the SLNB procedure.

The handling and preparation of sentinel nodes is described in a paper from the Philadelphia Consensus Conference on sentinel nodes in breast cancer [8]. Lymph node metastases are defined according to the Philadelphia Consensus Conference on sentinel nodes in breast cancer as macro metastases (tumour deposits > 2mm), micro metastases (MM) (tumour deposits between 0.2 and 2 mm) and isolated tumour cells (ITC) or sub-micro metastases (tumour deposits < 0.2mm). Low volume disease (LVD) include both MM and ITC [8].

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The prognostic significance of LVD has been described by Cibula et al in a retrospective multi-institutional review involving 645 women with early stage cervical cancer who underwent surgical treatment, SLNB followed by pelvic lymph adenectomy and pathological ultrastaging [9]. While the presence of ITC did not influence recurrence free and overall survival, MM significantly reduced overall survival with a similar hazard ratio as the presence of macro metastases.

Histological or pathological ultra-staging in SLN biopsy in both early stage cervical and endometrial cancer may increase the likelihood of identifying metastatic disease that would otherwise be missed by conventional histological examination [10-13]. Reports suggest that between 4% and 29% of additional metastases (MM and/or ITC) are found in SLN ultra-staging where conventional histology was reported to be negative [14-17]. A literature review on ultrastaging by Bezu et al suggests ultrastaging of regional lymph nodes is the preferred technique to detect micro metastases in women with uterine cancer [18].

The use of pathologic ultrastaging of SLNB seems to form an integral part of the SLNB procedure if this technique is to be used as part of treatment and surgical staging in women with early stage cervical and endometrial cancer.

3. Aims and objectives

3.1. Aims

3.1.1. To describe the characteristics of women who had successful SLN mapping and to investigate the ability of different histological examinations performed on SLNs to accurately predict the regional nodal status.

3.2. Objectives

3.2.1. To compare characteristics of successfully mapped women with those who did not map for SLNs;

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3.2.2. To investigate different disease characteristics of women who mapped successfully for SLNs;

3.2.3. To describe the anatomical distribution of mapped SLNs;

3.2.4. To compare the efficacy of different mapping techniques;

3.2.5. To determine the sensitivity, specificity, PPV, NPV and FNR of frozen section examination, SLNB and the SLNB algorithm.

4. Materials and methods

4.1. Study design and setting

This is a prospective cohort study conducted at the Gynaecological Oncology Unit, Department Obstetrics and Gynaecology, University of Pretoria. The study was performed at the Kalafong Provincial Tertiary Hospital (KPTH) and Steve Biko Academic Hospital (SBAH).

Women with early stage cervical (clinically staged as FIGO stage IA to IIA) and apparent early stage endometrial cancer (FIGO stage I and II) were eligible for recruitment to the study [19-21]. Surgical treatment consisted of laparoscopic or open radical hysterectomy and pelvic with or without para-aortic lymph adenectomy for cervical cancer [22-24], and of laparoscopic or total abdominal hysterectomy, bilateral salpingo oophorectomy (BSO) and pelvic with or without para-aortic lymph adenectomy for women with endometrial cancer. Laparoscopic or open SLNB procedure was performed during these surgical procedures.

4.2. Histological evaluation

Sentinel lymph node detection was done using ^{99m}Tc-Nanocolloid, indocyanine green (ICG) and blue dye labelling prior to surgery. ^{99m}Tc-Nanocolloid was injected in the cervix of women scheduled for surgery one day before the procedure, and blue dye and ICG was injected pre-operatively after induction of general anaesthesia. After identifying and removal of all SLNs a total pelvic

lymph node dissection was performed in addition to the appropriate type of hysterectomy with or without bilateral salpingo-oophorectomy. Para-aortic lymph adenectomy was performed at the discretion of the treating gynaecological oncologist according to the Gynaecologic Oncology Unit protocol.

The SLNs and the remainder of the pelvic lymph nodes were sent separately for histological examination. Each sentinel lymph node was bisected into two halves. The one half underwent FSE, while the other half was processed and stained using H&E as per normal routine. If after H&E examination the SLN was found to be negative for metastases, the second half of the node was ultra-staged by performing level sections as well as immunohistochemistry staining.

4.2.1. Frozen section examination

All SLNs collected underwent FSE. For the purpose of the study and for logistical reasons FSE was performed in the laboratory and not in the theatre. After removal, SLN specimens for FSE were not placed into formalin solution, but placed in a container with a saline drenched swab and couriered to the anatomical pathology department.

In the laboratory, the pathologist evaluated the SLNs macroscopically to identify any possible metastasis. The half of the lymph node in which a suspicious focus of macroscopic metastasis was identified, was used for the FSE component.

The lymph node was fitted onto the chuck used in the laboratory and placed in the frozen section machine. After positioning on the chuck, the tissue was covered by a gel-like medium called OCT (optimal cutting temperature compound) consisting of poly ethylene glycol and polyvinyl alcohol, placed in liquid nitrogen that froze the gel in less than one minute. The chuck was placed on the microtome in the cryostat set between -20 to -30°C. Histology sections were cut between 3 - 5 μm and put through the staining process to obtain H&E sections on the frozen section.

The sections were evaluated for the absence or presence of metastases, macro metastases, micro metastases or isolated tumour cells. After the FSE

the lymph node was submitted for H&E evaluation after routine processing.

4.2.2. Routine haematoxylin and eosin

All SLNs were marked and sent separately for histological evaluation. SLNs were divided in two parts. One part was examined using routine H&E staining. Ultrastaging was performed in all cases where the SLN was reported to be negative following FSE and routine H&E staining. Ultrastaging consisted of cutting two adjacent 5-µm sections from each paraffin block at two levels 50 µm apart. At each level, one side was stained with H&E and the other with immunohistochemistry (IHC) using the anti-cytokeratin AE1:AE3 (DAKO) for a total of five slides per block [13,25].

The presence of macro metastases, micro metastases and ITC were recorded. Women with micro metastases and ITC were regarded as having nodal metastases and were considered for appropriate post-operative adjuvant treatment.

4.3. Data collection

The following data were collected:

Patient characteristics: age, ethnicity, BMI

Medical history: HIV status (CD4 count, viral load and treatment if applicable), history of TB, history of PID, history of STD (Chlamydia, gonorrhoea, genital warts), history of cone biopsy

Tumour characteristics: FIGO stage, lymphovascular space invasion (LVSI), size of tumour, histology

Surgical characteristics: grossly enlarged lymph nodes, adhesions (signs of previous PID), total blood loss during surgery

SLN characteristics: number of SLN found (hot and/blue), location of SLNs, unilateral/bilateral detection

Final histology characteristics: involvement SLN, involvement non-SLN, final

histology cervix specimen final histology endometrial tumour, parametrial involvement, involvement of grossly enlarged lymph nodes

Adverse events: allergic reactions to technetium or blue dye.

4.4. Statistical analysis

4.4.1. Sentinel lymph node biopsy

SLNs were considered positive if they contained macro metastases micro metastases or ITC. False-positive results were per definition not possible. A true positive test was defined as at least one SLNB with metastatic disease regardless of the histology report of the non-SLNs of the same region. A false negative test was defined as a SLNB that was histologically negative for metastatic disease with histologically proven metastatic disease in non-SLNs from the same region. If no SLNs were identified in a hemi-pelvis, a full lymphadenectomy was performed and this area was not taken into account for the calculation of the false-negative rate. A true negative test was defined as a SLNB histologically negative for metastatic disease with non-SLNs also histologically negative for metastatic disease.

4.4.2. Calculations

The detection rate was calculated as the number of patients with at least one detected pelvic SLN divided by the total number of patients who underwent SLN mapping. Diagnostic performance was calculated for hemi-pelvises with at least one SLN harvested. Sensitivity was calculated as the proportion of true positives (patients with positive pelvic SLNs) among the patients with pelvic lymph-node metastases. NPV was calculated by dividing the number of true negatives (patients with negative pelvic SLNs) by the number of all patients without pelvic lymph-node metastases. Exact 95% confidence intervals (CI) for the proportions were calculated, and subgroup analysis was done using a two-sided $\chi 2$ test or Fisher's exact test ($\alpha = 0.05$).

4.5. Ethical considerations

Participation in the study was on a voluntary basis and all women agreeing to participate in the study signed an informed consent form. Consented patients who have initially agreed to participate were allowed to withdraw consent should they wish to do so.

Patients who declined the option of participation were in no way disadvantaged and received standard treatment according to the guidelines of the Gynaecological Oncology Unit or as decided by the attending gynaecological oncology team. Patients participating in the trial did not receive any financial or material benefits or rewards.

The University of Pretoria Faculty of Health Sciences Research Ethics Committee approved the study as well as amendments to the protocol (434/2014).

5. Results

Of the 100 patients recruited to the study, data of 94 were suitable for analysis. SLNs were detected in 57 patients (60.6%) and there was bilateral detection in 28 patients (29.8%).

The results of the 57 patients in whom SLNs were detected will be reported and discussed in this chapter.

5.1. Demographic data

The mean age of this cohort was 50.5 years. The demographic data is shown in Table 1.

Table 1: Demographic data of women in whom sentinel nodes were detected

n = 57	Mean	SD	Range
Age	50.53	12.27	32 – 81
Parity	3.58	2.15	1 – 12
Gravidity	3.77	2.12	1 – 12
BMI	27.71	5.89	18.37 – 43.72

SD = Standard deviation

Thirty-two women (56.1%) were HIV-infected and the mean CD4 count in this group was 448.47 cells/µl (SD = 235.47; range 43 - 950). Thirty-one of the 32 women (96.9%) were using ARV therapy, of which only four (12.5%) were using it for less than six months. Four patients (7.0%) reported previous TB infection, of which three (5.3%) had pulmonary TB and one (1.7%) had abdominal TB. Ten women 17 (17.5%) reported previous episodes of PID. Eleven women (19.3%) had previous abdominal surgery.

5.2. Disease characteristics

Ten patients (17.5%) had endometrial cancer and 47 (82.5%) had cervical cancer. The FIGO stage distribution of women with endometrial cancer is shown in Figure 1 and for women with cervical cancer in Figure 2.
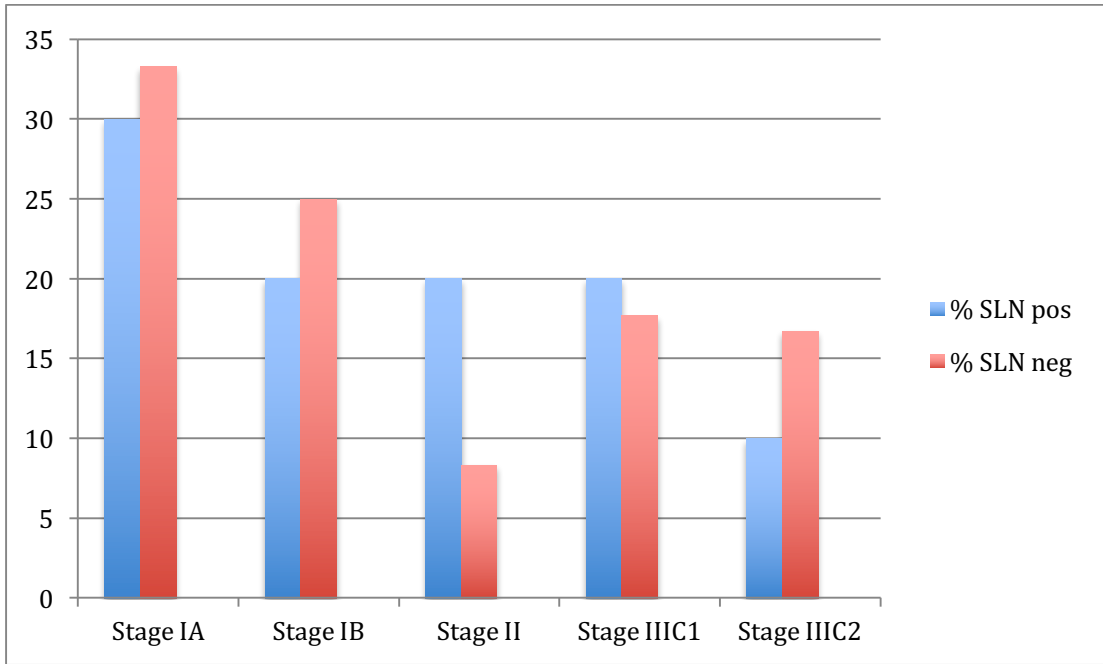
Figure 1: FIGO stage distribution women with endometrial cancer
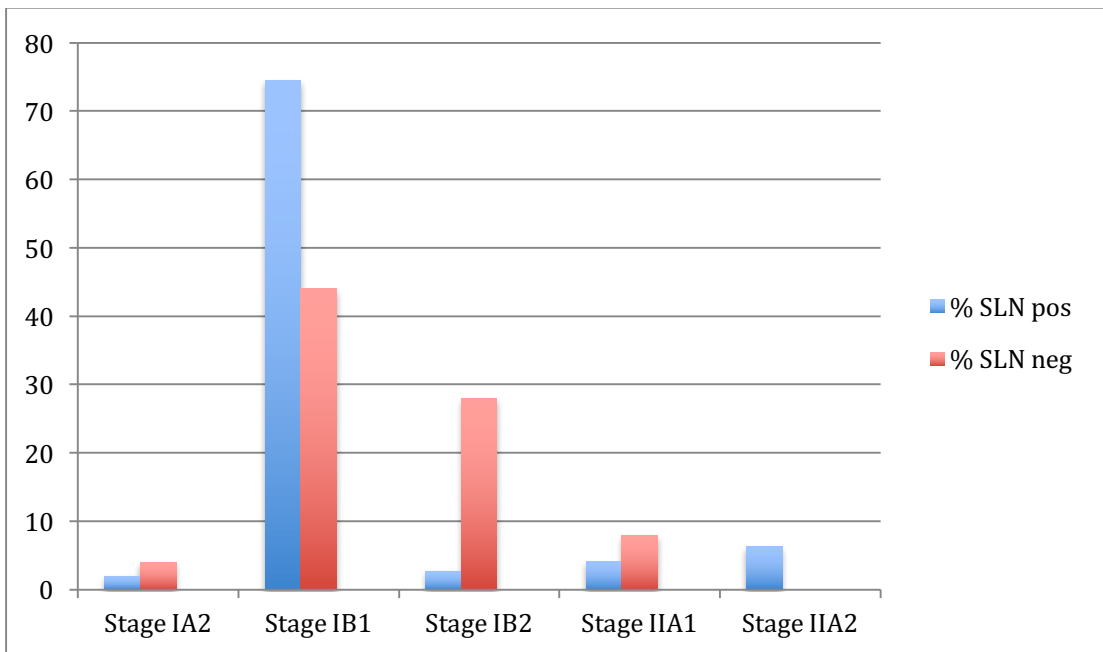
Figure 2: FIGO stage distribution of women with cervical cancer

In 20 women with cervical cancer (43.5%) the tumour diameter was ≥ 2 cm. The mean cervical tumour diameter was 26.41 mm (SD = 19.67; SEM = 3.37; range: 0.8 - 78; 95% CI = 19.54 - 33.28). The mean endometrial tumour diameter was 65.60 mm (SD = 20.09; SEM = 6.35; range: 30 - 95; 95% CI = 51.23 - 79.97).

5.3. Comparative data

Data comparing the demographic information of women with sentinel nodes to those who did not map is shown in Table 2, with stage distribution comparative data for cervical and endometrial cancer shown in Table 3 and Table 4 respectively.

Table 2: Comparative data of women with and without sentinel lymph nodes

	SLN negative n = 37		SLN positive n = 57		p-value
	Mean	SD	Mean	SD	
Age (years)	54.49	12.69	50.53	12.27	0.1359
Parity	3.14	1.76	3.58	2.15	0.3017
Gravidity	3.33	1.79	3.77	2.12	0.2995
BMI (kg/m^2)	29.68	6.19	27.72	5.89	0.1258
Tumour size (mm)					
Cervix	34.01	28.51	26.41	19.67	0.2383
Endometrium	63.00	19.38	65.60	20.09	0.7526
	n	%	n	%	
HIV infected	15	50.5	32	56.1	0.5966
Previous TB	0	0	5	8.8	0.0651
Previous PID	4	10.8	10	17.5	0.3749
Previous surgery	7	18.9	11	19.3	0.9618
Tumour ≥ 2 cm	16	43.2	20	43.5	0.9772
Enlarged nodes	16	43.2	13	22.8	0.0374
Nodal metastases	14	37.8	13	22.8	0.1182
Adhesions	10	27.0	10	17.5	0.2738

SD = Standard deviation

Table 3: Comparative stage distribution for cervical cancer women with and without sentinel nodes

FIGO Stage	SLN positive n = 47		SLN negative n = 25		p-value
	n	%	n	%	
IA1	1	2.1	1	4	0.6417
IB1	35	74.5	11	44	0.0108
IB2	6	12.8	7	28	0.1131
IIA1	2	4.2	2	8	0.5044
IIA2	3	6.4	4	16	0.1939

Table 4: Comparative stage distribution for endometrial cancer women with and without sentinel nodes

FIGO stage	SLN positive n = 10		SLN negative n = 12		p-value
	n	%	n	%	
IA	3	30	4	33.3	0.8830
IB	2	20	3	25.0	0.7854
II	2	20	1	8.3	0.4363
IIIC1	2	20	2	16.7	0.8453
IIIC2	1	10	2	16.7	0.6561

5.4. Surgical access

Laparoscopic surgery was performed in 20 women (35.1%) while 37 women (64.9%) had open surgery and this included 5 patients (8.8%) where the initial laparoscopic procedure was converted to open surgery.

5.5. Lymph nodes

Ten of the 47 cervical cancer patients (21.3%) had pelvic lymph node metastases, and three of the ten patients (30.0%) with presumed early stage endometrial cancer had pelvic nodal metastases, including one patient (10%) with pelvic and para-aortic nodal metastases. In this cohort therefore, 13 of the 57 patients (22.8%) had pelvic lymph node metastases.

Thirteen patients (22.8%) had grossly enlarged lymph nodes. Four of the thirteen (30.8%) had lymph node metastases, and nine of the thirteen patients (69.2%) with enlarged pelvic nodes did not have pelvic lymph node metastases.

5.5.1. Sentinel lymph nodes

A total of 164 pelvic sentinel nodes were removed, of which 77 were removed from the left side and 87 from the right side.

Fifty-one SLNs (31.1%) were located in the obturator bin, 47 (28.7%) in the external iliac bin, 29 (17.7%) in the internal iliac region, 20 (12.2%) in the common iliac region and 17 (10.4%) in the presacral area.

The location distribution of pelvic sentinel nodes is shown in Figure 3.

5.5.2. Detection of sentinel lymph nodes

MB was administered to all 94 women with data for analysis. In 43 patients (44.8%) [99]Technetium nanocolloid was used with MB, and a further 10 patients (10.4%) received MB, ^{99}Tc, as well as ICG.

MB detected SLNs in 50 (53.2%) from 96 women, ^{99}Tc detected SLNs in 28 women from 43 (65.1%), while ICG detected SLNs in 9 patients from 10 (90%). The combination of MB and ^{99}Tc detected SLNs in 36 from 43 women (83.7%). The comparative detection rate data is shown in Table 5.

5.6. Histology

In this cohort 13 of the 57 patients (22.8%) had pelvic lymph node metastases.

5.6.1. Frozen section examination

Frozen section examination was performed on all SLNs. In one of the 13 patients, the specimen sent for frozen section underwent autolysis and was therefore not examined. FSE showed metastases in 5 of the twelve patients (41.7%). In one of these cases the SLN was the only positive node.

There were six true positive, 73 true negative and three false negative SLNs on frozen section examination. The sensitivity, specificity, PPV and NPV for

FSE is 66.67%, 100%, 100% and 96.05% respectively. The FNR for FSE was 23.1%

Figure 3: The location distribution of pelvic sentinel lymph nodes
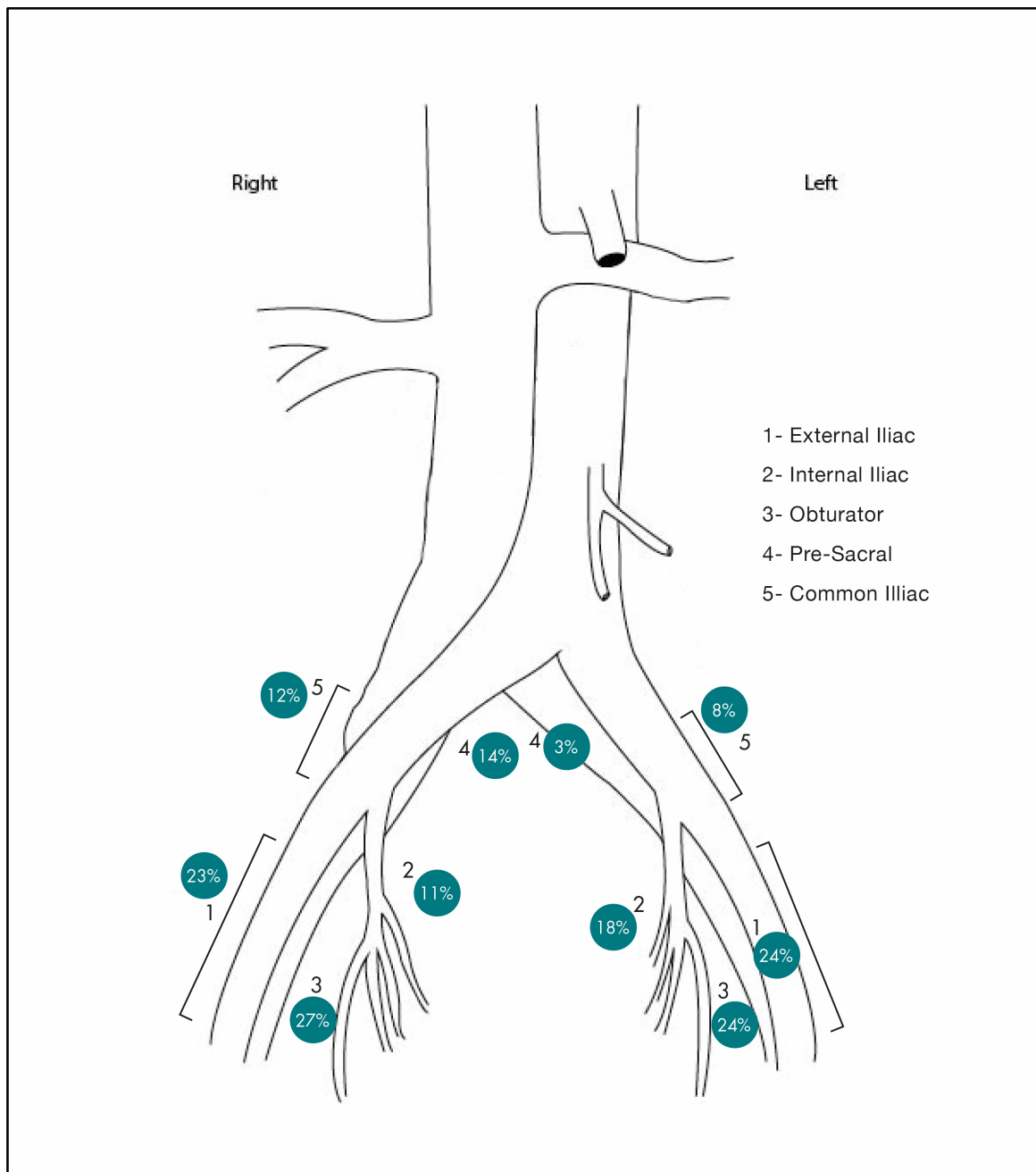
Table 5: Comparative sentinel lymph node detection rates between MB, ^{99m}Tc, ICG, and ^{99m}Tc + MB

MB only		^{99}Tc only		MB +^{99m}Tc		ICG only		p-value
Detect rate%	n	Detect rate%	n	Detect rate%	n	Detect rate%	n	
53.2	94	65.1	43					0.1934
53.2	94			83.7	43			0.0006
		65.1	43	83.7	43			0.0495
53.2	94					90	10	0.0263
		65.1	43			90	10	0.1260
				83.7	43	90	10	0.6197

5.6.2. Sentinel lymph nodes

For SLNB as group following FSE, H&E and ultrastaging, there were nine true positive, 75 true negative and one false negative. The sensitivity, specificity, PPV and NPV for the SLNB is 90%, 100%, 100% and 98.68% respectively. The FNR was 7.7%.

There was one case with a false negative sentinel lymph node. However, this patient had enlarged pelvic nodes on both sides that were removed, and if the SLN algorithm were evaluated there would not have been a false negative node. The sensitivity, specificity, PPV and NPV for the SLN algorithm is 100%, 100%, 100% and 100% respectively with a FNR of 0%.

The comparative findings of the performance of the SLNB alone with the SLNB algorithm are shown in Table 6.

5.6.3. Ultrastaging

Of the 13 women with nodal metastases, the SLNB identified two women with only micro metastases (15.4%). In one additional patient with bilateral nodal disease there was nodal disease in the SLN only on one side.

5.6.4. Anatomical sites

In five women (8.8%) in this group SLNs were present in the presacral lymph node basins. None of these SLNs contained metastatic disease

Table 6: Comparison of the SLNB and the SLNB algorithm

Histological examination	Sens	Spec	PPV	NPV	FNR
SLNB without the algorithm	90%	100%	100%	98.68%	7.7%
SLNB algorithm	100%	100%	100%	1000%	0

6. Discussion

More than half of the 57 patients in whom SLNs were detected were HIV-infected with a mean CD4 count of 448.47 cells/μl, which probably reflects the high rate of ARV use.

This data also confirms the poor predictive value of enlarged lymph nodes to predict nodal metastatic disease, with 69% of cases with enlarged nodes not having metastatic disease.

There were no statistical differences between the demographic data of women who mapped successfully compared with women who did not map. The statistically significant differences between the two groups included more TB infections in the group with SLNs, while the group who did not map had statistically significantly more enlarged lymph nodes compared to those who mapped successfully. Statistically significantly more women with FIGO stage IB1 cancer of the cervix mapped successfully compared to the group where no SLNs were detected. The rest of the disease characteristics were similar for the two groups.

The location distribution of SLNs in this study compared well, but differed slightly with published literature on this subject. The percentage of pre-sacral and common iliac nodes was slightly higher compared to what others found. The fact that sentinel nodes were found in regions that might not form part of routine systematic

lymphadenectomy, could explain the finding that the SLN algorithm detected higher rates of pelvic lymph node metastases compared to systematic lymphadenectomy [26].

Table 7 shows a comparison with published data on the location of sentinel nodes in the pelvis.

Table 7: Comparison of sentinel lymph node location with other publications

	Current study %	Wuntakel [27] %	Abu Rustum [28] %	Diaz [16] %
External iliac	29	39	30	35
Internal iliac	18	23	36	30
Obturator	31	25	24	20
Pre-sacral	10	1.4	7	
Common iliac	12		7	8

The role of FSE in the SLNB procedure is somewhat uncertain, but it might have a role in cervical cancer. If the policy is to not operate patients with pelvic nodal metastases, intra-operative FSE might assist in identifying this group of patients. In this scenario, FSE would have altered the treatment of 4 from 47 women (8.5%) with cervical cancer. It must be kept in mind when this strategy is employed that the sensitivity is only about 67% and the FNR is 23%. The data from the current study is comparable to findings reported by others [4,5].

The role of intra-operative FSE in endometrial cancer is less clear and it would probably not influence the surgical management of these women, and therefore it probably has very limited value in the surgical management of women treated for presumed early stage endometrial cancer.

Ultrastaging of sentinel lymph nodes seems to be one of the procedure's biggest advantages in that it detects more women with nodal disease compared to H&E examination following standard lymphadenectomy. In the current study ultrastaging detected disease in 15% of women who would otherwise have been diagnosed as

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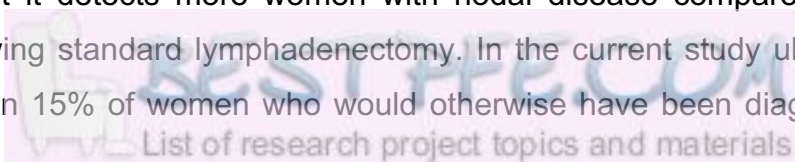
not having nodal metastases. The main reasons for this is the detection of micro metastases and removal of nodes from sites not routinely sampled [26]. The negative prognostic implication of micro metastases has been well described [9].

7. Conclusions

It appears from these results that a history of TB is not associated with not detecting sentinel nodes, and enlarged lymph nodes do not accurately predict the presence of nodal metastases.

In women with cervical cancer, more women with FIGO stage IB1 disease were in the group of women who mapped positive for sentinel nodes. Intra-operative FSE might have a place in cervical cancer if used to identify women who should rather be referred for primary cCRT. The sensitivity, NPV and FNR are similar in the current study than what has been published previously.

When the SLN algorithm is applied to this population it has a sensitivity of 100%, NPV of 100% and a false negative rate of zero.

References

1. Landoni F, Colombo A, Milani R, Placa F, Zanagnolo V, Mangioni C. Randomized study between radical surgery and radiotherapy for the treatment of stage IB-IIA cervical cancer: 20-year update. J Gynecol Oncol. 2017;28(3):e34.

2. Yamashita H, Okuma K, Kawana K, Nakagawa S, Oda K, Yano T, et al. Comparison between conventional surgery plus postoperative adjuvant radiotherapy and concurrent chemoradiation for FIGO stage IIB cervical carcinoma: a retrospective study. Am J Clin Oncol. 2010;33(6):583-6.

3. Bats AS, Mathevet P, Buenerd A, Orliaguet I, Mery E, Zerdoud S, et al. The sentinel node technique detects unexpected drainage pathways and allows nodal ultrastaging in early cervical cancer: insights from the multicenter prospective SENTICOL study. Ann Surg Oncol. 2013;20(2):413-22.

4. Martinez A, Mery E, Filleron T, Boileau L, Ferron G, Querleu D. Accuracy of intraoperative pathological examination of SLN in cervical cancer. Gynecol Oncol. 2013;130(3):525-9.

5. Slama J, Dundr P, Dusek L, Cibula D. High false negative rate of frozen section examination of sentinel lymph nodes in patients with cervical cancer. Gynecol Oncol. 2013;129(2):384-8.

6. How J, Lau S, Press J, Ferenczy A, Pelmus M, Stern J, et al. Accuracy of sentinel lymph node detection following intra-operative cervical injection for endometrial cancer: a prospective study. Gynecol Oncol. 2012;127(2):332-7.

7. Mosgaard BJ, Skovlund VR, Hendel HW. Promising results using sentinel node biopsy as a substitute for radical lymphadenectomy in endometrial cancer staging. Dan Med J. 2013;60(7):A4665.

8. Schwartz GF, Giuliano AE, Veronesi U. Proceedings of the consensus conference on the role of sentinel lymph node biopsy in carcinoma of the breast, April 19-22, 2001, Philadelphia, Pennsylvania. Cancer. 2002;94(10):2542-51.

9. Cibula D, Abu-Rustum NR, Dusek L, Zikan M, Zaal A, Sevcik L, et al. Prognostic significance of low volume sentinel lymph node disease in early-stage cervical cancer. Gynecol Oncol. 2012;124(3):496-501.

10. Van Trappen PO, Gyselman VG, Lowe DG, Ryan A, Oram DH, Bosze P, et al. Molecular quantification and mapping of lymph-node micrometastases in cervical cancer. Lancet. 2001;357(9249):15-20.

11. Gortzak-Uzan L, Jimenez W, Nofech-Mozes S, Ismiil N, Khalifa MA, Dube V, et al. Sentinel lymph node biopsy vs. pelvic lymphadenectomy in early stage cervical cancer: is it time to change the gold standard? Gynecol Oncol. 2010;116(1):28-32.

12. Raimond E, Ballester M, Hudry D, Bendifallah S, Darai E, Graesslin O, et al. Impact of sentinel lymph node biopsy on the therapeutic management of early-stage endometrial cancer: results of a retrospective multicenter study. Gynecol Oncol. 2014;133(3):506-11.

13. Kim CH, Soslow RA, Park KJ, Barber EL, Khoury-Collado F, Barlin JN, et al. Pathologic ultrastaging improves micrometastasis detection in sentinel lymph nodes during endometrial cancer staging. Int J Gynecol Cancer.2013;23(5):964-70.

14. Cormier B, Diaz JP, Shih K, Sampson RM, Sonoda Y, Park KJ, et al. Establishing a sentinel lymph node mapping algorithm for the treatment of early cervical cancer. Gynecol Oncol. 2011;122(2):275-80.

15. Darai E, Rouzier R, Ballester M, Barranger E, Coutant C. Sentinel lymph node biopsy in gynaecological cancers: the importance of micrometastases in cervical cancer. Surg Oncol. 2008;17(3):227-35.

16. Diaz JP, Gemignani ML, Pandit-Taskar N, Park KJ, Murray MP, Chi DS, et al. Sentinel lymph node biopsy in the management of early-stage cervical carcinoma. Gynecol Oncol. 2011;120(3):347-52.

17. Cibula D, Abu-Rustum NR, Dusek L, Slama J, Zikan M, Zaal A, et al. Bilateral ultrastaging of sentinel lymph node in cervical cancer: Lowering the false-negative rate and improving the detection of micrometastasis. Gynecol Oncol. 2012;127(3):462-6.

18. Bezu C, Coutant C, Ballester M, Feron JG, Rouzier R, Uzan S, et al. Ultrastaging of lymph node in uterine cancers. J Exp Clin Cancer Res. 2010;29:5.

19. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet. 2009;105(2):103-4.

20. Benedet JL, Bender H, Jones H 3rd, Ngan HY, Pecorelli S. FIGO staging classifications and clinical practice guidelines in the management of gynecologic cancers. Int J Gynaecol Obstet. 2000;70(2):209-62.

21. Snyman LC, Zondagh BA, Dreyer G, Lindeque BG, Louw M. Urine cytology as a screening test for bladder infiltration in cervical cancer. Int J Gynecol Cancer. 2006;16(4):1587-90.

22. Cibula D, Abu-Rustum NR. Pelvic lymphadenectomy in cervical cancer--surgical anatomy and proposal for a new classification system. Gynecol Oncol. 2010;116(1):33-7.

23. Cibula D, Abu-Rustum NR, Benedetti-Panici P, Kohler C, Raspagliesi F, Querleu D, et al. New classification system of radical hysterectomy: emphasis on a three-dimensional anatomic template for parametrial resection. Gynecol Oncol. 2011;122(2):264-8.

24. Querleu D, Morrow CP. Classification of radical hysterectomy. Lancet Oncol. 2008;9(3):297-303.

25. Kim CH, Khoury-Collado F, Barber EL, Soslow RA, Makker V, Leitao MM, Jr., et al. Sentinel lymph node mapping with pathologic ultrastaging: a valuable tool for assessing nodal metastasis in low-grade endometrial cancer with superficial myoinvasion. Gynecol Oncol. 2013;131(3):714-9.

26. How J, Boldeanu I, Lau S, Salvador S, How E, Gotlieb R, et al. Unexpected locations of sentinel lymph nodes in endometrial cancer. Gynecol Oncol. 2017;147(1):18-23.

27. Wuntakal R, Papadopoulos AJ, Montalto SA, Perovic M, Coutts M, Devaja O. Location of sentinel lymph node in cervical carcinoma and factors associated with unilateral detection. Int J Gynecol Cancer. 2015;25(9):1663-8.

28. Abu-Rustum NR. Update on sentinel node mapping in uterine cancer: 10-year experience at Memorial Sloan-Kettering Cancer Center. J Obstet Gynaecol Res. 2014;40(2):327-34.

Chapter 7

Summary and recommendations

1. Chapter 1: Introduction

This PhD research study set out to investigate the efficacy of functional imaging and a less invasive diagnostic procedure, to reliably predict the regional lymph node status in the rest of the pelvis, in women with early stage cervical cancer and women with presumed early stage endometrial cancer. The research question is set in the milieu that the only currently available method of reliably assessing lymph node status is through systematic full pelvic lymphadenectomy, a procedure not without morbidity that will not benefit the vast majority of women with early stage cervical and endometrial cancer.

The methods assessed were ^{18}Fluoro-deoxcy-glucose positron emission tomography/computed tomography scans as well as the sentinel lymph node biopsy algorithm. Both these modalities have been evaluated previously, mainly on women residing in well-resourced settings, and there is no data on the performance of either these diagnostic modalities in African or South African women. It is well known that South African women face unique challenges as they have the highest prevalence HIV infection, and they have high prevalence of tuberculosis and pelvic inflammatory disease. All of these factors can theoretically influence the lymphatic system, and therefore we regarded this study as essential to validate the performance of these modalities, prior to it being implemented in clinical practice as a treatment option based on results produced elsewhere.

In addition to the abovementioned modalities, we also set out to determine the usefulness of the presence or absence of high-risk HPV DNA in sentinel nodes in predicting the presence or absence of metastatic disease in the non-sentinel nodes of the pelvis.

One hundred women with early stage cervical cancer and presumed early stage endometrial cancer were eligible for inclusion following obtaining of written informed consent.

2. Chapter 2: Sentinel lymph node biopsy procedures in women with presumed early stage endometrial cancer

2.1. Summary

Endometrial cancer is the second most common cancer in South African women. This disease however differs from the same disease in developed countries. South African women are less likely to be diagnosed with stage one disease and are more likely to have histological subtypes other than endometrioid adenocarcinoma. These high-risk subtypes are associated with higher recurrence rates and poorer prognosis.

The treatment for endometrial cancer is surgical and minimally invasive surgery is fast becoming the standard of care with high quality data suggesting minimally invasive surgery has equivalent oncological outcomes compared to open surgery. The sentinel lymph node biopsy algorithm fits in well with minimally invasive surgery and is most probably a safer alternative than omission of lymphadenectomy in patients pre-operatively perceived to be at low risk for pelvic nodal metastases.

There are substantial data published on the sentinel lymph node biopsy and algorithm in endometrial cancer, and the vast majority of this is on women with early stage disease endometrioid adenocarcinoma.

The cohort of women with endometrial cancer in the current study consisted of only 22 patients, of which all were HIV negative. The findings confirmed high prevalence of high-risk histological subtypes, large tumours and poor correlation between pre-operative and post-operative final histology. Just over 50% had FIGO stage one disease and 27% had FIGO stage three disease.

Thirty-two percent of women had lymph node metastases. Sentinel lymph node detection was low at 45% and bilateral detection in only 18%. FDG-PET/CT scans performed in four women and it did not detect nodal metastases.

Although the detection rate of sentinel lymph nodes in this group was very low, the sensitivity, specificity, PPV and NPV of the SLNB as well as the SLN algorithm in this group was 100%, 100%, 100% and 1000% respectively. Applying the SLNB procedure to this group would have avoided full pelvic lymphadenectomy in 18% of women.

2.2. Recommendations

With the available knowledge and from the limited information provided from this small cohort, the following recommendations are made:

2.2.1. Sentinel lymph node procedures can be considered as a treatment option in selected South African women with presumed early stage endometrial cancer, specifically those with grade I and II endometrioid histological subtypes;

2.2.2. Although there are studies supporting the use of sentinel lymph node procedures in women with endometrial cancer with high-risk histology sub-types, there is a need for more research with regard to the effectiveness and reliability of sentinel lymph node procedures in African women with endometrial cancer, as this population have a different risk profile compared to women living in well-resourced countries;

2.2.3. Prospective studies are required before sentinel lymph node procedures can be implemented as standard of care in all women with endometrial cancer;

2.2.4. Sentinel lymph node procedures should be considered as a safer option compared to a policy of omission of lymphadenectomy in women with endometrial cancer, where these decisions are made in the absence of reliable intra-operative frozen section examination;

2.2.5. FDG-PET/CT scan should not be used in the pre-operative assessment of pelvic lymph nodes in women with endometrial cancer.

3. **Chapter 3: Sentinel lymph nodes in women with early stage cervical cancer**

Cervical cancer remains the most common gynaecological cancer in South African women, with at least at least nine deaths per day from this disease. Although a substantial volume of data has been published, there are no prospective data on sentinel node procedures in cervical cancer, and it seems to be less implemented as standard treatment compared to endometrial cancer.

3.1. Summary

Data of 72 women with operable stages of cervical cancer (FIGO stage IA – stage IIA2) were available for analysis. Sixty-five percent of this cohort was HIV positive and 67% were FIGO stage IB1. Twenty-five percent had lymph node metastases. The finding of enlarged lymph nodes did not accurately predict the presence of metastatic disease, with results showing 52% of women with enlarged nodes had metastatic disease.

The sentinel lymph node detection rate was 65% and the bilateral detection rate was 30%. Indocyanine green and the combination of methylene blue and [99]Technetium nanocolloid had the best detection rates and achieved better results than either methylene blue or [99]Technetium nanocolloid on its own.

Sentinel lymph node detection rate was influenced by four factors. The detection rate was significantly higher in women with no nodal metastases, tumour smaller than 2 cm, FIGO stage IA2 – IB1 (compared to stages IB2 – IIA), and BMI < 25 kg/m^2 (compared to women with BMI > 30 kg/m^2). History of TB, PID and the presence of adhesions did not influence the detection rate. HIV status did not influence the detection rate of sentinel lymph nodes.

The overall SLN detection rate of 65% in this study population was much lower than the detection rates published in international literature. In selected sub-groups of women, the detection rate was comparable to that reported elsewhere.

Although the detection rate of sentinel lymph nodes in South African women with early stage cervical cancer was lower than reported in the literature, the sentinel lymph node biopsy algorithm had a sensitivity of 100%, NPV of 100% and a false negative rate of 0% in this study.

The most significant contribution of this project to the current sentinel lymph node knowledge base is the fact that the results reported here is the first set of results of the sentinel lymph node procedure in South African women with cervical cancer who have a high prevalence of HIV, tuberculosis and pelvic infections. This is the first report of sentinel lymph node procedure performed in a substantial group of HIV infected women.

3.2. Recommendations

3.2.1. With the current knowledge, sentinel lymph node procedures should currently not be offered as the standard of care;

3.2.2. Sentinel lymph node procedures can be considered in selected women with small tumours as part of individualised early stage cervical cancer treatment, provided they are counselled on the experimental nature of the procedure;

3.2.3. Multicentre prospective trials are needed to assess the impact of sentinel lymph node procedures in women with cervical cancer;

3.2.4. HIV status is probably not a contra-indication for sentinel lymph node procedures in women with cervical cancer.

4. Chapter 4: The ability of [18]Fluoro-deoxy-glucose positron emission tomography/computed tomography scan to accurately predict pelvic nodal status in women with uterine cancer

The identification of metastatic lymph nodes using computed tomography scans and magnetic resonance imaging is mainly based on assessment of nodal size, and therefore these imaging modalities are not very reliable predictors of lymph node metastases.

FDG-PET/CT scan is a functional scan with a reported sensitivity to detect metastatic nodes in women with cervical cancer of around 75%. Patients with tuberculosis and HIV infection will have increased FDG uptake in involved lymph nodes, thereby adversely affecting the sensitivity and specificity of the procedure.

4.1. Summary

This group of patients was selected on the basis of the availability of resources to perform FDG-PET/CT scans and lymphoscintigraphy.

Results of 28 women who underwent FDG-PET/CT scan were available for analysis. Fifty percent of this cohort of women was HIV positive. Twenty-one percent had lymph node metastases.

The sentinel lymph node detection rate was 86% and the bilateral detection rate was 43%, mainly a result of using methylene blue with [99]Technetium nanocolloid in 93% of the cohort. Fourteen percent had sentinel lymph node metastases.

The sensitivity, specificity, positive and negative predictive values of FDG-PET/CT scans to accurately predict nodal status, were 66.67%, 82%, 30.77% and 95.38% respectively. The false negative rate of FDG-PET/CT scans was 33.3%.

It was possible to perform FDG-PET/CT scans in only 32 women (32%) from the entire cohort of 100 patients over a period of twenty months. This is an indication of the pressure on scarce commodities in healthcare such as FDG-PET/CT scan.

The sensitivity, specificity, PPV and NPV of FDG-PET/CT scan performed on South African women with high prevalence of HIV, TB and PID in this study is comparable to that published in international literature.

The findings of this study support the Radiology Society of South Africa PET/CT guidelines.

4.2. Recommendations

4.2.1. PET/CT should not be used as part of the primary diagnosis and staging investigations in women with uterine cancer, and is recommended only in selected cases for initial staging of locally advanced cervical cancer being considered for radical chemoradiation therapy.

5. Chapter 5: High-risk human papilloma virus DNA in sentinel lymph nodes in women with cervical cancer

Cervical cancer is caused by persistent infection with high-risk human papilloma virus. Some data suggest the presence of hrHPV in non-sentinel nodes is associated with poorer prognosis and risk of recurrence. A few publications in the recent literature have suggested the presence or absence of high-risk HPV types in the sentinel lymph nodes of women with cervical cancer may further assist in the assessment of the oncological status of pelvic lymph nodes in women treated for cervical cancer.

5.1. Summary

This cohort consisted of 42 patients treated for cervical cancer, of which 74% had FIGO stage IB1 disease, and 88% were squamous carcinoma. In 31 women HPV data were available for the tumour as well as the SLN. Sixty-nine percent of this group was HIV positive. Twenty four percent of this group had lymph node metastases.

HPV DNA was isolated in 46% of the sentinel lymph nodes, with HPV 16 the most common type encountered with HPV 33, 52, and 66 in second place.

The most common HPV type isolated from cervical cancer tumours was HPV 16 followed by HPV 52, 18 and 58.

In 57% of women with metastases, no hrHPV DNA was isolated. High-risk HPV DNA was isolated in 29% of women without pelvic lymph node metastases.

The sensitivity, specificity, PPV and NPV of sentinel lymph node HPV DNA to predict metastases was 50%, 69.6%, 30 and 84.2% respectively, with a false negative rate of 42.8%.

Findings in this study differ from the published data on this topic. This might be as a result of different tests that were used to detect HPV DNA in sentinel nodes in different studies cited from literature. It is also possible that the high HIV prevalence in this group impacted significantly on these results.

In the current study, testing for the presence of hrHPV DNA in the sentinel lymph nodes was not useful as a predictor of pelvic lymph node status. The presence of hrHPV in SLNs did not accurately identify patients with lymph node metastases, while the absence of hrHPV in SLNs did not accurately predict the absence of lymph node metastases. The combination of negative FSE and negative hrHPV in the SLNs did not have a reliable negative predictive value for the absence of pelvic nodal metastases.

5.2. Recommendations

5.2.1. The presence or absence of high-risk HPV DNA should not be used on its own or in combination with FSE to predict the status of non-sentinel nodes in the pelvis in women treated with early stage cervical cancer.

6. **Chapter 6: Analysis of women in whom sentinel nodes were detected and the role of different histological examinations of sentinel lymph nodes**

Intra-operative frozen section examination can be performed on sentinel lymph nodes. Sentinel nodes reported to be negative for metastatic disease after examination following routine H&E staining, must undergo ultrastaging consisting of serial sectioning and immunohistochemistry.

It has been reported in the literature that ultrastaging of sentinel lymph nodes results in higher detection of nodal metastatic disease compared to systematic lymphadenectomy and H&E examination, mainly due to the ability of ultrastaging to detect micro metastases. Examination of sentinel nodes in sites not routinely involved in systematic lymphadenectomy may further contribute to higher detection rates of nodal metastases.

6.1. Summary

The cohort of patients in whom sentinel nodes were detected consisted of 57 women of which 56% were HIV positive. Ten women had endometrial cancer and 47 had cervical cancer.

Twenty-one percent of this cohort with cervical cancer and 30% with presumed early stage endometrial cancer had pelvic lymph node metastases.

Statistically significantly less women who mapped successfully had enlarged lymph nodes compared to women who failed to map, while significantly more women in this group had FIGO stage IB1 cervical cancer.

Indocyanine green and the combination of methylene blue and [99]Technetium nanocolloid had significantly better sentinel node detection rates compared to methylene blue on its own. The location distribution of SLNs in this study compared well, but with a slight difference to other published literature with higher detection in the pre-sacral and common iliac regions. In 9% of this group SLNs were present in the presacral lymph node regions. None of these SLNs contained metastatic disease.

The sensitivity, specificity, PPV and NPV for FSE in this cohort was 66.67%, 100%, 100% and 96.05% respectively. The FNR for FSE was 23.1%. For SLNB as group following FSE, H&E and ultrastaging, the sensitivity, specificity, PPV and NPV for the SLNB was 90%, 100%, 100% and 98.68% respectively. The FNR was 7.7%, while the sensitivity, specificity, PPV and NPV for the SLNB algorithm was 100%, 100%, 100% and 100% respectively with a FNR of 0%.

Of the 13 women with nodal metastases, the SLNB identified two women with only micro metastases (15.4%). These women would not have been identified with systematic lymphadenectomy and H&E examination.

It appears from this data that a history of TB is not associated with not detecting sentinel nodes, and enlarged lymph nodes do not accurately predict the presence of nodal metastases.

The most important advantage of sentinel lymph node procedures is the improved detection of nodal metastases compared the standard technique in current use.

6.2. Recommendations

6.2.1. Intra-operative frozen section might have a role in identifying patients with early stage cervical cancer whom might benefit from primary concurrent chemoradiation;

6.2.2. FSE on its own is not a reliable predictor of non-sentinel lymph node status;

6.2.3. Intra-operative FSE has a limited role in the management of women with presumed early stage endometrial cancer;

6.2.4. Ultrastaging should form part of the investigations performed on sentinel lymph nodes

7. Conclusion

7.1. Limitations of the study

The sample size was based on what was convenient and realistic to achieve in the limited time available to complete this project, and the endometrial cancer cohort had a very small sample size.

Due to logistical challenges, it was not possible to perform [99]Technetium-nanocolloid and lymphoscintigram examinations and FDG-PET/CT scans on all women recruited to the study. The same is true for HPV DNA detection in all removed sentinel nodes.

The patients in this study were not followed up to determine the effect on prognosis of some of the findings in the study.

7.2. Contributions of this study to current knowledge on sentinel nodes

The main contribution in this regard is the knowledge obtained from this study on HIV positive women. This is the first sentinel lymph node study to our knowledge reporting on a significant number of HIV positive women.

The study also provides valuable insights in the challenges experience in low-resource settings and the pressure on available resources, as can be seen on the lower than expected proportion of women on whom all planned investigations was performed.

The best mapping techniques seem to be the combination of ^{99}Tc with methylene blue or ICG with near infrared mapping.

The SLNB algorithm performed perfectly well in this high risk population

7.3. Some ethical considerations

An important ethical consideration in sentinel lymph node procedures in low-resource settings in low and middle-income countries such as South Africa, is the use of expensive resources and treatment modalities. The ethical principle of

distributive justice is relevant and needs to be balanced with the other bio-ethical principles of beneficence and non-maleficence.

Sentinel lymph node procedures require investment in equipment, skills training, and is associated with additional expenses for tracers and ultrastaging procedures in the laboratory. This procedure fits very well into the concept of minimally invasive procedures, which are fast becoming the standard of treatment in the surgical treatment of uterine cancers.

Benefits to women treated for uterine cancer are substantial. Minimally invasive procedures are associated with shorter hospital stay, quicker recovery and less morbidity and pain. Sentinel lymph node procedures can further reduce morbidity.

The investment in equipment such as laparoscopy equipment, is also beneficial with regard to treatment of benign gynaecological conditions as well as use by other disciplines in health care, and its use is not exclusive to gynaecologic cancer care.

The abovementioned benefits of these procedures are more important to women in low resource settings, who do not receive financial benefits when in hospital, and who, as a result of socioeconomic factors seems to do less well and are more prone to complications when undergoing extensive surgery.

The investment and expenses associated with these technologies should be considered against this background and I am of the view that the cost benefit ratio for these procedures is a highly favourable one.

Sentinel lymph node procedures appear to be offering more lymph node status information with less invasion, and we might indeed be "getting more for less" using this procedure. However, these potential benefits must be balanced against the remaining uncertainties regarding the influence of this procedure on clinical outcome if complete lymphadenectomy is omitted, and more prospective studies are required, especially in women with early stage cervical cancer.

7.4. Final conclusions

The SLNB algorithm is a feasible option in South African women, including HIV positive women, with early stage cervical cancer and presumed early stage endometrial cancer. Although the detection rate is lower than anticipated, the procedure has a very good sensitivity, specificity, NPV, PPV, with a false negative rate of zero in this study

[18]FDG-PET/CT scan as a stand-alone test does not meet the requirements to be safely used as a reliable alternative test to assess the lymph node status of the pelvis.

Assessment of hrHPV DNA did not contribute to the SLNB algorithm.

Pathological ultrastaging contributes to detection of lymph node metastases compared to routine systematic pelvic lymphadenectomy.

7.5. Future research

7.5.1. There is still a lack of prospective data on the use of SLN procedures in women with early stage cervical cancer and it is unsure what the effect on disease free and overall survival will be should this concept be implemented in routine care;

7.5.2. More research is necessary to investigate the effectiveness and reliability of sentinel lymph node procedures in South African women with endometrial cancer, as this population have a different risk profile compared to women living in well-resourced countries;

7.5.3. More research is required on identifying factors that would determine the most appropriate patients for use of the SLNB procedure

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