A TWO PHASE LOCAL STUDY, COMPARING LIQUID-BASED CYTOLOGY TO THE CONVENTIONAL CERVICAL PAP SMEAR.

By

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A dissertation submitted to the University of Cape Town in fulfillment of the requirements for the degree of Masters of Medicine (Obstetrics and Gynaecology) Part III.

June 2005

DECLARATION

I, IA. VAN REENSBURG, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise), and that the whole work has not been, is being or is to be submitted for another degree in this or any other university.

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ACKNOWLEDGEMENTS

The nursing and medical staff of the Department of Obstetrics and Gynaecology at Groote Schuur Hospital who contributed to this trial.

Mareza van Rensburg, the research assistant, for her thorough and exact data keeping and preparation of this manuscript.

Carl Lombard, statistician at the Medical Research Council, who helped with the statistical analysis.

Finally, I would like to thank my supervisor, Prof Lynn Denny, for her support, enthusiasm and advice, which made this dissertation possible.

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ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
AGC	Atypical Glandular Cells
AGUS	Atypical Glandular Cells of uncertain significance
ASC	Atypical Squamous Cells
ASCUS	Atypical Squamous Cells of uncertain significance
ASIR	Age standardised rates of cervical cancer
CI	Confidence Interval
CIN	Cervical Intra-epithelial lesion
E	Early
FDA	Food and Drug Administration
GSH	Groote Schuur Hospital
HIV	Human Immunodeficiency Virus
HPV	Human Papilloma Virus
HSIL	High grade squamous intra-epithelial lesion
IARC	The International Agency for Research into Cancer
L	Late
LBC	Liquid-based cytology
LLETZ	Large loop excision of the transformation zone
LSIL	Low grade squamous intra-epithelial lesion
OR	Odds Ratio
ORF	Open reading frame
PAP	Papanicolaou
RR	Relative risk
SBLB	Satisfactory but limited by
SIL	Squamous intra-epithelial lesion
TP	ThinPrep
WHO	World Health Organization

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Chapter 1

INTRODUCTION

Cancer of the cervix is the most common cancer in women in developing countries and constitutes 33% of all malignant tumours in black women in South Africa.[1] Four out of every 5 cases of cervical cancer occur in developing countries, where only 5% of women receive a repeat PAP smear within a five year period.[2] The lifetime risk for the development of cervical cancer in black South African women is an estimated 1:26 compared to 1:83 for white South African women, the majority of whom have been screened.[3] The age standardised incidence rates (ASIR) of cervical cancer are expressed as the number of cases of cervical cancer per 100 000 women in the population. In 1992, the South African pathology-based Cancer registry reported the ASIR of cervical cancer as 35/100 000 for black women and 12/100 000 for white women. It is believed that many women who die of cervical cancer in developing countries do not reach hospitals and do not have their disease diagnosed histologically, so the rates quoted are probably an underestimate of the true incidence.[4, 26] (See Table 1)

Table 1. Age standardised rates of cervical cancer in different countries

Country	Rate
USA (1985)	9/100 000
Australia / New Zealand (1985)	11/100 000
Northern Europe (1985)	11/100 000
Eastern Africa (1985)	45/100 000
South America (1985)	45/100 000
Zimbabwe (1996)	67/100 000
South Africa (1992)	35/100 000
England (1997)	9,3 /100 000

Chapter 2

THE ROLE OF SCREENING IN THE REDUCTION OF CARCINOMA OF THE CERVIX

Cervical cytology screening using the Papanicolaou smear is regarded as an effective cancer screening tool.[4,5,7.] In the USA, deaths from cervical cancer declined by 74% between 1955 and 1992.[2] This decline has been largely attributed to widespread screening.[6, 9, 8]

There are no randomised, controlled trials of cervical screening.[4;7] Evidence confirming the reduction in the incidence of and mortality from cervical cancer is derived from non-experimental cohort and case-control studies.[9] The International Agency for Research into Cancer (IARC), estimated in 1986 based on data from 8 developed countries that had organised screening programmes, that cervical cancer mortality declined by approximately 30% between 1960 and 1980. The major cause of this decline has been attributed to mass screening.[4,8,10.] The IARC collaborative study suggested that an approximate 90% protective effect can be achieved at the individual level, while experience from the Nordic countries indicate that organised programmes in such populations can achieve an 80% protective effect at the population level in the targeted age groups.[9,11]

However, screening is one link in the chain of cancer control which extends from inviting women for screening, to screening, to diagnosis, treatment and follow-up. Therefore screening should be a part of the total healthcare delivery system and should not exist in isolation from that system.

Certain criteria apply for a screening program and can be separated in three parts namely the disease, the policy and the test where:

- The condition (cervical cancer) should impose an important health problem.
- The natural history of the disease should be well understood.
- The disease should be detectable at an early stage.
- The disease must have a premalignant state
- The result of treating the disease at the stage detected by screening, must be superior to treatment at a later stage.
- The test must be sufficiently sensitive to detect the disease in early stage.
- The test must be sufficiently specific to distinguish non-specific changes from disease.
- The test must be cost effective.
- The test must be acceptable to the population.
- Adequate facilities for diagnosis and treatment must be available.

Cervical cytology as a screening test meets the above requirements. [12,76]

i) Natural History:

Screening and treatment policies are based on the understanding of the natural history of cervical neoplasia. The current understanding of the pathogenesis of cervical cancer and its precursors is that invasive cervical cancer arises from intraepithelial lesions, which have been known by various terminologies. These lesions which are thought to be the precursors of cervical cancer, can be easily removed to prevent the development of invasive disease. In the last 3 decades it has also been widely accepted that CIN I, CIN II, and CIN III represent

progressive successive stages in the development of invasive cervical cancer.[13] It is clear however, that this does not occur invariably. Spontaneous regression of CIN I is a well-documented phenomenon. Most studies suggest that 10 to 15% of women with CIN I who are followed without biopsy will subsequently develop CIN II or CIN III. At least 60% will have smears that regress to normal and approximately 30% will have persistent CIN I.[13]

The role of Human papilloma virus in cervical cancer:

Epidemiological, clinical and molecular evidence strongly suggest that HPV infection of the cervix plays a central aetiological role in the pathogenesis of cervical cancer and its precursors.[13, 14, 15]

HPV infection of the cervix may regress, persist, or progress into CIN II and CIN III and eventually to cervical cancer. High risk types of HPV, e.g. 16 and 18, have consistently been associated with persistent infection of the cervix and progression to CIN and cancer.[4, 13, 15, 16, 17.] Walboomers[14] reported the worldwide prevalence of HPV in cervical cancer as 99.7%. However, there is still a limiting understanding of the natural history of genital HPV infections.[18, 19]

HPV is sexually transmitted[18] and the vast majority (over 80%) are transient, being cleared by the immune system within a few months without any residual detectable lesion [15, 18]. This correlates with a gradual decrease in HPV prevalence with age as reported in the United Kingdom from 20% between 20 and 25 years of age to 5% by the age of 50 years [15, 18].

On molecular level the HPV is a non-enveloped double stranded DNA virus (genome) with an icosahedral outer capsid. This genome is divided into approximately 8000 base pairs.[15] Each genome can be divided into early (E) and

late (L) regions.[15,18] The early region contains 7 early (E_{1-7}) open reading frames (ORF) and the late region contains 2 late (L_{1-2}) ORF. The early genes are responsible for DNA replication, transcriptional regulation and transformation. The late genes control the formulation of the capsid coat.[15] HPV are epitheliotropic viruses and infect the basal cell of sqaumous epithelium through minor injuries [15]. The ensuing virus life cycle is then linked to keratinocyte differentiation as viruses in general have no metabolism of their own and cannot reproduce independently of the host cell.[20] At the onset of infection expression of the early genes occur.[21] In high risk HPV infection, the ORF are effectively integrated into the host chromosomes. The E_6 and E_7 ORF of the HPV genome appear to be principally responsible for HPV neoplastic oncogenic effects. E_6 binds to and inactivates the p53 tumor suppressor protein.[15] The p53 suppressor protein protects the integrity of the genome [20]. The E_7 binds to the pRb suppressor protein and the expression of these early genes results in the initiating event in the molecular pathogenesis of cervical cancer. Thereafter, somatic genetic mutations occur that may be involved in cervical cancer development. [18]

ii) Cytopathology reporting:

During the past two to three decades, terminology for reporting on premalignant lesions of the cervix has changed. (Table 2a) Unfortunately previous reporting systems created substantial confusion for family physicians as these systems lacked descriptive evaluations and therefore obscured the exact cytologic findings.

Table 2a: Terminology in reporting premalignant lesions of the Cervix.

1970's	1980's	1990's
Dysplasia	Cervical intra-epithelial neoplasia (CIN)	Squamous intra-epithelial lesions (SIL)
Atypia;Mild dysplasia	Atypia; CIN I	SIL low-grade
Moderate dysplasia	CIN II	SIL High-grade
Severe Dysplasia	CIN III	SIL High-grade

Bethesda System:

The Bethesda system of cytopathological reporting was introduced in 1988 and revised in 1991 and 2001. It was designed to improve communication between pathologists and clinicians. It also aimed to standardize reporting, with a descriptive interpretation of cytology and comment on the adequacy of the sampling. Evaluation of specimen adequacy is considered by many physicians to be the single most important quality assurance component of the Bethesda system.[22] Criteria for a satisfactory PAP smear in the 1991 Bethesda System include the presence of an adequate sample of the transformation zone, sufficient squamous cellularity and minimal obscuring factors.[23]

According to the 2001 Bethesda system, a notation regarding the presence of an endocervical component is recommended but not required. This contributed to the elimination of the "satisfactory but limited" category. (Table 2b).[22]

Table 2b. Bethesda System for specimen adequacy:

1991	2001
Satisfactory Satisfactory but limited by Unsatisfactory	Satisfactory Unsatisfactory

In contrast to the other classifications, the Bethesda System:

- separates infection related changes and reactive or reparative changes, including inflammation, from squamous cell atypia and higher- grade lesions.[24]
- evaluates specimen adequacy as a quality assurance component [22].
- includes HPV infection and CIN I in low-grade squamous intra-epithelial lesions.
- mirrors closely what is understood about the behavior of low- and high- grade lesions. [4]

Infection and reactive/reparative changes fall into the category of benign cellular changes while more advanced lesions are classified as epithelial cell abnormalities. Reporting changes have been made in the category of epithelial cell abnormalities at the Bethesda workshop, including for ASCUS and AGUS. The classification of AGUS has been changed significantly. (Table 2c) [24, 22]

There is still controversy about implementing the Bethesda system. At the 2001 workshop, 44 professional societies who represented more than 20 countries, were co-sponsors. More than 20 national and international societies endorsed the 2001 Bethesda system at that time.[24, 22]

TABLE 2c

The Bethesda system for the classification of cytology of the cervix

1991	2001
Within normal limits	**Negative for Intra-epithelial lesion or malignancy**
Benign epithelial changes: • inflammatory • reparative • reactive • atrophy	**Benign epithelial changes:** • inflammatory • other non-neoplastic changes (optional): - reactive - atrophy
Epithelial Cell abnormalities: • ASCUS - atypical cells of uncertain significance • AGUS - atypical glandular cells of uncertain significance • LSIL - low-grade squamous intra-epithelial lesion • HSIL - high-grade squamous intra-epithelial lesion • suspicious malignant lesion	**Epithelial Cell abnormalities:** • ASC - atypical squamus cells ASC-US (ASC undetermined significance) ASC–H (cannot exclude HSIL) • AGC - atypical glandular cells (specify origin) AGC - favour neoplastic AIS - endocervical adenocarcinoma in situ • LSIL - low-grade squamous intra-epithelial lesion • HSIL - high-grade squamous intra-epithelial lesion • suspicious malignant lesion

iii) Sensitivity, Specificity of cervical cytology and the False negative smear:

Despite the success of some screening programs, cervical cytology has a wide range of reported sensitivities between 20 - 85% [7, 25] Fahey et al used a summary receiver operating characteristic curve in his metanalysis of 59 studies to estimate the accuracy of the PAP test. On average he concluded a sensitivity of 55 – 65% and 65 – 75% specificity for the conventional PAP smear. [26, 27] High sensitivity and specificity are prerequisites for a successful screening program, as they are indicators of test performance.[12,28,29.]

Sensitivity is defined as the probability of the test being positive in patients with the condition and is calculated as: true-positive / (true-positive + false-negative).

Specificity is defined as the probability of the test being negative in the absence of the condition and is calculated as: true-negative / (true-negative + false-positive)

The issue at stake around the PAP smear in developed countries or in countries where the majority of women are enrolled in screening programs, is the false negative PAP smear. A universally accepted definition for a false negative PAP smear has not been established. [23]

Errors in screening can occur in obtaining, processing, screening and interpreting PAP smears and in the follow-up of patients with abnormal PAP smears.[24] Therefore it is necessary to specify the variables, which include sampling versus laboratory false negative errors and define what constitutes disease and the time interval from screening to detection of the disease.[23] Richard et al defined a false negative Pap smear in a patient who has a neoplasm with a negative cytological smear, or if a neoplasm has been found shortly after a smear was taken . This may occur as a result of lack of diagnostic cells in the specimen obtained or as a result of observer error when the diagnostic cells are present but they are overlooked or misinterpreted by the examiner.

Wilkinson[12] subdivided false negative cervical and/or endocervical cytology into three main categories:

1. **Sample error**: 60% of false negative smears occur due to sample error. This implies that the diagnostic cells are not on the slide.

2. **Screening error:** 40% of false negative smears occur due to screening error. This implies that the cells are on the slide, but are missed by the cytotechnologist when screening the smear.
3. **Interpretation error:** The pathologist examined the cells in question and judged them benign when in fact they were malignant.

In addition to sampling error, 80% of cells are reported to remain on the spatula after the Pap smear was made.[30, 31] Causes of inadequate sampling include:

- scant cellularity
- poor fixation or preservation (air drying)
- presence of foreign material (lubricant)
- obscuring inflammation
- obscuring blood
- excess cytolysis
- no metaplastic or endocervical component.....[24,32]

Other limitations of the PAP smear leading to false negative smears include failure to detect adenocarcinoma or advanced squamous carcinoma due to low exfoliation of cells or necrotic debris respectively.[33.] As a consequence of the false negative PAP smear, it should not be used as a diagnostic tool. Also, carcinoma of the cervix may develop with associated morbidity and mortality and the original false negative PAP used as a diagnostic tool may lead to increased litigation [34, 22.]

Litigation around the false negative PAP smear includes the following reasons:

- The understanding of the individual woman that a positive or a negative Pap smear indicates disease or non-disease. [35]

- A subtle shift over the last half century from Papanicolaou's original vision of an inexpensive, widely accessible screening test to greater emphasis on the diagnostic component of the PAP smear.

The Insurance industry looked at summaries of all new pathology claims at one malpractice insurer and found that in 56 (17%) out of 335 claims, the interpretation of gynaecological smears was the central issue. [36]

iv) Screening in South Africa:

At present there is no national screening program for cervical cancer available in South Africa. However, the South African government has adopted a policy of three free smears per women, at the ages of 30, 40 and 50 years.[3] Assuming a 100% coverage of the population, this policy is expected to reduce the incidence of cervical cancer by 64%, based on an IARC analysis in 1986.[37,8] However this may not apply to the HIV positive population. HIV is associated with increased incidence of HPV, infection, SIL and cervical cancer. [3, 38]

Cervical cancer is an AIDS defined illness since 1993.[39] Wright et al[40] reported LSIL to be present in 13% of 398 HIV positive women and 4% of 307 HIV negative women which was statistically significant. For HSIL, 7% was detected in the HIV positive women and 1% in the HIV negative women. This was also statistically significant. Strong and consistent associations between HIV infection, high risk HPV types and pre-invasive cervical SIL have been demonstrated in developed countries.[41] In developing African countries, a similar association between HIV and pre-invasive cervical SIL was reported. Hawes et al[42] reported LSIL in 4% of HIV negative women, 17.2% in HIV -1 infected women, 19.5% in HIV-2 infected

women and 34% in women with dual infection. HSIL was detected in 1.4% HIV negative women versus 4.5 % of HIV-1 infected women, 10.5% in HIV-2 infected women and 13.8% of women with dual infection.

Conflicting reports exist regarding the relationship of HIV infected women and invasive cervical cancer.[41, 42, 44] Data from Africa in the early 1990s failed to document an increase in invasive cervical cancer. More recent studies reported an increased prevalence of HIV amongst women with invasive cervical cancer. Also, the mean age of occurrence in the HIV-positive women compared to the HIV negative women was 10 to 15 years younger.[43, 44] This risk associated with HIV for cervical cancer was also confirmed by Sitas et al.[45]

In the developed countries a clear relationship between HIV infection and invasive cervical cancer has not been established.[41, 43, 44] The results of the CDC Sentinel Hospital Surveillance Study demonstrated a modestly increased risk for cervical cancer among HIV-infected(10.4 cases /1000 women vs. 6.2 /1000 women) women admitted to the participating hospitals.[41] The Cancer Institute AIDS-Cancer Registry Match Study found a five fold elevated risk of invasive cervical cancer during the early pre-AIDS period but non significant elevated risks after AIDS.[41] However, aggressive screening and precancerous treatment programs may mask associations between HIV infection and invasive cervical cancer particularly if the HIV-related disease progresses.[41] Massad et al [46] reported as part of the Women's Interagency study invasive cervical cancer is uncommon in HIV-infected united States women participating in a regular prevention program involving HAART.

While there is limited data available from developing countries, the sensitivity of cytology appears to be lower than that reported by studies performed in developed countries. [47]

The explanation may be that:

- Cervical smears are taken opportunistically and often not under ideal circumstances, increasing the likelihood of sampling error.
- Women in developing countries have a significantly higher incidence of lower genital tract infections, with the result that smears are often inflammatory.
- The lack of training and human resources

Within the context of a screening policy, which in all likelihood will have a wide interval between smears, combined with the expectation of sub-optimal quality smears, there may be a role for using newer, technologically more advanced methods of cervical sampling in an attempt to overcome the limitations of such a screening policy.

v) New technologies:

Cytology automation has been developed over the last decade as a potential solution to the false negative PAP smear. Current automated cytology devices can be broadly categorized in three groups:

1. improving the sampling process:(liquid based sampling collection with thin -layer preparation)
2. screening process control : improving the detection process (automated screening)

3. automated preparation technology : improving the detection and characterization of the main causative agent of cervical cancer, the human papilloma virus.

At present only two automated preparation technologies (ThinPrep, AutoCyte) have been cleared by the Food and Drug Administration (FDA) in the USA as a replacement for the conventional method of PAP smear preparation.[48] The ThinPrep system was developed by CYTYC Corporation with approval in 1996 and begins with the collection of a sample, using a plastic spatula, into liquid medium instead of smearing the cells onto a glass slide. In the laboratory, an automated processor (The Thin Prep 2000) is used to collect cells from the sample and then deposit the cells in a thin layer on a glass slide.

Three key phases are involved:

1. Dispersion - cell clumps and mucus are broken up to produce a randomized cell suspension
2. Cell collection - a robotic processor filters out unsuitable cellular material (e.g. inflammatory cells) to optimize cell collection.
3. Cell transfer – The cellular material on the filter is transferred on a 20mm circle of a glass slide and then deposited into a vial of fixative.

This slide is stained with the conventional PAP stain for examination by the laboratory staff in a similar manner to a conventional PAP smear.[49, 48] Cytyc Corporation developed the above system to improve diagnostic accuracy.[50]

The following advantages of ThinPrep have been claimed:

- decreased number of unsatisfactory smears. [50, 26]

- decreased screening time due to monolayer and smaller screening area on slide.[51,26]
- increased detection of abnormal cells in smears with decreased false negative rate due to easier visualisation of cells with less cellular overlap. [50, 51, 26]
- slide ideal for automated screening techniques.
- co-collection for HPV DNA testing.[52]

Disadvantages of ThinPrep include:

- Retraining for cytotechnicians and redevelopment of standardisation
- Fewer diagnostic cells than conventional PAP smear. [51; 53]
- Possibly scattered abnormal cells rather than clustered in an area of the slide. [51]
- Increased cost and a new infrastructure required. [51]

Several studies have demonstrated increased adequacy and detection of SIL and carcinoma using ThinPrep technique compared to conventional cytology.[31;50;54;55;56]

In a study by Lee et al [54], the cytological diagnosis and specimen adequacy of ThinPrep were compared with the conventional PAP smear. The FDA approval for the use of the Thin Prep 2000 processor, was based on this study.[57] A total of 6747 women from 3 screening centers and 3 hospitals participated in this split sample / matched pair, double masked trial. For the split sample method, a single cervical sample is taken from the cervix. First, a conventional smear is prepared and then the collection device with the remaining sample (that otherwise would have been discarded) is rinsed in PreservCyt solution for later preparation of a ThinPrep slide. The conventional and ThinPrep slide were read independently and

cytologic diagnosis and specimen adequacy were classified using the Bethesda system.

There was 84% agreement between the conventional slide and ThinPrep. For the three screening centers there was a 65% higher rate of detection of LSIL and higher, with the ThinPrep method ($P < .001$) For the three hospital sites only 6% more cases were detected. Comparison of detection of benign cellular changes with the conventional and ThinPrep methods showed no significant differences. The difference in results was speculative. A possible explanation given was that the two hospital sites serve inner-city clinic population with much higher rates of gynaecological infections.

The specimen adequacy determination for conventional and ThinPrep slide from all 6 sites showed:

- 11% increase in number of satisfactory smears with ThinPrep method.
- 29% reduction in slides classified as Satisfactory but Limited by (SBLB) with ThinPrep method.

In the SBLB and unsatisfactory category, the conventional slides displayed a full range of sampling and preparation problems. The most common were obscuring inflammation, lack of endocervical component, obscuring blood, or drying effect and cytolysis. By comparison, in the ThinPrep slides classified as SBLB, 80% were so classified because of lack of endocervical component. In the unsatisfactory category, ThinPrep was so classified because of scant quamous epithelial component.

Wilbur et al[53] compared the accuracy of the conventional cervical PAP smear (CCP) with the ThinPrep method. A total of 3218 patients from 5 centers took part

in the study, in which a single cervical sample was split into a matched pair. Diagnostic findings identified on the two preparations were compared in a blinded fashion. The screening procedure was designed to eliminate intra and inter observer screening error. In no case did the cytotechnologist see the ThinPrep and conventional smear on the same day, but eventually did examine both slides from the same patient. Each conventional smear with a negative diagnosis was examined twice to establish the accuracy of the negative diagnosis. All slides of either type with a diagnostic interpretation of atypical or higher were examined by supervisory technologist and a pathologist. If there was discrepancy in the diagnosis between the two slides, both slides were evaluated by the supervisory technologist and pathologist.

Of the 3218 patients, the conventional smear and ThinPrep diagnosis agreed in 88.3%. In 117 non-correlating cases, 101 cases showed SIL with ThinPrep and atypical or negative with conventional. The remaining 16 cases were reported HSIL by ThinPrep and LSIL in conventional. Conversely, in 26 of 36 non-correlating cases, the conventional smear showed SIL and ThinPrep was interpreted as negative or atypical.

A total of 673 cases out of 3218 patients studied were diagnosed with SIL and higher by ThinPrep and conventional. Using the non-correlating, the inferred false-negative rate for the conventional smear to detect SIL and higher was 15% (101/673), compared to 3.9% (23/673) for ThinPrep.

The inferred false-negative rates were based on the number of patients in the study with a known positive cytological evaluation from one or both of the paired slides. As with many other studies, the diagnosis of SIL in the two arms was made

on the basis of cytology alone. In secondary research review studies few studies have correlated the cytological diagnosis with the recognized gold standard of colposcopy and histology.[26; 48]

Payne et al then emphasized in their review that the split sample studies are not the ideal study design.[26] They argued that the split sample technique might be:

- unfair as less material is available for both techniques and biased against the LBC preparation as the conventional smear is prepared first.
- the liquid based method clearly regarded as the "research" method and conventional PAP the "standard", which by itself can introduce bias.
- an increase in the abnormal results may not mean abnormal histology.

Looking at studies using colposcopy and histology as the "gold standard" for abnormal results, Hutchinson et al reported on 8636 patients where the split sample technique was used. Colposcopy was done for ASCUS or higher. In addition they conducted a validation sub-study among a random sample of 150 women with negative screening results. Among this group no abnormal histology was found. The assumption was made that all negative screening tests were negative with no false-negatives. For the diagnosis of LSIL and higher, a sensitivity of 68.7% for the conventional PAP and 87.9% for Thin Prep was reported.[26]

This study of a small number of women suggested that ThinPrep had a higher sensitivity than conventional, but the proportion of women referred for colposcopy was greater.

With this background, our study was undertaken to compare the performance of conventional vs. LBC in a South African setting.

Chapter 3

AIMS

The aim of the study in Phase I and II, was to compare the cytological diagnosis and specimen adequacy of a fluid-based, thin layer preparation (ThinPrep 2000) with a conventional Papanicolaou smear. The outcome measures were :

Phase I study:

1. specimen adequacy
2. comparing the rate of SIL and cancer between Thinprep I and the conventional PAP smear I.
3. correlation of the cytological diagnosis of SIL or cancer by ThinPrep I and conventional PAP I smear with colposcopy directed histology.

Phase II study:

1. specimen adequacy
2. comparing the rate of SIL and cancer between a direct to vial ThinPrep II and a historically conventional PAP I smear.
3. correlation of the cytological diagnosis of SIL or cancer by ThinPrep II with colposcopy directed histology.

Chapter 4

MATERIALS AND METHODS

The study protocol was approved by the Ethics and Research Committee of the University of Cape Town.

1) Summary:

Women were recruited from 3 Gynaecology clinics at GSH. The study was performed in two phases:

1. . The cervical smear was obtained by using a plastic spatula and if indicated, also an endocervical brush. Indications for the use of the endocervical brush included poor visualization of the squamous-columnar junction as in the postmenopausal women and a previous inadequate PAP smear. First the collected sample was used to prepare a conventional smear and then the spatula (and brush,if used) was placed in the vial with PreservCyt solution for later preparation of a ThinPrep slide. The split-sample design allowed the ThinPrep to be compared directly to the conventional smear prepared from the same patient. This first phase was performed as a matched-pair concurrent control design with a split-sample methodology
2. Prospective historically controlled design with "direct-to-vial" collection method. With the direct vial method, ThinPrep was compared to the conventional PAP smears done in phase I.

The 1991 Bethesda classification of cervical cytology, was used to evaluate the cytological diagnosis and adequacy of the slides. In addition, the accuracy of the cytological diagnosis was measured by the comparison with histological findings after colposcopy and biopsy.

2) Patient Population:

The study was conducted at Groote Schuur Hospital. Women were recruited from the following three clinics: the Gynaecological Outpatient Clinic, the Colposcopy Clinic and the Cytology Follow-up Clinic. Women who required a cervical smear as part of opportunistic screening, routine screening, workup or post treatment for CIN were eligible to participate. Pregnant women and women who had a hysterectomy were excluded from the study.

3) Specimen collection:

The clinicians who collected the samples for this trial, were trained and informed about the technique for ThinPrep 2000. Once a trial patient was identified, a cervical cytology request form was completed which recorded the clinical history, previous cervical treatment and cervical cytology and histological history.

In **Phase I**, specimens for the conventional cervical smear as well as the ThinPrep preparation were obtained during the same gynaecological examination. The samples were collected with a plastic spatula and when indicated (squamo-columnar junction not visible as in post menopausal women or previous inadequate smear), also with an endocervical brush. The conventional smear was prepared in the standard method. The spatula was rotated through 360° with firm

contact to the cervix. The specimen taken was smeared directly onto the conventionally labeled glass slide. This slide was immediately sprayed with cytofix, unless the use of an endocervical brush was indicated. Thereafter the spatula was rinsed in the PreservCyt solution in the vial by swirling it vigorously about ten times. The spatula was discarded. If the use of an endocervical brush to sample the endocervix was indicated, the brush was inserted into the endocervix until the bottom-most fibres were exposed. The device was rotated a quarter to a half turn in one direction. The brush was then rolled on to the same glass slide over the ectocervical smear. The slide was then immediately sprayed with cytofix. The endocervical brush was then placed in the same ThinPrep vial and swirled vigorously for about ten times. The PreservCyt vial was labeled with the same patient information as the conventional slide.

In **phase II**, the ThinPrep preparation was done directly as above and no conventional smear was prepared.

4) Cytology Laboratory Procedures:

1) Conventional slide preparations:

The conventional PAP smear slides were stained with Papanicolaou stain in routine fashion.

2) ThinPrep processing:

To make a slide, the operator placed the ThinPrep vial, a TransCyt filter and a glass microscope slide into the ThinPrep processor. The automated process was started.

This process involved three steps:

1. In the first step, the filter cylinder was inserted into the vial and spun at a relatively high speed. The spinning dispersed the cell sample.
2. After the mixing and dispersion, cells were collected onto the filter while a cell count was performed. This was done by applying a vacuum to the cylinder while a micro-processor monitors the rate at which the pressure difference across the filter changed. By monitoring the change, an estimate could be made of the percentage of coverage of the filter, in turn estimating the number of cells.
3. When sufficient cells had been drawn onto the filter, the instrument removed the cylinder from the vial while maintaining a vacuum. The cylinder was then turned upside down while the fluid that had passed through the filter was evacuated into a waste container. The filter was then touched against the glass slide. Mechanical pressure and positive air pressure within the cylinder helped to transfer the cells from the filter to the slide. After the cells were transferred, the slide-holding mechanism ejected the slide into a vial, containing an alcohol fixative.

The user then placed the slide into a multiple rack containing alcohol, thus completing the preparation of the slide for later staining with conventional PAP stain.

5) Slide Evaluation:

The conventional slides were screened according to the routine protocol of the Cytology Laboratory at the University of Cape Town. According to the protocol the following slides were reviewed by the control technologist:

- all post menopausal women
- all diagnosis of AGUS and pre-invasive lesions (low and high grade)
- any suspicion of cancer
- a benign diagnosis in a woman with a previously abnormal smear
- clinically suspicious but benign cytology
- rapid re-screening of the rest of the slides in a cross check manner.

All the slides reviewed by trainee technologists or any reported ASCUS slides were rechecked by a senior technologist. If ASCUS slides were suspicious of neoplasia it was referred for review by the pathologist. Criteria for the referral of a slide from the control technologist to the pathologist were evidence of the following:

- ASCUS suspicious of neoplasia
- Human Papiloma Virus (HPV) infection without dysplasia
- LSIL
- HSIL
- Suspicion of Cancer
- AGUS

The screening of the ThinPrep slide in Phase I was done by one control cytotechnologist who was blinded to the results of the conventional PAP. The control cytotechnologist received one week of training at the Cytyc headquarters in Boston, USA. The control cytotechnologist referred the ThinPrep slides to a specific cytopathologist who received the same training to screen ThinPrep slides. The referral criteria were the same as for the conventional Papanicolaou smears.

6) Colposcopy:

All patients with the following cytological abnormalities were notified to attend the colposcopy clinic within 3 months of being enrolled into the study:

- AGUS
- Low grade squamous intraepithelial lesion (LSIL)
- High grade squamous intraepithelial lesion (HSIL)
- Any suspicion of cancer
- ASCUS slides reviewed by the pathologist favoring neoplasia were also evaluated by colposcopy

At colposcopy, if a significant lesion suggestive of SIL or cancer was seen, a biopsy was performed. If the lesion was considered suitable for treatment, a large loop excision of the transformation zone (LLETZ) was performed or a cone biopsy if further diagnostic information was required. The requirements for LLETZ were:

- Parity between cytology and histology
- Entire extent of the abnormal area identified including the transformation zone
- No evidence of microinvasion of the cervix
- Endocervical cells reported normal on cytology/no evidence of glandular disease

Cone biopsy was performed under the following circumstances:

- Disparity between cytology and histology
- Entire lesion could not be visualized at colposcopy/inadequate colposcopy
- Colposcopy evidence of occult microinvasion
- Endocervical cells reported as abnormal on cytology

The initial evaluation for AGUS reported patients required colposcopy and endocervical sampling. If atypical endometrial cells were reported, an endometrial sample was taken at the same time as the colposcopy. A cold knife cone biopsy as a diagnostic modality was taken if the above was unrevealing.

The results of the colposcopic exam were recorded in a computerized data sheet.

7) Biopsy Histology:

Histology was reviewed according to routine protocol of the Laboratory and was not blinded. The histopathologist reported the histological diagnosis on the biopsies taken at colposcopy. Histology from a LLETZ conducted within three months of the patient's initial trial visit, was also included in the trial. The study coordinator identified the patients who had histological evaluation and recorded the histological diagnosis on a biopsy/histology outcome case report form.

8) Statistical Methods:

All data was entered into a computerised data using Microsoft Access. The null hypothesis for the evaluation of the trial data was that the ThinPrep and conventional PAP smear methods were equivalent for the detection of positive cytological diagnosis. McNemar's statistical method was used as a test of significance to determine if the null hypothesis can be rejected. To measure the agreement in diagnosis, the Kappa statistic was used.

Paired data:

McNemars test was used to test for marginal homogeneity of the cytological diagnosis for the total sample as well as for subsets. To determine which

cytological categories contribute to cases of marginal heterogeneity, the difference in the paired proportions was estimated together with a 95% confidence interval. Diagnostic measures such as sensitivity and specificity were also calculated for ThinPrep diagnosis conditional on the conventional and biopsy diagnosis taken as the gold standard.

Unpaired data:

In comparing the performance of ThinPrep II in two independent samples with respect to cytological diagnosis, relative risks (RR) were calculated with 95% confidence intervals. The RR is the ratio of the proportions observed in a specific diagnosis category. The confidence intervals can be used to judge significance and in the case of RR intervals, including one indicate no difference.

Chapter 5

RESULTS

Phase 1:

1) Study group:

Of the 804 patients enrolled in the study, 26 were excluded from the data analysis for the following reasons:

- 2 were duplicated
- 1 vault smear was done
- 3 conventional slides got lost
- 6 ThinPrep slides went missing
- 14 discrepant administrative paperwork

This left 778 cases for inclusion in the analysis. Of the 778 cases, 120 cases were seen at the Colposcopy clinic, 409 at the general Gynaecology outpatients and 249 at the Cytology clinic.

2) Comparison of cytological diagnosis between ThinPrep and Conventional Smear :

Table 1 shows the cytological diagnosis by ThinPrep I and conventional smears in each category in the Bethesda system. The diagnosis of ASCUS was statistically higher in ThinPrep I vs. conventional smear with 13.2% vs. 6.4% cases respectively. However, LSIL was diagnosed almost equally in the two groups with 3.9% cases by ThinPrep I and 4.2% cases by conventional smear.

For the diagnosis of HSIL, ThinPrep I identified 5.8% cases compared to 3.6% cases identified by conventional smear and this difference is statistically significant. The diagnosis of cancer was made almost equally by ThinPrep I and conventional smear with 0.8% and 0.9% cases respectively (Table1).

When ThinPrep I and the conventional smear was compared for cytological diagnosis of LSIL and higher, the difference was 1.7% (95%CI – 3.5% to 0.1%) and this is not statistically significant.

Table 1: Overall diagnostic categories (all sites)

Cytological diagnosis	TP I (%)	Conv I (%)	Difference	(95% CI)	
				Lower	Upper
Negative	75.8%	84.2%	8.4%	5.5%	11.2%
ASCUS	13.2%	6.4%	-6.8%	-9.6%	-4.1%
AGUS	0.5%	0.6%	0.1%	-0.8%	1.0%
LSIL	3.9%	4.2%	0.4%	-1.3%	2.1%
HSIL	5.8%	3.6%	-2.2%	-3.8%	-0.6%
Cancer	0.8%	0.9%	0.1%	-0.5%	0.9%

3) Diagnostic concordance:

The overall diagnostic concordance using the seven major categories in the Bethesda classification system is shown in Table 2.

The diagnosis by ThinPrep I and the conventional smear concurred in 603 (77%) of the 778 cases (Kappa = 0.554, 95% CI: 0.48 - 0.63) which indicates moderate agreement.

Comparing ThinPrep I and the conventional smear for LSIL and higher, the concordance is improved to 93.7% (Kappa = 0.637 95% CI: 0.54 - 0.73).

When the diagnosis of ASCUS/AGUS and higher is compared, the concordance is decreased to 82.9% (Kappa = 0.471 95% CI: 0.4 - 0.55).

Table 2: ThinPrep vs Conventional diagnostic concordance (7x7), all sites.

	Conventional							
ThinPrep	Negative N %	ASCUS N %	AGUS N %	LSIL N %	HSIL N %	Squamous Carcinoma N %	Glandular Cancer N %	Column Totals N%
Negative (75.8)	556(71.5)	23 (3.0)	2 (0.3)	6 (0.8)	3 (0.4)	0 (0.0)	0 (0.0)	**590 (75.8)**
ASCUS (13.2)	77 (9.9)	15 (1.9)	2 (0.3)	8 (1.0)	0 (0.0)	1 (0.1)	0 (0.0)	**103 (13.2)**
AGUS (0.5)	2 (0.3)	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**4 (0.5)**
LSIL (3.9)	12 (1.5)	2 (0.3)	0 (0.0)	10 (1.3)	6 (0.8)	0 (0.0)	0 (0.0)	**30 (3.9)**
HSIL (5.8)	8 (1.0)	8 (1.0)	1 (0.1)	9 (1.2)	18 (2.3)	1 (0.1)	0 (0.0)	**45 (5.8)**
Squamous Ca (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	4 (0.5)	1 (0.1)	**6 (0.8)**
Glandular Ca (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**0 (0.0)**
Row Total (100.0)	**655(84.2)**	**50 (6.4)**	**5 (0.6)**	**33 (4.2)**	**28 (3.6)**	**6(0.8)**	**1(0.1)**	**778**

4) Use of the endocervical brush:

The cytological diagnosis of SIL was higher when the endocervical brush was used for both ThinPrep I and conventional smears (Table II page 37). The cytological diagnosis of HSIL was more commonly made when the endocervical brush was used in both ThinPrep I and conventional smear. Of the 28 cases of HSIL diagnosed by conventional smear, 22/28 (78.5%) were diagnosed when the endocervical brush was used compared to 6/28 (21%) when not used. Similar findings were noted with ThinPrep I.

5) Specimen adequacy:

There were 7 unsatisfactory smears diagnosed by both ThinPrep I and conventional smears. Overall 76.9% (n=605) of the conventional smears were considered satisfactory for evaluation compared to 70.3% (n=553) of ThinPrep I smears.

In the category of SBLB, 28.8% was reported for ThinPrepI vs. 22.2% for conventional smears with a difference of 6.6%, which is statistically significant. Of the ThinPrep I smears called SBLB, 96% (n=218) had absent endocervical cells. By comparison 62.3% (n=109) of SBLB noted on conventional cervical smears were diagnosed with absent endocervical cells.

Of note, the rate of SBLB at the cytology and colposcopy clinics where women are examined in the lithotomy position, was equivalent at about 18% of all ThinPrep I and conventional smears. At Gynae OPD where women are examined in the supine position, the rate of SBLB was 38.2% of all ThinPrep I and 25.5% of all conventional smears, which is statistically significant (Table 3).

Table 3:Specimen adequacy

Site		Satisfactory but Limited by (SBLB)		Difference	(95% CI)	
		TP (%)	Conv (%)		Lower	Upper
all sites	(n = 787)	28.8%	22.2%	-6.6%	-10.2%	-3.0%
Gynae OPD	(n = 416)	38.2%	25.5%	-12.7%	-18.3%	-7.0%
Colposcopy clinic	(n = 122)	17.2%	17.2%	0%	-8.5%	2.5%
Cytol clinic	(n = 249)	18.9%	19.3%	0.4%	-4.4%	5.2%

6) Colposcopy/ Biopsy outcome results:

Of the 104 women with LSIL or higher who were referred for colposcopy, histological diagnosis is available for 83 (79.8%). This was due to 5 inadequate

biopsies, 12 normal colposcopies and repeat PAP smears taken, 1 pregnant women, 1 histology lost and 2 unexplained. The ThinPrep smear accurately identified 44 of the 57 histologically confirmed cases of LSIL or higher, which is equivalent to a sensitivity of 77.2% for LSIL or higher (Table 4). Using histology as the gold standard, ThinPrep was falsely positive in 14/26 cases where no SIL was confirmed histologically (Table 4).

Conventional smears correctly identified 39 of the 56 histologically confirmed cases of LSIL or higher which is equivalent to a sensitivity of 69.6%. Conventional Cytology was falsely positive in 12/26 cases where no disease was confirmed histologically, which is similar to the false positive rate of ThinPrep cytology (Table 4).

Table 4: ThinPrep and Conventional PAP (LSIL or higher) vs Biopsy diagnostic concordance (2x2), all sites.

ThinPrep	Biopsy		
	Negative N %	Positive N %	Column Totals N %
Negative or ASCUS /AGUS	12 (14.5)	13 (15.7)	25 (30.1)
Positive (LSIL or higher)	14 (16.9)	44 (53.0)	58 (69.9)
Row Total:	**26 (31.3)**	**57 (68.7)**	**83 (100.0)**
Conventional			
Negative or ASCUS /AGUS	14 (17.1)	17 (20.7)	31 (37.8)
Positive (LSIL or higher)	12 (14.6)	39 (47.6)	51 (62.2)
Row Total:	**26 (31.7)**	**56 (68.3)**	**82 (10.0)**

Table 5 summarises the estimated positive predictive value of cytology for LSIL or higher and shows that it is similar on ThinPrep and conventional smear.

While the sensitivity of ThinPrep is marginally higher than conventional cytology, the estimated specificity is marginally lower but these differences are not statistically significant.

Table 5: Sensitivity and Specificity and Positive predictive value of ThinPrep and conventional PAP smear for LSIL and higher.

	ThinPrep	Conventional
Sensitivity (95%CI)	77.2% (0.663 – 0.881)	69.6% (0.576 - 0.817)
Specificity (95%CI)	46.2% (0.270 - 0.653)	53.8% (0.347 - 0.730)
Positive predictive value	75.9% (0.648 – 0.869)	76.5% (0.648 - 0.881)

RESULTS : PHASE II

1) Studygroup:

In phase II, 414 patients were enrolled.

The following patients were excluded:

- 2 pregnant
- 1 bottle was damaged
- 5 slides had insufficient liquid left in vial
- 3 duplicated in phase II
- 12 duplicated in phase I

That left 391 eligible patients after evaluating the slides. Another 3 were reported unsatisfactory and 2 results went missing, leaving a total of 386.

Table I: Comparison of cytological diagnosis between ThinPrep II and Conventional I.

Cytological diagnosis	TP II (n = 386)	Conv I (n = 778)	RR (95% CI)
Negative	293 (75.9%)	655 (84.2%)	0.9 (0.85 – 0.96)
ASCUS	63 (16.3%)	50 (6.4%)	2.54 (1.79 – 3.61)
AGUS	1 (0.3%)	5 (0.6%)	A (0.05 – 3.64)
LSIL	12 (3.1%)	33 (4.2%)	0.73 (0.38 – 1.40)
HSIL	15 (3.9%)	28 (3.6%)	1.08 (0.58 – 2.0)
Cancer	2(0.5%)	7(0.9%)	0.58 (0.12- 2.75)

ThinPrep II diagnostic profile differed significantly from the conventional profile for all cases (Table I). Significantly more cases of ASCUS were found in ThinPrep II versus Conventional I (RR 2.5; 95% CI : 1.79 – 3.61). This means ThinPrep II had a high rate of ASCUS diagnosis of 16% which was more than 2 times greater than conventional.

There was no statistical difference in the diagnosis of LSIL or higher between ThinPrep II (7.5%) and Conventional I (8.4%) (RR 0.86; 95%CI: 0.57 – 1.3).

There was also no statistical difference in the individual diagnosis of LSIL or HSIL when ThinPrep II was compared to Conventional I smear (Table I).

Use of Endocervical brush:

The diagnosis of ASCUS was significantly increased for ThinPrep II, whether the endocervical brush was used or not, when compared to conventional I (RR 3.09; 95%CI: 1.68 – 5.69 and RR 2.25; 95%CI: 1.46 – 3.46 respectively). The use of the endocervical brush increased the diagnosis of LSIL and higher for ThinPrep II and Conventional I, but it was not statistically significant.

In ThinPrep II, there was no significant increase for the diagnosis of HSIL alone, when no brush used was compared to brush used (Table II).

When ThinPrep II was compared to conventional I, for the diagnosis of SIL and higher and the endocervical brush used, there was no statistical difference in the detected cases (RR 0.84; 95% CI: 0.51 – 1.39).

Table II: Comparing Endobrush vs no Endobrush for all sites and for both ThinPrep and Conventional:

Cytol diag.	ThinPrep I			Conventional I			ThinPrep II		
	Endo used (n=444)	Endo not used (n=316)	RR (95%CI)	Endo used (n=444)	Endo not used (n=316)	RR (95%CI)	Endo used (n=206)	Endo not used (n=177)	RR (95%CI)
ASCUS	61(13.7%)	41(13%)	1.06 (0.73-1.53)	35(7.9%)	15(4.7%)	1.66 (0.92-2.66)	37 (17.7%)	26 (14.2%)	1.21 (0.76-1.91)
LSIL	18(4.1%)	11(3.5%)	1.16 (0.56-2.43)	21(4.7%)	11(3.5%)	1.36 (0.66-2.78)	10 (4.8%)	2 (1.1%)	4.23 (0.94-19.07)
HSIL	38(8.6%)	7(2.2%)	3.86 (1.75-8.54)	22(5%)	6(1.9%)	2.61 (1.07-6.36)	8 (3.83)	7 (4.0%)	0.97 (0.36-2.62)
CA	4(0.9%)	2(0.6%)	1.42 (0.26-7.72)	5(1.1%)	2(0.6%)	1.78 (0.35-9.11)	1	3 (0.6%)	0.85 (0.05-13.44)

2) Specimen adequacy:

There were 261 (66.75%) satisfactory, 127 (32.48%) SBLB and 3 (0.8%) unsatisfactory cases reported.

There was no statistical significant difference between ThinPrep I and ThinPrep II for specimen adequacy (RR 1.13; 95%CI: 0.94 – 1.35).

However, for SBLB there were more cases diagnosed within ThinPrep II than Conventional I (RR 1.46; 95% CI: 1.2 – 1.77), as was the case in phase I.

Table III: Specimen Adequacy

	ThinPrep II	Conventional I	RR(95% CI)
Satisfactory	261 (66.8%)	605 (76.9%)	0.87 (0.8 – 0.94)
SBLB	127 (32.5%)	175 (22.4%)	1.46 (1.2 – 1.77)
Unsatisfactory	3 (0.8%)	7 (0.9%)	0.86 (0.22 – 3.32)
Total	391	787	

Of ThinPrep II results called SBLB, 93.7% (n = 119), had absent endocervical cells. By comparison, 62.3% (n = 109) of SBLB in Conventional I, were diagnosed with absent cells and this was statistically significant (RR 1.5 with 95%CI 1.33 – 1.7).

Endocervical brush:

In ThinPrep II, of the 119 cases reported SBLB with absent endocervical cells, 43 (20.4%) cases had a brush used vs. 76 (42%) who had no brush used.

Colposcopy / Biopsy outcome results:

29 Colposcopies were requested of which 22 (75.8%) were performed. ThinPrep II accurately identified 14 of 20 (70%) cases by histology. Poor agreement was observed between ThinPrep II cytology and biopsy outcome results. (Kappa = 0.1363; 95%CI: 0.14 – 0.5) (Table IV). Sensitivity could not be determined due to too small numbers.

Table IV: ThinPrep II (LSIL and higher) vs Biopsy Diagnostic concordance:

all sites

	Biopsy		
ThinPrep	Negative N %	Positive N %	Column Totals N %
Negative or ASCUS /AGUS	1 (4.8%)	0	1 (4.8%)
Positive (LSIL or higher)	6 (28.6%)	14 (66.6%)	20 (95.2%)
Row Total:	7 (33.4%)	14 (66.6%)	21 (100.0)

Kappa = 0.1818 . The 95% CI for Kappa = 0.1363 to 0.4999

Chapter 6

DISCUSSION

This study was conducted in 2 phases at 3 outpatient clinics at a tertiary hospital. Phase I was designed to compare specimen adequacy, the rate of the cytological diagnosis of SIL and cancer between ThinPrep and conventional PAP. It also compared the cytological diagnosis SIL and cancer by ThinPrep and conventional cytology to colposcopic directed histology as a gold standard. Phase II was designed to evaluate ThinPrep as a direct to vial using historical controls in the form of conventional cytology from Phase I.

The main findings can be summarised as follows:

1. The diagnosis of SBLB was significantly more commonly made by ThinPrep compared to conventional cytology in phase I and II. The most common reason for the diagnosis of SBLB was absence of endocervical cells.
2. In phase I and II ASCUS was diagnosed statistically significant more commonly with ThinPrep than with conventional cytology. In phase I, a similar rate of LSIL for ThinPrep and conventional cytology was found and HSIL was more commonly diagnosed with ThinPrep, which is statistically significant. The use of the endocervical brush in phase I and II, improved the detection rate of SIL and higher for both ThinPrep and conventional cytology. However, in phase II there was no statistical significant difference for the diagnosis of HSIL or SIL and higher, between ThinPrep and the conventional smears.
3. In phase I, the diagnosis for LSIL and higher was almost equally confirmed with colposcopic directed histology for ThinPrep and conventional cytology, with 53% vs. 47.6%.

The sensitivity of a positive diagnosis for LSIL and higher was 77.2% compared to conventional cytology which was 69.6%, but it was not statistical significant.

The finding of an increased number of SBLB diagnosed with ThinPrep compared to conventional cytology is in contradiction to the literature.

Payne et al[26] reviewed 23 studies and reported that liquid-based methods had a larger proportion of specimens classed satisfactory. When the data was combined the liquid-based method seem to have an unsatisfactory rate of half compared to conventional smears. (RR 0.54, 95%CI 0.51 – 0.56)

Lee et al[54] reported a study sample of 6747 women in whom 1431(19.85%) were diagnosed as SBLB with ThinPrep compared to 2008(27.8%) with conventional cytology, a statistically significant difference. However, similar to our study the main reason for the diagnosis of SBLB was lack of endocervical cells. Lee et al found absent endocervical cells in 15.8% ThinPrep smears compared to 9.4% in conventional smears. Bur et al[51] found that 38% ThinPrep smears had absent endocervical cells compared to 27.4% conventional smears. In the phase I study, the diagnosis of absent endocervical cells was higher than most studies reported in the literature at 26.7% for ThinPrep and 13.8% for conventional cytology.

It could be argued that the split sample technique is biased against ThinPrep in phase I as the conventional slide was prepared prior to the ThinPrep slide using the same cervical collection device. In the direct-to-vial study by Corkhill et al the cervical sample was collected with a broom type device and directly rinsed into a vial of PreservCyt preservative solution for the preparation of the ThinPrep slide. She reported an absence in endocervical cells in 4.96% of cases.[58]

However, our phase II direct to vial study did not confirm the bias with the absent endocervical cells reported to be 24.5%. This was much higher than the 4.96% reported by Corkhill.[58]

Mcgoogan et al reported on criteria for sample adequacy. Factors that play a role include the clinician responsible for taking the sample, the collecting instrument and laboratory evaluation of adequacy.[59]

Over time spatulas have been modified to improve the sampling of the transformation zone and the collection of endocervical cells.[26; 59] When the spatula and endocervical brush are used in combination, it has been reported to perform better.[59; 60]

In a prospective randomized trial comparing ThinPrep with the conventional PAP, Obwegeser et al[67]reported improved specimen adequacy due to better sampling. In their study of 1999 patients, the Szaley spatula (collecting cells from the endocervical canal) was used for the conventional smears. All conventional PAP smears were collected under colposcopic guidance after mucus and debris had been removed from the cervical surface with a cellulose swab. The ThinPrep sample was obtained with a broom type device combined with a plastic spatula, also under colposcopic guidance. Specimen adequacy for SBLB in the ThinPrep group was (5.5%) significantly higher compared to the conventional PAP (2.5%). Absent endocervical cells responsible for SBLB in the conventional PAP was 56% and 54% for ThinPrep.

This emphasizes the importance of the sampling technique and device used. The use of the endocervical brush clearly increased the presence of endocervical cells in our study. The endocervical brush was used in 53% of cases to collect the

sample in the TP II direct to vial study and the absent endocervical component decreased from 42% to 20.4%. It can be postulated the use of the endocervical brush in every patient would have contributed to increase our specimen adequacy and decrease the SBLB due to absent endocervical cells. This concept has also been supported by Cheung et al[61] in a comparison study of 382 000 where the broom type device was used.

Another possibility for the lack of endocervical cells could be that they were present on the slide, but not recognized by the cytopathologist. Bur et al describe initial discrepancies between ThinPrep and conventional PAP related to inexperience with ThinPrep. He claims there is a learning curve in the recognition of abnormal cellular morphology on ThinPrep which is in agreement with the review by Payne et al.[51; 26]

The reason why the GOPD clinic performed worse compared to the cytology and colposcopy clinics for specimen adequacy is speculative. Considering that cervical samples were taken in the lithotomy position by dedicated sample takers, it could have contributed to improved specimen adequacy.

Looking at cytological diagnosis, ASCUS/AGUS was significantly more likely to be diagnosed by ThinPrep than by conventional cytology. Different studies have reported different rates of ASCUS diagnosis. For instance Lee et al, similar to this study, showed ASCUS to be less commonly diagnosed by conventional cytology compared to ThinPrep method.[54] In contrast however, Hutchinson et al reported ASCUS/AGUS to be more commonly diagnosed by conventional cytology compared to ThinPrep. [55] Bernstein et al[62] in a metanalysis of 25 studies reported no difference in the rate of ASCUS for TP versus conventional PAP smear. The

reason for the higher ASCUS rate in phase I and II of our study in which all smears were read by a senior cytotechnologist, is not clear. It may be due to alteration in the appearance of cells due to the ThinPrep process and the learning curve required to adjust to the appearance of the ThinPrep slide. Payne et al also reported a decrease in ASCUS diagnosis as experience is gained by the cytologist in LBC.[26]

In our phase 1 study, LSIL was diagnosed equally by ThinPrep and conventional cytology. This is contrary to previous studies which have shown for instance, in the study by Linder et al, an average improvement of 65% using ThinPrep compared to conventional cytology.[63]

In agreement with some studies, phase I illustrated an increased detection rate of HSIL. However in phase II, there was no statistically significant increase. In phase I, the use of the endocervical brush improved the detection rate of HSIL.

In the study by Corkhill et al, a cytobrush (endocervical brush) and plastic spatula were used to prepare the slides. It was found that the ThinPrep method detected 54% more HSIL compared to conventional cytology, but because of small numbers it was not statistically significant. (20 slides vs. 13 slides) For the diagnosis of LSIL and higher, Corkhill also reported the ThinPrep method performed statistically significant better, when compared to conventional PAP.[64]

To determine the accuracy of the two different preparations, the cytology diagnosis was compared to colposcopy directed histology findings. Phase I shows a higher sensitivity and a lower specificity for ThinPrep compared to conventional cytology, but the difference was not statistically significant. These findings

compare equally to the performance of other studies for example the study by Sheets et al. The study by Sheets et al reported a sensitivity of 73.6% for ThinPrep and 67.3% for conventional cytology and almost equal specificity of 76.9% and 76.2% respectively.[65] Comparing phase I, with the study done by Sheets, sensitivity was almost equal but specificity was decreased.

Caution should be exercised with the interpretation of above sensitivity and specificity. Sensitivity and specificity requires a reference diagnosis to be defined for positive and negative results. In cervical cytology screening, no consistently used reference exists. Ideally one would compare against biopsy diagnosis, but this raises the ethical implications of carrying out an invasive procedure on women with negative cytology. Therefore, even when using the colposcopy/histology as gold standard for abnormal cytology, it is still required to presume the negative cytology does not include any false negative results [26; 48] This causes verification bias.

It should be mentioned that Koss[25] emphasized comparing cytologic with histologic diagnosis is only a secondary method of quality control. The concordance between cytologic and histologic findings or lack thereof, may also depend on the skill of the observers, the adequacy of the sample and the perception of the level of abnormalities. Many studies have shown lack of agreement among pathologists in the interpretation of cytologic and histologic samples.[25]

Hopman et al also reported observer variability where the histopathological diagnosis reached may be based on a sampling error. This means the histology result is only as good as the ability of the colposcopist who identifies the abnormal

area for biopsy. However he concluded that the levels of agreement among experienced colposcopists increase as the cervical lesion becomes more severe.[66]

In our study the cytopathologist and pathologist had one week of training in reading ThinPrep slides and it might well be that they are still on the learning curve interpreting the ThinPrep slides.

In Costa Rica, a population based cohort of 8636 Costa Rican women had conventional PAP smears prepared and diagnosed in Costa Rica. The residual material was shipped to the USA where the liquid based ThinPrep slides were prepared and diagnosed. In this study ThinPrep cytology demonstrated significantly increased sensitivity for the detection of HSIL and Carcinoma.[56]

It could be that in our study the adequacy and cytological diagnosis will improve with independent re-evaluation familiar with liquid-based cytology preparations in the USA.

Chapter 7

CONCLUSION

Based on our data, in the routine clinical setting conventional cytology performed better in terms of smear adequacy. This is only applicable to the SBLB category. It was mainly due to absent endocervical cells which is in agreement with other studies.[51; 54; 58] When the endocervical brush was added to collect the sample in the TP II direct to vial study the absent endocervical component decreased from 42% to 20.4% This decrease shows a significant advantage in collecting endocervical cells.

The colposcopy clinic performed better for SBLB category compared to the general gynae OPD for both the TP I and conventional I smears. However when TP I and conventional I smears were compared for the SBLB category in the colposcopy clinic only, they performed equally. The explanation of this finding is not clear. It can be speculated that dedicated health care workers exercised a skillful sampling collection technique, which is in agreement with the findings of Obwegeser et al.[67]

The limitation in the study design of the split sample technique, could have introduced bias towards ThinPrep, due to the potential lack of transfer of cells to the TP slide. However when TP I split sample is compared to TP II direct to vial sample for the SBLB category, it shows no statistical significant difference and therefore make this limitation unlikely.

According to the new 2001 Bethesda system, the SBLB category has been eliminated.[68] They still recommend the presence of endocervical cells, but it is no

longer required.[22; 68] The Cochrane[46] metanalysis on collection devices report that abnormal cytology and HSIL are more commonly enhanced if endocervical cells are present. Therefore there is still controversy how patients whose smears lack endocervical cells should be followed.

The diagnosis of ASCUS was statistically higher in TP I and TP II when compared to the conventional smear which is in contradiction to equal rates reported by Bernstein et al.[62] In our study the experience of the cytotechnologist and the pathologist as part of a learning curve could have contributed to this discrepancy for ASCUS diagnosis.[26] A potential advantage of the Thinprep sample, is the option to do additional HPV testing for the diagnosis of ASCUS.[52; 69; 70] The ASCUS-LSIL Triage study (ALTS) group[71] reported on their randomized trial on the management of ASCUS. The two year cumulative diagnosis of Cin III was 8 to 9% in all three triage options. They concluded HPV triage is as sensitive as immediate colposcopy for detecting CIN III and refers only half as many women for colposcopy, while follow-up that used repeat cytology requires two follow up visits to reach the same sensitivity as HPV triage. However this view is not shared by all research studies. Cösta et al[72] for the French Society of Clinical Cytology study group, reported increased ASCUS/AGUS for TP vs. conventional PAP smears and no benefit from HPV testing in the ASCUS group.

In a pilot study, Swierszynski et al,[73] reported retrospectively on 358 HIV positive women where LBC (SurePath) was used. ASCUS was diagnosed in 9% of the LBC preparations. They reported 65% SIL diagnosed within 7 months of follow-up of those after colposcopy and biopsy. They advise a more aggressive approach with immediate colposcopy and biopsy rather than follow-up cytology. In our study

HIV status was unknown, but as a weakness in our study design ASCUS could have been used as a primary inclusion criteria for colposcopy referral.

The only advantage of the ThinPrep over the conventional smear, was the increased diagnosis of HSIL which was confirmed by colposcopy and histology in phase I. This advantage was improved when the endocervical brush was used. However, this was not replicated in phase II.

In the diagnosis of SIL and higher, the sensitivity of ThinPrep compared to conventional cytology was higher but not statistically significant.

Discrepancy in our cytological results might be explained due to a learning curve involved in interpreting the morphology of cells on the ThinPrep slide. Ideally the issue could be resolved by having all slides independently reviewed by a cytopathologist familiar with ThinPrep.

The phase II study in a direct-to-vial fashion did not confirm the issue of bias towards ThinPrep as a result of the limitation of the split sample technique.

From this study the use of the endocervical brush combined with a spatula in all cases of cervical sampling, is recommended. It increased both adequacy and cytological diagnosis. This is in agreement with metanalysis of randomized control-trials supporting the combined use of the spatula for the ectocervix and the endocervical brush (cytobrush) for the endocervix.[65; 8; 46] The Cervexbrush has been used in many of the split sample studies. It has the advantage of sampling the endocervix and ectocervix simultaneously. However, it is expensive and when compared to the spatula and endocervical brush (cytobrush), the spatula and endocervical brush performed superior. (OR 2.31, 95%CI 2.06-2.56) [60; 46]

Since this local study has been performed, several institutions, including the NHS, replaced the conventional cervical PAP smear with LBC.[74] However this has not been universally accepted. The new Zeeland Health Technology Assessment (NZHTA) concluded that the introduction of new devices for cervical screening cannot be recommended for New Zealand at the time.[75] Grimes[76] reported on the increased cost associated with the newer technologies to screen for cervical cancer which aims for better sensitivities. However, paradoxically these higher costs could make screening unattainable by low socio economic communities who are at highest risk[76]

Thus, if we take the above in consideration as well as the fact that LBC requires new equipment, retraining of cytotechnologists involves a learning curve as well as higher cost, it would be premature to change our current practice in a developing country such as South Africa.

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Listing of corrections/changes

iii).The author was a co-investigator and was involved in the design and implementation of the study.

iv).The author aimed to compare the performance of the two screening modalities in a normal gynaecological clinical setting. The study did attempt to evaluate screening strategies in R.S.A.

v). There was no internal audit done as the aim was to do it in the normal clinical setting.

vi). It could be legitimate criticism, but the design of the study was to evaluate the two screening modalities in the normal clinical setting. The samplers were all qualified gynaecologists and registrars in the department of obstetrics and gynaeclogy. The smear analysis was done by the UCT pathology department. Only two personnel, namely a senior cytotechnologist and a pathologist evaluated the ThinPrep smears. Both of them had I week of training in the USA. All atypical ThinPrep slides were evaluated by the pathologist.

Major changes

1. Inserted the HPV cell cycle and its role in cervical cancer comes within the text page 5 line 7
2. The information pertaining to the role of the HIV in cervical cancer was added to the text on page 12 line 15
3. The use of the endocervical brush was left to the own discretion of the clinician. Indications for the use of the endocervical brush were:
    - The squamous-columnar junction not visualized as in the postmenopausal patient

- Previous inadequate smear

See: page22 line 7

4. All ASCUS slides were read by the senior cytotechnologist. Only ASCUS slides suggestive of neoplasia were referred to the registrar/ pathologist. This is the routine of the laboratory. See: page 26 line 7
5. The routine practice of the Gynaecology clinic was to follow ASCUS reported smears with follow-up cytology in 3 – 6 months. They were not primarily referred for colposcopy. In retrospect , it can be seen as a weakness in the study design. See page 49 line 1
6. The management of AGUS entered in the text on page 28 line 1
7. The reasons for the 20% loss of histology is explained on page33 line18
8. Explanation given page 42 line24
9. Please see page 49 line 19

MINOR CHANGES

1. Phase I of this 2 phase study was submitted by the author to The College of Medicine as a commentary to enter the part 2 examination in 2000.

The smaller corrections as suggested have been made except suggestions 8, 32.

8. See: page 4 line1

11. See : page 11 line 18

24. Ethics approval is included.

32. The statistics for phase I was done in the U.S.A. Thus, the tables, including table 4, presented are correct if it is accepted their entry in the U.S.A. were correct. The sensitivity and specificity were then calculated using the figures as presented in the dissertation.