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The diagnosis and management of cervical intraepithelial neoplasia in women with a transformation zone type 3

The diagnosis and management of cervical intraepithelial neoplasia in women with a transformation zone type 3.

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A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Philosophy in the Faculty of Translational Health Sciences.

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Thesis Abstract

Twenty percent of colposcopic assessments are inadequate due to a type 3 transformation zone (TZ3). Despite this, the literature relating to this finding is sparse. Management is guided by the referral screening test and, in this thesis, I have shown that the presence of a TZ3 is the strongest predictor of false positive cervical screening results.

Analysis of colposcopists' decision-making, both locally and nationally, identified heterogeneity of care in women with low grade cytology; there was disparity in the total length, clinical setting and technique of cytological follow-up. These areas of discordance were affected by anxiety of missing a cancer and paucity of guidance, suggesting a need for a national consensus opinion.

To date, no study has evaluated the effectiveness of different cytological sampling techniques in a TZ3 assessment. In the UK, routine cervical screening is completed by a Cervex-Brush alone. In my thesis, the addition of a cytobrush increased the yield of endocervical cells but this was not associated with increased predictability of CIN2+ (high grade dysplasia). I propose that cytological follow-up with a Cervex-Brush alone can be safely undertaken in a primary care setting. This finding is relevant for resource allocation which is particularly pertinent in the current economic climate within the NHS.

Given the significant risk of CIN2+ in women with high grade cytology and a TZ3 (80%), it is appropriate to offer LLETZ first line. My results have also shown, for the first time, that women with low grade cytology, high risk HPV and a TZ3 have double the risk of CIN2+ (36.7%) when compared to women where the TZ is visible. In these women, I propose the use of surrogate biomarkers for HPV infection (p16 and Ki-67) in combination with liquid based cytology; these biomarkers provide a >99% sensitivity for CIN2+ and improve the specificity (decrease false positive screening) from 19.3% to 71.7%. When compared to dual-stained cytology, neither HPV 16/18 genotyping nor p16 & Ki67 in combination with endocervical curettings demonstrated an equivocal sensitivity.

The continued investigation of adjuncts which can improve the diagnostic accuracy of cervical screening will help;

i. achieve the World Health Organization's 2018 global priority of reducing the incidence of cervical cancer and
ii. decrease the morbidity of treatment-related preterm birth by reducing false positive screening.

Dedications and Acknowledgement

The ideas for this thesis were developed as a consequence of my own concerns, as a colposcopist, that this cohort of women are sub-optimally managed; so I dedicate this thesis to women affected by the morbidity and mortality of false negative and false positive cervical screening.

My supervisors have been invaluable. Professor López-Bernal stepped into the breech when I lost my clinical supervisor two months after starting my studies; he provided indispensable academic support and advice. Dr Andrew Wills provided innumerable hours of discussion and practical support. His dedication and commitment to learning a specialty that bore no relation to his previous work was instrumental to my projects and I look forward to future collaborations. Dr Rachna Bahl developed my understanding of qualitative methodology and a commitment to helping me to succeed. My first and second year reviewers, Dr Naomi Crouch and Professor David Cahill, gave me constructive feedback and, in the absence of a clinical supervisor, this was invaluable in helping refine my ideas.

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Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University’s Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate’s own work. Work done in collaboration with, or with the assistance of others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: DATE:

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ACORN study – Endocervic**a**l **c**urettage and cytology with p16/Ki-67 to diagn**o**se p**r**e-cancerous cervical cha**n**ges in women with a TZ3

AR – absolute risk

AUC – area under the curve

BSCC – British Society for Clinical Cytology

BSCCP – British Society of Colposcopy and Cervical Pathology

BNC – borderline nuclear change

cGIN – cervical glandular intraepithelial neoplasia

CDB – colposcopic directed biopsy

CIN – cervical intraepithelial neoplasia

COCP – combined oral contraceptive pill

ECC – endocervical cell curettage

H&E – haematoxylin and eosin staining

HC2 – hybrid capture 2 (pooled high risk HPV test)

IFCPC – International Federation of Cervical Pathology and Colposcopy

MDT – multidisciplinary team

NHS CSP – National Health Service Cervical Screening Programme

HPV – Human Papillomavirus

LBC – liquid based cytology

LLETZ – large loop excision of the transformation zone

LC – Langerhans cell

MACC – mean average cell count

NLR – negative likelihood ratio

NPV – negative predictive value

OR – odds ratio

PIS – participant information sheet

PLR – positive likelihood ratio

PPV – positive predictive value

pRb – retinoblastoma protein

RCT – randomised control trial

ROC – receiver operator curve

RR – relative risk

SCC – squamous cell cancer

SCJ – squamocolumnar junction

SD – standard deviation

TA – thematic analysis

TZ3 – transformation zone type 3 (or unsatisfactory colposcopy)

VAIN – vaginal intraepithelial neoplasia

VLR – virus like particles

Publications arising from work in this thesis

Manley KM, Wills AK, Morris CG, Hogg JL, Lopez-Bernal A, Murdoch JB (2016). The impact of HPV cervical screening on negative large loop excision of the transformation zone (LLETZ): A comparative cohort study. *Gynecologic Oncology* 141(3): 485 - 491

Manley KM, Simms RA, Platt S, Patel A, Bahl R (2017). Unsatisfactory colposcopy: clinical decision-making in conditions of uncertainty. *BMC Medical Informatics and Decision Making* 17(1): 125

Manley KM, Wills AK, Villeneuve N, Hunt K, Patel A, Glew S (2018). Comparison of the Cervex-Brush alone to Cytobrush plus Cervex-Brush for detection of cervical dysplasia in women with a transformation zone type 3. *Cytopathology* (ePub ahead of print).

Presented abstracts arising from this thesis

Manley KM, Wills AK, Morris CG, Hogg JL, Lopez-Bernal A, Murdoch JB. *The impact of HPV cervical screening on negative large loop excision of the transformation zone (LLETZ): A comparative cohort study* –

- Poster presented at the BSCCP Annual Conference, April 2015

Manley KM, Simms RA, Platt S, Patel A, Bahl R. *Unsatisfactory colposcopy: clinical decision-making in conditions of uncertainty* -

- Poster presented at the BSCCP Annual Conference, April 2016

Manley KM, Wills AK, Crouch N, Lopez-Bernal A, Patel A. *Current Practices in the Management of a Type 3 Transformation Zone: Results of a UK Survey* –

- Poster presented at the BSCCP Annual Conference, April 2017
- Oral presentation at the South West Colposcopy Study Day, September 2017

Manley KM, Wills AK, Pawade J, Hunt K, Villeneuve N, Sargent A, Lopez-Bernal A, Patel A, Glew S. *The use of biomarkers and HPV genotyping to improve diagnostic accuracy in women with unsatisfactory colposcopy: A diagnostic accuracy study* –

- Winner of the Academy of Medical Sciences Early Researcher Prize, May 2018. Four PhD students (including myself) were shortlisted from 50 submissions across the South West. We presented our work in front of an AMS committee and audience. I was selected as the finalist.
- Oral presentation at the South West Colposcopy Study Day, June 2018

Manley KM, Wills AK, Hunt K, Villeneuve N, Patel A, Glew S. *Comparison of the Cervex-Brush alone to Cytobrush plus Cervex-Brush for detection of cervical dysplasia in women with a TZ3* -

- Oral Presentation at the South West Cervical Screening Network Meeting, March 2018

Chapter 1 **Introduction**

1.1 Background

In the United Kingdom, the introduction of a national cervical screening programme has reduced the overall mortality rate from cervical cancer by 70% to 2.8 per 100,000 women[3]. Despite this, cervical cancer is still the most common cancer in women under the age of 35 and one-third of these women will die within five years of diagnosis[3]. Diagnosing and preventing progression of the precursor lesion, cervical intraepithelial neoplasia (or CIN) continues to be a national priority. When women with a positive cervical screening result are referred to colposcopy, the purpose of this assessment is to visualize the area infected by the Human Papillomavirus (HPV), the virus responsible for 99% of cervical cancers. This assessment is crucial in helping determine who requires treatment and who can be safely managed with cytological follow-up. Management difficulties arise when the epithelium of interest is 'tucked inside' the cervix and not visible for assessment. This is known as unsatisfactory colposcopy or a transformation zone type 3 (TZ3), the incidence of which is approximately 20%[4], potentially accounting for more than 34,500 women seen in UK colposcopy clinics each year[5].

There is a paucity of national and global guidance in this cohort, which can lead to uncertainty in decision-making. Novel interventions which improve the diagnosis of CIN in this cohort and national recommendations which improve homogeneity of care are needed to reduce the morbidity of false positive and false negative screening.

1.2 Aetiology of cervical cancer

The pathogenesis of cervical cancer has been studied in depth and although the primary aetiological factor, infection with HPV, has been well documented, integration of this virus into the host's genome is a multifactorial process. When considering the aetiology of cervical cancer it is useful to study the structure of the normal cervix, as this influences the pathogenesis of HPV infection.

1.2.1 The contribution of the cervical epithelium

The ectocervix (external, vaginal portion of the cervix) is covered by stratified, non-keratinising, glycogen containing squamous epithelium. In the neonatal period the endocervical canal is lined by columnar epithelium, which is non-stratified and mucus secreting. Folding of the epithelium forms crypts which can extend 5-8mm into the stroma. The Squamocolumnar Junction (SCJ) is where these two epithelia meet and histologically this appears as a step due to their differing heights. This distinctive feature allows histopathologists to identify that sampling of the SCJ has occurred, as cervical neoplasia usually begins here[6, 7].

The structure of the epithelium (Figure 1.1: Mature squamous epithelium) is integral to the development of dysplasia (abnormal maturation). At the bottom of the squamous epithelium a basement membrane separates the stroma below from the epithelium above. Attached to the basement membrane is a single layer of basal cells which contain large nuclei and little cytoplasm. The basal cells divide mitotically under the influence of oestrogen to form further basal (or immortal) cells and these differentiate to form the higher cell layers of the epithelium. These upper cell layers lose mitotic capability and become terminally differentiated (mortal cells) to form:

- Parabasal cells
- Intermediate cells; polygonal cells with increased cytoplasm:nuclei ratio.
- Superficial cells; large, flattened and terminally differentiated cells containing small dense nuclei and abundant cytoplasm.

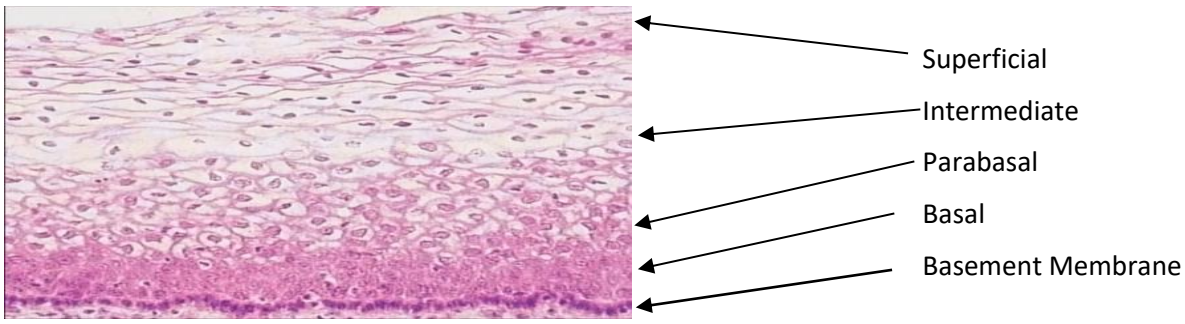


Figure 1.1: Mature stratified squamous epithelium[8]

1.2.2 The Transformation Zone

Under the influence of oestrogen (adolescence, pregnancy and use of the combined oral contraceptive pill), there is an increase in the number and size of the endocervical glands, the vascularity of the cervix and stromal oedema. This expansion in cervical volume leads to eversion of the columnar epithelium onto the ectocervix, secondary to fixation of the lateral cervix.

Following puberty, the relative acidity of the vagina is increased (pH 4), promoting lactobacilli which stimulate the protective conversion of columnar cells into squamous – a process known as metaplasia[9]. Metaplasia begins in the crypts and the tips of the endocervical villae and occurs in three distinct histological stages[10]:

1. Reserve cell hyperplasia – small round cells with hyperchromatic nuclei appear next to columnar cell nuclei. Their origin is thought to be either from subepithelial stromal cells or more likely columnar cells adapting to the acidic vaginal environment.
2. Immature squamous metaplasia – the reserve cells proliferate to form a multicellular epithelium with no stratification. These epithelial cells are susceptible to carcinogens (HPV) and most squamous cell cervical cancers (SCC) arise here.
3. Mature squamous metaplasia – immature cells have differentiated into cells which are difficult to distinguish from the original squamous epithelium.

The presence of crypt openings on the ectocervix demarcates the extent of the metaplastic epithelium and the original SCJ. The area which is bounded distally by the original SCJ and medially by the new SCJ is called the Transformation Zone (TZ). The TZ is the area colposcopists need to visualize when women are reviewed in the colposcopy clinic as this is where HPV may have invaded during immature metaplasia. Figure 1.2 represents the SCJ through reproductive life.

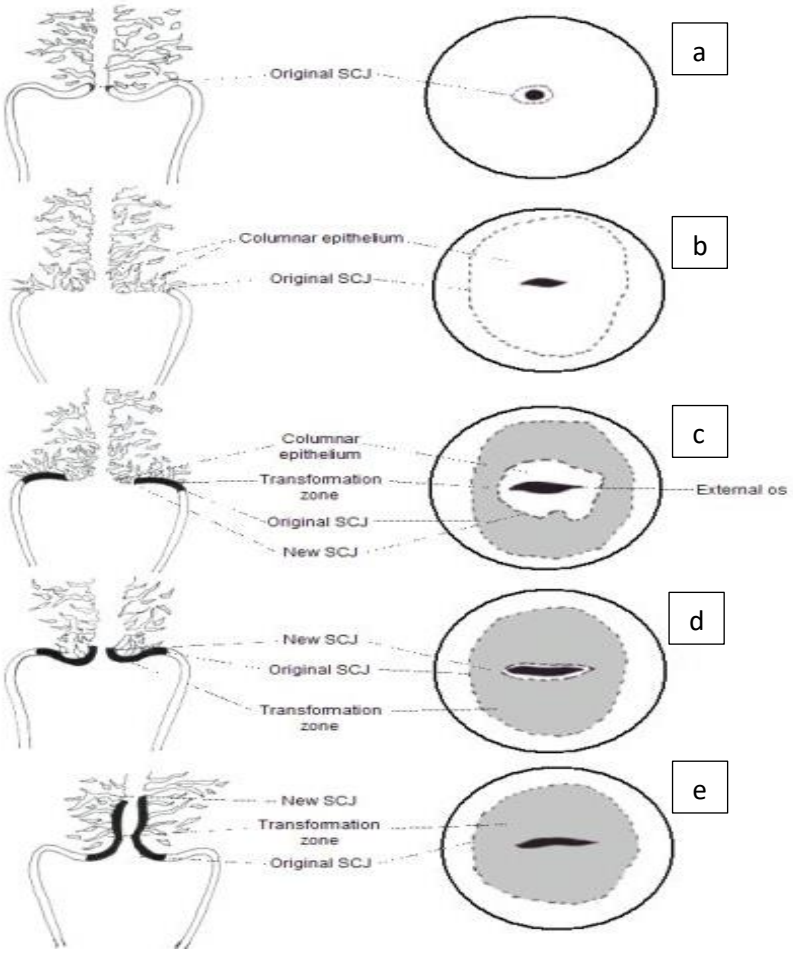


Figure 1.2*:* Taken from the International Agency for Research on Cancer[2]; '*Location of the SCJ and TZ (a) before menarche, (b) after puberty, (c) during a woman's 30s, (d) in the perimenopause and (e) after the menopause'.*

1.2.2.1 Transformation Zone Nomenclature

The International Federation of Cervical Pathology and Colposcopy (IFCPC) Classification is based on the location of the transformation zone in relation to what is visible during the colposcopic examination. The TZ can either be visible on the ectocervix (Type 1 TZ: Figure 1.3), partially within the endocervical canal but visible (Type 2 TZ) or entirely within the endocervical canal and not visible (Type 3 TZ or unsatisfactory colposcopy: Figure 1.4). According to a consensus guideline written by the American Committee for Colposcopy and Cervical Pathology, a TZ3 occurs in approximately 20% of women assessed in colposcopy[4] and can make the diagnosis of cervical cancer and CIN problematic (See Section 1.5.2.2 and 1.5.3.2).

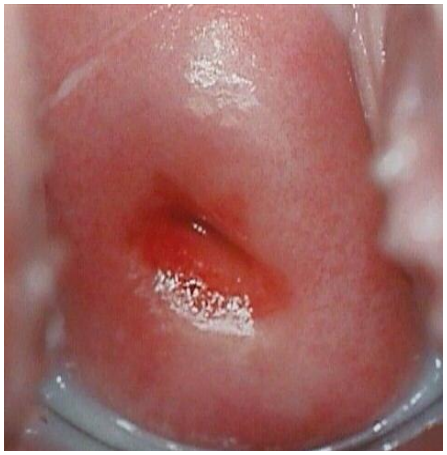


Figure 1.3: Type 1 TZ

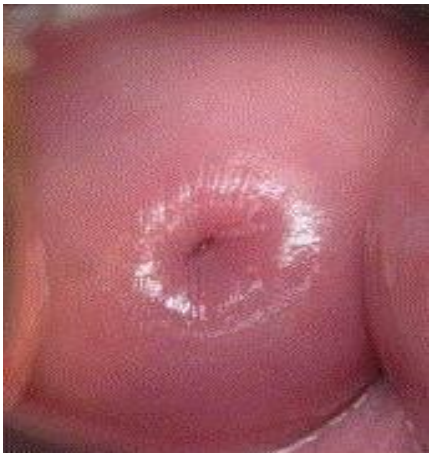


Figure 1.4: Type 3 TZ (unsatisfactory colposcopy)

1.2.3 Human Papillomavirus (HPV)

In 1983, Harald zur Hausen examined the histology samples of 60 women with cervical cancer. His monumental discovery that HPV subtypes have differing carcinogenicity with high risk subtypes present in 93 – 99% of cervical cancers[11] led to the publication of many large meta-analyses which have corroborated the global epidemiology[12, 13], and reported the cellular pathogenesis and immunology of this virus.

1.2.3.1 Epidemiology

A meta-analysis spanning five continents and over a million women with normal cervical cytology estimated a worldwide prevalence of 11 - 43%, except in sexually active adolescents and young women where it can reach 50%[14]. In young women, exposure to HPV is high but natural immunity low. Transmission is by sexual contact and the lifetime risk of infection for women and men is 80% but this is usually transient and the clearance rate in the immunocompetent is 80-90% within two years of infection[15]. It is unclear whether the host antibody response completely eliminates HPV and provides lasting immunity to the subtype (immunoclearance) or whether viral DNA is kept at an undetectable level by immunologic control (latency). The reappearance of HPV genotypes has been reported in women over 40 years of age but these infections are generally benign[16]. Progression from infection to invasive cancer usually occurs over 10 - 15 years and the likelihood of a cancer diagnosis before the age of 30 is rare[3], except in women who are immunocompromised or have their first sexual contact at a young age.

1.2.3.2 Classification

HPV is an 8000 base pair double stranded, circular DNA virus. 150 different types of HPV have evolved over millions of years, of which the main genera are known to cause genital warts, commensal infections and infect anal, oral and cervical epithelia. The 40 types which infect the cervical epithelium originate from the alpha 5, 6, 7, 9 and 11 species which have been subdivided by the International Agency for Research on Cancer according to their carcinogenic potential: *'1 - carcinogenic, 2a - probably carcinogenic and 2b - possibly carcinogenic'*[17]. This carcinogenic grading of the HPV subtypes was based on studies like that of Shiffman *et al* who recruited 10,000 women into a population-based prospective study[18].

However, the prevalence and carcinogenicity of HPV subtypes can differ dependent upon specific populations which may result in underestimation of the risks associated with the rarer subtypes. HPV 16 is reported to be responsible for the greatest proportion of cervical, anogenital and oral SCCs (50%) due to a reduced rate of immunoclearance (Section 1.6.4). HPV 18 is found in 35% of adenocarcinomas and although responsible for a lower proportion of SCCs, 10 - 15%, some studies suggest it is a more aggressive phenotype. Of the remaining oncogenic subtypes HPV 45 accounts for 7% of cervical SCCs, HPV 31 for 3% and the rest combined (33, 35, 39, 51, 52, 56, 58, 59, 66 and 68) are accountable for less than 2%. HPV 6 & 11 are low risk subtypes associated with condylomata acuminata which are benign exophytic papillary lesions (genital warts). HPV genotypes 42, 44, 53, 54, 55 and 66 are also low risk and associated with benign or low grade pre-invasive lesions[18, 19].

1.2.3.3 Pathogenesis

The interaction between HPV and the host genome has been extensively studied over the past 20 years. Low risk HPV subtypes are seen as extra-chromosomal DNA whereas high risk subtypes integrate into the host's genome producing early and late viral genes which have different functions. Late genes encode the envelope proteins L1 and L2, whilst early genes encode proteins E1, E2, E4, E5, E6 and E7 which affect cell function and replication[20] (Figure 1.5). It is believed that sexual intercourse traumatizes the cervical epithelium at the transformation zone, allowing the virus to bind to the basement membrane via the envelope protein, L1, and pass into the nuclei of keratinocyte receptors in the basal layer via L2[20]. As discussed in 1.2.1, the basal cells are the immortal cells and viral integration at this level compromises all higher cell layers within the epithelium.

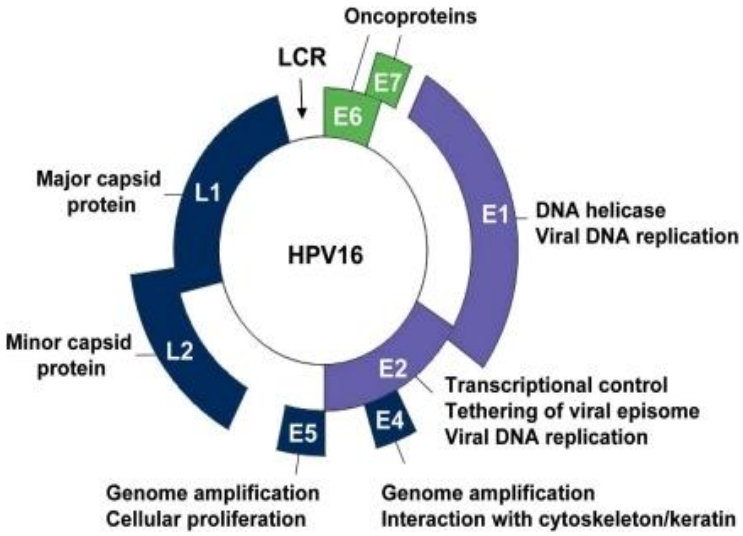


Figure 1.5: HPV genome[21]

The HPV derived proteins E1 and E2 are responsible for early transcription and viral replication. E5, E6 and E7 proteins interfere with cell cycle control causing instability at 3p. E6 binds to E3 ubiquitin ligase leading to degradation of p53, an oncogenic regulator. As a consequence, apoptosis of DNA damaged cells is prevented. During integration, viral DNA is disrupted and recombination usually occurs with deletions at E1-2, providing E6 and E7 direct access to the viral promoter and enhancer sequencing[22].

In an attempt to increase viral replication, E6 activates cyclin D and cyclin dependent kinase (CDK) 4/6 which triggers the G1 stage of the cell cycle pathway. This action, combined with the degradation of p53, causes exponential cell proliferation (and the release of the proliferation marker Ki-67 (Section 1.6.3.1.2). To further increase viral replication, HPV protein E7 inactivates retinoblastoma protein (pRb), another oncogenic regulator. In its absence activation of E2F occurs; this cell cycle regulatory gene codes transcription factors which, following the loss of the oncogenic regulators, are no longer counteracted by cell apoptosis

In an attempt to slow cell proliferation the activation of E2F triggers the production of a cell cyclin-dependent kinase inhibitor, $p16^{INK4a}$ (p16)(Section 1.6.3.1.1). p16 blocks the CDK 4/6 (cyclin D) proliferation which is promoted by HPV E6 (Figure 1.6). Secondary to this blockade, another cyclin dependent pathway (cyclins A/E: CDK2) is activated promoting the G1/S transition and the S phase to G2 stage of the cell cycle; this continues the exponential proliferation that is associated with neoplastic lesions[23]. An

understanding of the molecular changes that occur with HPV infection is integral to planning diagnostic and screening tests which detect viral integration.

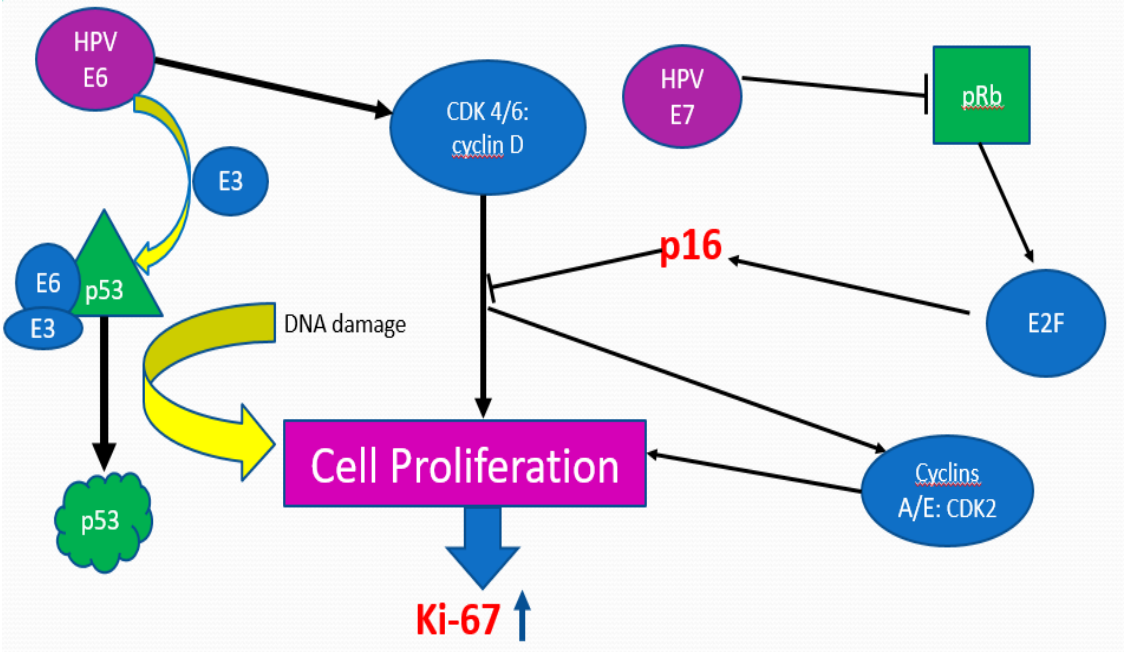


Figure 1.6: The cellular expression of p16 and Ki67 (diagram author's own).

Infected cells can remain in a quiescent stage in the basal layer or, when activated, move over 4 - 6 weeks from the basal layer to the superficial, terminally differentiated cells of the epithelium. HPV replication occurs in the nuclei here, which through constant sloughing of the upper layers of the epithelium, allows HPV to go unnoticed by the host's immune system, permitting large scale viral replication[24]. It is still unclear, other than in those with a dampened immune system, why viral integration occurs in some women and not others.

1.2.3.4 The immune response to HPV

Antibody-mediated (humoral) and cell-mediated immune responses combat current viral infections and prevent future re-infection. The humoral response to HPV is slow and results in antibodies against the L1 capsid protein which provides protection for at least 10 years after sero-conversion[25]. Consideration of these pathways is important when addressing the potential for HPV vaccination (Section 1.3.1).

Langerhans' cells (LCs) are immune cells which are found in the epidermis and help prevent infection by presenting antigens to T-lymphocytes[26]. Studies have looked at the density of LCs within the normal ectocervix and found a mean of 8 per 100 basal cells in the TZ, predominately clustered at the basement membrane. Uniform distribution of LCs has been described in the TZ and the rest of the ectocervix but T-lymphocytes are not uniformly distributed and studies have been unable to accurately measure these on tissue biopsies[27, 28]. Infections and malignancy change the density and distribution of LCs: an increase in density and dendritic branching is reported in direct relation to the severity of CIN[29]. Conversely, in the presence of HPV there is a decrease in these cells, potentially due to a cytotoxic effect[30].

The immune response during the progression from HPV to cervical cancer has been studied and the evidence indicates that blockade of immune signaling pathways is integral to this progression, as outlined below[31];

1. *Cell mediated pathways:*

- The intracellular control mediated by cyclin-dependent kinase inhibitors ($p16^{INK4a}$ and $p14^{ARF}$) is blocked.
- Suppression of viral oncogene transcription by paracrine control (specifically macrophages and TNF-α) is blocked.
- E6 and E7 proteins inhibit the interferon response which is a key antiviral defense mechanism.

2. *Humoral pathways:*

- Human leucocyte antigen presentation of viral antigens is inhibited.

1.2.4 Cervical Intraepithelial Neoplasia

The discovery of a cervical cancer precursor lesion, which occurs after integration of persistent high risk HPV into the host's genome, delineated the importance of screening for and managing this precursor lesion. This was termed cervical intraepithelial neoplasia (CIN). Consideration of the structural changes which occur with CIN is helpful when exploring and interpreting new investigative avenues. An understanding of the natural history and factors which can affect progression of CIN is also vital when planning screening and treatment algorithms.

1.2.4.1 Histological diagnosis

Histological assessment interprets the degree of dysplasia within the cervical epithelium by identifying nuclear abnormalities such as enlarged nuclei, a raised nuclei:cytoplasmic ratio, increased hyperchromasia, nuclear polymorphism and variations in nuclear size (anisokaryosis). Mitotic figures are usually only seen in the parabasal layer but as the severity of the dysplasia increases these figures are observed throughout the epithelial layers. There is a large body of evidence, with consistent findings across the published data, which have reported the structural changes observed with different histological grades of CIN. This grading is determined by the degree of disturbance of cellular maturation and stratification (Figure 1.7).

- *CIN 1:* Undifferentiated cells are confined to the lower third of the epithelium, with scarcely populated mitotic figures. Koilocytes are seen throughout the epithelium.
- *CIN 2:* Dysplasia is observed in the lower two thirds of the epithelium. Increasing nuclear abnormalities and mitotic figures are seen throughout the lower third.
- *CIN 3:* Loss of stratification and differentiation of cells throughout the full thickness of the epithelium. Numerous mitotic figures with abnormal morphology are seen.

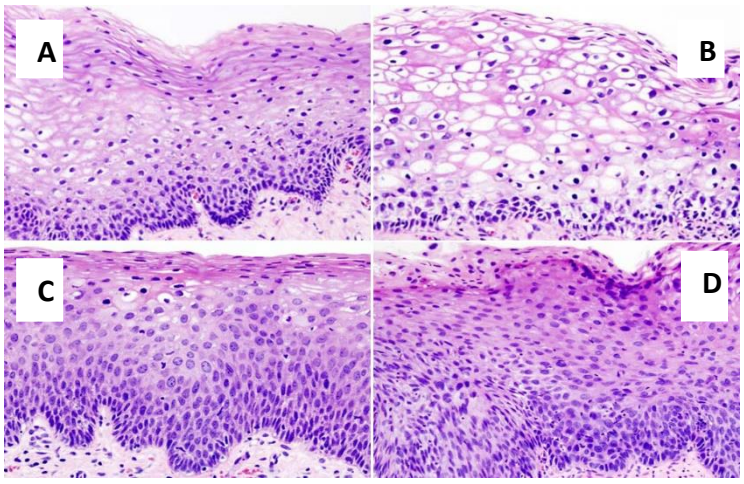


Figure 1.7: Histological grading of CIN[1]:

A - Normal epithelium

B - CIN1

C - CIN2

D - CIN3.

Routine immunohistochemistry for CIN consists of Haematoxylin and Eosin (H&E) staining. Eosin stains acidophilic structures (cytoplasm) pink and haematoxylin (in combination with aluminum salts) stains basophilic structures (DNA in nuclei, RNA in ribosomes and endoplasmic reticulum) purple-blue. Many benign changes can be mistaken for dysplasia[32] such as reserve cell hyperplasia where the nuclei become crowded and larger but retain their normal shape and morphology. With immature metaplasia there is a high nuclear:cytoplasmic ratio and in atrophic epithelium the parabasal cells predominate and appear hyperchromatic with an increased N:C ratio. These benign changes can make differentiation from dysplasia difficult with routine H&E staining, leading to the development of novel biomarkers in an attempt to improve the diagnostic accuracy of the screening tests (Section 1.6.3).

1.2.4.2 Natural history

Many studies have evaluated the natural history of CIN but much of the evidence available is limited by short (less than 6 months) follow-up because, ethically, observing the potential progression of CIN3 to cancer cannot be sanctioned. The exception was a clinical study in New Zealand between 1965 - 1974, where consent was not gained to withhold treatment in women with CIN3[33]. Ostor AG, 1993[34] pooled and critically reviewed studies between 1950 and 1993 to approximate the regression and progression of CIN. It is considered the seminal paper in this area and the findings are outlined in Table 1.1. The outcomes from this study are the basis for the current UK recommendation to treat CIN2+[35].

Table 1.1: Natural History of CIN[34]

CIN grade	Regression	Persistence	Progression to CIN3	Progression to Cancer
1	60%	30%	10%	1%
2	40%	40%	20%	5%
3	32%	56%	-	12%

1.2.4.3 Risk factors for CIN progression

The following risk factors are not an exhaustive list but give an indication of how complex the progression of CIN can be and the factors which colposcopists need to consider when evaluating appropriate management options. The difficulty is that many of these co-variables are related and although interventional studies could help assess the independent effect of these factors, this may not always be ethically possible.

- Cross-sectional and case-control studies in different continents have reported *early onset of **intercourse***, *multiple sexual partners and partner's number of sexual partners* have a strong association with HPV acquisition and persistence[36, 37]. Integration of HPV following early age of first intercourse may be mediated by a large, metaplastic ectocervical TZ or an immature immune response[38, 39].
- ***Smoking:*** There is a significant body of evidence which, having adjusted for covariables such as sexual behavior, support the association of smoking with the development of cervical cancer. A large meta-analysis reported that smokers are 1.6 times more likely to develop a cervical SCC when compared to never smokers (95% CI: 1.48 – 1.73, p<0.001) and if they smoke fifteen or more per day their risk further increases (RR 1.98, CI 1.78-2.21, p<0.001)[40]. Tobacco metabolites such as Benzo[a]pyrene, cotinine and nicotine are found within smokers' cervical mucus and it is thought they enable expression of cytochrome P450 enzymes which activate carcinogenic nitrosamine leading to DNA damage and immunosuppression[41]. Furthermore, the frequency of smoking increases the viral load and longevity of HPV by dampening Langerhan cell mediated immune responses[30]. A prospective intervention study, which adjusted for confounders, showed the benefits of promoting smoking cessation in 82 women with biopsy proven CIN1: at six months of cessation 82% of women had ≥20% (4mm^2) reduction in low grade (CIN1 / HPV) lesion size with a corresponding reduction in tertiary follow up compared to 28% of smokers (OR 12.0, 95% CI 3.9 – 32.7)[42].
- ***Combined oral contraceptive pill (COCP) use:*** Large epidemiological and case-control studies, which adjusted for other co-variables, have demonstrated an association between use of the COCP and cervical cancer. In women who are high risk HPV positive, the risk of cervical cancer increases three-fold if the COCP is used for 5 - 9 years and four fold if use is >10 years (OR 4.03, 95% CI 2.09 - 8.02), when compared to never users[43, 44]. COCP use has been shown to increase the size of the ectocervical TZ (OR 1.8, 95% CI 1.0 - 3.3)[45] and this may facilitate HPV acquisition. Some studies

have postulated that COCPs induce folate deficiency, which affects DNA synthesis and repair, but a link between women with folate deficiency and an increased risk of cervical cancer has not been shown[46].

- ***Immunocompromise:*** Despite resolution of an active infection (negative serum samples), HPV DNA can still be detected in the skin, oral cavity and female genital tract[47]. Reactivation of a DNA virus has been reported for hepatitis B, herpes simplex, EBV and CMV in immunocompromised patients[48, 49]. It is logical that persistence or reactivation may occur in women who are immunocompromised if HPV clearance is mitigated by the host's immune response. Patients who have undergone renal transplantation have a five-fold increased risk of cervical dysplasia, a 15% incidence of HPV and higher rates of false negative cytology[50]. Women who are HIV positive have a five-fold increased risk of cervical SCC, a higher risk of false negative cytology and an increased risk of both progression and recurrence of low grade lesions[51, 52]. These studies were published before HPV testing and the false negative rates may be associated with the quick progression of HPV infection in these patients.
- ***Other infective agents:***
    - *Chlamydia trachomatis* (an obligate intracellular bacteria) increases the risk of cervical SCC (OR 1.8, 95% CI 1.2 - 2.7) but not adenosquamous cervical cancer (OR 1.0, 95% CI 0.53 – 1.9). This risk is increased in women with elevated antibody titres (>128)[53]. Possible reasons include a humoral rather than cell-mediated response (which may reduce clearance of HPV[54]), the bacteria may affect the structure of the epithelial cell cadherin-catenin junctions - increasing susceptibility to HPV infection[55] - or inflammation secondary to chronic infection may produce reactive oxygen species which damage cellular DNA[56].
    - *Herpes Simplex* (HSV): A meta-analysis of seven case-control studies (2000 women) reported that HSV-2 (genital infections) increases the risk of cervical SCC (OR 2.19, 95% CI 1.41 - 3.40) and adenosquamous cancer (OR 3.37, 95% CI 1.47 - 7.74)[57]. No link was made between HSV-1 antibodies (non-genital infections) and cervical cancer. Similar pathogenic mechanisms to *C. trachomatis* have been proposed, as well as facilitation of HPV to the basal layer secondary to ulcerative lesions[58].
- ***Nutrient deficiency:*** A meta-analysis of case-control studies, which included 10,000 women, reported that deficiency in folic acid and vitamins A, E and C may affect immune status and increase the risk of cervical cancer[59].

- ***Parity:*** Many studies have suggested an association with cervical SCC. A large multicentre case-control study reported, that in high risk HPV positive women, parity greater than four doubles the risk of SCC when compared to nulliparous women (OR 2.3, 95% CI 1.6 - 3.2)[60, 61]. No correlation was noted with adenocarcinomas[60]. Postulated theories include trauma to the cervix and / or hormonal effects which evert the TZ.
- ***Genetic:*** Current evidence suggests cervical cancer is not hereditary, but women with an affected 1st degree relative have a two-fold increased risk. This may be secondary to similar lifestyles, such as high risk sexual behavior or smoking, or they may have a genetically dampened immune response to HPV[62].

1.3 Prevention of cervical cancer

1.3.1 Vaccination

A prophylactic HPV vaccine would have many applications; it would be beneficial for women at risk of cervical, vulval and vaginal cancer, for women and men at risk of genital warts or cancer of the larynx and anus and for infants who contract HPV laryngeal infections - which need to be surgically excised to prevent tracheal occlusion.

Despite a global effort, development of the vaccine was protracted for a number of reasons: HPV infections are species specific (they cannot be studied in mice as they do not contract the virus), the viral particles are sparse in lesions, low antibody levels to the L1 capsid protein are produced during seroconversion and live attenuated or killed vaccines cannot be generated because in vitro cultures cannot support the complete cycle. Moreover, the virus is classified according to genotype (genetic composition) rather than serotype (classification of a virus based on their surface antigens)[63]. This has prevented the development of a vaccine which provides broad spectrum immunity to all high risk types (targeted at the L2 envelope proteins).

In 2006 a bivalent vaccine targeted at HPV 16 & 18 (Cervarix) was licenced, as was a quadrivalent vaccine to HPV 6, 11, 16 & 18 (Gardasil). These are given as intramuscular injections at 0, 2 and 6 months. Early studies demonstrated that when eukaryotic vectors expressed HPV L1 proteins, virus like particles (VLPs) were generated and an antibody response was produced[64]. Intramuscular injections allow the VLPs to enter the

vasculature and lymphatics, starting a T helper response with antibody mean titres 14-24 times higher than natural concentrations one month after the 3rd injection. In contrast, a poor inflammatory response is seen with cervical infections as HPV is contained within the surface epithelium, thereby reducing the antigen presenting response from macrophages and Langerhans' cells [65].

Trials have been undertaken to assess the efficacy of the vaccines; in women who are HPV naïve the bivalent vaccine confers a higher and more sustained immune response than the quadrivalent vaccine, with immunogenicity so far demonstrated for 8.4 and 5 years respectively[66]. Randomised control trials[67] and a recent observational study have demonstrated that the prevalence of CIN2+ is lower in women vaccinated against HPV 16 & 18 (19% vs 36%, p=0.006)[68]. Moreover, the specificity and NPV of screening were higher in women who were negative for HPV 16 (92.4 vs 75% and 94.6 vs 64.9% respectively)[68].

These findings suggest the introduction of a vaccine which provides protection against HPV 16 and 18 will be of benefit but cervical screening is still required as older women will not be vaccinated, the efficacy of the vaccine in women who are not HPV naïve is only 30%[69] and although some cross-protection is provided against HPV 31, 33 and 45, other high risk subtypes are not covered[70]. Recent studies indicate that high risk subtypes not covered by the vaccine, HPV 51[71], 52, 56 & 58[68] and 59[70], are increasing in incidence; the introduction of a nine-valent vaccine in 2019 will help address this but modelling studies have suggested the full effects of this new vaccine will not be seen until 2035 - 2040[72].

What has been reported since the introduction of the quadrivalent vaccine is a failure to decrease low grade screening abnormalities[73] and the potential decreased PPV of screening from 70% to 20%, by reducing the prevalence of cervical cancer[74]. As the vaccine provides protection against HPV 16 and 18, the subtypes currently responsible for 65% of cervical cancers, a reduction in the prevalence of these subtypes within a vaccinated population will influence the number of true positives in women who have a positive screening test. The trade-off, as discussed, is the improved NPV of screening as more people who test negative for high risk HPV will actually be negative. These studies delineate the importance of future research which targets improved triage of vaccinated (and unvaccinated) women who have positive screening results.

1.4 Screening for cervical cancer

As previously discussed, the progression to high grade CIN can be multifactorial and regression is possible, even with CIN3. However, the mortality from cervical cancer is high and excision of high grade CIN can prevent progression to cancer. To improve detection of CIN the UK cervical screening programme was introduced in 1988; the mortality rate from cervical cancer since its introduction has reduced by 70% from 8.9 per 100,000 women to 2.6 per 100,000 women in 2016[75]. In women with a TZ3, who have a positive screening test, colposcopic assessment is not possible and management is guided by the screening result alone. When evaluating methods which may increase diagnostic accuracy or when formulating potential treatment algorithms, an understanding of the current UK cervical screening programme is necessary.

1.4.1 Cytology

1.4.1.1 Sample taking, processing and adequacy criteria

The cervical epithelium is routinely screened in a primary care setting using a Cervex-Brush alone which 'brushes' and collects cells from the ectocervix and 0.5cm of the endocervix. With liquid based cytology (ThinPrep or SurePath), the cells are dislodged from the Cervex-Brush into a pot of preservative. Liquid based cytology (LBC) superseded conventional cytology in 2008 throughout the UK as it has a higher sensitivity (83.9% vs 72.7%) for squamous cell lesions albeit a slightly lower specificity (82% vs 76%)[76]. The improved filtration of blood and mucus from LBC led to a national decrease in inadequate samples (9% to 2.8%). Moreover, processing of the slides is automated, which has increased lab turnover times[77].

Currently, the British Association for Cytopathology suggests the following criteria for confirming adequacy of an LBC sample: a minimum average cell count (MACC) of 5 - 10,000 squamous cells and the sample taker must have visualized the cervix. The presence of endocervical cells is now only reported in women with previous glandular intraepithelial neoplasia (cGIN)[78]. In theory, if the cervix is visualized and the transformation zone is on the ectocervix, TZ1 or 2, sampling should be adequate. In women with a TZ3, colposcopists may not be reassured by the cytology result if the presence of TZ sampling (endocervical cells) is no longer reported.

1.4.1.2 Other cytology collection devices

There is a large body of evidence comparing combinations of Cervex-Brush, Cytobrush, Spatula and cotton swabs (Figure 1.8) to determine cytological adequacy rates and detection of dysplasia[79]. The evidence about the contribution of endocervical cells in predicting high grade disease is contradictory, with some studies promoting their importance in reducing false negatives[80, 81] whilst other studies refute this risk[82]. A study of 20,000 LBC samples reported improved detection of endocervical cells (TZ) with combined Cervex-Brush and cytobrush sampling when compared to the Spatula plus Cytobrush or Cervex-Brush alone (89.8%, 86.9% and 77.1% respectively, $p<0.001$)[83]. Although no difference was observed in the diagnosis of high grade lesions between devices, improved detection of low grade dysplasia was noted with the combined Cervex-Brush and cytobrush (4.2%, 2.4% ($p<0.001$) and 2.9% ($p=0.003$) respectively). It should be noted that the rate of inadequate samples was doubled with the Cervex-Brush + cytobrush when compared to the Cervex-Brush alone (1.6% vs 0.8% respectively, $p<0.001$); this may be due to order of device sampling, which was not reported, as a cytobrush can increase blood loss which may reduce subsequent cytological yield.

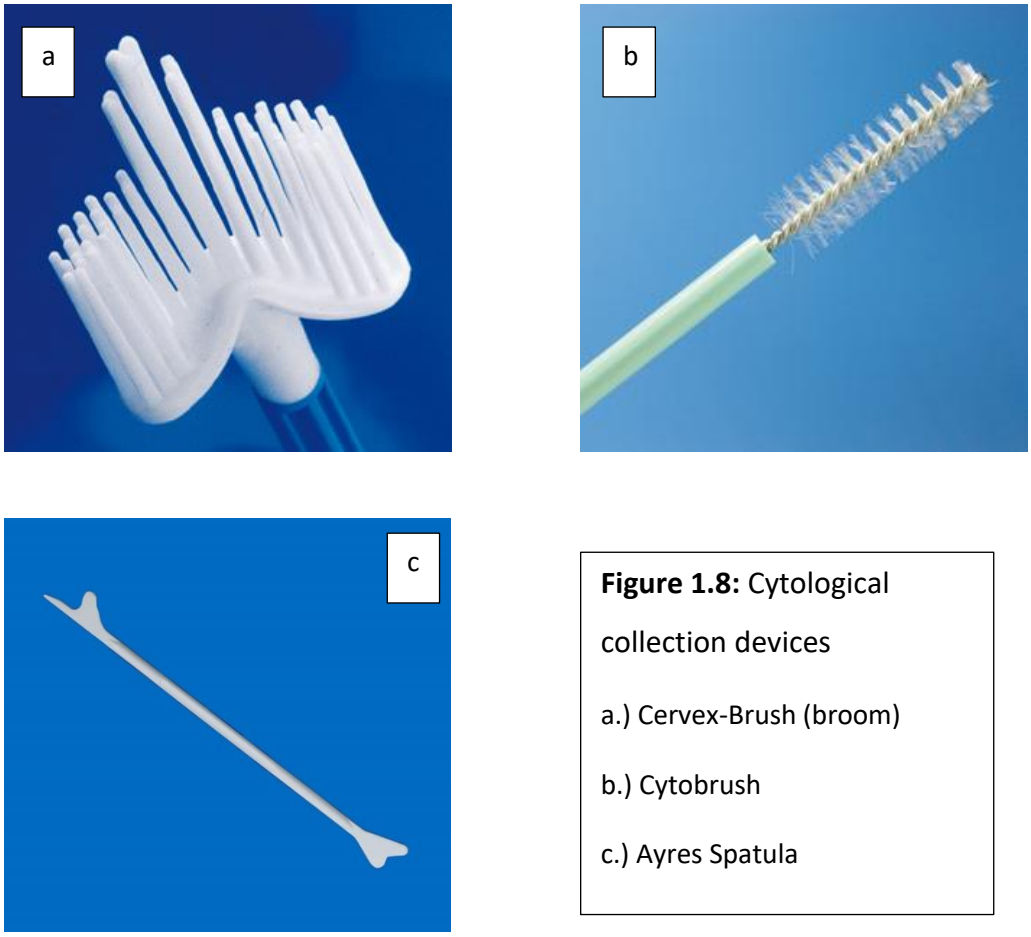


Figure 1.8: Cytological collection devices

a.) Cervex-Brush (broom)

b.) Cytobrush

c.) Ayres Spatula

Of the studies which have assessed adequacy of LBC collection devices, none have correlated their findings with topographical position of the TZ[79]. Furthermore age, parity and hormonal status were not adjusted for and these factors may affect the MACC. In my thesis I will compare the use of a Cervex-Brush alone to a Cervex-Brush + Cytobrush in women with a TZ3, whilst adjusting for co-variables which may affect the cytological yield, to try and address this shortfall in the literature.

1.4.1.3 Interpretation

In the UK, cytological grading uses the revised 1986 British Society for Clinical Cytology (BSCC) classification system[84]. Samples are divided into high grade cytology (moderate or severe), high grade possibly invasive, low grade (mild or borderline nuclear change (BNC)) and borderline, high grade not excluded. Referral to colposcopy should be within two weeks for possible invasion or high grade cytology and six weeks for low grade cytology[85]. Unlike squamous cell cancers, sensitivity and specificity of cytology for glandular lesions is poor at 32.7 - 48.1% and 69.4 - 94.4% respectively[86]. Multi-focal lesions may be missed and it can be difficult to distinguish benign lesions such as tubo-endometrial metaplasia from cGIN.

Of the 172,776 women referred to colposcopy with abnormal screening results in 2015 - 2016, 77.5% (133,859) were for a low grade result and 22.5% (38,917) were for a high grade result. In women with high grade cytology, 87.6% were of reproductive age (25 - 44 years) and of those with low grade cytology, 63.3% were aged 25 – 44[87].

1.4.1.4 Frequency of screening

In the UK alone, £175 million is spent annually on cervical screening and colposcopic assessment – the majority of lesions reviewed are benign and likely to regress[88]. A UK based case-control study[89] looked at the screening histories of 1300 women with invasive cancer and 2500 matched controls to evaluate the 'protection' (efficacy of cytological screening to prevent cervical cancer) that yearly, 3 yearly and 5 yearly testing would provide. Under the age of 25, 60% of women infected with HPV exhibit low grade cytological and histological changes and, as previously discussed, with high rates of HPV clearance in this cohort, the cost of screening and treatment is not currently thought to be economically viable.

In women aged 55 - 69, five yearly screening is recommended as protection is 87% for 1 and 3 years and 83% for five years. For women 40 - 54, screening is 3 yearly as protection is 88% at 1 year, 84% at 3 years and 73% at 5 years. In women aged 20 - 39, screening is three yearly as protection is 76%, 61% and 30% respectively[89]. These results suggest screening in women <40 is not as accurate as in women ≥40. This indicates cancer develops faster in these women and findings such as this have led to the recent change in cervical screening, HPV testing, to improve the performance and accuracy of screening.

1.4.2 HPV testing

1.4.2.1 Triage of low grade cytology and test of cure

Randomised control trials have reported that compared to cytology alone, HPV DNA testing has better sensitivity (94.6% vs 83.9%) and specificity (94.1% vs 69.3%) for squamous cell cervical lesions[90]. In 2007 six 'sentinel sites' in the UK (including Bristol, the setting for the studies in this thesis) instituted management protocols separate to the rest of the UK; these sites used HPV testing in 10,051 women with low-grade cytology to determine who requires colposcopic assessment and who can be safely returned to routine recall (3 or 5 yearly screening). Women who were HPV negative had a 0.5% chance of developing SCC between screening intervals; this finding led to recommendations that HPV positive women should be referred to colposcopy[91]. Since 2013 HPV triage of low grade cytology has been part of routine screening within the UK.

Although the Sentinel Sites Study reported that the negative predictive value (NPV - probability that women with a negative screening result do not have CIN2+) of HPV testing in women with low grade cytology is high (95.6%), the positive predictive value (PPV - probability that women with a positive screening result do have CIN2+) is low at 16%. This poor PPV indicates that HPV testing may not differentiate between transient and transforming infections; given that at least 50% of women under the age of 30 years will have a transient HPV infection, the PPV and specificity for CIN2+ will be lower in younger women[92].

1.4.2.2 Primary HPV screening

In the UK, primary HPV screening will be introduced nationally by 2019. If women test positive for high risk HPV their screening sample will be further triaged by a cytology test. The HPV DNA test currently approved is Hybrid Capture 2 (HC2) as the use of PCR has been shown to give a lower sensitivity[93]. HC2 recognizes the gene which codes for the HPV L1 protein and gives a pooled result for high risk HPV subtypes, namely HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. Subtyping is not possible with this probe set – only a high or low risk result is given. This inability to subtype prevents persistent and new infections from being distinguished and stratification by risk of persistence (HPV 16 & 18). The use of genotyping may therefore be of benefit in women where the PPV of screening is poor, such as a TZ3 (Section 1.6.4).

1.4.3 Correlation of screening results with histological outcome

Recent UK cervical screening statistics reported that women with high risk HPV and high-grade cytology have an 82% chance of the excised tissue containing CIN2+ and a 2.6% chance of cancer. In women with high risk HPV and low grade cytology, this risk is 15.9% and 0.1% respectively[5]. The limited data assessing the significance of a HPV positive, cytology negative result appears to suggest that risk is dependent on genotype; although these women are at low risk for CIN2+ this is not a negligible risk – a UK colposcopy clinic assessed 1076 women referred with negative cytology and high risk HPV of whom 355 had HPV 16, 86 had HPV 18 and 441 had other high risk subtypes. Of these women the risk of CIN2+ was 10% in women with HPV 16, 3.3% with HPV 18 and 3.5% with other high risk subtypes[94].

1.4.4 The reliability of colposcopy and directed punch biopsy

The literature reviewed so far suggests a cervical screening test result can stratify a woman's need for treatment but it is not a definitive determinant of outcome. Colposcopic assessment aims to differentiate between women with transient and persistent infections by visualizing the area infected by HPV and histologically confirming or refuting the presence of CIN2+. The use of colposcopy and colposcopic directed biopsy (CDB) has been shown to reduce the mean rate of negative excisional histology from 6% to 1.9% ($p<0.001$) and the mean rate of positive margins following treatment from 28.2%

to 21.7% (p=0.002)[95]. Although CDB has a higher PPV for CIN3 (86%) than for CIN 1 or 2 (16% & 32% respectively)[96], the diagnostic accuracy is still more than double that of the screening test for CIN2+.

In women with a TZ3 colposcopic assessment cannot be completed and the screening test is relied upon to guide decision-making. There is currently no evidence which has assessed outcomes in women with a TZ3 who have had HPV testing. In my thesis I will initially evaluate the incidence of false positive screening (negative LLETZ histology) and histological outcomes in women with a TZ3 to determine if improved diagnostic accuracy is required in this cohort.

1.5 Management of abnormal screening results

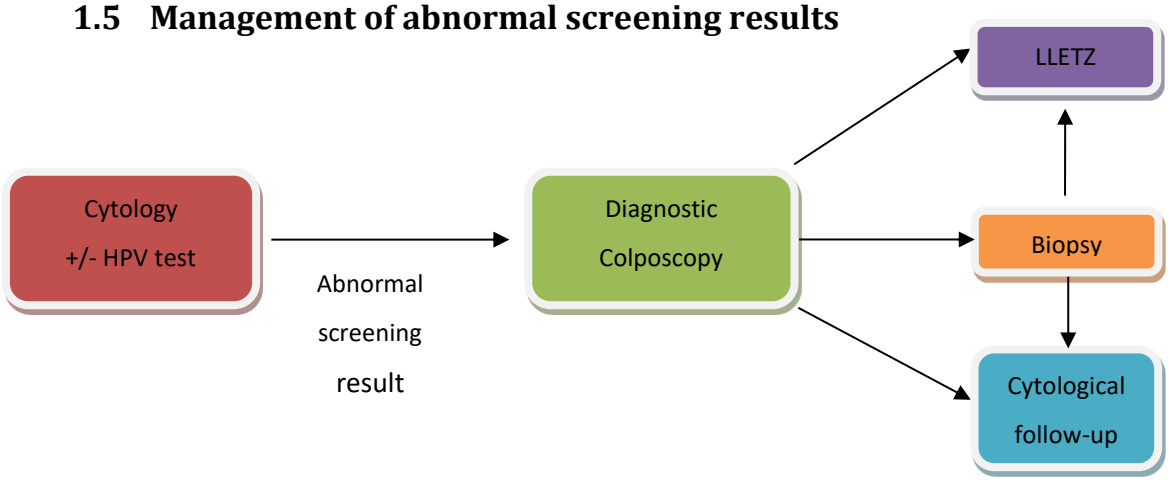


Figure 1.9: Cervical screening and colposcopic assessment algorithm

1.5.1 Repetition of the referral cytology

Current UK guidance denotes, *'Cervical cytology should not be repeated at the first colposcopy appointment following a referral for cytological abnormality. Where an initial cytology sample is inadequate, the repeat cytology sample should be taken no less than three months after the date of the first sample'*[85]. This recommendation aims to reduce the risk of false negative screening as a previous cytology test may have denuded the cervical epithelium. Of note, the evidence this recommendation is based upon did not correlate outcomes with topographical position of the TZ nor absence of TZ sampling.

1.5.2 High grade cytology

1.5.2.1 TZ 1 or 2

Although the time of progression from CIN3 to invasive cancer is slow, the progression from HPV to CIN3 is not, and as yet it is not possible to predict when CIN3 will invade the basement membrane and stroma beneath. Other than the previously outlined risk factors (section 1.2.4.3), we do not know what specific factors are associated with the progression to cancer. For this reason the NHS Cytology Screening Programme (NHS CSP) currently recommends treatment if CIN2+ is identified colposcopically at the first appointment ('a see and treat') or confirmed with a biopsy[35].

1.5.2.2 TZ3

There is a lack of guidance and expert opinion within the available literature for this cohort[35, 97, 98]. Although women with a high grade screening result have an 82% chance of CIN2+ and a 2.6% risk of cancer (Section 1.4.3), these histological outcomes were not adjusted for TZ type. In this thesis I aim to assess histological outcomes in women who are offered a LLETZ for high grade cytology and a TZ3; I will evaluate if their outcomes are comparable to women where the TZ is visible and assess if the use of surrogate markers for HPV or HPV genotyping improve the accuracy of their screening test result.

1.5.3 Low grade cytology

1.5.3.1 TZ type 1 or 2

Women with low grade screening results are referred to colposcopy for assessment as this has been shown to detect more cases of CIN2+ than cytological surveillance alone[99]. However, due to the moderate specificity of the colposcopic examination, CIN2+ should be confirmed histologically prior to excision[85]. If the colposcopic examination is normal or ≤CIN1 is detected on biopsy, UK guidance states these women can be safely offered cytological follow-up over 24 months as low grade abnormalities have a high rate of regression[100] (Table 1.1). However, this policy relies on initial colposcopic visualisation or histological confirmation of the lesion, which cannot be undertaken with a TZ3.

1.5.3.2 TZ3

A TZ type 3 in combination with low grade cytology is a common area of clinical uncertainty due to the lack of clear evidence and guidance[35, 97, 98]. As with high grade cytology, there are no NHS CSP management recommendations and it is my experience that recommendations for cytological follow-up versus excisional treatment are hospital specific. When cytological follow-up is offered in this cohort, decisions on total length, clinical setting and technique vary dependent upon the colposcopist. Health care professionals rely on their own experience of managing these women, and the advice of the multidisciplinary team (MDT) to determine who requires treatment and this may lead to disparities in care[101].

Attendance rates for colposcopy and loss of patients to follow-up are affected by service inefficiencies[102], anxiety[103] and poor accessibility to targeted information[77]. With non-attendance rates for colposcopy in the UK documented at 24.4%, of which 46.1% are follow-up appointments[5], areas of heterogeneity in service provision need to be improved.

Studies which have assessed histological outcomes but not adjusted for TZ type report a 15.9% risk of CIN2+ in women with low grade cytology. Although smaller than the chance conferred with high grade cytology, this is not negligible. It would be reasonable to assume that patient and health care provider anxiety may deter conservative follow-up and lead to higher rates of excision when compared to women where the TZ is visible. Clear guidance and enhanced diagnostic accuracy is needed to improve patient outcomes in women with a TZ3 and low grade cytology.

In this thesis I will evaluate the current management of this cohort within the UK and the factors which affect the decision-making process. These outcomes may aid in the formation of recommendations on which to base a consensus opinion, potentially improving homogeneity of care. Assessment of the histological outcomes in women who have a LLETZ for low grade cytology and a TZ3 would help stratify this risk and provide data on which to base recommendations for cytological follow-up versus excisional treatment.

1.5.4 Large loop excision of the transformation zone (LLETZ)

Loop excision of the cervical transformation zone (LLETZ) was introduced to provide accurate histological assessment of CIN[104]. LLETZ uses a monopolar energy source and a high frequency current to provide cutting and coagulation. It is the gold standard at present for excision of high grade cervical disease (CIN2+ / cGIN) and for diagnosis in women with persistent low grade CIN or a TZ3[85]. It is cheap and easy to use, providing a 98% 'success' rate (removal of all dysplasia) after one treatment[105].

Squamous epithelium located in the endocervical canal and ectocervix can be affected by CIN. This can extend into the crypts, millimetres below the surface. To plan an excisional treatment it is important to know the maximum depth of the crypts and the mean topographic position that the transformation zone can take. If the excised portion of the cervical epithelium does not include the underlying crypts there can be delayed recognition of CIN that may become invasive. A meta-analysis assessed the risk of CIN2+ recurrence following excisional treatment in 35,000 women and showed a pooled prevalence of 18% if the margins were incomplete vs 3% if complete[106].

Papoutsis *et al* assessed the effect of excision depth, volume and ratio of cone base to ectocervical surface and found that a depth <10mm was the most sensitive of the three in predicting positive margins[107]. This finding is supported by other studies which have reported an increased risk of recurrence if the excision depth is <10mm in women >35 years[107]. Boonstra *et al* assessed the maximum proximal distance that CIN3 extended from the ectocervix; it was reported as 13.3 +/- 3.7mm. Although women with a TZ type 2 and 3 were included in these studies, maximum proximal distance was not correlated with TZ type[108].

As a result of these studies, national NHS CSP guidance for excision depth in women with a TZ1 is 7-10mm. For women with a TZ type 3, a deeper excision is recommended - albeit with the caveat of considering the higher risk of treatment related morbidity in childbearing women[85]:

> *'Type III cervical transformation zone: excisional techniques should remove tissue to a depth/length of 15mm to 25mm.* Evidence: *…….in women under the age of 35, excisions >10mm in depth are not associated with improved*

recurrence rates. There is, however, an increased risk of preterm delivery after loop treatments >10mm in depth.'

The predicament, as stated, is the treatment related morbidity as the risk of preterm labour has been correlated with the depth of excision: a case control study of 11471 women reported a baseline absolute risk (AR) 6.7% with a 7 - 10mm LLETZ the AR is the same as a punch biopsy (7.5%) and the relative risk (RR) 0.98; if 10 - 14mm depth RR 1.28, AR 9.6%; and for the depth recommended for a TZ3, RR 2.04, AR 15.3% for 15 -19mm and RR 2.40, AR 18% for ≥20mm[109]. This risk does not decrease with time.

After-effects following LLETZ such as pain and bleeding were compared to punch biopsy by the Tombola Study group. Questionnaires revealed that more women reported pain as a consequence of a LLETZ than biopsy (67% vs 53%) and also more bleeding (87.3% vs 79.1%: $p<0.001$). Moreover, 52.9% of the LLETZ cohort reported severe bleeding compared to 21.4% in the biopsy cohort[110].

Compared to the background population, LLETZ increases the risk of cervical stenosis (3%) and this can cause infertility and an inability to take future cytological samples[111, 112]. In these women, a second LLETZ may be advised if a test of cure cannot be undertaken or mild cytological abnormalities cannot be assessed colposcopically. Even after dilatation of the cervix, re-stenosis can occur in 77%[113]. After adjusting for a range of clinical variables, age over 50 and a TZ3 (where rates of up to 25% have been reported), are the only independent risk factors for cervical stenosis in women who have a LLETZ.

The introduction of cervical screening and treatment of CIN has reduced the mortality rate from cervical cancer but the treatment-related morbidity is considerable, particularly in women with a TZ3 where the depth of LLETZ is double that undertaken with a TZ1. Improving the diagnostic accuracy of cervical screening in this cohort may decrease false positive results and unnecessary treatments. I aim to evaluate the use of adjuncts such as HPV genotyping and surrogate markers for HPV to help stratify who may require treatment and who can be safely offered conservative management.

1.6 Improving diagnostic accuracy in women with a TZ3

About 80% of HPV positive women referred to colposcopy will have low grade cytological changes. Distinguishing between integration and potential regression is difficult even when colposcopic assessment is possible. There is a plethora of studies assessing the use of molecular and biochemical tests which may improve the diagnostic accuracy of punch biopsies and LLETZ histology (section 1.6.3) but these adjuncts have not been studied in women with a TZ3.

1.6.1 Mechanical and pharmacological methods

Before evaluating novel biomarkers it is important to consider mechanical and pharmacological methods that may convert a TZ3 to a TZ1 or 2. Completing an assessment at specific times during the menstrual cycle has been unsuccessful[114]. Vaginal misoprostol has varying success (20 - 78.9%)[115] but patients report nausea, abdominal pain and fever. Hygroscopic cervical dilators have a reported success rate of 79 - 94%[116], but only women with a TZ2 were included in these studies.

It is logical given the effect of oestrogen on eversion of the TZ in puberty that the use of systemic and topical oestrogen may convert a TZ3 to a TZ1. A randomised control trial assessed the use of ten days of ethinyl estradiol (30mg) versus placebo and found that eversion of the TZ occurred in 12/17 (70%) of women with a TZ3 (OR 7.8, 95% CI 1.6 - 36; P<0.01)[117]. These results were supported by an observational study which gave 178 postmenopausal women with a TZ3 three months treatment with oestrogen replacement therapy and found 130 (73%) had a visible TZ[118]. Furthermore, success rates of 64% have been reported in studies using topical oestrogen[119]. It is my experience that this practice has not been routinely adopted by colposcopists and this may be due to the side effect profile, contraindications or patient acceptability. In this thesis, to aid the development of a consensus management guideline, I will assess colposcopists' experience with oestrogen in women with a TZ3.

1.6.2 Techniques for sampling the endocervical canal

Endocervical canal curettage (ECC) can be used to obtain ~1mm^3 tissue samples of squamous epithelium from inside the cervical canal. The side effects of ECC can include mild suprapubic pain and per vaginam spotting. Whilst its use can increase the detection rate of squamous dysplasia by up to 18% with a TZ1 or 2, this diagnostic yield is higher with a TZ3 (~83%). Specificity is also high at 84 - 97%[120-123]. Despite this, due to the small and fragmented nature of the samples inadequacy rates can be up to 19%, inter-observer agreement moderate (k = 0.58, 95% CI 0.52 – 0.63) and diagnosis underestimated in up to 16% of squamous cell lesions[124-126]. Most of these authors agree that ECC does not improve diagnostic accuracy with a TZ1. As discussed in 1.2.4.1, distinguishing benign changes from dysplasia can be difficult even when the epithelium is intact and copious. I aim to assess whether the use of surrogate biomarkers for HPV can improve the diagnostic accuracy of these fragmented samples.

In regards to the assessment of glandular lesions via ECC, there is a paucity of true endocervical disease such as adenocarcinoma in situ reported[120, 127]. The American College of Obstetricians and Gynaecologists have concluded that positive curettings may be inadvertent contamination of a squamous lesion near the external os rather than dysplasia of endocervical cells[128]. Although it has been suggested that ECC can affect the distinction between adenocarcinoma *in situ* and invasive adenocarcinoma[129], similar effects on squamous lesions have not been reported[125].

The use of the endocervical cytobrush (Figure 1.8) was developed to decrease the discomfort of sampling the epithelium within the endocervical canal. Despite the ease of use, lower cost and 77 - 93% sensitivity, there is a moderate false positive rate (specificity 63% - 75%)[129-131]. Boardman *et al* compared endocervical curettings to cytobrush samples, in 62 women who either had a cone biopsy or hysterectomy, and randomised the order of the sampling procedures. There was no difference in sensitivity (32% vs 44%) and although the curettings were more specific than the cytobrush (100% vs 88%), the adequacy was poorer (78% vs 98%). The order of sampling did not affect the adequacy rates of either specimens[132]. Furthermore, the sensitivity of the cytobrush sample was lower in Boardman's study than has previously been reported; stripping of focal dysplastic epithelium by the curette prior to the cytology sample may account for this and suggests cytological sampling should occur first.

None of these studies correlated their outcomes with topographical position of the TZ and it would be of interest to assess the diagnostic accuracy of methods which sample the epithelium within the endocervical canal in combination with HPV genotyping and/or surrogate biomarkers for HPV. I aim to evaluate whether this combination improves the adequacy rates, inter-rater reliability and accuracy of the cytobrush cytology and endocervical curettings.

1.6.3 Surrogate markers for integrated HPV

Investigators over the past decade have studied surrogate markers for HPV integration to help differentiate benign from pathological aetiology and to improve the specificity of HPV testing. Research has focused on four main areas of which only studies assessing points 1 and 2 below have used large clinical sample sizes. These are the areas I will focus on:

1. Cellular proteins which are over-expressed by cells infected with HPV
2. E6 and E7 HPV mRNA transcripts
3. Alterations of viral and host genomes
4. Gene methylation pattern alterations

1.6.3.1 Cellular proteins over-expressed by cells infected with HPV

1.6.3.1.1 p16^{INK4a}

This cellular protein has been the subject of considerable recent study. p16^{INK4a} protein (p16) is a cell cycle regulatory protein – a cyclin-dependent kinase inhibitor - which is strongly expressed in the majority of CIN2+ lesions following inactivation of pRb by the HPV derived E7 protein (Section 1.2.3.3). Benign changes such as squamous metaplasia, tubal endometrial metaplasia, nonmucinous secretory endocervical cells and cervical endometriomas can sporadically express p16[133, 134]. This does not affect the interpretation of histological samples but to assess p16 stained cytology, morphological examination of the cells is required[135].

Studies have shown the use of this biomarker in:

1. Differentiating benign histological lesions from precancerous changes with good sensitivity (98.5%)[136].

2. Differentiating low grade histological lesions (HPV / CIN1) from high grade. The intensity of staining increases with the severity of CIN ($p<0.001$)[136].
3. Improving the sensitivity of low grade cytology samples (92.6%).

In a study of 12,000 cervical biopsies, inter-observer agreement in diagnosing high grade CIN was improved with the addition of p16 stained slides to the routine H&E slides (*k* 0.74 vs 0.56 respectively; $p<0.001$). Sensitivity for all grades of CIN was improved ($p=0.0004$) and false negative rates were reduced by 45%[137]. The disadvantages are the low sensitivity and specificity of p16 for adenocarcinomas[138] and the poor specificity for squamous lesions (74.8%). There is also a lack of longitudinal studies assessing the long term predictive potential of p16 for development of high grade CIN and there are no studies assessing the use of this marker in small and fragmented epithelial samples like endocervical curettings.

1.6.3.1.2 Ki-67(MIB-1)

Ki-67, as outlined in section 1.2.3.3, is a proliferation marker and uncontrolled proliferation is a marker of neoplasia. Ki-67 is elevated in HPV infected squamous tissue but also in regenerating epithelium and metaplastic tissue which are HPV-negative. The sensitivity of Ki-67 for CIN in histological samples is 92.2% but the specificity is 56.6%[136, 138]. Neikerk *et al*,[139] analysed staining in the different epithelial layers and found that high risk HPV positive samples showed Ki-67 staining in the basal, intermediate and superficial layers whilst HPV negative samples stained only in the basal layers. To the best of my knowledge, due to the poor specificity in histological samples, the sole use of Ki67 immunocytochemistry has not been assessed. As with p16, its use in conjunction with fragmented samples like endocervical curettings has not been evaluated.

1.6.3.1.3 Dual staining with p16 and Ki-67

It is hypothesized that the concurrent detection of p16 and Ki-67 within the same cell should differentiate transient from transforming infections; during the normal cell cycle a proliferation marker and a protein which inhibits cyclin dependent proliferation should not co-exist. The use of p16 alone and in conjunction with Ki67 in 1450 cervical (histological) biopsies was compared by Galgano *et al*[136]; the addition of Ki-67 did not significantly improve the sensitivity (99.2% vs 98.5%) nor the specificity (78.1% vs 74.8%) when compared to p16 alone. However, as stated, the predictability of these biomarkers for CIN2+, individually or in combination, have not been evaluated in fragmented tissue

samples such as endocervical curettings, where individual scattered cells, as seen on a cytology slide, may need to be interpreted.

When dual staining is used in conjunction with cytological samples, positivity increases with the histological grading of CIN; 39.4 - 58.8% staining with CIN1, 70.8 - 91.9% with CIN2 and 86.5 - 100% with CIN3[135, 140-142]. Dual stained cytology has been shown to have an equivalent sensitivity to HPV testing but higher specificity - positivity increases with high risk HPV genotypes (when compared to HPV negative samples); OR 1.0 for low risk HPV, OR 6.86 for high risk HPV and OR 9.92 for HPV 16 & 18[135, 140-142]. Dual stain specificity is higher in women over the age of 30 years when compared to women less than 30 (60% versus 46.1%) and this may be secondary to the transient nature of HPV infection in younger women[135, 143]. Furthermore, 12% of samples which contain CIN1-2 can be dual stain negative[141] and 12 - 44.1% of negative biopsies can be dual stain positive[109, 135, 140]. It has been postulated that these outcomes are potentially identifying transient HPV infections or early transforming infections respectively.

Table 1.2: Sensitivity and specificity of p16, Ki67 and HPV testing

	Borderline Cytology		Low grade Cytology		High grade Cytology		Histology (CIN2+)	
	Sens	**Spec**	**Sens**	**Spec**	**Sens**	**Spec**	**Sens**	**Spec**
p16	92.6%	63.2%-71.1%[1]	92.6%	37.3% -53.3%[1]	100%	91% – 95%[2]	98.5%	74.8%[3]
Ki-67	-		-		-		92%	56%[4]
Dual-stain	92.2%	80.6%[5]	94.2%	68%[5]	94.6%	16.6%[6]	99.2%	78.1%[3]
HPV	90.1%	37.8%[1]	95.7%	18.5%[1]	97%	86.2% - 94%[2]	-	

1. Denton *et al*, 2010[144] (n = 810)
2. Gustinucci *et al*, 2012[145], (n = 578) & Zhao *et al*, 2012[146], (n = 13,000).
3. Galgano et al, 2010[136], (n = 1450).
4. Kruse *et al*, 2001[138], (n = 65).
5. Schmidt *et al*, 2011[147], (n = 776).
6. Wentzensen *et al,* 2015[148] – calculated from the raw data (n = 1509).

1.6.3.2 mRNA

Direct detection of viral gene expression may increase predictability of persistent HPV infections when compared to DNA assays. Detection of E6/E7 mRNA may improve predictability of dysplasia as these proteins deregulate p53 and pRb. Routine cytology samples preserved in PreservCyt produce high yields of mRNA and do not deteriorate over time[149, 150]. The SurePath LBC samples of twenty women with known dysplasia were compared to RNA removed from fresh cells; the RNA extracted from the samples fixed in the SurePath medium produced small diagnostic yields and short storage times (days) did not reduce the degradation effect [151, 152].

There is a paucity of data from clinical studies and those that are published have used different detection assays and targeted different transcripts. Data from cross-sectional studies suggests HPV DNA is detected in more benign and low grade samples than RNA – indicating that mRNA may have a higher specificity for high grade disease[153, 154]. For CIN2+ lesions, 95% were DNA positive and 77% RNA positive[154]. mRNA negative samples may identify lesions which are likely to regress but prospective long term follow-up is needed to corroborate this. p16/Ki-67 dual-staining is reported to have an equivalent sensitivity to mRNA testing for CIN3 (96%) but a higher specificity (48.2% vs 33.8%)[143] – although the retrospective nature of this study may have led to mRNA degradation.

For these reasons I aim to evaluate whether p16 and ki67 (individually or in combination), rather than mRNA, can increase the reliability and diagnostic accuracy of methods which sample the endocervical canal.

1.6.4 HPV genotyping

CIN in women infected with Group 1 carcinogenic HPV subtypes is more likely to progress to cancer than in women infected with non-oncogenic subtypes. As previously discussed, this finding led to the introduction of a pooled high risk HPV DNA cervical screening test in the UK. The inability of these pooled tests to genotype the more aggressive subtypes, such as HPV 16 and 18, which cause 65% of cervical cancers, may account for the poorer specificity and PPV of HPV testing when it is used to triage low grade cytology (86.5%[155] and 16%[156] respectively). When adjusted for age, and compared to low-risk subtypes, HPV 16 and associated subtypes (HPV 31, 33, 35, 52 and 58) have, respectively, 53% and

38% less chance of immuno-clearance (RR 0.47, 95% CI 0.32 - 0.72 and RR 0.62, 95% CI 0.47 - 0.94 respectively)[157]. Multiple studies support the lower clearance rate of HPV 16 and also of HPV 18[15, 158] but concurrent infection with multiple HPV subtypes does not appear to have this effect[158].

A prospective diagnostic test study reported the 10 year cumulative incidence rate of CIN3 as 17% if HPV 16 was identified, 14% if HPV 18, 3% with other HR subtypes and 1% with low risk subtypes. These findings suggest HPV subtyping may be of benefit for women with a TZ3 to help triage who requires treatment and who can be conservatively managed.

1.7 Null hypotheses and aims of this thesis

The information outlined in this chapter suggests improving the diagnosis of CIN in women with a TZ3 is an important area of clinical research. The aim of this dissertation is to improve the management of women with a TZ3 and the objectives were to:

i. Determine the impact of HPV testing on false positive screening in women with a TZ3.
ii. Investigate factors which affect colposcopists' decision-making when applied to management of a TZ3.
iii. Formulate management recommendations for women with a TZ3, where appropriate, aimed at improving service efficiency and homogeneity of care.
iv. Investigate the predictability of CIN2+ with surrogate biomarkers for HPV and HPV genotyping, in combination with techniques which sample an endocervical transformation zone.
v. Compare the Cervex-Brush alone to a cytobrush and Cervex-Brush to identify the optimal cytological collection device in women with a TZ3.

To address these aims, clinical studies in humans will be used:

i. A retrospective cohort study of 800 women using a clinical database was established and utilized to investigate the incidence of negative LLETZ histology (false positive screening) before and after the introduction of HPV testing. Potential confounders were adjusted for and potential predictors of negative LLETZ evaluated.
ii. Focus groups in one English healthcare region were undertaken to evaluate colposcopists' decision-making when applied to the management of women with a TZ3.
iii. All accredited UK colposcopists were asked to participate in a national survey which aimed to ratify areas of consensus in the management of a TZ3 to aid in the development of guideline recommendations.
iv. A prospective diagnostic accuracy study, the ACORN study, examined;
    a. The predictability for CIN2+ of HPV genotyping and p16/Ki67 in combination with cytological and histological samples in 101 women with a TZ3.
    b. The effect of different immunostaining and diagnostic categories on the predictability of p16 and Ki67 stained histology and cytology slides for CIN2+.

v. Finally, I aimed to evaluate whether the addition of a cytobrush to a Cervex-Brush improves the accuracy of cervical screening in 105 women with a TZ3

The null hypotheses tested are:

i. The incidence of negative LLETZ (false positive screening) is not affected by HPV testing nor TZ type.
ii. In the absence of national guidelines, decision-making in women with a TZ3 is not determined by colposcopists' affect.
iii. In the absence of national guidelines, decision-making in women with a TZ3 is homogenous.
iv. a) The diagnostic accuracy of a routine cervical screening test in women with a TZ3 cannot be improved by the use of (I) HPV genotyping, (II) p16 / Ki67 in combination with cytological samples and (III) p16 / Ki67 in combination with endocervical curettings.

    b) Inter-rater reliability of the cytological and histological samples are not affected by different diagnostic categories or immunohistochemistry categories.
v. There is no difference in diagnostic accuracy nor adequacy rates when a Cervex-Brush alone is compared to a cytobrush and Cervex-Brush in women with a TZ3.

Chapter 2 **Methodology**

2.1 The impact of HPV cervical screening on negative LLETZ

In women with a TZ3 the diagnostic accuracy of the cervical screening test is used to guide management. The 2013 UK introduction of HPV triage of low grade cytology aimed to improve the accuracy of this cytology result and although the NPV has been improved, the PPV is still poor. The aim of this novel study was to evaluate the impact of HPV testing on the incidence of negative LLETZ (false positive screening) and to assess potential predictors such as the presence of a TZ3.

This comparative cohort study was completed at University Hospitals Bristol NHS Foundation Trust, a sentinel site for HPV testing (Primary and HPV triage of low grade cytology). It was a retrospective cohort design; this is defined by the absence or presence of an exposure or intervention (HPV screening in this instance), rather than the absence or presence of an outcome, as with a case-control study. In comparison to a prospective study, this method is achievable within the study time constraints as the outcome and the intervention have already occurred. The disadvantages of this design can include the impact of missing data (accurate documentation is required), selection bias, the need for a large sample size and confounding factors. The use of a randomized control trial which, in this study, would compare women who are offered a screening test which is not as sensitive or specific as the reference standard, would not be ethically appropriate.

I applied for and was granted R&D approval by University Hospitals Bristol on 20th July 2014 (ref OG/2014/4626) and ethical approval by NRES Committee South West – Cornwall and Plymouth, on 30th May 2014 (ref 14/SW/0127); see Appendix 2.

2.1.1 Sample size calculation

To determine the sample size before and after the institution of HPV screening I worked with my statistical supervisor (AW) to perform a power calculation. Alpha or the type 1 error rate is the probability of rejecting the null hypothesis when it is actually true; this was set at 0.05. Beta or the type 2 error rate denotes the power of the study and is the

probability of accepting the null hypothesis when it is actually false. Detecting a true difference >80% of the time is considered satisfactory for medical research[159].

A study can be powered to detect a difference of any size but due to the novel nature of this study clinically relevant mean values and standard deviations were not available to calculate effect sizes. Several power calculations were undertaken assuming a negative LLETZ rate of 14% from Livasay *et al*[160] and examining reductions of negative LLETZ to 7% and 9% (my alternative hypothesis (H_1)). The incidence from Livasay *et al* was used as they reported the mean incidence of comparable studies (retrospective cohort studies prior to HPV testing). The estimated effect size of 50% was based on the improved sensitivity of HPV testing; although I considered that the poor specificity of HPV testing may increase the negative LLETZ rate, the addition of a cytology result in women who test positive for high risk HPV improves this specificity[155].

As I needed to extract the pre-HPV screening cohort data manually, different sampling ratios were assessed (Table 2.1); to show an effect size of 7%, with alpha at 0.05 and power at 90%, I would need to analyse clinical records from 401 women in both cohorts.

Table 2.1: Power calculation for different sampling ratios

EFFECT SIZE	SAMPLE RATIO	POWER	
p1 - p2*	pre:post	80%	90%
9%	1:1	166:166	221:221
7%	**1:1**	**300:300**	**401:401**
9%	1:2	117:234	163:320
7%	1:2	216:432	294:588
9%	1:3	100:300	139:417
7%	1:3	187:561	258:774

**p1 is the proportion of women with negative LLETZ histology in the pre-HPV testing cohort and p2 is the proportion in the post-HPV testing cohort.*

2.1.2 Population

Women aged 25 – 64 who had a LLETZ before the introduction of HPV testing (2007) and after the introduction (2012) were eligible for inclusion. Women outside of this age range are currently not eligible for cervical screening in England. Women who had a LLETZ during the Sentinel Sites Study (2008 – 2010)[91] were excluded; Bristol was a pilot centre for HPV triage in the UK and management of these women, including frequency and type of follow up, was different to national protocols instituted after the publication of this study.

2.1.3 Exposure

Women in the pre-HPV testing cohort were referred to colposcopy for all grades of cytological abnormalities. Women in the post-HPV testing cohort were referred to colposcopy with; i) a positive high risk HPV result as part of primary HPV testing which was then triaged by a cytology test or ii) HPV triage of a low grade or negative cytology result (section 1.4.2). Testing for HPV was by Hybrid Capture 2 which provides a pooled result of 13 high risk subtypes.

2.1.4 Outcome

The definition of a positive LLETZ was a histological specimen which contained CIN of any grade, cGIN or cancer. The definition of a negative LLETZ varies between studies[160-164]; following a review of the evidence base and discussion with the colposcopy multidisciplinary team the definition for a negative LLETZ in this study was *'a histological specimen in which there is no evidence of CIN'*, rather than no CIN / no HPV, because high risk HPV positivity is not a definitive determinant of outcome. As part of routine practice, LLETZ samples reported as negative are reviewed independently by two consultant histopathologists and additional blocks processed to confirm the absence of CIN.

2.1.5 Potential confounders

A confounder is an influence which is separate to the exposure which can account for the outcome. Potential patient confounders which could affect the LLETZ histology by increasing the chance of CIN regression or progression, as outlined in section 1.2.4.3,

include age, smoking status, parity and contraceptive use. Differences in the incidence of negative LLETZ may have been affected by policy changes rather than HPV testing so variables that relate to this were also collected. National guidelines in the UK[85] provide recommendations on intervals from cytological screening to colposcopic assessment, diagnostic standards for colposcopy and criteria for LLETZ. The indications for LLETZ were divided into those who had had a biopsy prior to LLETZ (persistent CIN1 for greater than 24 months, CIN2 / 3 or cGIN) and those who had a see and treat LLETZ (no prior histology). Indications for 'see and treat' LLETZ included high grade cytology with confirmatory high grade colposcopic findings, high grade cytology with a TZ3 or persistent (≥12 months) low grade cytology with a TZ3.

Clinical records and colposcopic images were assessed, by me, to confirm the TZ type and, as specified by the IFCPC nomenclature (Section 1.2.2.1), this was coded as a TZ type 1, 2 or 3 [165]. The size of the lesion (coded out of four quadrants) and to improve analysis, the interval in weeks from cytology to colposcopy and from colposcopy to treatment were coded as 0 - 4 weeks, 5 - 8 and more than nine.

Negative LLETZ histology can be reported if dysplasia is present deeper in the endocervical canal than the LLETZ sampled or if vaginal intraepithelial neoplasia (VAIN) 'contaminates' the cervical cytology. To assess for this, and to validate the negative LLETZ outcome, I recorded and evaluated any post-LLETZ cytological or histological results.

2.1.6 Data collection

To ensure that selection bias is not introduced a target population should be defined and the sample should match that target population. Participants were selected from a colposcopy database by an independent Information Analyst using 'treatment type - LLETZ' and 'appointment date' as search terms. There are different methods of sampling aimed at reducing bias, for pragmatic reasons, I chose cluster sampling. This is where a group from the target population was chosen at random by the Information Analyst and all of that group was used for the sample. I initially searched for the medical records using the list of hospital numbers and then entered the participant's information under an anonymous study number in a separate database.

From 2011 onwards, colposcopist's manually entered all clinic data onto an electronic colposcopy specific database which incorporated all relevant cytology, histology and clinic

findings including photographs of the TZ. The direct electronic transfer of data into Excel should have prevented any transference errors as the quality of data is monitored by the Information and Performance team. Pre-2008, I found data collection more problematic and time consuming; cytology and histology results were stored on an electronic system but clinic findings were stored either in paper medical records or archived discs.

To reduce transference errors and improve the quality of the data collection, double data entry was completed, the first worksheet by myself and the second by two O&G Registrars. To ensure we all entered the same field values and to reduce erroneous or missing data both the registrars were made familiar with the study protocol and I provided training on where to find the data and how to enter it. I constructed a table of definitions (potential confounders, intervention and outcomes – Appendix 4, Table S4.1) and generated an Excel Spreadsheet which included the code definitions for each variable. 'Cleaning' the data in this way simplifies the data and facilitates data analysis. Numerical data was stored as raw data, continuous variables were grouped for ease of analysis and categorical data was given a numerical code. For example, referral cytology was coded as Negative ('0'), Low grade ('1') or High Grade ('2') according to the revised 1986 BSCC Classification System[84]. Two databases were kept; one included the raw data and the second the coded data.

2.1.7 Analyses

To analyse the collected data I compared the double entered data to identify and correct missing or erroneous entries. I described and compared the demographic and clinical characteristics of the participants and calculated the incidence of negative LLETZ in the two cohorts, along with the risk ratio and absolute risk difference.

To assess whether the association between the introduction of HPV testing and the incidence of negative LLETZ histology could be explained by the differences in potential confounders between the two cohorts, multivariable regression models were used. My statistical supervisor guided me through this data analysis to improve my understanding of the complexities of these statistical models and to facilitate discussions on how the data should be processed and analysed.

Relative Risk (RR) and Odds Ratio (OR) are both used to measure the association between an exposure or independent variable (HPV testing) and a binary outcome or dependent variable (negative LLETZ). A relative risk estimates the ratio of two probabilities - the probability of the outcome occurring in the exposed population divided by the probability of the outcome occurring in the unexposed population. So a relative risk of 1.2 would mean that the risk is 20% higher in the people exposed compared to the people unexposed. An odds ratio estimates the ratio of odds in the exposed versus unexposed groups. The odds of an outcome occurring is calculated as the probability of the event divided by the probability of the event not occurring. Most people find odds more difficult to interpret compared to risk, however, the odds ratio and risk ratio are approximately the same when the prevalence of the outcome is low (<10%). When the prevalence (negative LLETZ) is higher than approximately 10% (as in my study), the odds ratio will be further away from the null value of one compared to the risk ratio, making the association appear larger. I have presented the confidence interval for the RR as the inclusion of the null value (1.0) within the confidence interval is a strong indicator of an underpowered study or a lack of evidence to support the alternative hypothesis[166].

When adjustment for potential confounders is needed (as is the case in most observational research), regression models are often used. Logistic and Poisson regression are two types of models that can be used for binary outcomes. Logistic regression allows the association to be presented as an odds ratio whereas a Poisson model will allow a risk ratio to be presented. Logistic regression is most commonly used, almost as a default, because of its statistical properties, for example, sometimes a Poisson regression model will not be possible to estimate in situations where a logistic model will be. Nonetheless, when the outcome is relatively prevalent and it is important to convey risk clearly, a Poisson regression may be preferred.

Two sets of poisson regression models were estimated: the first adjusted for each of the potential confounders in turn and the second adjusted for all potential confounders. I also explored predictors of negative LLETZ in the HPV testing cohort. First, in unadjusted models I examined the association between each of the following with negative LLETZ: age, parity, contraceptive, cytology result, interval from cytology to colposcopy and TZ type. Next, I included those variables where there was some evidence of an association with negative LLETZ ($p<0.05$). All approaches resulted in the same final model. Stata v13.1 (Statacorp) was used for all analyses.

2.2 Evaluation of decision-making in women with a TZ3

This was a qualitative study, utilising a series of focus groups. I applied for and was granted ethical approval by NRES Committee South West – Frenchay on 14th April 2015 (ref 14/SW/0128 - Appendix 2) and gained a small grant (Appendix 1) to cover the cost of the qualitative software (Nvivo; QSR International), expenses such as petrol and parking at the individual trusts and refreshments for the colposcopists.

2.2.1 Method of data collection

Focus groups are used to investigate views or experiences and are distinguished from interviews through the examination of the interaction between participants[167]. The interaction between colposcopists will be useful in providing a broad understanding of the issues involved in managing women with a TZ3. Focus groups can also be useful in stimulating discussion from quiet participants and reducing the interaction of the facilitator[168].

The methodology applied to the focus groups was adapted from the available literature[168-170]:

- Three groups or more.
- Preferably four to six participants per group – smaller groups can decrease group interaction but increase facilitator involvement if there are quiet or disruptive individuals. Large groups (more than 10) can be difficult to analyse if multiple conversations occur simultaneously.
- Homogenous participants; it may be preferable to have groups consisting of strangers to reduce unspoken assumptions that occur within workplaces that will not be recognised or reported by the researcher[171]. However, with a small population this is not always practical. Moreover, grouping cohorts into general population groups such as geographical units (block sampling) can improve interaction by replicating social dynamics[170]. Backgrounds may be similar but attitudes, which is the focus of interest in this study, may not be.
- A semi- structured interview with moderator involvement.

2.2.2 Selection of participants

Sampling was primarily criterion based; I included participants if they were active accredited members of the British Society for Colposcopy and Cervical Pathology (BSCCP). As a Colposcopist, membership is a pre-requisite to practice as the BSCCP standardizes training and audits quality of service provision. The secondary sampling strategy was purposive in nature; this is a selective, non-probability sampling method which is used when the population to be sampled is small. In two waves of recruitment, I emailed the lead colposcopist in each South West of England NHS trust my study information sheet and consent form (Appendix 3), and followed this up two weeks later if I had not received a response. Lead colposcopists are responsible for quality assurance within their department and if they agreed to take part they forwarded the information to all other colposcopists within the unit to request participation (Appendix 4, Figure S4.1).

Colposcopists' intention to participate was confirmed during the emails to organise the focus groups. This allowed an abundant cooling off period (2 -3 weeks) prior to signing the consent form. Immediately prior to the focus groups, I gained written consent from all participants to be audio-recorded and for anonymized quotes to be utilized in publications. The participants were made aware that they could withdraw their involvement at any time but data up until the point of withdrawal would be kept for analysis.

To assess heterogeneity within my study population, such that a range of demographics and opinions were included to improve the generalizability of the findings, data regarding the number of patients assessed in each unit per annum and colposcopists' years of experience and job title were collected. Different health care professionals such as nurses, oncologists, GPs and general gynaecologists can accredit as colposcopists; their background experience may shape their attitudes and opinions and affect the decisions they make. As management protocols and educational experience may vary within the different units, I collected the demographic location of the colposcopists' training centre. To maintain anonymity, age and gender were not collected.

Data saturation[172], such that no new opinions or attitudes were identified, was achieved with a total of twenty-three colposcopists from four centres. At this point, recruitment ceased.

2.2.3 Interview procedure

Free flow conversation provides a wealth of knowledge but to ensure that the research agenda was addressed, a topic guide was used to improve comparisons between groups, prompt the flow of conversation and focus the discussion to allow an exploration of the decisions that are made when reviewing women with a TZ3 and a range of clinical and cytological variables[169]. I designed the topic guide (Table 2.2) following a literature review and discussions with my qualitative supervisor (RB) and three experts in the field (who did not participate in the focus groups). I defined an expert as a colposcopist who was respected and nominated by their peers for their expertise in colposcopy, as these practitioners manage complex as well as routine cases.

These semi-structured focus groups enabled me to cover the core set of questions but also allowed for a flexible and dynamic discussion that could be expanded upon by the participants. To understand the colposcopists' decision-making process I asked the participants to identify the criteria they used in management decisions by asking 'why' and 'how'.

Table 2.2: Focus group topic guide

Unsatisfactory Colposcopy (TZ3) Topic Guide

Conservative management
- Why
- Effect of age
- Effect of parity
- Effect of HR HPV
- Length of follow up
- Place of follow up
- Technique of follow up (what)

Who to treat?
- How
- Where
- Why
- Effect of age
- Effect of parity
- Effect of HR HPV

Oestrogen
- Who?
- How?
- Alternative uses
- Other methods of everting the TZ?

Depth of LLETZ
- How deep?
- Why?
- Who?

Adjuncts to improve diagnosis

Quality monitoring
- Reporting methods
- Interpreting the reports
- Colposcopists' HPV knowledge

Issues regarding colposcopy management
- National guidance
- Patient focused.

Due to the amount of technical content discussed within the focus group, the challenge of conducting these sessions was reduced by being an accredited colposcopist myself. A facilitator (my supervisor or a gynaecology registrar) were also present to aid transcription by recording the speaker order and noting non-verbal communication. The focus groups were conducted in private rooms at the participants' hospital and lasted 40 - 50 minutes. Refreshments were provided to facilitate participation during colposcopists' lunch times.

I wrote my field notes immediately after the focus group to aid in the interpretation of the transcripts and to contextualise the discussion – these, for example, included notes on the environment, the atmosphere and the interaction between participants. All of the interviews were transcribed by myself with the aid of the speaker list. It takes approximately one hour to transcribe ten minutes of audio; in total, 20 hours to transcribe all audio files and a further four hours per transcript to validate, format and correlate with the field notes. This process was important to maximise my immersion within the data.

All participants were anonymised and I sent the transcripts to the respondents and the facilitators for validation. One participants' statement was refined to expand upon how their anxiety of missing a cancer affected their decision-making, but this clarification of their reasoning did not change my original interpretation of the dataset.

2.2.4 Analyses

There are multiple methods of analyzing qualitative data and to find the best fit analysis I undertook a literature review and discussed my findings with an experienced qualitative researcher at the University of Bristol. I selected thematic analysis (TA) as it is one approach that can be used to identify, analyse and organise patterns of opinions within a data set[173]. The experiences of participants can be analysed without evaluating how they experience reality (such as with interpretive phenomenological analysis (IPA)). TA provides the flexibility to allow participants to expand upon their concerns without deviating from the decision-making process (which was the aim of this study). Narrative analysis provides depth on a specific area but not the breadth of information I required from my participants[173].

TA was chosen in preference to grounded theory as data was collected through the use of focus groups (rather than interviews) and the emphasis was on decision-making and not on social processes[172]. Although grounded theory is inductive in nature and useful for areas not previously researched, researchers do not access the literature prior to analysis to prevent it being shaped by preconceptions (reflexitivity) rather than being 'grounded' in the data[174, 175]. However, to design the topic guide and assess whether previous studies had already investigated my area of interest, I completed an extensive literature review. To reduce the reflexitivity of my own experiences and knowledge of colposcopy, my inclusion of qualitative researchers who had no training within colposcopy, nor the existing literature, should have balanced my own unknown preconceptions during data analysis[176].

To complete the thematic analysis I used the six stage TA process, as described by Braun and Clarke[173]. Analysis was conducted iteratively after each interview so that future focus group questions were informed by prior analysis. This helped ensure saturation was reached because the same opinions and adequate depth was attained. This process was used rather than a rigid pre-designed coding structure or framework of behavioural determinants (as with framework analysis) to reduce bias. After familiarization with the data set and extensive discussion with my supervisor and a second postdoctoral qualitative researcher (who had no clinical involvement in the approached colposcopy units), I developed a coding list and we individually coded the first transcript. This coding framework (Chapter 4, Table 4.1) was then applied to future transcripts and revised once more as further datasets were analysed.

To achieve a rigorous analysis, consistency of interpretation was assessed; a qualitative researcher and I independently coded the last three transcripts, compared results, discussed divergences and if disagreements arose, settled these with my qualitative supervisor (RB). The qualitative software package NVivo 10 was used to aid analysis by improving organisation of the data. I then compared the transcripts with the field notes to define tone and potential meaning to the words transcribed. On completion of the coding, I met with the research group to discuss and refine key themes following in-depth consideration of potential alternative interpretations, through the use of mind maps and iterative lists (Chapter 4, Figure 4.1). Illustrative quotes and descriptive accounts were linked to these themes, interpreted in relation to the literature (a semantic approach)[173] and mapped to the relevant theoretical constructs within this framework.

2.3 Developing a questionnaire for evaluating TZ3 management

To expand upon the outcomes from the focus groups I developed a questionnaire which aimed to assess UK colposcopist's decision-making when applied to women with a TZ3. The incorporation of questions (items) which have been developed for questionnaires which evaluate different areas (domains) of interest - for example using items which assess gynaecologist's management of women with menorrhagia - may not measure what I intend them to measure. Furthermore, the use of items which assess the domain of interest but are developed for a different population can reduce the validity of the questionnaire as the items may not be interpreted by the new population in the same way[177].

I applied for and was granted ethical approval by NRES Committee South West – Frenchay on 14th April 2015 (ref 14/SW/0028). To develop my knowledge base I attended a University of Bristol *'questionnaire design and validation'* course, I completed an extensive literature review and discussed my study methodology with a statistician and a trial methodologist whose research has focused on the development and application of patient-reported outcome measures.

2.3.1 Questionnaire design

To form a conceptual framework I collated the domains and corresponding items from the focus group outcomes and from the existing literature. The black script in Figure 2.1 illustrates the initial conceptual framework. Following review of this initial framework by experts in the field (section 2.3.2.1 – content validity), I developed the items after assessing the literature which describes this methodology[178-181].

Items were written in one sentence where possible, asked for one piece of information and ambiguous words such as 'frequently' and 'regularly' were excluded. Closed responses were used to facilitate coding and analysis. The format of the items included binary responses (Yes/No), multi-nominal or discrete responses and a matrix of dropdown options so that respondents could evaluate several items using the same set of measures. Initially I did not include Likert scales, as although easy to understand and analyze, the aim of this study was the development of national guidance based on consensus opinion. To facilitate this, ordinal scales can provide less ambiguous answers (Section 2.3.3:

Reliability). To triangulate the outcomes with the focus groups, I included a freehand textbox with each question.

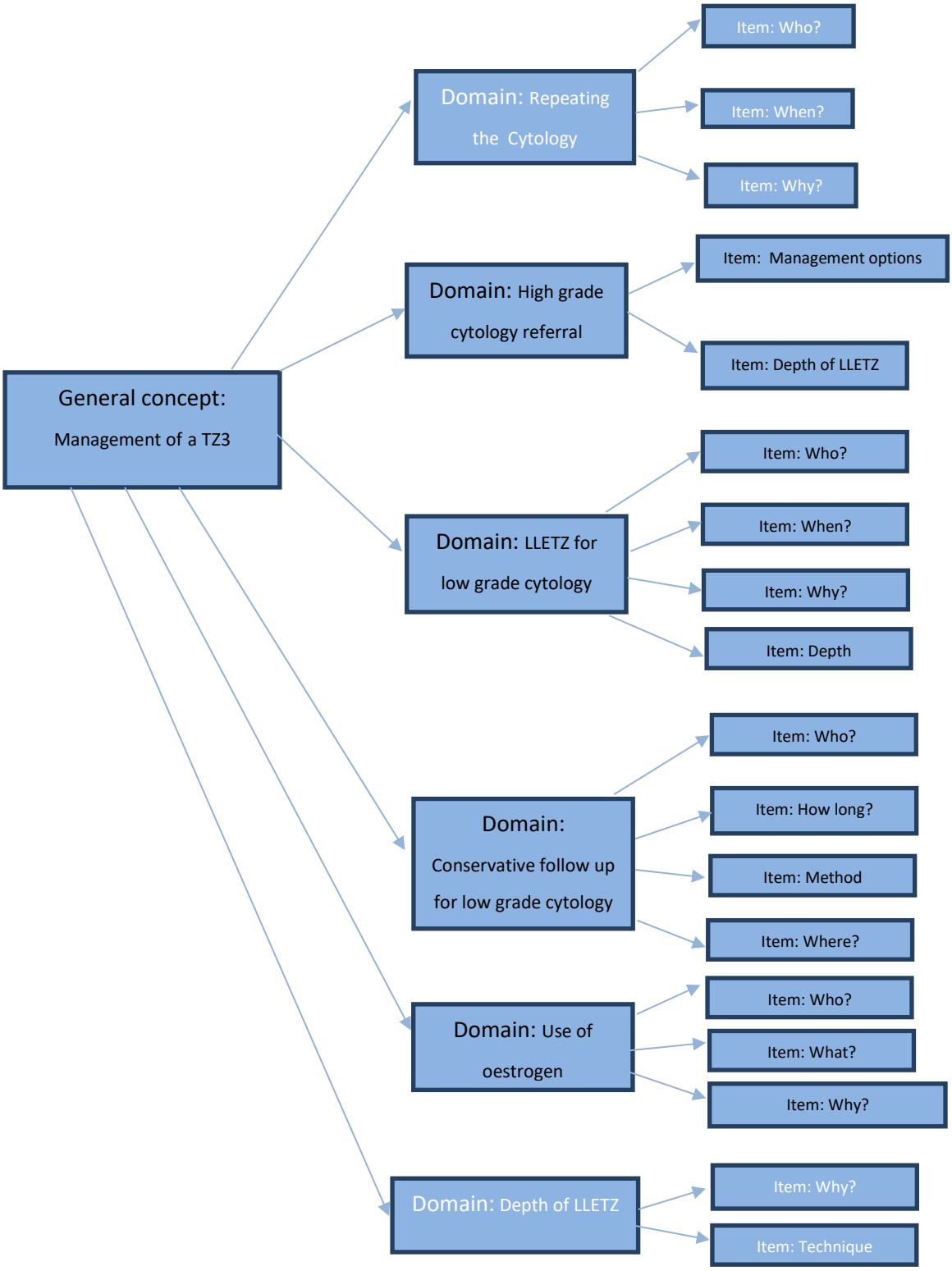


Figure 2.1: Questionnaire conceptual framework: The initial framework (black script), the domains that were added following the Delphi consensus (white script) and the corresponding items which assessed each domain.

2.3.1.1 Potential predictors of decision-making

Patient age and parity were major factors which had affected decision-making in the focus groups and I deliberated on how to categorize these variables for the survey. Although progression of CIN is comparable in older and younger women with low grade cytology, this risk is increased in older women with high grade cytology[182]. Parity is a risk factor for progression and this tends to be higher in older women[60]. Furthermore, the RCOG classification of 'advanced maternal age' is women aged ≥40[183] and the HPV primary screening proformas used in the sentinel sites advised LLETZ, irrespective of the cytology result, in women aged ≥40 who had a TZ3 and high risk HPV (Appendix 4, Figure S4.2). For these reasons the final demographic categories were:

- 25 - 39, no children
- 25 - 39, family not complete
- 40 years or older, family not complete
- Completed Family, any age

A sub-theme identified in the focus groups was the endorsement of a shallower LLETZ than nationally recommended[35] (Section 4.3.4.5). To provide targeted guidance I wanted to clarify if this was restricted to regional practice and/or limited to a specific patient demographic. Categories for depth of LLETZ included ≤6mm, 7-10mm, 11-15mm and ≥16mm; these were based on the outcomes reported by Castanon *et al*, 2014[109] who linked depth of LLETZ to risk of preterm labour (Section 1.5.4).

Respondent demographics included job title, years of experience in colposcopy and gender. The initial categories for job title were general gynaecologist, gynaecological oncologist, nurse colposcopist and gynaecology registrar. In the focus groups these professionals placed different emphasis on the factors that affected their decision-making and this information may be useful for guideline implementation.

Correlation of experience with management decisions may be valuable for guideline development and it could be argued that more weight should be applied to recommendations from health care providers who have more experience in this area. However, measuring competence and experience can be problematic; most decision-making in colposcopy contains limited variables and is formulaic, all colposcopists have to

attain the same basic competencies and re-accredit three yearly. If multiple items that assess competence are included, the rate of missing items would increase.

Health Care Professional's gender has been shown to influence clinical decision-making; female clinicians can have longer consultation times, especially when speaking to other women[184, 185], and more of this time is devoted to counselling the patient whereas male colleagues may spend more time discussing technical aspects[186]. As a major outcome of this study was to assess whether conservative or surgical management options are initially offered, evaluating the association between gender and management choice would be useful for guideline implementation.

2.3.1.2 Structure of the questionnaire

The most important items were placed at the beginning of the questionnaire. Demographic questions, such as gender, can be considered intrusive if they are not contextualised. They are also the least interesting questions for respondents who may become disengaged if they are answered first. A long questionnaire with multiple items testing the same domain can reduce response rates and increase the risk of false positives[180] as respondents may become 'tick happy'. To adjust for this, the questionnaire was initially restricted to 14 items with a maximum of three items testing the same domain; see Table 2.3

Table 2.3: The domains and items for the first version of the questionnaire

DOMAIN	Corresponding Items
Initial management of low grade cytology and a TZ3	*Practice:* 1 multi-nominal
	Attitude: 1 multi-nominal
Repeating the referral cytology	*Attitude:* 1 discrete response
Conservative follow-up for low grade cytology	Practice: 1 multi-nominal
Depth of LLETZ in women with low grade cytology and a TZ3	*Practice and knowledge:* 1 discrete response *Attitude:* 1 multi-nominal
Initial management of high grade cytology and a TZ3	*Practice:* 1 multi-nominal
Depth of LLETZ in women with high grade cytology and a TZ3	*Practice and knowledge:* 1 discrete response *Attitude:* 1 multi-nominal
Non-routine measures to improve the adequacy of the examination	*Practice:* 1 discrete response
Use of oestrogen	*Attitude:* 1 discrete response
Respondent demographics	*Job title & gender:* 2 discrete responses *Years of experience:* 1 continuous scale

2.3.2 Validity

2.3.2.1 Content validity

This is the extent to which the questionnaire measures all domains relating to the construct of interest. Eight experts in the field reviewed the initial conceptual framework; colposcopists who were respected and nominated by their peers for their clinical or quality assurance experience. This panel consisted of six Consultants (three general gynaecologists and three gynaecological oncologists) and two nurse colposcopists from three different NHS Trusts within the South West England Region. Three were male and five were female, three were leads for quality assurance in their departments, two were lead colposcopists and two had published recent relevant literature in the field.

In a modified Delphi technique[187] (yes /no responses were used rather than a Likert scale) I asked these experts to include or exclude domains which they felt encompassed all possible decision-making options for this cohort of women. This method is a practical way of bringing together experts who have competing clinical duties and work in separate trusts. The first round of feedback added two additional domains which can be viewed as the white script in Figure 2.1.

Repetition of cervical cytology within three months of the referral sample is not recommended by the NHS CSP[35] but some of the experts felt there may be scenarios, such as absence of endocervical cells on the cytology report, when a repeat test may be taken. The second stage of feedback led to a clear consensus on the included domains.

Following development of the questionnaire, the first version was emailed to the BSCCP for review. The committee consists of experts in the field; health care providers with experience in colposcopy and related research, cervical screening quality assurance and questionnaire development. The second version, as accepted by the BSCCP committee, consisted of 14 items (Section 5.3.1.1).

2.3.2.2 Face validity

To improve the robustness of the study and response rates, face validity of the second version was assessed by colposcopists from the South West England Region, which I chose for logistical reasons. Face validity is the extent to which the items are interpreted as intended and incorporates clarity of wording and layout. There is no accepted consensus for sample size in this stage of psychometric evaluation (average 10-20)[178-181]. To reduce prior sensitization I aimed to recruit colposcopists who had not assisted with the conceptual framework or participated in the focus groups. I emailed these potential participants a PIS and consent form (Appendix 3) and followed this up two weeks later if I had not received a response.

In a private setting, I gained written consent and observed these colposcopists completing the questionnaire. I interviewed them to evaluate their interpretation of the items, the layout (spacing, font and graphics), sequencing, terminology and phrasing. 'Closed' items were evaluated for omnicompetence. Data was transcribed verbatim. Acceptability of the items was ratified by assessing the response rate and any missing data. Items were considered for deletion or rephrasing if 10% of participants did not complete an item or

more than 50% of participants scored either the highest or lowest values (floor/ceiling effects) i.e. responses were not evenly distributed.

2.3.2.3 Construct validity

This is the association between the questionnaire and evidence relating to a TZ3; it is another method of assessing *'how much the questionnaire measures what it intends to measure'*[188]. For example, current BSCCP standards[35] denote that cytology samples should not be repeated at the first appointment and a deeper excision is required to remove a TZ3 - albeit with the caveat of considering the higher risk of treatment related morbidity in childbearing women[85] (Sections 1.5.1 & 1.5.4). With a paucity of guidance relating to a TZ3, I compared respondent's choice of treatment depth and the proportion of respondents who repeated the referral cytology to the above BSCCP standards. To complete this psychometric component I used the datasets from the test-retest reliability participants (Section 2.3.3.1); Fisher's exact test was used to evaluate this unpaired categorical data.

2.3.2.4 Other considerations

- *Convergent validity:* This measures the relationship between the questionnaire and the hypothesis surrounding the construct it is measuring i.e. how well does the questionnaire and theory converge or reflect things that are known to be true. This could not be evaluated as there is no gold standard questionnaire or indeed any other questionnaire which assesses these concepts.
- *Criterion validity:* This is the association between the questionnaire and an existing gold standard. This could not be assessed as there is no gold standard to compare against.

2.3.3 Reliability

2.3.3.1 Test-retest (stability)

The COSMIN 2011 checklist, which evaluates the methodological quality of studies assessing measurement instruments, was used to determine if the final version of the questionnaire provided consistent and reproducible results [189]. Repeat measures can be by the same participant (intra-rater reliability), as in this study, or independent measures of the same variable (inter-rater reliability)[190]. Using Glimmpse online software, which

specializes in sample size calculations for repeated measures, 19 participants were required based on a two tailed test (negative values are also of clinical interest to determine if respondents are misinterpreting the questions) with an effect size of 0.8, alpha of 0.05 and power of 0.9.

To reduce sensitization of the data, I recruited colposcopists who had not attended the focus groups. These colposcopists were sent a PIS and consent form (Appendix 3) and those that agreed to participate were asked to complete the same questionnaire two weeks apart. Too short a timeframe may allow participants to remember their previous answers and too long may lead to a change in the stability of the participant's clinical practice. Two weeks has been reported as an optimal timeframe[191] and if participants reported a perceived change in their standard management practice prior to completion of the second questionnaire, they were excluded. Test conditions were similar for both questionnaires; participants were asked to complete them in a quiet environment at a time when they would not be rushed.

2.3.3.1.1 Analyses

When measuring levels of agreement that are not due to chance between discrete variables (ordinal or nominal data), a Kappa statistic is commonly used. For ordinal variables such as a Likert scale, a weighted Kappa was used; as unlike an unweighted kappa it does not treat all disagreements similarly. For example, in an unweighted kappa analysis of a 5 point Likert scale a score ratings of three on one day and two on another would be considered as complete disagreement whereas in fact they are close and may be considered to partially agree. A Kappa value of zero is agreement that occurs due to chance, values of 0.61 - 0.80 suggests 'very good' agreement and 0.81 - 1.00 'excellent' agreement[192]. A linear weighting scheme was used; this is common for ordinal data as it allows those observations that do not agree absolutely, but agree a little, to contribute some agreement. All analysis was done using Stata v13.1 statistical software.

2.3.3.2 Parallel forms reliability

This assesses whether the same outcomes are identified when multiple items that measure the same construct are administered to one group and the same number but a different variation of question on the same construct is given to a second group. The aim

is to observe similar scores between groups. This is a labour intensive approach as multiple items on the same construct need to be developed, validated and administered. It also assumes there is a large population to recruit respondents from - in addition to those who will be recruited to complete the final questionnaire. For these reasons, I chose to evaluate test-retest reliability.

2.3.4 Other considerations

2.3.4.1 Internal consistency

This is the extent to which items within a scale are inter-related. This methodology is less time consuming than parallel forms, inter-rater or test-retest reliability as only one data set is required. The disadvantage is that multiscale items (Likert scales) are required [193] and in my questionnaire discrete responses or multi-nominal scales were used to facilitate the identification of a consensus opinion.

2.3.4.2 Sensitivity

Some surveys are designed to detect a change in symptoms, attitudes or behaviour over time. To measure this, patients complete a questionnaire before and after an intervention or at different points in time (a cross-sectional design). As the focus of interest in this study was the opinions and behaviour of colposcopists at one set point in time, sensitivity analysis was not applicable.

2.4 Current Practices in the management of a TZ3: A UK Survey

Following development of the questionnaire I used quantitative analysis to describe the responses and to identify areas of consensus and discordance.

2.4.1 Administering the questionnaire

Survey Monkey software was used as the online platform for the final version of the questionnaire. Although formatting of the questionnaire for this software was time consuming, this platform maintained confidentiality and prevented breach of data protection laws as I sent a cover letter by email (Appendix 4), which contained the URL and study information, to the BSCCP secretariat who forwarded it to their members. Telephone conversations are time consuming and participants can be distracted and

terminate the call. Mailed questionnaires are also time consuming, expensive for the researcher and missing items will affect the statistical power.

Electronic methods are an effective way of surveying a large cohort, require minimal expense and can be designed so that all the items need to be completed. I presumed that all participants had access to a computer as email addresses are provided to the BSCCP. A Cochrane review[194] assessed 481 RCTs and concluded that survey response rates can be improved by the following variables (which were included); addition of the researcher's contact details, position and an explanation of the importance of the research in the cover letter, resending of the questionnaire at two weekly intervals, the use of text response rather than visual, a white background and a deadline.

1200 BSCCP accredited colposcopists were emailed the invitation to participate on March 24th 2016 and a reminder was sent by the BSCCP secretariat two weeks later.

2.4.2 Sample size

The larger the population sampled the smaller the sampling variation (margin of error) and the smaller the chance that the questionnaire results will differ from the true population average. Table 2.4 shows the association between the number of respondents and the margin of error. For example, if 500 respondents answered the question relating to repetition of the referral cytology and 90% adhered to national guidance I could be 85.5 – 94.5% certain this reflected practice within the UK.

Table 2.4 Association between number of respondents and margin of error

Respondents (n)	Margin of error (%)
50	14.1
100	10.0
200	7.1
500	4.5
1000	3.2

2.4.3 Data input and classification of variables

As with the database in the negative LLETZ study, to aid data input into the statistical software package, Stata, each item was coded and a table of definitions compiled. An ExCel database for entering the codes was designed prior to validation of the questionnaire. Before data was transported from Excel into Stata (for statistical analysis) I checked and 'cleaned' it; assessed for missing or incorrect entries. Variables were classified as nominal, ordinal or continuous to aid identification of the appropriate statistical tests.

2.4.4 Analyses

A range of descriptive statistics were used to describe the frequencies and percentages from each item. McNemar's test was used to compare paired categorical variables and a chi squared test or Fisher's exact test (for variables that had cell counts less than five) were used for unpaired categorical variables. Cochran's Q test was used to assess for equality of proportions. A risk difference with confidence intervals was used to describe the difference in proportions.

Multivariable logistic regression was used to assess the association between management decisions and respondent demographics such as years of experience, job title and gender. An odds ratio (OR) was used to report the strength of the association between the exposure and the outcome variables; it reports the odds that a specific outcome will occur in the presence of an exposure variable compared to the odds of the same outcome happening in its absence. If the ratio equals 1 there is no difference between the two arms. If the OR is <0.5 then, for example, the odds of an outcome, such as repetition of the referral cytology outcome in women with a TZ1 (exposure) is 50% less than in women with a TZ3.

An odds ratio was used as the information in this study is for clinicians and not patients; communicating risk is not as important as in 2.1.7. Furthermore, as lots of models will be required and poisson regression can be unstable, logistic regression (which uses OR to report association) was used.

2.5 The use of biomarkers and HPV genotyping to improve diagnostic accuracy in women with a TZ3

2.5.1 Study design and population

This prospective diagnostic accuracy study was conducted in a single NHS Trust in the South West England region from August 2014 – February 2016. To plan the methodology I used the QUADAS checklist for diagnostic studies, applied for and was granted ethical and R&D approval, respectively, by NRES Committee South West – Frenchay on 25th May 2014 (ref OG/SW/0028) and University Hospitals Bristol NHS Trust (ref OG/2013/4461) respectively (Appendix 2). To cover the cost of the immuno-stains, genotyping and supervision of a laboratory technician I applied for two grants (Appendix 1).

Women 25 - 64 years of age referred to the colposcopy clinic with a range of cytological squamous abnormalities, who were having a LLETZ for a TZ3, were approached for participation. All women had high-risk HPV as Bristol is an NHS CSP 'sentinel site'; chosen to institute Primary HPV Screening from 2012 prior to the national roll-out in 2019[195]. These excisions may have been a 'see and treat' LLETZ for high grade cytological abnormalities or a pre-booked LLETZ for persistent low grade screening.

Eligibility (presence of a TZ3) was assessed prior to LLETZ. Participants were excluded if;

- Endocervical sampling had occurred within the previous twelve weeks as this increases the risk of false negatives.
- The referral cytology was glandular - ECC can affect the distinction between adenocarcinoma *in situ* and invasive adenocarcinoma[129]
- A pregnancy test was positive - ECC can increase the risk of miscarriage, preterm labour and/or infection[196]. Although the use of a cytobrush in the first trimester is reported to be safe[131].
- The potential participant was immunocompromised - ECC is thought to increase the risk of ascending infection[120].

2.5.2 Sample size calculation

These are rarely reported in diagnostic studies as it is difficult to conceptualise the effect size in primary studies. My aim was to estimate accuracy with precision and therefore a power calculation was performed. As the NPV for HPV testing is >99%, the sample size was calculated to detect adequate test sensitivity of 0.95 - alpha was set at 0.05 and power at 95%. Outcome data from the negative LLETZ study (Chapter 3[197]), which assessed the histological outcome in women with a TZ3 and high risk HPV, was used to calculate the sample size. It was estimated that 97 complete data sets would be needed based on 47/72 (65.3%) of women who had CIN2+ with a TZ3 [198].

2.5.3 Recruitment

Women with high grade cytology were sent participant information sheets and consent forms (Appendix 3) two weeks before their appointment. I then checked the clinic lists daily for potential participants and approached them for written consent after they 'booked in'. In my unit the majority of women with low grade cytology and a TZ3 are offered cytological follow up and then a LLETZ 6 - 12 months later if dyskaryosis is still persistent. I kept a record of these women and sent information sheets with their appointment letters if a LLETZ was recommended (see Figure 2.2 for the recruitment flowchart).

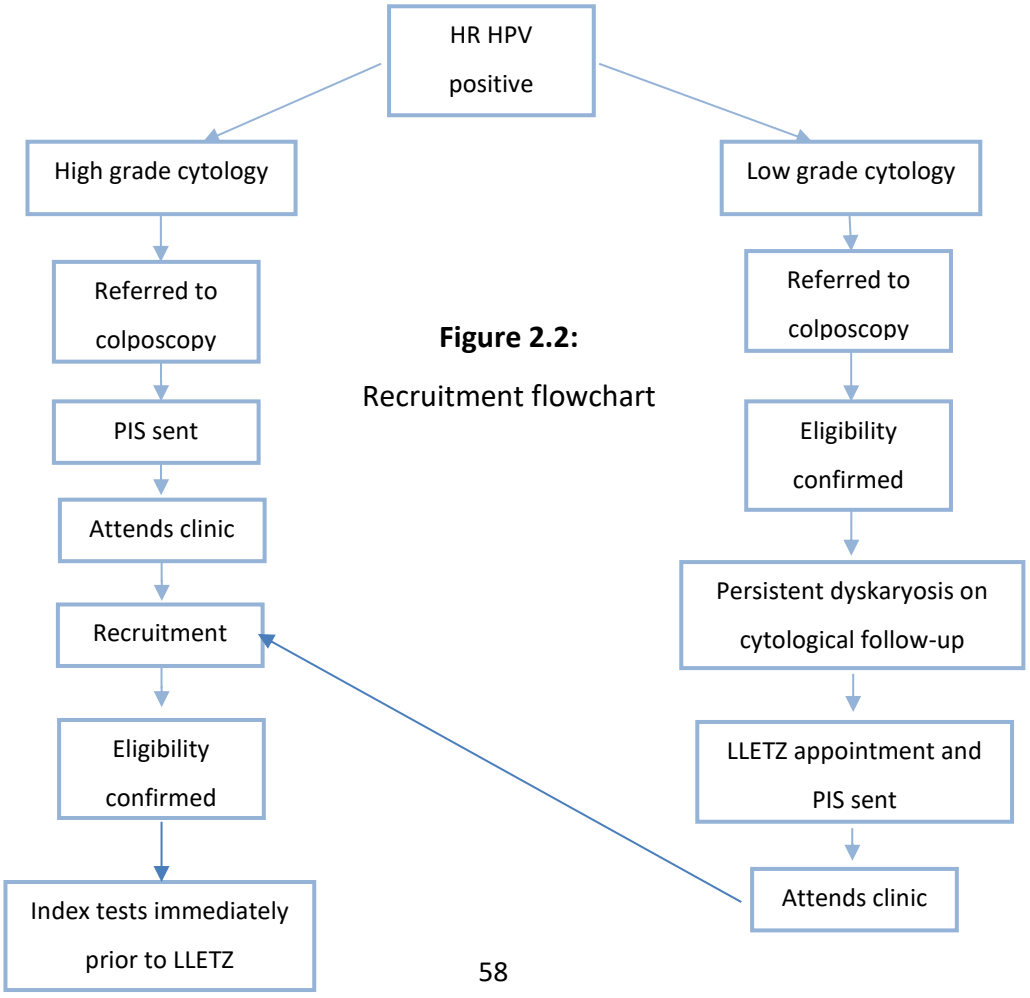


Figure 2.2:

Recruitment flowchart

Data collected on co-variables included age, parity and smoking status as these factors can affect HPV clearance (Section 1.2.4.3). Age was grouped as 25 – 39 years and ≥40 (based on the RCOG advanced maternal age criteria[183] and the 2012 HPV primary screening treatment protocol – Appendix 4). Hormonal status (atrophic changes can affect the cytology result), smoking status, the referral screening test result, ability to pass the cytobrush and curette, interval in weeks from the referral cytology to the index tests and the LLETZ result (including presence of the TZ, histological limiting factors and depth) were also recorded.

2.5.4 Sample taking

Figure 2.3 illustrates the techniques and order of endocervical sampling.

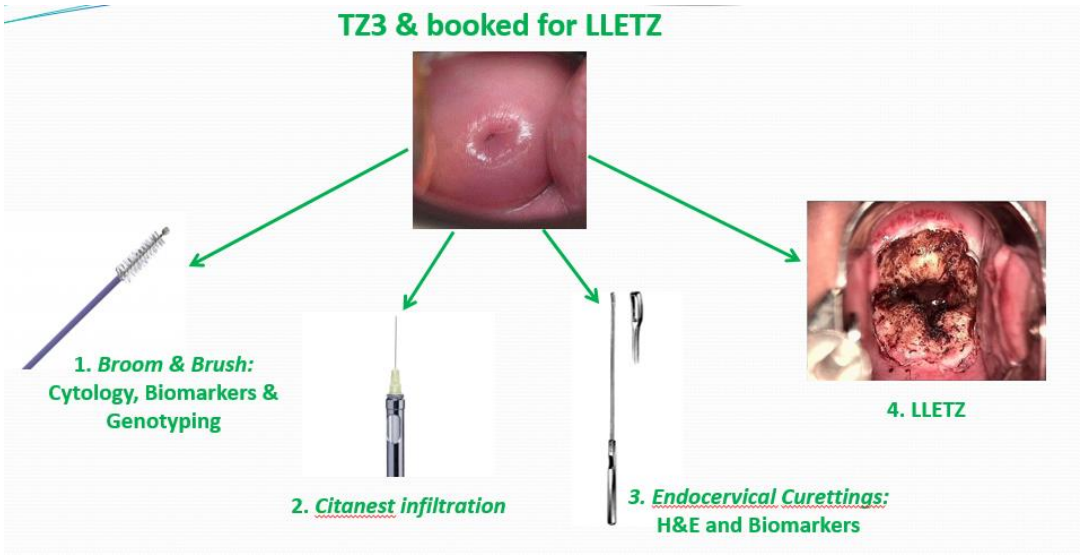


Figure 2.3: Order of endocervical sampling

Trauma to the fragile endocervical cells by a cytobrush can increase blood loss leading to false negative or inadequate cytology samples[199]. To reduce this risk I used a standard Cervex-Brush (Rovers^{R} Cervex-Brush; 70671-001) to sample the ectocervix prior to the use of a standard Cytobrush (Medscope Cytological Brush). The cytobrush was inserted in the endocervical canal to a depth of 2.0cm. Both cytology samples were stored in the same

LBC container (PreservCyt^{R} 20ml solution; 70097-003; Hologic UK) and labelled with the participant's study number only.

Citonest (x2-3 ampules) was infiltrated, as for standard LLETZ. ECC was performed with a Kevorkian Young Biopsy curette which incorporated a 12 x 3mm basket (Wholesale Surgical Instruments Inc; 6-12760) to improve collection of the epithelial fragments and to aid in gauging depth. Curettings were taken from four quadrants (anterior, posterior, right and left lateral), from 2.0cm distally to the external os. The curettings were stored in 4% formaldehyde (30ml CellStor Pot, CellPath) and labelled with the participant's ID.

Routine LLETZ was then performed in one pass (to reduce distortion) and to a depth of 15 - 20mm, in line with BSCCP recommendations[35], and to allow an accurate comparison with the curettings. LLETZ was chosen as the reference standard as this is the gold standard diagnostic test and treatment method for women with CIN. All index and reference standard tests were completed by myself.

Although I had chosen four quadrant ECC rather than circumferential sampling, to facilitate correlation with the LLETZ histology, the Ethics committee were concerned that the curettings would affect the integrity of the LLETZ, changing standard post-LLETZ management. I suggested to the committee the recruitment of five women booked for a hysterectomy who had a TZ3 and normal cytology. The histology could then be assessed for denudation before further participants were recruited. If more than two of the five samples showed epithelial stripping that prevented satisfactory analysis of the cervical epithelium, the study was to be terminated. Two participants were agreed upon as epithelial denudation of a LLETZ, secondary to handling of the tissue, is a recognized complication of standard excision.

The PIS, consent form, clinic advertising, GP letters, checklists and participant letters that I designed can be viewed in Appendix 3 and Appendix 4. In addition, data collection proformas, lab proformas, safety assessment forms and clinic folders all needed to be completed which meant the first patient was recruited in August 2014.

2.5.5 Sample processing

2.5.5.1 Referral, index and dual-stained cytology

The referral LBC sample was either initially tested for HPV DNA by HC2 which contains 13 high risk HPV DNA probes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and then had routine ThinPrep cytology processing as per the HPV primary screening protocols or had HPV triage (with HC2) if the sample was reported as low grade dyskaryosis.

The index cytology had routine Hologic ThinPrep processing and then immunostaining according to the manufacturer's instructions, using the CINtec (dual-stained p16 & Ki67) Cytology Kit (ref 9521, Roche, mtm laboratories). Both of these tests are completely automated. For dual-stained cytology the LBC bottle is centrifuged, an aliquot removed and 'stamped' onto a slide, minus the 'circle' that is synonymous with Thinprep. These slides can be stored at room temperature for three days before immunostaining to allow concurrent runs of 100 samples. The immunocytochemistry assays come in pre-prepared containers ('kit') which are slotted in prior to each run. This takes approximately 3 hours.

Primary mouse monoclonal antibody (E6H4) directed at p16 and a rabbit monoclonal antibody directed at Ki-67 are added at room temperature and processed over 60 minutes. Secondary reagents include horseradish peroxidase (HRP) which converts 3,3'-diaminobenzidine (DAB) to produce the brown p16 staining and a reagent which joins to alkaline phosphatase to produce the pink/red Ki-67 staining by converting Fast Red chromogen. To validate the procedure, a control slide (containing positive and negative p16 and Ki67 cells) was used for each staining run.

The only part of the process which is not automated is the cover slips; aqueous solution is applied to the slides to protect the cells from xylene / alcohol. The slides are dried in an oven as air drying can lead to cracking of the aqueous solution and difficulty in interpreting the slides (Appendix 7, Figure S7.1). The slides are then placed in xylene for a couple of minutes and glue is applied. Xylene is the final processing step and removes debris from the slide. This liquefies on top of the xylene allowing a cover slip to be applied and air bubbles to be 'brushed out'.

There were initial difficulties in acquiring the CINtec kits. ROCHE, an American company, is the producer of these kits and studies are currently in progress to assess the viability of this dual-staining process. Multiple correspondence was needed between myself and the

board at ROCHE to justify the purchase and use of their product before the company released their own studies. An agreement was reached that I could purchase immunostaining kits, which process 100 slides, at a cost of £3819.71 per kit. ROCHE would absorb the cost of the slips and any other reagents as part of their current study, 'The Sentinel Study', which is assessing the use of dual-staining in women with low grade cytology. In exchange, they requested that ROCHE be acknowledged on any future publications.

2.5.5.2 HPV genotyping

The virology department at Manchester Royal Infirmary is the only laboratory in England who currently test for the full HPV array. To facilitate testing, I organized a material transfer agreement between North Bristol Trust (where the cytology samples were stored) and Manchester Royal Infirmary.

As recent studies have shown that high risk HPV is detected in samples with scanty TZ sampling[200], I took a 0.5ml aliquot (Figure 2.4a) for HPV genotyping after the routine cytology and dual-stained slides were processed. I took these aliquots to Manchester and observed the genotyping process under the supervision of Dr Alex Sargent, Consultant Virologist. Papillocheck (Greiner Bio-one) is a micro-array based kit which is used to detect the E1 gene of the HPV genome. Target HPV DNA was amplified by PCR and hybridized to specific DNA probes (as outlined below) to detect 24 HPV genotypes (6 low and 18 high risk; 6, 11, 40, 42, 43, 44/55 & 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82). Analyte cutoff ratios of ≥1 were reported as positive.

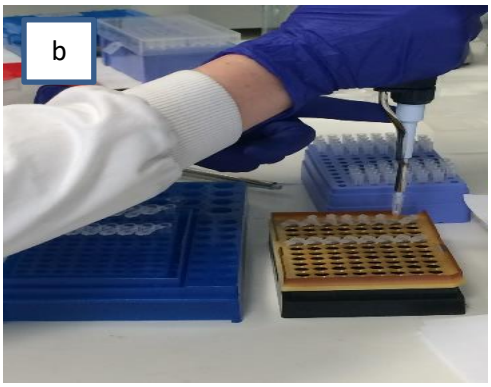
Viral and DNA extraction were done in air extraction stations in a 'clean' room to reduce contamination. 250µl of the study sample was added to a HPV primer mix which amplifies the DNA fragments. The first step is thermocycling of the sample/primer mix; BioMerieux EasyMag system has specific cycles (temperature / length) for different viruses – for HPV the cycle takes 3 hours. The PCR sample was then stored at 4^{o}C overnight to prevent degradation.

The following day the amplified PCR samples were vortexed and 5µl added to 30µl of hybridization buffer (Figure 2.4b). To prevent cross-contamination, the pipettes were disposed of between samples. The PCR / buffer mix was left for 15 minutes, during which

time a computer template of the PCR scanner 'chip' was produced to facilitate the automated transfer of results. Ten study samples could be processed in the same run.

To remove unbound DNA and reduce interference on the PCR scanner, the hybridized mix is buffered (washed vigorously) - Figure 2.4c. The sample is pipetted onto a chip containing DNA probes which hybridize to the corresponding amplified DNA, labelling it flourescently. The chip has 12 windows (DNA-microarrays) – 10 for study samples and a positive (HPV 16) and negative control (Figure 2.4d).

When pipetting the PCR sample onto the chip, if the pipette or technician touch the chip the process has to start again due to contamination. The chip is stored securely for 15 minutes to allow binding as even gentle vibrations of the bench can prevent this process. The chip is then inserted into an automated Greinier Bio-one PCR scanner (Figure 2.4e). The fluorescent light from the bound products is detected using the CheckScanner at excitation wavelengths of 532 and 635nm. The results are evaluated by CheckReport analysis software and a result provided for each of the 24 subtypes.



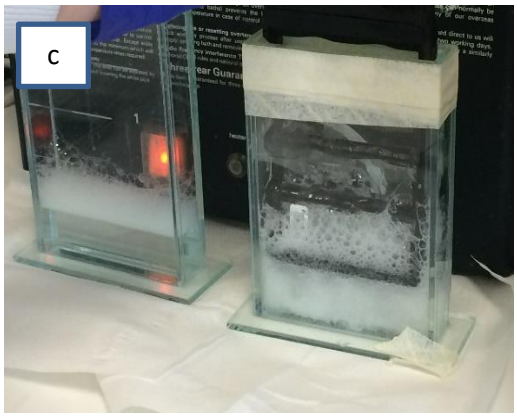


Figure 2.4: HPV genotyping

a) study aliquots for genotyping

b) hybridization of the PCR sample

c) buffering to remove unbound DNA

d) 'chip' containing DNA probes

e) PCR scanner

2.5.5.3 Endocervical curettings

The curettings were processed in batches of ten at University Hospitals Bristol NHS Trust, by myself, under the supervision of a senior laboratory technician. Processing was dependent upon size. In stage 1, samples <1mm were centrifuged, the formalin discarded, liquified agar jelly pipetted over the remaining pellet and the sample transferred into a cassette before the agar could solidify. If the pellet was poured directly into a cassette without the agar, the sample would fragment and dissipate during the dehydration process. A potential limitation of this stage was the speed in which the agar solidified within the pipette; if I was slow in transferal to the cassette, scattered (possibly dysplastic) cells could be retained within the pipette leading to false negative samples.

If the sample was ≥1mm it was poured directly from the histology pot through a funnel into a muslin bag. The funnel needed to be rinsed with formalin to ensure tiny fragments were not retained and to prevent cross contamination with any subsequent samples (Figure 2.5a). The bag was then placed in a cassette and put through the first automated processing run (fixation and dehydration) prior to impregnation with wax. This run consisted of:

- Formalin – 68 minutes - to fix the tissue
- 85% ethanol – 70 minutes – to remove the water in the tissue
- 80/20 Ethanol/Isopropanol – 140 minutes – to dilute the ethanol to isopropanol which is gentler on the tissue
- Isopropanol (IPA) – 210 minutes – to clear the ethanol from the tissue and make it miscible with wax
- Wax impregnation – 210 minutes

Stage 2 was the processing of the fixed and dehydrated tissues into a solidified wax block (Figures 2.5b and 2.5c). The cassettes containing the tissue suspended in agar jelly were covered with molten wax and placed on a cold plate to solidify. The cassettes containing the muslin bags were covered in molten wax and transferred to a warm plate to prevent total solidification. This allowed transfer of the wax encased tissue fragments from the bag to a second cassette. Another potential limitation of this processing method was the speed of wax solidification - to prevent dysplastic cells 'sticking' to the forceps and being excluded from the final sample, the forceps were warmed between the transfer of each tissue fragment and a fresh pair used for each participant to reduce the risk of cross contamination.

Stage 3 was slicing of the blocks to provide levels for staining. Due to the expert nature of this stage and the high probability of destroying the samples during slicing, this process was completed by the laboratory technician. The blocks were kept on a cold plate to improve handling and four levels (of 3μm depth) were cut (Figure 2.5d); the first for H&E, the second for p16 staining, the third for Ki-67 and the deepest level for a second H&E. The second H&E slide was to assess if dysplastic fragments were 'hiding' deeper within the block. To improve transfer of the wax slices onto the slide they were placed in a warm bath to prevent crinkling of the wax (Figure 2.5e) and baked at 60^{o} for 25 minutes to improve adhesion.

As with the cytology samples, a control slide was used for each staining run. Primary antibodies against p16 and Ki-67 were applied prior to the secondary antibody application, which resulted in the distinctive staining pattern. The slides were cover slipped by an automated machine. To assess the slide quality, I checked them against the block to ensure the general size of the sample was the same and to ensure the slides were numbered with the correct participant ID.

2.5.5.4 LLETZ

Routine processing and reporting of the LLETZ was undertaken and the six Histopathologists were unaware these women were enrolled in the study.

All samples were processed within two weeks of collection to allow speedy correlation of the study outcome and the LLETZ result. If a mismatch occurred, such as the index tests reported a higher grade of CIN than the reference standard, the case was reviewed at a multidisciplinary meeting.

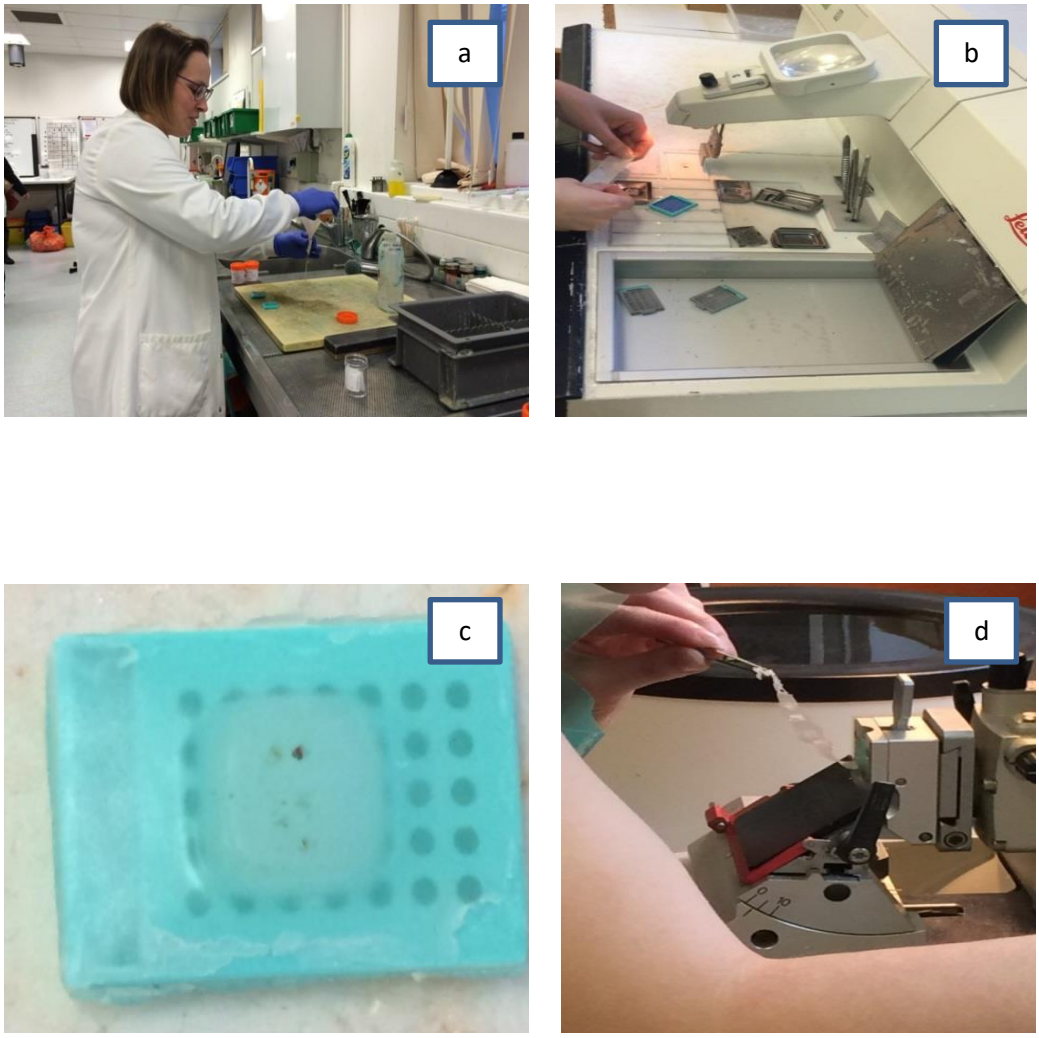


Figure 2.5: Processing of the endocervical curettings

a) transfer of the curettings into the cassettes

b) transfer of the fixed and dehydrated tissue into wax blocks

c) wax blocks containing tiny tissue fragments

d) cutting of the levels

e) transfer of the levels onto slides

2.5.6 Sample interpretation

2.5.6.1 Referral, index and dual-stained cytology

The NHS CSP's definition of adequacy and the UK's BSCC grading classification system were used to interpret the cytology[84]; slides were reported as borderline suspicious of high grade, low grade dyskaryosis or high grade. Standard interpretation of the referral cytology included three 'full screens' lasting 5 minutes by two cytologists and one consultant pathologist as all referral cytology samples were reported as dyskaryotic. Interpretation of the study slides (cytology and dual-staining) were done by two independent consultant cytologists, in two separate trusts, who were blinded to the participant's demographics, referral cytology and LLETZ result.

I adapted the scoring protocol for the dual-stained cytology slides from the manufacturer's handbook (CINtec Plus Cytology Interpretation, Roche 2013) who recorded a positive result as *'…one or more cervical epithelial cell(s) stained with a brown cytoloplasmic stain (p16) and a red nuclear (Ki-67) stain irrespective of morphologic abnormalities'*. Magnification was x10 to screen and x40 to allow assessment of the dual-stained positive cells in the same plane of focus. Although screening could stop at the first positive cell, as during a normal cell cycle pathway p16 and Ki67 expression is mutually exclusive (section 1.6.3.1.3), in practice, both cytologists continued screening until a second positive cell was identified. To develop my knowledge and to ground myself in the data, I watched all screening under a double headed microscope and recorded the results.

2.5.6.2 HPV genotyping

Samples were considered positive for high risk HPV if one of the 13 IARC classified type 1 carcinogens was identified (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68).

2.5.6.3 Endocervical curettings

Adequacy rates for endocervical curetting H&E slides are poorly defined in the literature. I initially used an adapted definition by Boardman *et al,* 2003[132] – *'at least one strip with at least ten endocervical cells that can be viewed at x10 magnification'* - in combination with the criteria for cervical punch biopsy by Heatley *et al*[201] - *'evidence of metaplasia, squamous cells and stroma'*. These samples were reported by participant number and interpreted as normal, CIN1, 2 or 3, cGIN (low or high grade) or carcinoma. All histological grades ≥CIN2 were grouped as CIN2+ for ease of analysis and because all results ≥CIN2 meet the national criteria for treatment. p16 histological staining was graded according

to an abbreviated version of Lesnikova *et al's*[202] definition; *'0 – no staining, 1 – weak staining and 2 – strong straining'*. Ki-67 nuclear staining was graded according to a classification adapted from Galgano *et al*[136] and van Niekerk *et al*[139]; *'0 – no staining, 1 – parabasal staining, 2 - full thickness staining'*. The curettings were interpreted by two consultant pathologists in two separate NHS trusts who were not aware of the participant's history or LLETZ result. Due to the expert/novel nature of interpreting these small and fragmented samples, I viewed the samples under a double headed microscope with these experts and recorded all outcomes.

2.5.6.4 LLETZ

Routine reporting of the LLETZ was undertaken and the histopathologists were unaware these women were enrolled in the study. Results were recorded as normal (which for the purposes of the study included metaplasia and inflammation), CIN1, 2 or 3, cGIN or SCC, but for analytical purposes and because results ≥CIN2 meet the national criteria for treatment, results <CIN2 and ≥CIN2 were grouped.

2.5.7 Analyses

Descriptive statistics, such as means & SD or median & IQR for continuous variables and frequency & proportions for categorical variables, were used to describe the study sample. To compare my study characteristics to national data (where 80% have a TZ1 or 2) differences in proportions and their confidence intervals were reported.

The inter-rater reliability of predicting CIN2+ by different diagnostic and staining categories was measured by a Kappa statistic, as explained in section 2.3.3.1.1. The most predictive immunostaining categories were then used to assess the diagnostic accuracy of the index tests.

To determine the diagnostic accuracy of the referral and index tests, sensitivity and specificity were calculated as well as PPV and NPV (Table 2.5). PPV and NPV relate to the performance of the test and will vary depending on the population screened; if the test is used in a population which has a high prevalence of disease (such as CIN) the chance of false positives will be lower for a given sensitivity. Sensitivity and specificity relate to the accuracy / characteristics of the test and are stable across different populations. To

calculate sensitivity, specificity, NPV and PPV a diagnosis of low grade cytology (<CIN2) or high grade cytology (CIN2+) by diagnostic test were reported as accurate if the LLETZ sample reported a corresponding histological diagnosis. Participants with inadequate samples were excluded. Where suitable, McNemar's test was used to compare the sensitivity and specificity of the index tests.

Table 2.5: Calculation of sensitivity, specificity, PPV and NPV

	LLETZ result		TOTAL
	CIN2+	**<CIN2**	
Index test positive	True Positive (a)	False Positive (b)	Test positives (a + b)
Index test negative	False Negative (c)	True Negative (d)	Test Negatives (c + d)
TOTAL	Total CIN2+ (a + c)	Total <CIN2 (b + d)	

Sensitivity = a / a + c PPV = a / a + b

Specificity = d / b + d NPV = d / c + d

Hypotheses for diagnostic studies can be tested using more than one accuracy measure. When this occurs adjustment for multiple testing needs to be completed to reduce potential type 1 errors. Overall accuracy of the tests (a + d / a + b + c + d) was not calculated as this gives an equal weighting to true positives and true negatives. This means a test which has better specificity than sensitivity could in theory have a better overall accuracy than a test which has a higher sensitivity than specificity. Although specificity is important to reduce treatment related morbidity, sensitivity in my study is of higher importance; missing a cancer carries a higher morbidity than is associated with the treatment. For these reasons the area under the curve was not measured when the index tests were evaluated and adjustment for multiple testing was not required[203].

Patient characteristics can affect the accuracy of diagnostic tests. To assess for this, logistic regression models were calculated with CIN2+ (as predicted by LLETZ) as the dependent variable and patient's age, parity, hormonal status, smoking status and time

between referral cytology and LLETZ as the independent variables. The analysis was restricted to complete cases (97/101) to allow comparison across tests and across models with different predictors.

Univariable models assessed whether each potential predictor explained the LLETZ outcome. A series of bivariable models then assessed the index tests in combination with each potential predictor. Wald tests were used to determine if their inclusion explained the LLETZ result over and above what would be expected from natural sampling variability.

A series of bivariable models which included (a) HPV 16/18, (b) dual-stained cytology and (c) immunostained curettings were evaluated to determine whether the inclusion of each potential predictor modified the prediction ability of the diagnostic test. Receiver operator curve (ROC) plots and area under the ROC curve (AUC) statistics for (a) initial models evaluating the individual tests and (b) models with the test and other significant predictors were produced. The area under the curve measures the ability of the test to identify women with and without CIN2+; values of 0.90 - 1 are considered excellent, 0.80 - 0.90 are good, 0.70 - 0.80 are fair and <0.70 are poor.

The most predictive tests were then assessed in combination with the screening test result to determine the impact on diagnostic accuracy. Only participants with complete (and adequate) datasets were used. Sensitivity, specificity, PPV, NPV and likelihood ratios for a positive and negative test were calculated.

Likelihood ratios are another method of assessing the usefulness of a test in identifying the probability of CIN2+. A positive likelihood ratio (PLR) is sensitivity / 1- specificity and a negative likelihood ratio (NLR) is 1 – sensitivity / specificity. A value greater than 1 implies the test result is associated with CIN2+ and a minus value implies the absence of CIN2+. They are slightly harder to interpret than sensitivity and specificity as the value needs to be correlated to a given probability of the presence or absence of disease (Table 2.6). For example, a PLR of 5.0 in women with a positive dual-stained cytology result implies a 30% probability of CIN2+

Table 2.6: Likelihood ratio and probability of disease

LIKELIHOOD RATIO	CHANGE IN PROBABILITY
Negative Values	
0.1	- 45%
0.2	- 30%
0.5	- 15%
1	0%
Positive Values	
1	0%
2	+ 15%
5	+ 30%
10	+ 45%

Chapter 3 **The impact of HPV screening on negative LLETZ**

3.1 Introduction

The current literature has focused on HPV screening test performance and referrals to colposcopy rather than the impact of a positive test on colposcopists' decision-making. Negative LLETZ is an important performance indicator in colposcopy and quality management of a cervical screening programme. It occurs when no CIN is identified in the histological specimen and the reported incidence in women with biopsy confirmed CIN2+ varies from 5.9% to 41%[105, 160-164]. This large variation is secondary to differing criteria for treatment. Given that 56% of women referred to colposcopy in England are aged 25 - 35 and 12.9% of these referrals will result in a LLETZ[87], a reduction in false positive cervical screening could reduce unnecessary treatments and the associated morbidity (See 1.5.4).

There is contradictory evidence following the introduction of HPV triage of low grade cytology as to the potential effect on the incidence of negative LLETZ. Women who are negative for high risk HPV can have higher rates of negative LLETZ[162, 163]. Conversely, HPV positivity does not differentiate between transient and transforming infections, which accounts for the poor specificity of HPV testing in women with low grade cytology[156].

The aims of this study were to:

(i) evaluate whether HPV testing, as undertaken in the sentinel sites, has reduced the mean rate of negative LLETZ histology and

(ii) examine predictors of negative LLETZ in women who test positive for high risk HPV.

It is my experience, as a colposcopist, that women with a TZ3 appear to have higher rates of negative LLETZ than women where the TZ is visible. By addressing this shortfall in the literature, this novel study will determine the clinical significance of negative histology after HPV testing and provide recommendations on whether women with a TZ3 should be targeted for improved screening test accuracy prior to LLETZ.

3.2 Methodology

This was a retrospective cohort study (as outlined in section 2.1). Two independent cohorts who attended for a LLETZ procedure, before and after the introduction of HPV testing, were compared. For each cohort, 401 individuals were randomly selected from a colposcopy database. Clinical and colposcopic variables were extracted. The incidence of negative LLETZ was estimated in each cohort. Regression analysis was used to adjust for potential confounders and to explore predictors of negative LLETZ.

3.3 Results

All 802 women had complete data sets for the clinical and colposcopic variables collected. The majority of women were aged 25 - 40, parous, non-smokers, had a high grade cytology referral and satisfactory colposcopy (TZ1 to 2). A full description of the clinical characteristics is given in Table 3.1.

Compared to the pre-HPV cohort, more women in the post-HPV screening cohort were younger than 30 and older than 50 years and more women used contraceptive. The two cohorts were similar with respect to parity, smoking habits, referral cytology, histological limiting factors and visibility of the TZ. However, in the pre-HPV cohort more women had an excision depth less than 7mm and the interval from cytology to colposcopic assessment was longer (mean 7 weeks (SD 4.21) vs mean 5.4 weeks (SD 4.44); $p<0.001$).

The LLETZ histology was also compared between cohorts in Table 3.1; the incidence of negative histology was higher in the pre-HPV testing cohort but there was no difference in the rates of CIN1-3, cGIN or invasion between cohorts. The criteria for LLETZ, as advised by the NHS CSP publication No 20[35], are described in Table 3.1; there was no observed difference in criteria based on the outcome of a punch biopsy but in the absence of confirmatory histology, twice as many women had a LLETZ in the post-HPV testing cohort for high grade cytology in the presence of a TZ3. Table 3.2 describes the criteria for treatment in the women with negative LLETZ histology; there was no evidence of a difference between cohorts.

Table 3.1: Description and comparison of patient and clinical characteristics in women who had a LLETZ pre and post HPV testing

	Combined cohorts (n=802)	Pre-HPV cohort (n=401)	Post-HPV cohort (n=401)	p-value
Patient Characteristics:				
AGE:				
25 - 30 years	350 (43.6%)	163 (40.7%)	187 (46.7%)	
31 - 40 years	275 (34.2%)	155 (38.7%)	120 (29.9%)	} 0.01
41 - 50 years	127 (15.8%)	65 (16.2%)	62 (15.5%)	
51+ years	50 (6.2%)	18 (4.4%)	32 (7.9%)	
PARITY:				
None	385 (48.0%)	195 (48.6%)	190 (47.9%)	
1	163 (20.3%)	68 (16.9%)	95 (23.7%)	} 0.04
2+	254 (31.7%)	138 (34.4%)	116 (28.9%)	
CONTRACEPTIVE:				
None	295 (36.8%)	173 (40.1%)	122 (30.4%)	
Oestrogen	177 (22.1%)	79 (19.7%)	98 (24.4%)	} 0.009
Progesterone	330 (41.1%)	149 (39.9%)	181 (45.1%)	
SMOKING:				
None	530 (66.8%)	251 (62.6%)	279 (69.6%)	
1 - 5 per day	81 (10.1%)	41 (10.2%)	40 (9.9%)	} 0.15
6 - 10 per day	91 (11.3%)	52 (12.9%)	39 (9.7%)	
11+ per day	100 (12.4%)	57 (14.2%)	43 (10.7%)	
Clinical Characteristics:				
Referral Cytology:				
Low Grade	232 (28.9%)	125 (31.2%)	107 (26.7%)	} 0.16
High Grade	570 (71.2%)	276 (68.8%)	294 (73.3%)	
Cytology to Colposcopy Interval:				
0 - 4 weeks	385 (48%)	134 (33.4%)	251 (62.6%)	
5 - 8 weeks	291 (36.3%)	183 (45.6%)	108 (26.9%)	} <0.001
9+ weeks	126 (15.7%)	84 (20.9%)	42 (10.8%)	
TZ type:				
Unsatisfactory (TZ3)	130 (16.2%)	58 (14.5%)	72 (17.9%)	} 0.17
Satisfactory (TZ1-2)	672 (83.9%)	343 (85.5%)	329 (82.1%)	
Excision Depth:				
0 - 6mm	339 (42.2%)	190 (47.4%)	149 (37.2%)	} 0.003
7+ mm	463 (57.7%)	211 (57.6%)	252 (62.8%)	
Limiting histological factors:				
No	578 (72.1%)	283 (70.6%)	295 (73.6%)	} 0.34
Yes	224 (27.9%)	118 (29.4%)	106 (26.4%)	
Criteria for LLETZ:				
Punch biopsy:		**179 (44.6%)**	**200 (49.9%)**	
Persistent CIN1 for >24 months	74 (9.2%)	40 (9.9%)	34 (8.5%)	
CIN2	156 (19.5%)	79 (19.7%)	77 (19.2%)	} 0.16
CIN3	138 (17.2%)	56 (13.9%)	82 (20.4%)	
cGIN	11 (1.3%)	4 (1%)	7 (1.7%)	
No prior histology:		**222 (55.4%)**	**201 (50.1%)**	
High grade cytology & TZ3	82 (10.2%)	24 (5.9%)	58 (14.5%)	
High grade cytology & HG colp	283 (35.3%)	163 (40.6%)	120 (29.9%)	} <0.001
Low grade cytology >12m & TZ3	58 (7.2%)	35 (8.7%)	23 (5.7%)	
LLETZ Histology:				
CIN1	85 (10.5%)	37 (9.2 %)	48 (11.9%)	
CIN2	182 (22.7%)	98 (24.4%)	84 (20.9%)	} 0.16
CIN3	351 (43.7%)	162 (40.3%)	189 (47.1%)	
cGIN	28 (3.4%)	13 (3.2%)	15 (3.7%)	} 0.86
Invasion	25 (3.1%)	11 (2.7%)	14 (3.4%)	
Negative Histology	134 (16.7%)	80 (19.9%)	54 (13.4%)	0.01

Table 3.2: Criteria for treatment in women with negative LLETZ histology in the pre- and post-HPV testing cohorts

	Pre-HPV testing cohort (n=80)	Post-HPV testing cohort (n=54)	p-value
Previous Punch Biopsy:			
• Persistent CIN1 for >24 months	10 (12.5%)	6 (11.1%)	} 0.22
• CIN2+	32 (40%)	16 (29.6%)	
- *CIN2*	*14 (17.5%)*	*3 (5.5%)*	
- *CIN3*	*18 (22.5%)*	*13 (24.1%)*	
No confirmatory histology *(See and Treat LLETZ):*			
• High Grade Cytology and a TZ3	9 (11.2%)	7 (12.9%)	0.97
• High Grade Cytology and high grade colposcopy	4 (5%)	3 (5.5%)	
• Low Grade Cytology >12m and a TZ3	25 (31.2%)	22 (40.7%)	

3.3.1 Association of HPV testing with negative LLETZ histology

The incidence of negative LLETZ was 19.9% (80/401) in the pre-HPV cohort and 13.4% (54/401) in the post-HPV screening cohort, giving an unadjusted relative risk of 0.68 (95% CI: 0.49 to 0.93); see Table 3.3. The largest confounder in Table 3.3 was the interval from cytology to colposcopy; after adjusting for this variable there was no evidence of an association between HPV screening and negative LLETZ (RR 0.83; 95% CI: 0.60 to 1.15). In the final fully adjusted model that controlled for differences in age, smoking, contraceptive use, parity, referral cytology, biopsy result prior to LLETZ and histological limiting factors, there was a 25% reduction in negative LLETZ in women who had HPV screening.

Table 3.3: The association between HPV screening and the risk of negative LLETZ (n=802). The crude association is provided along with the association after adjusting for each potential confounder and a final model adjusting for all possible confounders.

	Relative Risk (RR)	**95% CI**	**P-value**
Unadjusted	0.68	0.49 – 0.93	0.01
Adjusted for:			
Age	0.66	0.48 – 0.90	0.01
Smoking	0.68	0.49 – 0.94	0.01
Contraceptive	0.71	0.51 – 0.97	0.03
Parity	0.70	0.51 – 0.95	0.02
Referral Cytology	0.69	0.50 – 0.94	0.02
Cytology to Colposcopy Interval	0.83	0.60 – 1.15	0.26
TZ type	0.62	0.46 – 0.84	0.002
Biopsy result	0.66	0.48 – 0.90	0.01
Excision Depth	0.69	0.50 – 0.94	0.02
Limiting histological factors	0.69	0.50 – 0.94	0.01
Adjusted for all potential confounders	**0.75**	**0.55 – 0.97**	**0.04**

3.3.2 Predictors of negative LLETZ in HPV positive women

Table 3.4 reports the association of a range of variables with negative LLETZ among women who underwent HPV screening. In the unadjusted model, women >40 had 3 times the risk of negative LLETZ compared to women <30, women with two or more children had almost 3 times the risk compared to nulliparous women, women with low grade cytology had more than three times the risk compared to women with high grade cytology and women who had a TZ3 had four times the risk compared to women where the transformation zone was visible. The risk of negative LLETZ was reduced in women who had confirmatory histology prior to LLETZ and in women using the COCP.

In the final model in Table 3.4, the presence of CIN2 on punch biopsy reduced the risk of negative LLETZ by 75% when compared to women where prior histology was not available. After adjusting for parity, women with a TZ3 had almost three times the risk of a negative LLETZ when compared to women where the TZ was visible and women with low grade cytology had almost four times the risk when compared to women with high grade cytology. The risk of negative LLETZ was highest among women with low grade cytology and a TZ3 (RR 10.4, 95% CI 5.9 - 18.4, p<0.001). It should be noted that while this risk is high in both relative and absolute terms (+40%; 95% CI 27 - 54%, p<0.001), only 22/401 (5.5%) women who had HPV testing, had both low grade cytology and a TZ3.

Of note, the risk of negative LLETZ from a TZ3 was shown to be independent of a low grade or high grade cytology referral. The marginal probability of negative LLETZ with high grade cytology, based on the final model in Table 3.4 was 6% (0.06, 95% CI 0.04 - 0.09). Among women with low grade cytology this was 23% (0.23, 95% CI 0.09 - 0.21).

The association between clinical variables and a TZ3 was explored. When compared to women who were aged less than 30 years a strong positive linear relationship was observed between increasing age and a TZ3: among women aged 31 to 40 RR 1.26, 95% CI 0.69 – 2.29, among 41 to 50 year olds RR 2.72, 95% CI 1.57 – 4.73 and in women older than 50 years of age RR 4.17, 95% CI 2.41 – 7.21. Compared to women who did not use any form of hormonal treatment there was no difference in the incidence of a TZ3 and use of progesterone (RR 0.93, 95% CI 0.60 – 1.44) but use of oestrogen had a protective association (RR 0.25, 95% CI 0.11 – 0.60).

Table 3.4: Predictors of negative LLETZ in the post-HPV testing cohort (n=401)

	Unadjusted associations			Adjusted final model		
	RR	95% CI	p	RR	95% CI	p
Age						
≤30	Ref*					
31 to 40	1.36	0.69 - 2.69	0.37			
41 to 50	3.02	1.60 - 5.67	0.001			
51+	2.92	1.36 - 6.26	0.006			
Parity						
0	Ref*					
1	1.25	0.59 - 2.65	0.6	1.28	0.56 - 2.90	0.55
2+	2.87	1.62 - 5.07	<0.001	2.13	1.13 - 4.01	0.02
Smoking						
No	Ref*					
Yes	1.35	0.81 - 2.24	0.26			
Contraceptive						
None	Ref*					
COCP	0.40	0.18 - 0.89	0.02			
Progesterone	0.77	0.44 - 1.30	0.32			
Cytology						
High Grade	Ref*					
Low Grade	3.19	1.95 - 5.21	<0.001	3.60	2.18 - 5.97	<0.001
Cytology to Colp Interval						
0 to 4 weeks	Ref*					
5 to 8 weeks	1.61	0.82 - 2.81	0.09			
9+ weeks	2.30	0.98 - 4.42	0.06			
Colposcopy						
TZ1-2	Ref*					
TZ3	3.94	2.46 - 6.31	<0.001	2.88	1.76 - 4.72	<0.001
Biopsy prior to LLETZ						
None	Ref*					
CIN1 >24 months	1.15	0.52 - 2.54	0.7	0.68	0.31 - 1.50	0.34
CIN2	0.25	0.08 - 0.80	0.02	0.25	0.08 - 0.79	0.02
CIN3	1.03	0.57 - 1.86	0.9	0.92	0.49 - 1.73	0.8
Excision Depth						
>6mm	Ref*					
≥7mm	1.09	0.65 - 1.83	0.70			
Limiting Factors						
None	Ref*					
Present	1.17	0.68 - 2.01	0.60			

Ref* = Reference category

3.3.3 Follow up cytology in the negative LLETZ cohorts

In women who had HPV testing and negative LLETZ histology, follow up cytology was available for 83.3% (45/54); 35/45 were HPV negative six months after LLETZ and none developed dyskaryosis during the follow up period (mean 39.6 months, range 6 to 44 months). Of the 4/45 women who were HPV positive but cytology negative at their six month test of cure, one had CIN1 whilst the remainder had negative cytology during follow up (mean 30 months). Of the 6/45 women who had dyskaryosis at their 6 month test of cure, (two high grade and four low grade), two had vaginal intraepithelial neoplasia (VAIN) and the remainder HPV (mean follow up 33 months, range 28 to 36 months).

In the women who had a negative LLETZ prior to HPV testing, follow up cytology was available for 85% (68/80). Seven of the 68 women had dyskaryosis at their 6 month follow up, (one high grade and six low grade) of whom two had VAIN, one cGIN, one CIN1 and three HPV (mean follow up 7 months, range 6 to 18 months). 61/68 had negative cytology six months after LLETZ, of whom ten developed dyskaryosis (all low grade) during the follow up period (mean 66 months, range 6 to 102 months). Three of the ten women women had VAIN, one CIN2 and the remainder HPV.

In summary, 6% (3/45) of women with a negative LLETZ in the HPV testing cohort had positive histology (CIN1+) following excision and 11.7% (8/68) in the cytology only cohort (diff 5.7%, CI -6.6 – 16.2%, p=0.3).

3.4 Strengths

In this study, double data entry and a complete data set for both cohorts removed bias caused by erroneous and missing data. To control for confounding, variables which could explain the observed association between HPV testing and negative LLETZ were collected and controlled for. HPV screening results and the colposcopic assessment were recorded before the LLETZ outcome was known and clinical data was prospectively documented, minimizing recall bias. A sample size calculation, along with the strict triage described above, helped ensure sufficient power.

The study was timely; HPV testing had been in use long enough to meet the sample size but not too long for practice to change dramatically, as evidenced by the similarities

between the cohorts. Missing CIN during the treatment or when interpreting the histology could account for a negative LLETZ result. However, rates of positive histology following a negative LLETZ (an indicator of residual disease) were compared and there was no evidence of a difference between the cohorts. Furthermore, the negative histology samples were assessed by two independent histopathologists, with extra levels, to ensure that all cases met the inclusion criteria - indicating that the same proportion of CIN should be 'missed' between cohorts.

3.5 Limitations

In the pre-HPV testing cohort photographic images were not taken as part of routine practice prior to LLETZ, preventing assessment of lesion size. However local policy advocated, in both cohorts, a strict selection criteria for treatment by recommending confirmatory biopsies if a significant change in lesion size and / or grading occurred. In using historical controls it could be argued that the observed decrease in negative LLETZ histology was associated with a change in clinical practice rather than the introduction of HPV screening. To address this, potential confounders were collected and controlled for. Moreover, following the results of the sentinel sites study it would be unethical to conduct a randomized control trial when high quality studies have shown that cytology alone has poorer sensitivity for detecting CIN than HPV cervical screening.

Cluster sampling was used but the optimal method is randomisation; bias will however have been reduced by a clear definition of the target population and the avoidance of judgement sampling. Although a 25% reduction in negative LLETZ was identified between cohorts, the confidence interval was wide, as the effect size was smaller than predicted (section 2.1.1) suggesting larger sample sizes are needed to improve precision.

3.6 Conclusions

Despite a 25% reduction in negative LLETZ following the introduction of HPV cervical screening, the incidence is still high, at 13.4%. These results delineate the importance of improving the specificity of cervical screening in women with a TZ3 and the need for national guidelines to optimise their management. In the next three chapters, to aid guideline development, I will assess areas of consensus and discordance in the management of women with a TZ3, through the use of focus groups and a national survey.

Chapter 4 Assessment of colposcopists' decision-making in relation to a TZ3

4.1 Introduction

The PPV of detecting CIN2+ in women who have high risk HPV and low grade cytology is only 16%[91]. To differentiate between women with transient and transforming HPV infections, women with a positive cervical screening test are referred to the colposcopy clinic. Management difficulties arise in the presence of a TZ3. To provide diagnostic histology a LLETZ can be undertaken, but as shown in Chapter 3, women with low grade cervical screening have a 10 fold increased chance that the excised tissue will be normal when compared to LLETZ histology in women with a TZ1 -2.

There is little evidence to guide the management of women with a TZ3, specifically recommendations on when to offer cytological follow-up or excisional treatment [35, 97, 98], and this may lead to disparities in care.

Clinical decision-making is a complex process and the inconsistent nature of intuitive management has led to the development of evidenced based practice[204]; this aims to minimize morbidity and optimise outcomes. When a paucity of evidence exists, decision-making under conditions of uncertainty can be influenced by patient choice or risk factors and health care provider attitudes, experience, age, gender or culture[205, 206]. Colposcopists play an important role in leading research and policy change in cervical screening programmes and there is currently no literature to suggest how their opinions and experiences shape the management or counselling of patients with a TZ3.

The aim of this study was to identify factors that affect colposcopist's decision-making, specifically recommendations for excisional treatments over cytological follow-up, to interpret these findings in line with decision-making theory and to detect areas of consensus which may aid in the development of future guidelines.

4.2 Methodology

As outlined in Chapter 2, this was a multi-centre qualitative study utilizing a series of focus groups in an English healthcare region. This is the first study designed to assess these outcomes and was qualitative in nature to provide depth of information. Sampling aimed to ensure heterogeneity of experience and healthcare provider demographics. A topic guide covered a range of clinical and cytological variables and was compiled following a review of the literature and ratified by three expert colposcopists. Using an iterative approach, thematic analysis was selected as the most appropriate method to identify the factors affecting decision-making.

4.3 Results

4.3.1 Study population

Twenty-three of a potential twenty-eight colposcopists from four units participated in four focus groups. The colposcopists who declined gave conflict with their clinical workload as the reason for non-participation. The participants represented a range of years of experience, geographical training backgrounds and specialty. There were five nurse practitioners, four gynaecological oncologists, three lead colposcopists, seven gynaecology consultants, two pathologists and two gynaecology registrars. Years of experience in colposcopy ranged from 1 to 34 with the mean number of years 11.2. Fourteen participants trained in the South West England region, three in London, two in the West Midlands, two in the Northern region, one in the North East and one in the Eastern region.

Two of the units partook in the sentinel sites study and prior to recruitment they had eight years of experience managing women referred with HPV triage of low grade cytology and three years' experience with primary HPV testing. The remaining two units had two years' experience of HPV triage of low grade cytology following the national roll-out in 2013.

All of the transcripts were coded as outlined in Section 2.2.4 and the coding tree which links these codes to the themes identified is shown in Table 4.1. The themes were defined following in-depth consideration of potential alternative interpretations and these can be viewed in Figure 4.1: Mind Map of the Identified Themes and Subthemes.

Table 4.1: List of codes, subthemes and themes

Codes	Subthemes	Themes
Excisional Treatment	Impact of a high grade cytology result	**Anxiety of missing a cancer**
MDT		
Anxiety	Lack of confirmatory histology	
Laboratory protocols	A HPV positive status increases the risk of treatment.	**The screening test result**
False negative screening		
MDT		
Shift in pathology?	Repetition of the referral cytology	
Shift in opinion?		
Improves cytology		
Side effect concerns		
Assists excision margins		
Factors predisposing to excision	Stratifying risk factors for high grade disease	**Patient Characteristics**
MDT referral		
Risk of over-treatment		
Patient choice	Patient choice	
Paucity of evidence	A multidisciplinary approach	
Younger women		
Older women		
Community; lab reliable	Clinical setting for cytological follow-up	**Paucity of guidance engenders reliance on prior clinical experience**
Colposcopy; not lost to follow up		
Colposcopy; no diagnosis yet	Oestrogen use	
Community; save resources		
Colposcopy; cytobrush use	Depth of LLETZ	
Paucity of evidence		
12m: immuno-clearance	Length of cytological follow-up lacked consensus	
6m: no diagnosis		
12m: low risk of cancer		

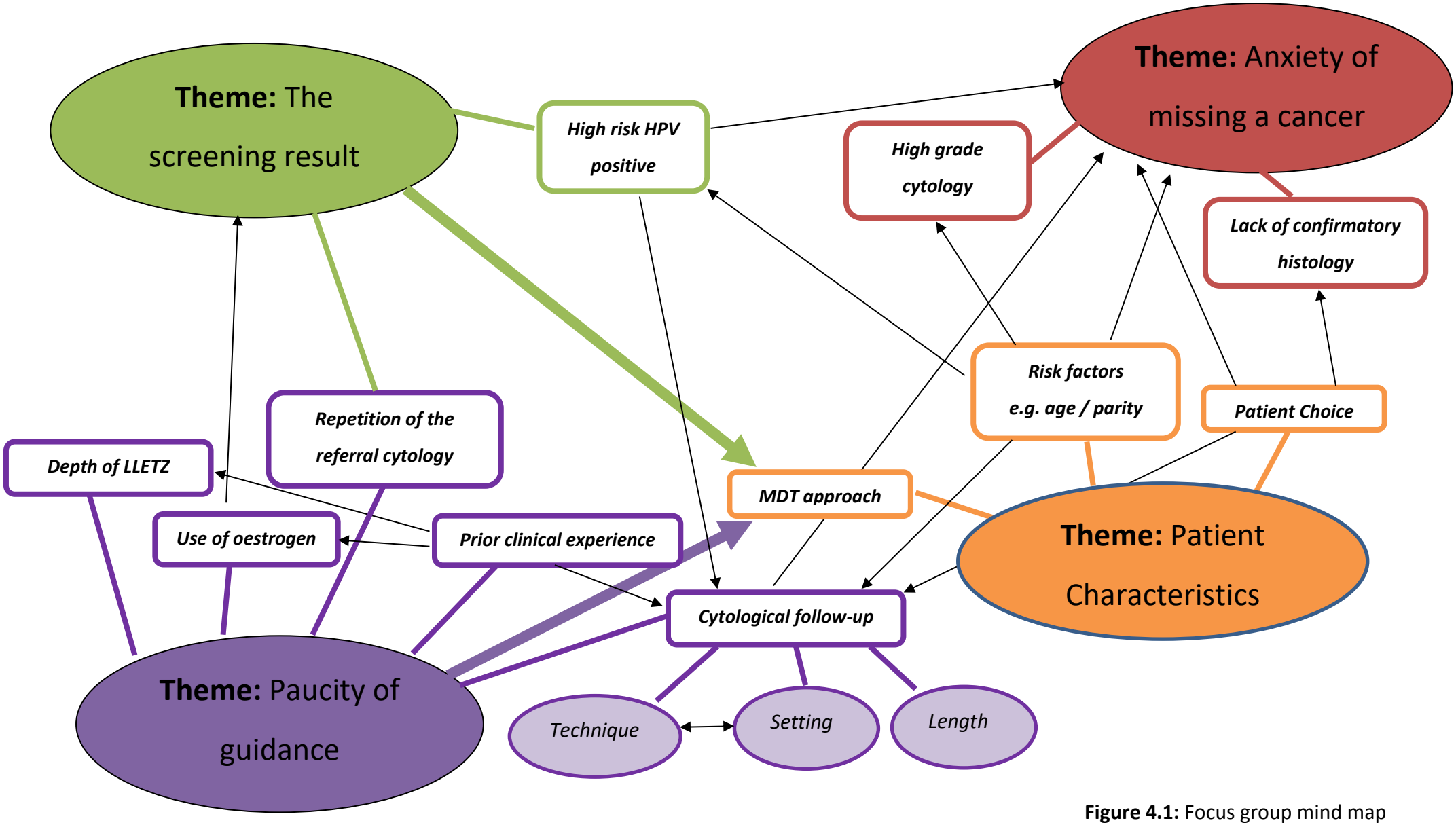


Figure 4.1: Focus group mind map

4.3.2 Theme 1: Anxiety of missing a cancer

4.3.2.1 Lack of confirmatory histology

In our study, irrespective of a high grade or low grade cytology result, if the TZ was not visible and a diagnostic biopsy not possible, most participants were deterred from advocating long-term cytological follow-up.

> **14:** *'They've come to colposcopy and it's been pointless because you're not getting the information you want from that examination. Yes, there is a good chance it could clear up but I just feel a bit nervous about leaving them because there could be high grade there that you can't see...Its knowing whether it's there or not, that's the nightmare.'*

> **03:** *'With unsatisfactory* (colposcopy) *I have no idea of what is going on in the canal. I can't see it, I can't biopsy it. I think that the reason that many of us do a LLETZ in this scenario is because we, and the patients, want information that the examination isn't giving us.'*

This suggests an affective component to decision-making; fear of missing high grade dysplasia (CIN2+) induces a pessimistic outlook of future events if women are not offered a LLETZ. It appears that this is of higher importance than concerns relating to treatment morbidity, even in women with a low grade cytology result.

4.3.2.2 Impact of a high grade cytology result

In these women most participants strongly advised, with patient consent, a LLETZ irrespective of age or family status. Affect and cognition had a major influence on decision-making; anxiety of missing a cancer if treatment was not undertaken was driven by the plethora of evidence which reports a significant risk of the excised tissue containing CIN2+. When making decisions that could result in dangerous outcomes and negative emotions, participants made safe choices.

> **02:** *'If you think of these women having a cancer, there's a cancer that we just can't see, it's tiny, it's inside the canal. If you repeat the cytology and you are giving it at least three months, then we don't know if those three months could make a 1a1 into a 1a2 or even a 1b1 - who knows that? So that's my worry, I look*

at what's the worst possible scenario here and with high grade cytology it's more than likely that there will be high grade disease inside.'

4.3.3 Theme 2: The screening test result

4.3.3.1 High risk HPV increases the risk of LLETZ

Although colposcopists did not reflect on the global impact of HPV testing within colposcopy, they did discuss how a high risk HPV result, in conjunction with low grade cytology, would influence their decision-making. Sixteen colposcopists suggested a high risk HPV result increased the chances of underlying high grade disease. This belief appeared to reduce the uncertainty in their decision-making as all participants agreed that this perceived increase in risk, with the potential of missing a cancer, was leading them to advocate LLETZ in this cohort.

22: *'I think, in the younger ones, how long do you wait to see if the HPV is going to resolve? And I think that now we are using HPV testing that has upped the ante. So, you know that they've still got active HPV and the longer that stays the more likely they are to have an abnormality. So, I do talk to them about having a LLETZ.'*

07: *'I think, you know, the sensitivity has gone up and you do see high grade histology with low grade smears...and I would probably, in the older women, I would be much more pushed to do just a small LLETZ.'*

Colposcopists appear to be highly risk adverse and choose, what they view, as the least dangerous outcome such as treatment morbidity over the chance (even if small) of missing a cancer. Being 'pushed' into advocating a treatment suggests they would have preferred to manage women with low grade screening results more conservatively but are worried about the potential risks of doing so. Pathologists and lead colposcopists (who are responsible for quality assurance) suggested that colposcopists' behaviour was shaped by their experience of reviewing women with high risk HPV. Rather than recognizing the benefits of an improved screening test, they suggested colposcopists believed women were at increased risk of CIN2+ and this decreased recommendations for long-term cytological follow-up.

14: *'There is a shift in expectations and opinion rather than pathology.'*

21: *'What you're doing is weeding out the women with borderline or low grade smears who have no pathology because they are HPV negative...a patient who has a mild smear with high risk HPV is at no greater risk than they were 10 years ago.'*

4.3.4 Theme 3: Patient characteristics

4.3.4.1 Stratifying risk factors for high grade CIN

When patients present with individual characteristics known to increase the risk of cancer such as smoking, poor attendance, older age and / or increased parity, behavioural decision-making is influenced by analytical thinking. Rational judgement denoted that in women with low grade screening, the decision to treat was easier if women were stratified as high-risk - the possibility of developing a cancer outweighed the risk of treatment-related morbidity.

14: *'If she's a heavy smoker and she's clearly never going to give up, then that predisposes me towards treatment'.*

04: *'...I think you're going to be more likely to do a small LLETZ on someone you're going to be concerned about their attendance. It's an individual thing.'*

01: *'If she had had her family or was an older lady, I would do a loop* (LLETZ) *for diagnosis.'*

4.3.4.2 A multi-disciplinary approach

Young women who have not started their families and present with low grade cytology and risk-adverse behaviour (non-smoker, safe sexual behaviour) are at low-risk of high-grade dysplasia but high risk for treatment-related morbidity. A multi-disciplinary approach was advocated by fourteen colposcopists in three focus groups for these women.

13: *'A young nulliparous woman I would bring to the MDT to get a consensus that it was the right thing to do a LLETZ, to be honest, in case they have problems in the future. If it was an MDT decision I'd feel happier.'*

This suggests participants are concerned that their affect is influencing their rational thinking and by sharing decision-making with an expert group they assuage this emotional response. The participants who advocated the MDT suggested this choice was influenced by the introduction of HPV screening. They indicated this had resulted in women being referred to colposcopy earlier, reducing the time needed to clear the infection and subsequently increasing the chance of over-treatment. Cognition (their knowledge of the natural history of HPV infection) and intuition (prior experience of reviewing women pre- and post-HPV screening) appears to affect behaviour.

16: *'We're treating them sooner because they are coming to us sooner.'*

14: *'But that's a potential disadvantage isn't it....?'*

16: *'Not if there's high grade in it.'*

14: *'But, we might be over-treating the women who potentially might get better.'*

17: *'CIN2 in a young girl has a 40% chance of regression, doesn't it.'*

4.3.4.3 Patient choice

This was discussed as a major factor affecting decision-making in all of the focus groups. Colposcopists acceded to patient treatment wishes, even in women at high risk of treatment morbidity and low risk of disease, if the woman was informed of and understood the potential implications of their chosen management option. This suggests that the cognitive factors influencing colposcopist's decision-making can be superseded by the patient's affect and cognition.

03: *'You know, it's a discussion with the patient explaining the pros and cons'.*

17: *'I had a woman the other day, actually, who wanted a LLETZ. Her mum died of cervical cancer, she actually had a low grade smear, but she said 'just cut it away'. I was like 'but you're 25, let's just do a couple of smears' and she said 'just cut it away'. So I spoke to the consultant on call and they said 'do what the patient wishes'.'*

None of the participants mentioned scenarios in which women had declined treatment and the subsequent impact of this choice.

4.3.5 Theme 4: Sparse guidance engenders reliance on experience

4.3.5.1 Clinical setting for cytological follow-up

In women with low grade screening results who were deemed suitable for cytological follow-up, there was a lack of consensus regarding where to review them. Eight participants (including four of the five nurse colposcopists) advocated GP follow-up.

> **09:** *'If they've got abnormalities already on the current smear, there's no good reason why you should have to bring them back to colposcopy. If it was a low grade smear I would probably send her back to her GP because we've got direct referral to colposcopy and we all use the same lab.'*

This suggests trust in the reliability of the laboratory and a belief that outcomes will be the same regardless of where the cytology is taken as the technique is standardized. Conversely, fifteen colposcopists, who were all doctors, differed in this opinion;

> **12:** *'I hear what you're saying about having a good reliable lab but patients aren't often very reliable and so I'd like to know that she's been followed up and make efforts to do so.'*

> **07:** *'We haven't made the diagnosis yet, she still sort of belongs to us. We can't discharge her to the community without working out whether there is something to get concerned about or not.'*

The doctors suggested they had a responsibility to ensure a decision / diagnosis was made and a cancer not missed by personally reviewing these women. Doctors tend to review more complex cases and the adverse outcomes of missed diagnoses. This may have influenced their management choice. It appears that emotion can be more influential that cognitive elements when the risk of cancer is factored into decision-making. The role of emotion and responsibility were strengthened by the paucity of guidance surrounding the optimal technique of smear taking in women with a TZ3.

> **17:** *'They don't do endocervical smears* (with the GP) *so how can you specify that? You see I would do a cytobrush and broom. With a type 3 transformation zone you're more likely to get a better specimen with a brush and broom, aren't you?'*

> **14:** *'I agree with you, but has anyone proven that.'*

17: *'No, not that I know of.'*

It appears that in conditions of clinical uncertainty, intuitive decision-making - affect, perception, rational judgement and prior experience – aids colposcopists in their assessment of risk.

4.3.5.2 Length of cytological follow-up

For those women whom participants recommended cytological follow-up, rather than LLETZ, there was a discrepancy in the number of months advocated before repeating the cytology. Thirteen colposcopists suggested six months and if at this time any grade of dyskaryosis was reported, and a TZ3 was still present, they would recommend a LLETZ.

16: *'I would prefer to see them in 6 months. They've come to colposcopy and it's been pointless because you're not getting the information you want from that examination. Yes, there is a good chance it could clear up but I just feel a bit nervous about leaving them because there could be high grade there that you can't see.'*

03: *'...I do think it's kind of two strikes and you're out, the referral cytology and a 6 month follow up, because they clearly still have got continuing HPV.'*

01: '*I can't see what's going on, so I think if there is still an abnormality on the referral back in, the 6 month follow-up smear, I would rather know what was going on in the canal…..do a LLETZ, because of anxiety and we are not getting to the bottom of it.'*

Anxiety about missing high grade disease, compounded by the perceived risk that persistent HPV confers, deterred long-term follow-up even in women with low grade screening. Conversely, six colposcopists discussed individualizing care based on patient risk factors. If women were young and/or nulliparous with low risk factors, participants recommended 12 month cytological follow-up.

09: *'The 12 month repeat allows the immune system to battle HPV, as studies showed there is a greater clearance at 12 months rather than 6 months.'*

14: *'The debate we're having here is whether 6 months or 12 months is better and the issue or question is whether this lady might have a high grade dysplasia underlying. The likelihood of that becoming a malignancy in the 6-12 month phase is* (pause) *in the order of a fraction of a percent.'*

This suggests a combination of cognitive and intuitive decision-making based on prior experience, perception of risk and knowledge.

4.3.5.3 Repetition of the referral cytology

Two of the colposcopists who could not attend the focus groups provided the researchers with a scenario they considered an area of clinical uncertainty; due to the topographic position of the TZ, would colposcopists repeat the referral cytology at the first colposcopy appointment? The majority of the colposcopists adhered to national guidance and did not repeat the cytology. However, some participants suggested they had concerns that the referral cytology collection device may not have adequately sampled the TZ due to its endocervical position.

01: *'I think if we speak to any cytologist they'll always say you should not repeat the smear within 8 weeks because you've already sampled it and you've already taken off the epithelium and then you really need to wait for it to re-grow or you're going to get a false positive / false negative and you're going to be back to square one.'*

16: *'I would wait three months. I know not everybody does.'*

17: *'If it was a poor sample with a TZ3 then I would re-smear.'*

4.3.5.4 Oestrogen use is based on prior experience

The use of oestrogen has been discussed in the literature as a potential pharmacological method which can convert a TZ3 to a TZ1. Colposcopist's discussed their recommendations for its use which appeared to be linked to prior experience.

12: *'The thing with oestrogen is you're never too sure about the compliance prior to it and whether that makes a difference.'*

01: *'If she has an atrophic cervix I would ask her, definitely, to have two weeks of oestrogen before her next cytology...not so much because you're going to pull out the transformation zone but, it can help the interpretation of the cytology. Also for her comfort...'*

Despite the evidence suggesting the potential benefits of oestrogen use, most participants felt, in practice, it did little to improve the examination findings. The majority of gynaecological oncologists did not advocate use but eleven participants, including all of the nurse colposcopists, used oestrogen to improve the smear quality and to reduce discomfort during the examination. Gynaecological oncologists manage women with oestrogen driven cancers and this may have affected their decision-making, particularly as they reported no real improvement in examination adequacy; the harm of oestrogen use may have outweighed the benefit in their minds.

Three of the four units used topical preparations and the reasons identified were the side effect profile and poorer efficacy of systemic hormone replacement therapy. As was seen in theme one, negative emotions led participants to make, what they considered to be, the safer management options.

06: *'Well I guess topical oestrogens are less harmful than actually giving HRT...and it works.'*

18: 'Because 30% of women with genitourinary atrophy don't respond to systemic HRT.'

4.3.5.5 Depth of LLETZ

Decision-making in this area was driven by prior experience, perceived individual risk and affect.

14: *'Greater than 7 less than 10mm, to reduce the risk of cervical dysfunction in pregnancy.'*

07: *'There's a chance there's absolutely nothing wrong with her cervix and you're chucking out a big bit of tissue and if you do really have something wrong with the cervix there's the option of doing a second LLETZ if you're really concerned.'*

02: *'I think the biggest problem is that because you can't see the TZ you don't know how far to go...If you do a deeper loop and it is negative that is much easier to criticize than if you do a smaller loop and then if it is positive, you do another one because that is much more targeted.'*

As seen in Theme 1 and with systemic oestrogen use, colposcopists make safe choices. Women at high-risk of treatment-related morbidity engender negative emotions which prompted participants to make autonomous choices and deviate from UK national recommendations for optimal depth (15-25mm)[35]. In older women, who are at reduced risk of treatment morbidity and increased risk of high grade disease, colposcopists adhere to national guidance. These data suggest cognition and rational judgement have a greater impact than affect in this patient demographic.

13: *'In an older woman I probably would go a bit deeper because they're more likely to have an adenocarcinoma than squames.'*

04: *'The older women, 15mm, what you want to avoid, if possible, is the inconvenience of bringing them back for a repeat LLETZ and risking non-attendance.'*

Conditions of clinical uncertainty can cause anxiety in both health care providers and patients. The use of rational judgement and a colposcopist's experience appear to aid in decision-making but affect also plays a strong (and sometimes more dominant) role when evaluating risk. The following quote most accurately reflected the overall findings of this study;

03: *'I think it's interesting. I think what we're all talking about is individualization of care . . . All you're trying to do is be safe to gain or achieve the information that you need and it does need to be individualized. And I think in our day to day practice that's what we all spend our lives doing.'*

4.4 Strengths

Focus groups, rather than interviews or questionnaires, were chosen as the method of study as numerous viewpoints on a specific issue can be studied in an interactive setting. In my study, comments made by individual participants stimulated group discussions, decreasing the interaction of the facilitator (me) and reducing researcher bias. Moreover, they provide richer data than a questionnaire by expanding upon the decision-making process and enabling targeted suggestions for guidance - which was the key component of interest in this study.

A heterogenous group ensured differing opinions were shared leading to lively debates in some of the units. Sensitization, with the possibility of pre-set answers which may reduce analytical thinking during the focus groups, was reduced by the provision of a general theme in the participant information sheet rather than set questions. Further strengths included the use of open ended questions, an extensive coding process and an iterative analysis which helped ensure saturation and depth of information was attained. Transcribing the audio files grounded me in the data and improved my interpretation of these transcripts (as suggested by Braun and Clarke)[207]. None of the participants withdrew their data and the respondents verified the validity of the transcripts. Double coding of the transcripts and a self-awareness of my own preconceptions by including both colposcopists and qualitative supervisors in the study group should have reduced reflexitivity[176].

4.5 Limitations

Assessing practice in one geographical region may increase the institutional bias but the inclusion of four centres with varying patient populations and participants who trained in six of the 12 English regions should improve the generalizability of the data. Moreover, there was no difference in opinion based on training location.

It is important to consider why participants agreed to take part; it could be argued that attendees did so to express a particular viewpoint and the data may not resonate with national opinions. However, only four of twenty eight colposcopists did not participate and this was due to conflicting clinical commitments and involvement with the topic guide. Furthermore, two of these gave written statements for clinical scenarios that they

wished to be discussed. Although these statements were not used in the analysis, they stimulated discussions on the optimal cytological collection device and the risks and benefits of repeating the referral cytology at the first colposcopy appointment.

Age and gender were not collected for confidentiality reasons but gender, as discussed in 2.3.1.1, can influence clinical decision-making. Assessing this association would be useful for guideline implementation and will be explored in Chapter 6.

4.6 Conclusions

In this study, anxiety of missing a cancer deterred long-term cytological follow-up, resulting in higher than anticipated excisional treatments in women with low grade screening and a TZ3. Moreover, when a LLETZ was offered, colposcopists undertook shallower excisions than nationally recommended as a result of treatment morbidity concerns. In areas of clinical uncertainty, when decisions are dominated by affect, clinical guidance and targeted evidence can reduce the difficulty and anxiety of decision-making. In Chapters 5 and 6, through the use of a national survey that was based on the themes identified in the focus groups, I will explore the frequency of these opinions and clarify areas of consensus.

Chapter 5 Development of a questionnaire for evaluating management of a TZ3

5.1 Introduction

Management of a TZ3, as identified in chapter 4, is an area of clinical uncertainty. Yet these patients account for 20% of the women reviewed in colposcopy annually. This area has been poorly researched with no studies to date assessing national consensus for management. Studies which investigate current management of a TZ3 may help to stimulate research in areas of discordance and provide guidance in areas of consensus.

Direct assessment of clinical care through first-hand observation would require extensive resources, can be observer subjective and patient specific. Indirect measurement can occur through the use of qualitative studies (chapter 4) or questionnaires (chapter 6). There are currently no validated tools for the assessment of TZ3 management and, as discussed in section 2.3, the use of items which are designed for different domains than those intended will reduce the validity and reliability of the questionnaire.

The aim of this chapter is to describe the development of a questionnaire that assesses UK colposcopists' decision making when applied to the management of women with a TZ type 3.

5.2 Methodology

As outlined in Chapter 2, a questionnaire was developed based on a conceptual framework, literature review, contributions from the focus groups and a Delphi consensus consisting of eight experts in the field. The psychometric properties were assessed:

(i) Content, face and construct validity through cognitive interviews with 12 colpocopists and an expert committee evaluation.

(ii) Test-retest reliability was estimated using 20 colposcopists who completed the questionnaire two weeks apart.

5.3 Results

5.3.1 Validity

5.3.1.1 Content Validity

Following the development of the items (section 2.3.1), the BSCCP committee reviewed the first version of the online questionnaire and made the following suggestions:

1. *'Change all questions that ask the participant to rank their options to a Likert scale as ranking can become a guessing game or a click a box exercise, is onerous to complete, may reduce response rates and gives a clustered layout. The board can understand the reasoning behind a ranking approach but consider a Likert scale instead'.*
2. *'Rather than using a drop down box for binary responses, use circles that people can click. The less clicks, the more likely people are to complete it. In general people are more willing to complete surveys if they can see the answers in front of them and click one box. If they have to make choices it is usually the 'quick' choices that increase completion of the survey. The options for the answers are good though'*.
3. *'Please add associate specialist to the demographic list'.*
4. *'One of the management options is to repeat the cervical cytology. The role of the BSCCP audit programme is to assess current practice against national standards. There is a national standard in regard to not repeating a cervical screening sample at colposcopy. We see no evidence that this should be challenged on the current peer reviewed evidence base. If you wish to ask about the role of repeating cytology in this situation, then please ask why a colposcopist would want to repeat the cytology and ask how a repeat test would change their practice'.*

Multi-nominal and discrete response items had originally been included to reduce ambiguity when analyzing management choices and to improve the reliability. Ranking of item stems can increase the depth of information provided but can also decrease the reliability of the item[179, 180]; increasing the number of variables can reduce response rates. Five point Likert scale response anchors were researched[208] and scales which assessed levels of importance *'not at all important, low importance'* and levels of concern *'not at all concerned, slightly concerned'* were incorporated.

The outcome from the focus groups and the Delphi consensus identified that colposcopists may not adhere to national guidance which recommends that cervical cytology should not be repeated within 3 months of the referral cytology. I was interested in clarifying whether this was a regional or national perspective as it may be a specific educational point that needs addressing in this cohort. To incorporate the BSCCP stipulations the survey was amended to include two additional discrete response items which asked respondents if and why they may want to repeat the cytology and how the outcome of this test would affect their management. The second version of the questionnaire now consisted of 15 items with a maximum of three items testing the same domain (Table 5.1).

Table 5.1: The domains and items for the second version of the questionnaire

DOMAIN	Corresponding Items	Item no
Initial management of low grade cytology and a TZ3	*Practice:* 1 multi-nominal	*1*
	Attitude: 1 ordinal scales	*2*
Non-routine measures to improve the adequacy of the examination	*Practice:* 1 discrete response	*3*
Use of oestrogen	*Attitude:* 1 discrete response	*4*
Conservative follow-up for low grade cytology	Practice: 1 multi-nominal	5
Depth of LLETZ in women with low grade cytology and a TZ3	*Practice:* 1 discrete response *Knowledge & Attitude:* 1 multi-nominal	*6* *7*
Initial management of high grade cytology and a TZ3	*Practice:* 1 multi-nominal	*8*
Depth of LLETZ in women with high grade cytology and a TZ3	*Practice:* 1 discrete response *Knowledge & Attitude:* 1 multi-nominal	*9* *10*
Repeating the referral cytology	*Practice & Knowledge:* 1 discrete response *Attitude:* 1 multi-nominal scale	*11* *12*
Respondent Demographics	*Job title & gender:* 2 discrete responses *Years of experience:* 1 continuous scale	*13 - 14* *15*

5.3.1.2 Face validity

Response rates for the qualitative interviews were excellent (100%, n=12). The socio-demographic characteristics can be viewed in Table 5.2; colposcopists from four units participated of whom four were male and eight female. Two nurse colposcopists, three gynaecological oncologists, two O&G registrars and five gynaecology consultants participated. The mean years of experience was 11.8 (range 2 - 30). English was the first language for 83.3% (n=10).

Table 5.3 describes the completed and missing items from the participants who were interviewed for face validity (the raw data of which can be viewed in Appendix 5, Table S5.1). Eleven participants (91.6%) completed all of the stems within the items. One respondent did not complete their gender nor one stem from the item assessing reasons for choice of LLETZ depth. When asked for reasons for non-completion the participant reported this was unintentional, supporting use of electronic software which requires respondents to complete all items.

Table 5.2: Sociodemographic information for cognitive interview participants

Participant	Unit	Job Title	Gender	Years of Experience	English First Language
1	1	Oncologist	M	15	N
2	1	Nurse	F	7	Y
3	1	Registrar	F	2	Y
4	1	Gynaecologist	F	4	Y
5	1	Gynaecologist	M	30	Y
6	2	Nurse	F	4	Y
7	2	Registrar	F	2	Y
8	2	Oncologist	M	30	Y
9	3	Gynaecologist	F	8	N
10	3	Gynaecologist	F	4	Y
11	4	Oncologist	M	20	Y
12	4	Gynaecologist	F	15	Y

Table 5.3: Missing item data from the cognitive interview participants

Item Number	Item Domain and Stems	Responses N	Missing Item (%)
1	Low grade cytology management		
	- 25-39 year old, nulliparous	12	0
	- 25-39 year old, parous	12	0
	- >40, family incomplete	12	0
	- Family complete, any age	12	0
2*	Why follow-up in colposcopy?		
	- To use a cytobrush	5	0
	- To prevent loss to follow-up	5	0
	- To perform a colposcopy	5	0
	- They are HR HPV positive	5	0
3	Possible adjuncts	12	0
4	Use of oestrogen	12	0
5	Total months follow-up before LLETZ		
	- 25-39 year old, nulliparous	12	0
	- 25-39 year old, parous	12	0
	- >40, family incomplete	12	0
	- Family complete, any age	12	0
6	LLETZ depth for low grade cytology		
	- 25-39 year old, nulliparous	12	0
	- 25-39 year old, parous	12	0
	- >40, family incomplete	12	0
	- Family complete, any age	12	0
7	Reasons for depth in Q6		
	- 25-39 year old, nulliparous	12	0
	- 25-39 year old, parous	12	0
	- >40, family incomplete	11	8.4
	- Family complete, any age	12	0
8	High grade cytology management		
	- 25-39 year old, nulliparous	12	0
	- 25-39 year old, parous	12	0

	- >40, family incomplete	12	0
	- Family complete, any age	12	0
9	LLETZ depth for high grade cytology		
	- 25-39 year old, nulliparous	12	0
	- 25-39 year old, parous	12	0
	- >40, family incomplete	12	0
	- Family complete, any age	12	0
10	Reasons for depth in Q9		
	- 25-39 year old, nulliparous	12	0
	- 25-39 year old, parous	12	0
	- >40, family incomplete	12	0
	- Family complete, any age	12	0
11	Repeat the cytology?	12	0
12*	Reasons for repeat in Q11	1	0
13	Demographics		
	- Job title	12	0
	- Years of experience	12	0
	- Gender	11	8.4

**Gated Responses*

Responses were assessed for floor or ceiling effects (Table 5.4). Item 4 was excluded from the analysis as it consisted of three discrete responses and the odds that more that 50% of participants would choose the lowest or highest value was greater than chance. Items 9 and 11 were excluded from the analysis as these evaluated colposcopists' adherence to national guidance; I would expect >90% of participants to choose a floor or ceiling answer. Item 8 does not relate to national guidance but it is expected that most colposcopists would offer a LLETZ to a woman who presents with high grade cytology and a TZ3. Of the included items, a floor or ceiling effect was not observed so no items were removed.

Table 5.4: Floor to ceiling effect

Item	Responses[a]	Lowest values	Highest values
1 – Low grade management	48	2 (4.2%)	0
2[b] - Why follow-up in colposcopy	20	2 (10%)	9 (45%)
3 – Possible adjuncts	12	1 (8.3%)	0
4 – Use of oestrogen	12	8 (66.7%)	2 (16.7%)
5 – Total follow-up before LLETZ	48	20 (41.6%)	4 (8.3%)
6 – LLETZ depth for low grade	48	0	2 (4.2%)
7 – Reasons for depth in Q6	48	11 (22.9%)	0
8 – High grade management	48	42 (95.5%)	0
9 – LLETZ depth for high grade	48	0	22 (50%)
10 – Reasons for depth in Q9	48	2 (4.7%)	0
11 – Repeat the cytology?	12	11 (91.7%)	0
12[b] - Reasons for repeat in Q11	1	0	0

(a)Total responses for the stems within an item *(b) Gated Responses*

All participants asked for the term *'diagnostic LLETZ'* to be changed to *'a standard LLETZ of 7-10mm'*. Seven Colposcopists suggested *'3 months cytological follow-up should be added to the management options for women reviewed with high grade cytology and a TZ3'*. Two participants felt the item discussing cytological follow-up was not omnicompetent as it should include 'never'. One participant suggested that the question evaluating management of women with high grade cytology should come first;

> **ID 10:** *'Whilst completing the questionnaire I felt that what initially had been routine management decisions were becoming daunting and problematic. I felt that this then influenced how I completed Q9* (Management of high grade cytology and a TZ3) *which I had always felt was a straight forward decision to treat'.*

All other participants felt the order was correct so this was not changed. One participant suggested 'years of experience' should be changed from a continuous to an ordinal scale in line with the 10K hour rule which links competence to hours of training. When changing a questionnaire based on the opinion of one participant, consideration must be given to how this advice may resonate with the rest of the cohort. On review of the literature I felt a trend may be more accurately pinpointed if categories with a limited number of variables were provided; the scale was subsequently revised in line with this recommendation. The amended questionnaire was sent to respondents for evaluation and no new comments were provided.

5.3.1.3 Construct validity

Of the twelve participants, 11 (91.7%) adhered to national guidelines[35] and did not repeat the cervical cytology at the first colposcopic assessment (p=0.99) and 100% chose ≥15mm depth of LLETZ when the family was complete. The items were not adjusted after assessment of this psychometric component.

5.3.2 Test-retest reliability

Twenty participants were recruited and completed 100% of the items. None of the participants felt their practice had changed in the two weeks between questionnaires. Table 5.5 outlines the socio-demographic characteristics of the respondents; 30% were male and 70% female, English was the first language for 85% and 11 were gynaecologists, four were oncologists, four were nurses and one was a registrar. The mean years of experience was 14.3 (range 2-36 years).

Table 5.5: Socio-demographics of the test-retest reliability participants

Participant	Job Title	Gender	Years of Experience	English First Language
1	Gynaecologist	F	8	Y
2	Oncologist	M	30	Y
3	Nurse	F	8	Y
4	Nurse	F	5	Y
5	Gynaecologist	F	7	Y
6	Registrar	F	4	Y
7	Gynaecologist	M	36	N
8	Oncologist	M	14	Y
9	Gynaecologist	F	32	Y
10	Oncologist	F	16	Y
11	Gynaecologist	F	3	Y
12	Gynaecologist	F	14	N
13	Gynaecologist	F	5	Y
14	Nurse	F	2	Y
15	Gynaecologist	M	17	Y
16	Oncologist	M	26	Y
17	Nurse	F	6	N
18	Gynaecologist	M	23	Y
19	Gynaecologist	F	18	Y
20	Gynaecologist	F	12	Y

Cross tabulations were produced to calculate the crude agreement; instability was observed in only 3 of 39 variables. Table 5.6 presents the crude agreement and kappa values for the test-retest questionnaires; perfect agreement was observed in eight items - Questions 1, 3, 4, 5, 6, 8, 9, 10 and 11. Agreement was very good, with kappa >0.84, for question 2. For some of the stems in questions 8 & 9 all respondents chose the same variable preventing the calculation of a kappa value but this strong consensus of opinion gives a stability of 100%. In question 7, kappa values were >0.90 for all responses.

Table 5.6: Kappa values for the test-retest reliability

	ITEM	n	Crude Agreement	Kappa	P value
Q1	***Initial management for low-grade cytology***				
	• 1a (25-39 yo, nullips)	20	100%	1.0	<0.001
	• 1b (25-39 yo, family incomplete)	20	100%	1.0	<0.001
	• 1c (>40 yo, family incomplete)	20	100%	1.0	<0.001
	• 1d (Family complete)	20	100%	1.0	<0.001
Q2	***Reasons for colposcopy follow-up***				
	• 1a (25-39 yo, nullips)	10	100%	1.0	0.008
	• 1b (25-39 yo, family incomplete)	10	100%	1.0	0.008
	• 1c (>40 yo, family incomplete)	9	96.3%	0.84	0.006
	• 1d (Family complete)	9	96.3%	0.84	0.006
Q3	***Alternative methods of diagnosis***	20	100%	1.0	<0.001
Q4	***Reasons for use of oestrogen***	20	100%	1.0	<0.001
Q5	***Length of cytology follow-up***				
	• 1a (25-39 yo, nullips)	20	100%	1.0	<0.001
	• 1b (25-39 yo, family incomplete)	20	100%	1.0	<0.001
	• 1c (>40 yo, family incomplete)	20	100%	1.0	<0.001
	• 1d (Family complete)	20	100%	1.0	<0.001
Q6	***Depth of LLETZ for low grade cytology***				
	• 1a (25-39 yo, nullips)	20	100%	1.0	<0.001
	• 1b (25-39 yo, family incomplete)	20	100%	1.0	<0.001
	• 1c (>40 yo, family incomplete)	20	100%	1.0	<0.001
	• 1d (Family complete)	20	100%	1.0	<0.001
Q7	***Reasons for depth chosen in Q6***				
	• 25-39 yo, nullips				
	- 8a1	8	100%	1.0	<0.001
	- 8a2	15	100%	1.0	<0.001
	- 8a5	6	100%	1.0	<0.001
	- 8a6	14	100%	1.0	<0.001
	• 25-39 yo, family incomplete				
	- 8b1	8	100%	1.0	<0.001
	- 8b2	15	100%	1.0	<0.001
	- 8b5	6	100%	1.0	<0.001
	- 8b6	14	100%	1.0	<0.001
	• >40yo, family incomplete				
	- 8c1	18	100%	1.0	<0.001
	- 8c2	9	95%	0.90	<0.001
	- 8c3	19	100%	1.0	<0.001
	- 8c4	10	100%	1.0	<0.001
	- 8c5	19	100%	1.0	<0.001
	- 8c6	16	100%	1.0	<0.001
	• Family complete, any age				
	- 8d1	18	100%	1.0	<0.001
	- 8d2	9	95%	0.90	<0.001
	- 8d3	19	100%	1.0	<0.001
	- 8d4	9	100%	1.0	<0.001
	- 8d6	16	100%	1.0	<0.001

Q8	***Initial management for high-grade cytology***				
	• 1a (25-39 yo, nullips)	20	100%	1.0	<0.001
	• 1b (25-39 yo, family incomplete)	20	100%	1.0	<0.001
	• 1c (>40 yo, family incomplete)	20	100%*		
	• 1d (Family complete)	20	100%*		
Q9	***Depth of LLETZ for high grade cytology***				
	• 1a (25-39 yo, nullips)	20	100%	1.0	<0.001
	• 1b (25-39 yo, family incomplete)	20	100%	1.0	<0.001
	• 1c (>40 yo, family incomplete)	20	100%*		
	• 1d (Family complete)	20	100%*		
Q10	***Reasons for depth in Q10***				
	• 25-39 yo nullips				
	- 11a1	10	100%	1.0	<0.001
	- 11a2	16	100%	1.0	<0.001
	- 11a4	10	100%	1.0	<0.001
	- 11a5	12	100%	1.0	<0.001
	• 25-39yo, family incomplete				
	- 11b1	10	100%	1.0	<0.001
	- 11b2	16	100%	1.0	<0.001
	- 11b4	10	100%	1.0	<0.001
	- 11b5	12	100%	1.0	<0.001
	• >40 yo, family incomplete				
	- 11c2	12	100%	1.0	<0.001
	- 11c3	11	100%	1.0	<0.001
	- 11c4	18	100%	1.0	<0.001
	- 11c5	11	100%	1.0	<0.001
	• Family complete, any age				
	- 11d2	12	100%	1.0	<0.001
	- 11d3	10	100%	1.0	<0.001
	- 11d4	19	100%	1.0	<0.001
	- 11d5	11	100%	1.0	<0.001
Q11	***Repeating the referral cytology***	20	100%	1.0	<0.001

*all participants chose the same response so there was no variation to estimate a kappa statistic upon.

5.4 Strengths

The extensive psychometric evaluation of the questionnaire helped ensure it was interpreted as intended and covered all the domains of interest. The sample size for the cognitive interviews was large enough to ensure a diverse range of views and to provide valuable information on which to evaluate and improve the content and face validity. Likewise, the reliability study was sufficiently large enough to give a precise estimate for this aspect of the questionnaire. Although the cognitive interviews were conducted in one English healthcare region, the BSCCP committee consists of members from across the UK

which will improve the generalizability of the questionnaire. Finally, the questionnaire performed well across genders, years of experience and job title.

5.5 Limitations

To reduce ambiguity and improve the likelihood of a consensus opinion, items which tested the same construct were multi-nominal or discrete responses rather than ordinal. The limitation of these designs, are the inability to assess internal consistency which can affect the reliability of the questionnaire. However, the kappa values for the items which were evaluated for test-retest reliability were all >0.90 (very good). There were unfortunately two items which could not be measured – gated questions (items 2 and 12 in Table 5.3) – so although the test-retest sample size calculation was achieved, the power was limited as only five and one participants respectively answered these gated questions.

Evaluation of self-perceived practice is easier when the domains of investigation have clear guidance but can be more problematic in areas of clinical uncertainty, reliability could be reduced if there is no 'correct' answer, but this was the focus of the study and as such the questionnaire was designed to evaluate this.

Some of the items are labour intensive and this may reduce response rates. However, in comparison to many validated questionnaires it is relatively brief and designed to be completed in only 15 minutes.

5.6 Conclusions

The third, and final, version of the questionnaire can be viewed in Appendix 5 (Figure S5.1). The data from this chapter suggests the final questionnaire is a suitable measure of the management of women with cytological abnormalities and a TZ3. It will enable the evaluation of colposcopist's practice in Chapter 6, facilitating the identification of a consensus opinion on which to formulate guidance.

Chapter 6 Current practices in the management of a TZ3: Results of a UK survey

6.1 Introduction

Colposcopists lead research and policy change in cervical screening and pathology. Their attitudes and knowledge are likely to have a significant effect on national recommendations and guideline implementation in a clinical setting. The outcomes from the focus groups in chapter 4[101] identified that management of a TZ3 is an area of clinical uncertainty due to the lack of clear evidence and guidance. Anxiety was the primary factor affecting decision-making and led to heterogeneity in decisions relating to length, clinical setting and technique of cytological follow-up.

Service inefficiencies and poor access to condition specific information can lead to patient anxiety and failure to attend follow-up appointments[77, 102, 103, 209, 210]. With non-attendance rates for follow-up Colposcopy documented at 11.2% in the UK[3], homogeneity of service provision needs to be improved. The development of evidence based guidelines would optimize outcomes by minimizing the unreliability of intuitive management. The aims of this study were to:

(i) evaluate UK colposcopists' decision-making when applied to the management of women with a TZ3

(ii) identify areas of concordance to inform a national consensus opinion.

6.2 Methodology

This was a cross-sectional design. All UK colposcopists (1200) were invited to complete an online questionnaire. Two invitations were sent one week apart. The questionnaire was developed following a literature review and evaluation by an expert body; this consisted of colposcopists who are leaders in research and policy making in this area. The final questionnaire contained 15 items which covered a range of clinical and cytological variables, oestrogen use, techniques to improve diagnostic yield, cytological follow-up and depth of LLETZ in women with a TZ3.

6.3 Results

6.3.1 Sample

Of the 1200 emailed colposcopists, 205 participated providing a 17.1% response rate. Participant demographics are presented in Table 6.1 and Appendix 6, Tables S6.1 & S6.2. Of the 200 respondents who provided demographic information, 133 (65.2%) respondents had more than 11 years of experience. General gynaecologists made up half of the participants and there were 27% more female than male respondents. This distribution was similar to that seen in the focus groups.

Table 6.1: Participant demographics

Demographic Information	Respondents (n, %)
Gender:	
Male	73 (36.5%)
Female	127 (63.5%)
Years of Experience:	
0-2	11 (5.4%)
2-4	11 (5.4%)
5-10	48 (23.5%)
≥11	133 (65.2%)
Job Title:	
Nurse Colposcopist	32 (15.7%)
Gyanecology Consultant	112 (55.2%)
Gynaecological Oncologist	27 (13.3%)
Associate Specialist	24 (11.8%)
O&G Registrar	8 (3.9%)

6.3.2 Initial management of low grade cytology

For this item, complete datasets were available for 203/205 respondents (Table 6.2). The most frequent initial management choice, irrespective of the patient's age or parity, was cytological follow-up (mean 148 (72.9%), range 112 - 171). When compared to 25 - 39 year olds there was a greater preference for LLETZ in women older than 40 (diff 8.8%, 95% CI 5.3 - 16.1%, p<0.001) and in women of any age who had completed their family (diff 21.7%, 95% CI 18.4 - 33.5%, p<0.001).

When adjusted for years of experience and compared to gynaecological consultants, who were the largest proportion of respondents, there was no evidence of an association between colposcopist's demographics and choice of initial management (Table 6.3). Indeed, for patients aged 25 - 39 years old, when comparing management choices of gynaecological oncologists and associate specialists to gynaecology consultants, the odds ratio could not be calculated as all respondents chose cytological follow-up. These data and free-text comments suggest colposcopists perceive increasing age to be a risk factor for high grade CIN;

> **ID 133:** *'I would not perform a LLETZ in a 25 year old, even if family complete, as low grade changes are likely to go back to normal. Over 40 I would perform a LLETZ whatever the fertility wishes'*

> **ID 135:** *'I would manage LSIL/HPV+ conservatively and review colposcopy in 6 - 12 months, becoming less conservative with increasing age and persistence of abnormality.....'*

Referral to the MDT was recommended by an average of 11.9% of respondents with no evidence of a difference in this choice between patient demographics (p=0.16). Free-text comments suggest colposcopists are concerned about missing high grade disease but also about the treatment-related morbidity;

> **ID 202:** *'I would like to do a LLETZ because the colp is unsatisfactory but not wanting to risk obstetric complications I would want to discuss with colleagues.'*

> **ID 123:** *'Discuss at MDT to confirm low grade changes and to ascertain that endocervical cells are present i.e. TZ is sampled'*.

Table 6.2: Management choices for low grade cytology and a TZ3 by age and family situation

	25-39, nulliparous N; % *(95% CI)*	**25-39, family incomplete** N; % *(95% CI)*	**≥40, family incomplete** N; % *(95% CI)*	**Family complete, any age** N; % *(95% CI)*
LLETZ	3; 1.5 *(0 – 9%)*	4; 1.9 *(0 – 9%)*	21; 10.3 *(3.2 – 17.4%)*	24; 23.2 *(16.1 – 30.3%)*
6m colposcopy follow-up	79; 38.5 *(31.4 - 45.6%)*	84; 41.4 *(34.3 - 48.5%)*	69; 34 *(26.9 – 41.1%)*	46; 22.6 *(15.5 – 29.7%)*
12m colposcopy follow-up	49; 23.9 *(16.8 – 31%)*	46; 22.7 *(15.6 - 29.8%)*	34; 16.7 *(9.6 – 23.8%)*	34; 16.7 *(9.6 – 23.8%)*
6m community cytology	20; 9.8 *(2.7 - 24%)*	19; 9.4 *(2.3 – 16.5%)*	15; 7.4 *(0 – 14.5%)*	14; 6.9 *(0 – 14%)*
12m community cytology	23; 11.2 *(4.1 - 18.3%)*	21; 10.3 *(3.2 – 17.4%)*	21; 10.3 *(3.2 – 17.4%)*	18; 8.9 *(1.8 – 16%)*
MDT	20; 9.8 *(2.7 - 16.9%)*	19; 9.4 *(2.3 – 16.5%)*	32; 15.8 *(8.7 – 22.9%)*	26; 12.8 *(5.7 – 19.9%)*
Other	9; 4.4 *(0 - 11%)*	10; 4.9 *(0 – 12%)*	11; 5.4 *(0 – 12.5%)*	18; 8.9 *(1.8 – 16%)*
TOTAL	203	203	203	203

Table 6.3: Association of management for low grade cytology and TZ3 with respondent demographics when adjusted for experience, job title and gender

	25-39 nulliparous[a] OR (95% CI), p value	**25-39 family incomplete**[b] OR (95% CI), p value	**≥40 family incomplete**[c] OR (95% CI), p value	**Family complete**[d] OR (95% CI), p value
LLETZ vs cytological follow-up				
Gynaecology consultant	Ref	Ref	Ref	Ref
Gynaecology oncologist	(e)	(e)	2.92 (0.84 - 10.0), 0.90	1.26 (0.46 - 3.42), 0.65
Nurse colposcopist	1.91 (0.15 - 23.9), 0.62	2.04 (0.17 – 25.1), 0.57	0.99 (0.24 - 4.09), 0.99	0.56 (0.17 – 1.84), 0.34
Associate specialist	(e)	2.92 (0.25 - 34.3), 0.39	1.18 (0.24 – 5.99), 0.84	0.44 (0.90 – 2.17), 0.90
LLETZ vs cytological follow-up				
0 - 10 years[f]	Ref	Ref	Ref	Ref
≥ 11 years	1.04 (0.92 – 11.7), 0.97	1.57 (0.16 – 15.4), 0.7	0.87 (0.34 - 2.24), 0.77	1.64 (0.78 - 3.46), 0.19
LLETZ vs cytology follow-up[g] Male	Ref	Ref	Ref	Ref
Female	0.26 (0.02 - 3.20), 0.3	1.01 (0.09 - 10.9), 0.99	0.62 (0.23 – 1.73), 0.36	1.53 (0.69 – 3.36), 0.29
6 vs 12 month follow-up				
Gynaecology consultant	Ref	Ref	Ref	Ref
Gynaecology oncologist	0.67, CI 0.25-1.82, p=0.43	OR 0.72, CI 0.27-1.97, p=0.53	OR 0.97, CI 0.31-3.04, p=0.96	OR 0.59, CI 0.17-2.04, p=0.40
Nurse colposcopist	OR 1.60, CI 0.67-3.85, p=0.29	OR 1.96, CI 0.81-4.77, p=0.14	OR 1.86, CI 0.69-4.99, p=0.22	OR 1.66, CI 0.57-4.85, p=0.35
Associate specialist	OR 2.07, CI 0.73-5.93, p=0.17	OR 2.11, CI 0.72-6.19, p=0.17	OR 1.21, CI 0.38-3.83, p=0.75	OR 1.07, CI 0.28-4.12, p=0.92
6 vs 12 month follow-up				
0 - 10 years[g]	Ref	Ref	Ref	Ref
≥ 11 years	1.06 (0.56 - 2.02), 0.85	1.39 (0.72 - 2.69), 0.32	1.53 (0.74 - 3.17), 0.25	1.68 (0.78 - 3.64), 0.16
6 vs 12 month follow-up[g] Male	Ref	Ref	Ref	Ref
Female	1.28 (0.65 – 2.49), 0.47	1.64 (0.83 – 3.26), 0.16	1.69 (0.79 - 3.59), 0.17	2.18 (0.94 – 5.05), 0.07
Colposcopy or GP follow-up				
Gynaecology consultant	Ref	Ref	Ref	Ref
Gynaecology oncologist	0.66 (0.23 – 1.95), 0.46	0.56 (0.19 – 1.71), 0.32	0.43 (0.13 – 1.49), 0.19	0.67 (0.18 – 2.55), 0.56
Nurse colposcopist	0.75 (0.28 – 2.02), 0.57	0.81 (0.29 – 2.26), 0.69	0.49 (0.17 – 1.43), 0.19	0.45 (0.15 – 1.39), 0.17
Associate specialist	1.26 (0.33 – 4.84), 0.57	1.04 (0.27 – 4.04), 0.96	0.89 (0.22 – 3.62), 0.88	0.69 (0.16 – 3.04), 0.63
Colposcopy or GP follow-up				
0 - 10 years[f]	Ref	Ref	Ref	Ref
≥ 11 years	1.86 (0.92 – 3.85), 0.08	1.93, (0.93 – 4.01), 0.08	2.05 (0.94 – 4.46), 0.07	1.29 (0.56 – 2.97), 0.54
Colposcopy or GP follow-up Male	Ref	Ref	Ref	Ref
Female	1.22 (0.51 – 2.95), 0.66	1.34 (0.53 – 3.38), 0.53	1.68 (0.61 – 4.64), 0.31	1.43 (0.48 – 4.21), 0.52

(a): *3/170 selected a LLETZ* ***(b):*** *4/170 selected a LLETZ* ***(c):*** *19/156 selected a LLETZ* ***(d):*** *43/155 selected a LLETZ* ***(e):*** *All respondents chose cytological follow-up preventing estimation of an odds ratio i.e. perfect prediction.* ***(f):*** *Categories were combined to help identify a trend.* ***(g):*** *Adjusted for years of experience but we could not adjust for job title because of perfect prediction*

6.3.3 Cytological follow-up for low grade cytology

6.3.3.1 Frequency of follow-up and clinical setting

Table 6.2 describes colposcopists' management choices for women with low grade cytology by age and family situation. There was a preference for follow-up to be 6 rather than 12 monthly (58.1% vs 42.1%; diff 16%, 95% CI 11 – 22.5%, p<0.001) and a strong preference for this to occur in the colposcopy clinic rather than the community (76.4% vs 25.8%; diff 50.6%, 95% CI 43.7 – 53.9%, p<0.001). Table 6.3 shows the association between these management choices and a range of respondent demographics, of which there was no evidence of an association.

The importance of the factors which influenced a colposcopist's decision to recommend colposcopy clinic follow-up, rather than primary care, were assessed (Figure 6.1). 'Extremely important' factors were evaluated; the odds of colposcopists recommending follow-up in colposcopy to use a cytobrush and Cervex-Brush (broom and brush) was twice the odds of colposcopists offering this clinical setting to perform a colposcopy (n=72 vs 46; OR 2.15, 95% CI 1.29 – 3.57, p=0.003). In comparison to offering colposcopy clinic follow-up to reduce loss to follow-up (n=64), colposcopists were slightly more likely to offer colposcopy clinic follow-up to use a broom and brush (OR 1.24, 95% CI 1.02 – 2.85, p=0.05). There was no evidence of an association between the factor affecting this decision-making and years of experience or gender but nurses, when compared to gynaecology consultants, were three times more likely to request colposcopy follow-up to use the colposcope (Table 6.4).

Free-text explanations for choice of colposcopy clinic follow-up suggests the absence of cytobrush sampling in the community deters colposcopists from recommending follow-up in this setting and that colposcopists' feel a responsibility to make a diagnosis.

> **ID 124:** *'I would be happy to discharge to GP cytology if I was sure endo & ecto-cervical cytology would be done'*

> **ID 21:** *'By this visit we will have 2 smears to look at'*

Figure 6.1: Factors affecting choice of colposcopy follow-up in women with low grade cytology and a TZ3

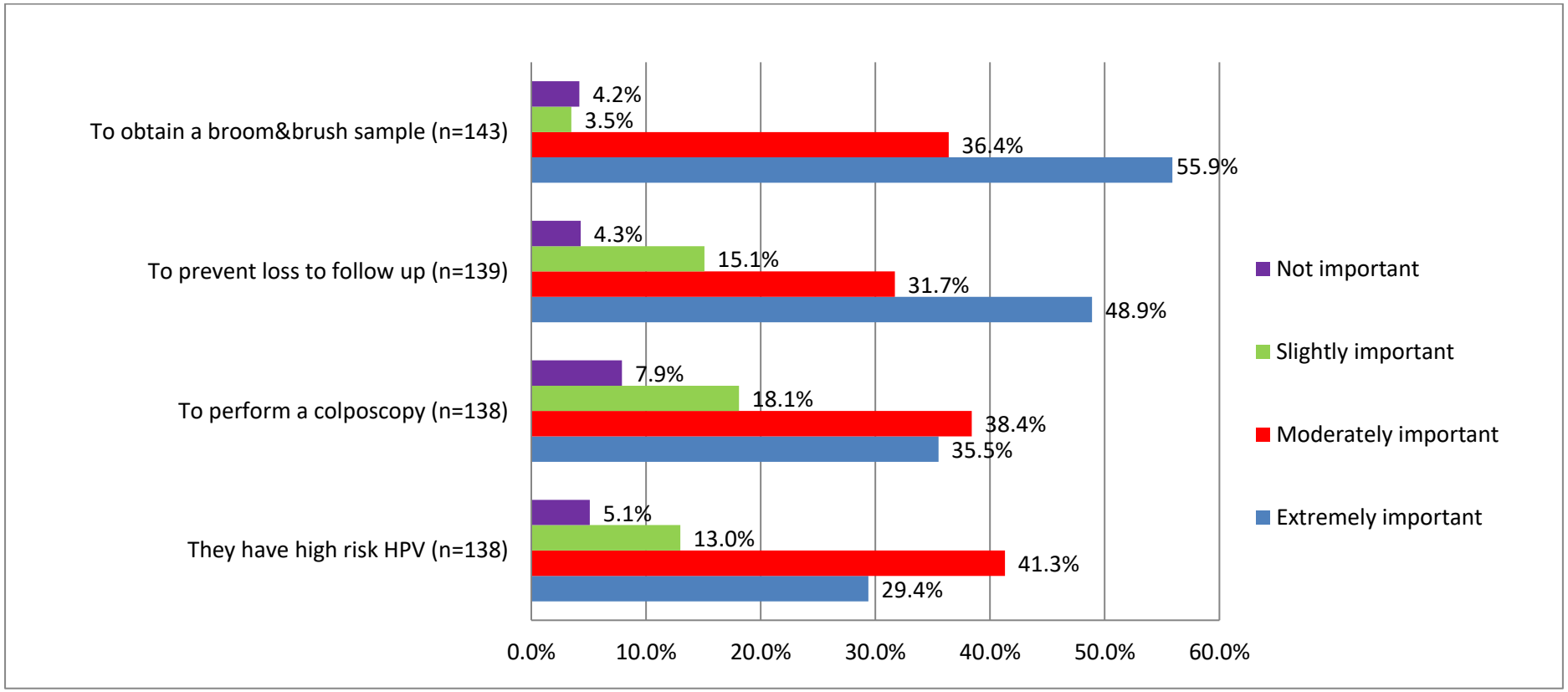


Table 6.4: Association between respondent demographics and factors affecting choice of colposcopy clinic follow-up

Respondent Demographic	To use a broom and brush OR (95% CI), p value	To prevent loss to follow-up OR (95% CI), p value	To do a colposcopy OR (95% CI), p value	They have high risk HPV OR (95% CI), p value
Job Title:[(a)]				
Gynaecology consultant	Ref	Ref	Ref	Ref
Gynaecology oncologist	0.32 (0.10-1.03), 0.06	0.78 (0.25-2.45), 0.68	0.53 (0.14-2.09), 0.34	0.29 (0.08-1.12), 0.07
Nurse colposcopist	1.26 (0.47-3.37), 0.65	1.91 (0.72-5.06), 0.19	3.20 (1.18-8.73), 0.02	1.33 (0.50-3.53), 0.57
Associate specialist	0.71 (0.24-2.09), 0.53	2.05 (0.68-6.19), 0.2	1.74 (0.58-5.23), 0.32	0.88 (0.28-2.71), 0.82
Years of Experience:[(b), *]				
0 - 10 years	Ref	Ref	Ref	Ref
≥ 11 years	0.37 (0.35-1.48), 0.37	1.04 (0.51-2.14), 0.9	0.86 (0.39-1.81), 0.67	1.09 (0.52-2.29), 0.82
Gender:[(a), *]				
Male	Ref	Ref	Ref	Ref
Female	0.75 (0.36-1.56), 0.4	0.88 (0.42-1.83), 0.74	0.53 (0.25-1.12), 0.09	1.35 (0.64-2.83), 0.43

(a) Adjusted for years of experience
(b) Adjusted for gender.
** Unable to adjust for job title as co-linear with years of experience*

6.3.3.2 Total length of follow-up before recommending a LLETZ

Figure 6.2 summarises the colposcopists' preference for total length of cytological follow-up before a LLETZ is offered for women with low grade cytology. The most frequent observation was 24 months of cytological follow-up in nulliparous and parous 25-39 year olds (n=76, 38% and n=74, 37% respectively). However, when pooling the 6, 12 and 18 month responses, this combined proportion was equivalent to the proportion of colposcopists who selected 24 months (n=74, 35% and n=75, 37% respectively): p=0.72 for nulliparous and p=0.99 for parous women.

Table 6.5 describes the association between respondents' demographics and the total length of follow-up they would recommend before offering a LLETZ. Nurse colposcopists had approximately three times the odds of waiting 24 months if the family was complete before offering LLETZ when compared to general gynaecologists. When compared to the choice of offering LLETZ at 24 months, doctors are more likely to recommend LLETZ by 12 months in the family complete group (n=45 vs n=115) and in women ≥40 (n=60 vs n=103), p<0.001 and p=0.003 respectively.

Explanations for choice of less than 24 months were not provided. Reasons for 24 months included;

> **ID 196:** *'They can be safely monitored with cytology and HPV test'*

Colposcopists who ticked 'other' preferred individualized care rather than a prescribed timescale;

> **ID 128:** *'Other factors would influence my decision, such as prior screening history, patient choice, if they are immuno-compromised (less likely to treat due to high risk of persisting HPV) or post-menopausal (risk of stenosis and difficult follow up post LLETZ)'*

> **ID 144:** *'As per MDT decision with pathologists and other colposcopists. No timescale'.*

Figure 6.2: Maximum length of cytological follow-up, in months, before a LLETZ is offered for persistent low grade cytology and a TZ3

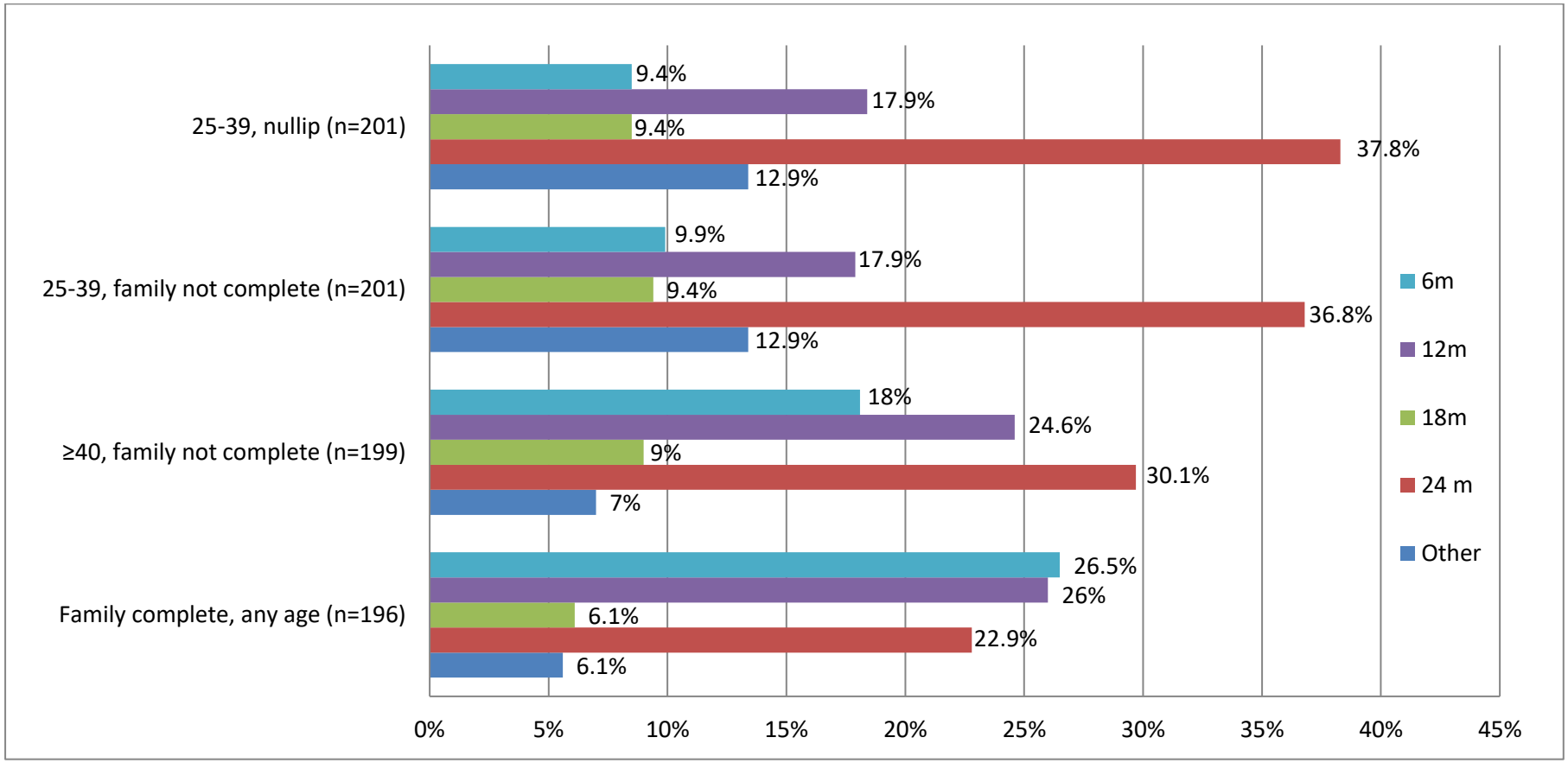


Table 6.5: Association between respondent demographics and choice of 24 months cytological follow-up (compared to choice of <24 months)

	25-39 nulliparous OR (95% CI), p value	**25-39, family incomplete** OR (95% CI), p value	**≥40, family incomplete** OR (95% CI), p value	**Family complete, any age** OR (95% CI), p value
Job Title[(a)]				
General gynaecologist	Ref	Ref	Ref	Ref
Gynaecology oncologist	1.26 (0.52 - 3.05), 0.6	1.12 (0.46 - 2.74), 0.79	1.33 (0.51 - 3.49), 0.55	1.71 (0.62 - 4.75), 0.29
Nurse colposcopist	1.24 (0.53 - 2.88), 0.6	1.42 (0.61 - 3.30), 0.41	1.95 (0.81 - 4.71), 1.49	3.16 (1.25 - 7.97), 0.02
Associate specialist	0.67 (0.25 - 1.76), 0.42	0.73 (0.27 - 1.91), 0.52	1.41 (0.54 - 3.66), 0.47	1.57 (0.55 - 4.51), 0.39
Years of Experience[(b)]				
0 - 10 years	Ref	Ref	Ref	Ref
≥ 11 years	0.89 (0.48 - 1.66), 0.73	0.95 (0.51 - 1.79), 0.24	1.25 (0.64 - 2.44), 0.51	1.32 (0.63 - 2.77), 0.45
Gender:[(a)]				
Male	Ref	Ref	Ref	Ref
Female	1.57 (0.84 - 2.95), 0.15	1.45 (0.77 - 2.72), 0.24	1.30 (0.67 - 2.51), 0.43	1.04 (0.51 - 2.13), 0.90

(a) Adjusted for years of experience
(b) Adjusted for gender.

6.3.4 Techniques used to obtain a diagnosis

Table 6.6 describes the non-routine techniques which are used to improve diagnosis in women with a TZ3. The majority of colposcopists, 93.6%, reported use of at least one method.

Topical oestrogen is prescribed more than systemic in postmenopausal (n=182 (89.7%) vs n=16 (7.9%); diff 81.8%, 95% CI 75 - 86.8%, p<0.001), and premenopausal women (n=93 (45.8%) vs n=18 (8.9%); diff 36.9%, CI 28.4-44.8%, p<0.001)**.** Comments included;

> **ID 196:** *'systemic may not be enough'*
>
> **ID 70:** *'I have never used the COCP but I suppose it is logical'*

When compared to postmenopausal women, colposcopists were 43.9% less likely to prescribe topical oestrogen in pre-menopausal women (95% CI 35.2 – 51.8%, p<0.001). The reasons for this were not provided.

When adjusted for years of experience general gynaecologists had 2.26 times the odds of prescribing topical oestrogen when compared to gynaecological oncologists (90.2% vs 66.7%; OR 2.26, 95% CI 1.15 – 6.9, p=0.005). Compared to gynaecological oncologists, nurses were also more likely to prescribe topical oestrogen (100%; p<0.001), as were associate specialists (91.7%; OR 2.18, CI 1.09 – 7.95, p=0.04). Furthermore, women had four times the odds of prescribing topical oestrogen when compared to men (OR 4.07, CI 1.56 - 10.6, p=0.002).

Although HPV genotyping is used, other surrogate biomarkers for HPV infection were rarely advocated (n=35 (17.2%) vs n=7 (3.4%); diff 13.8%, 95% CI 7.8 - 20%, p<0.001). Use of these techniques may be affected by resources;

> **ID 158:** *'We do not have HPV triage in Scotland but can request if agreed at MDT'*.

Only 35 respondents used endocervical curettage and reasons for this were not elaborated upon. There was no evidence of an association between years of experience and these methods, although the sample sizes may be too small to detect a trend (Table 6.6 and Appendix 6; Tables S6.3 & S6.4).

Table 6.6: Non-routine methods used to improve diagnosis of dysplasia and association of choice with respondent demographics

TECHNIQUE:	**Response rate** n, % (95% CI)	**Effect of experience** (p value)	**Effect of job title** (p value)	**Effect of Gender** (p value)
None	13, 6.4% (3.4 - 10.6)	0.07	0.18	0.003
HPV Genotyping	35, 17.2% (12.3 - 22.9)	0.37	0.35	0.69
Endocervical Curettage	24, 11.8% (7.7 - 16.9)	0.67	0.46	0.92
Biomarkers combined with cytology	7, 3.4% (1.4 - 6.9)	0.28	0.56	0.27
Topical oestrogen if postmenopausal	182, 89.7% (84.1 - 92.8)	0.10	<0.001	0.002
Topical oestrogen if premenopausal	93, 45.8% (38.6 - 52.4)	0.09	0.03	0.39
Systemic oestrogen (HRT) if postmenopausal	16, 7.9% (4.5 - 12.4)	0.41	0.76	0.92
Systemic oestrogen (COCP) if premenopausal	18, 8.9% (5.3 - 13.5)	0.36	0.73	0.21
TOTAL	204			

6.3.5 Main reasons for using oestrogen

The majority of respondents answered this question (n=196, 95.6%) of whom 141 (71.9%; 95% CI 65 - 78.1%) used oestrogen to improve the adequacy of the colposcopy in women with low grade cytology, 44 (22.4%; 16.8 – 28.9%) to improve the adequacy of the repeat cytology and 7 (3.5%; 1.4 – 7.2%) to make the examination more comfortable. There was no evidence of an association between respondent demographics and their reasons for use of oestrogen (Table 6.7).

Table 6.7: Association (OR) between a colposcopist's demographics and their use of oestrogen to improve the cytology when compared to use of oestrogen to improve the colposcopic adequacy

Respondents Demographics	OR (95% CI), p value
Gender:	
Male (n= 64)	Ref
Female (n=118)	0.89 (0.43 - 1.85), 0.7
Years of Experience:	
0 - 10 (n=65)	Ref
≥11 (n=117)	1.02 (0.48 - 2.13), 0.9
Job Title:	
General gynaecologist (n=100)	Ref
Nurse colposcopist (n=30)	0.49 (0.15 - 1.60), 0.2
Gynaecological oncologist (n=21)	0.80 (0.26 - 2.44), 0.7
Associate specialist (n=23)	1.07 (0.38 - 3.02), 0.8

6.3.6 Initial management of high grade cytology

Data was available for 205 respondents (Figure 6.3 and Appendix 6, Table S6.5). In nulliparous women aged 25 – 39, 46.7% (n=96) chose LLETZ, 34.6% (n=71) MDT review and 11.1% (n=24) three month colposcopy follow-up. In comparison to nulliparous women aged 25 - 39, more colposcopists recommended a LLETZ in parous women aged 25 - 39 (57.6%, n=118; diff 10.8%, 95% CI 0.79 - 20.6, p=0.03), women >40 years whose family was incomplete (69.3%, n=142; diff 22%, 95% CI 12.4 – 30.9%, P<0.001) and women of any age who had completed their family (87%, n=179; diff 40.3%, CI 31.6 – 48%, p<0.001). One third of colposcopists advocated referral to the MDT in women where the family was incomplete, irrespective of age. There was no evidence of an association between gender and years of experience with initial management choice (Table 6.8) but nurses, when compared to general gynaecologists, were three times more likely to refer to the MDT than recommend LLETZ when the family was incomplete.

Figure 6.3: Respondent's management choices for women with high grade cytology and a TZ3

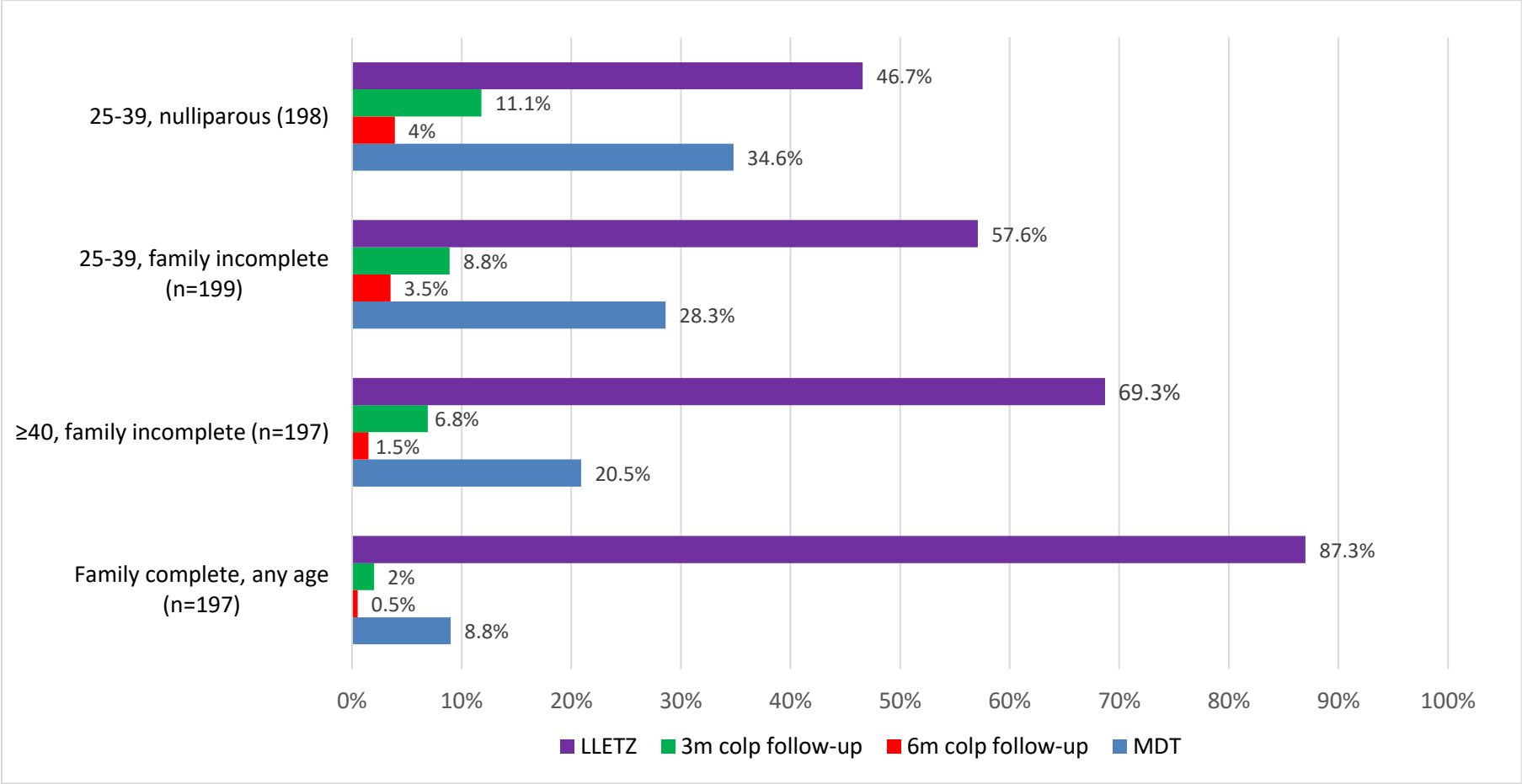


Table 6.8: Association between colposcopist's demographics and choice of LLETZ vs 3 month follow-up or LLETZ vs MDT in women with high-grade cytology

	25-39, nulliparous OR (95% CI), p value	**25-39, family incomplete** OR (95% CI), p value	**≥40, family incomplete** OR (95% CI), p value	**Family incomplete** OR (95% CI), p value
LLETZ vs 3 month colposcopy follow-up				
Gender: [a]				
Male (n=73)	Ref	Ref	Ref	Ref
Female (n=127)	0.98 (0.37 - 2.56), 0.97	1.19 (0.41 - 3.44), 0.74	1.11 (0.35 - 3.48), 0.85	*
Years of Experience: [b]				
0 - 10 (n=68)	Ref	Ref	Ref	Ref
≥11 (n=131)	2.42 (0.83 - 7.05), 0.1	5.07 (0.99 - 23.1), 0.05	7.38 (0.98 - 58.1), 0.06	*
Job title: [a][b]				
General Gynaecologist (n=110)	Ref	Ref	Ref	Ref
Nurse Colposcopist (n=31)	0.76 (0.15 - 3.92), 0.74	1.92 (0.45 - 8.03, 0.37	2.76 (0.60 - 12.6), 0.18	*
Gynaecology Oncologist (n=26)	0.37 (0.08 - 1.85), 0.23	1.13 (0.22 - 5.82, 0.87	0.86 (0.16 - 4.46), 0.85	1.41 (0.12 - 16.3), 0.78
Associate Specialist (n=24)	1.61 (0.42 - 6.15), 0.48	0.68 (0.07 - 5.86), 0.73	0.53 (0.06 - 4.59), 0.56	*
LLETZ vs MDT review				
Gender: [a]				
Male (n=73)	Ref	Ref	Ref	Ref
Female (n=127)	0.94 (0.48 - 1.83), 0.86	0.85, CI 0.43 - 1.68, 0.65	1.18, CI 0.55 - 2.51, 0.66	0.76, CI 0.26 - 2.16, 0.61
Years of Experience: [b]				
0 - 10 (n=68)	Ref	Ref	Ref	Ref
≥11 (n=131)	1.10, CI 0.58 - 2.08, 0.76	1.11, CI 0.58 - 2.14, 0.74	0.83, CI 0,41 - 1.69, 0.61	0.64, CI 0.24 - 1.71, 0.37
Job title: [a][b]				
General Gynaecologist (n=110)	Ref	Ref	Ref	Ref
Nurse Colposcopist (n=31)	2.67 (1.04 - 6.84), 0.04	3.11 (1.22 - 7.94), 0.01	2.60 (0.98 - 6.94), 0.05	1.94 (0.57 - 6.56), 0.28
Gynaecology Oncologist (n=26)	0.90 (0.35 - 2.32), 0.83	1.14 (0.43 - 3.01), 0.78	1.47 (0.50 - 4.27), 0.47	0.50 (0.05 - 4.25), 0.52
Associate Specialist (n=24)	1.94 (0.70 - 5.41), 0.20	1.29 (0.46 - 3.60), 0.61	1.72 (0.59 - 5.02), 0.31	1.62 (0.40 - 6.54), 0.49

(a) Adjusted for years of experience (b) Adjusted for gender

**Unable to calculate OR because all of the respondents in this category chose LLETZ.*

6.3.7 Depth of LLETZ

6.3.7.1 Low grade cytology

Table 6.9 describes the preferred depth of LLETZ in women with low grade and high grade cytology in the presence of a TZ3. Where the family was incomplete the majority of colposcopists chose 7-10mm depth. The strength of the consensus was similar between 25 - 39 year old nulliparous and parous women (diff 0.5%, p=0.99) but was weaker in women ≥40 (diff 10.8%, p=0.002). Although the most frequent observation was 11 - 14mm in women who had completed their families, this consensus was weak at 50%. The association of colposcopist's demographics with choice of ≥15mm LLETZ, in comparison to choice of ≤14mm, could not be calculated as most respondents chose a depth ≤14mm in all patient demographics (99%, 98.5%, 94.9% and 87.7% respectively).

Of the colposcopists (n=164) who chose a depth ≤10mm in any of the patient demographics (Table 6.9 and Appendix 6, Table S6.6), *'future fertility is an issue'* (n=95, 58%) was the primary factor affecting choice. In comparison, the following factors were less likely to influence this decision; *'I can repeat the LLETZ if diagnostic for CIN'* (n=67, 41.2%; diff 16.8%, p<0.001), *'these women have reassuring histology'* (n=55, 33.4%; diff 24.6% p<0.001) and *'the risk of cervical stenosis'* (n=34, 21%; diff 37%, p<0.001). Of the colposcopists who chose a LLETZ ≥11mm (n=123), *'a deeper LLETZ excises an endocervical TZ'* was the primary reason for this choice (n=72, 58.8%). In comparison, *'fertility in no longer an issue'* (n=53, 42.7%; diff 5.1%, p=0.03) and *'they have high risk HPV'* (n=20, 16.5%; diff 42.3%, p<0.001) were less likely to affect this choice.

6.3.7.2 High grade cytology

Irrespective of parity, the majority of colposcopists recommended 7-10mm depth in 25 - 39 year olds and this proportion was similar to that observed with low grade cytology (Table 6.9). In women ≥40 whose family was incomplete, although more chose 11 - 14mm when compared to choice in women aged 25 - 39, the greatest proportion still chose 7-10mm (diff 9.6%, p=0.06). Where the family was complete the most frequent observation was 11-14mm, but this consensus was weak at 55%. Compared to low grade cytology, respondents were more likely to choose 11-14mm depth in women ≥40 and in those whose family was complete. Respondents were also less likely to perform a 6mm LLETZ and more likely to complete an 11-14mm LLETZ in 25 - 39 year olds with high grade cytology when compared to women aged 25 - 39 with low grade cytology.

Table 6.9: Colposcopists' recommendations for depth of LLETZ in women with low grade or high grade cytology and a TZ3

	Low grade cytology (n=195) N (%, 95% CI)	**High grade cytology (n=199)** N (%; 95% CI)	**Difference in proportions** % (95% CI), p value
25-39 nulliparous:			
≤6 mm	22 (11.2; 7.2 – 16.7)	8 (4; 1.9 – 8.1)	7.2 (1.7 - 12.9), 0.007
7-10 mm	142 (72.9; 66.2 – 78.9)	130 (65.3; 58.2 – 71.8)	7.6 (1.8 - 16.9), 0.1
11-14 mm	29 (14.8; 10.1 – 20.7)	54 (27.1; 21.2 – 33.9)	12.3 (3.9 - 20.5), 0.003
≥15mm	2 (1.0; 6.4 – 15.6)	7 (3.5; 1.6 – 7.4)	2.5 (-0.8 - 6.2), 0.1
25-39 family incomplete:			
≤6 mm	20 (10.2; 6.5 – 15.6)	6 (3; 1.2 – 6.8)	7.2 (2.0 - 12.7), 0.004
7-10 mm	142 (72.4; 65.9 – 78.8)	130 (65.3; 58.2 – 71.8)	7.1 (-2.4 - 16.5), 0.13
11-14 mm	30 (15.3; 10.7 – 21.4)	56 (28.1; 22.1 – 35)	12.8 (4.3 - 21.1), 0.002
≥15mm	3 (1.5; 3.2 – 4.8)	7 (3.5; 1.6 – 7.4)	2 (-1.6 - 5.8), 0.21
≥40, family incomplete:			
≤6 mm	10 (5.1; 2.6 – 9.4)	3 (1.5; 0.4 – 4.7)	3.6 (-0.3 - 7.9), 0.05
7-10 mm	121 (61.7; 54.7 – 69.2)	98 (49.3; 42.1 – 56.4)	12.4 (2.2 - 22.3), 0.01
11-14 mm	54 (27.6; 21.7 – 34.5)	79 (39.7; 32.9 – 46.9)	12.1 (2.4 - 21.5), 0.01
≥15mm	10 (5.1; 2.6 – 9.4)	19 (9.6; 6.0 – 14.7)	4.5 (-1.0 - 10.1), 0.09
Family complete, any age:			
≤6 mm	2 (1; 1.8 – 3.9)	0 (0; 0 – 0.2)	1 (-1.0 - 3.6), 0.16
7-10 mm	70 (35.7; 29.1 – 42.9)	50 (25.1; 19.4 – 31.9)	10.6 (1.2 - 19.9), 0.02
11-14 mm	98 (50.0; 42.8 – 57.2)	111 (55.8; 48.6 – 62.8)	5.8 (-4.4 - 15.9), 0.25
≥15mm	25 (12.8; 8.8 – 18.7)	38 (19.1; 14 – 25.4)	6.3 (-1.3 - 13.8), 0.09

Of the colposcopists who preferred a ≤10mm LLETZ in women with high grade cytology (n=189 - Table 6.9 and Appendix 6, Table S6.7), *'fertility is an issue'* was the primary reason (n=105, 55.6%) when the family was incomplete. Other factors which influenced choice in all patient demographics included; *'I can repeat the LLETZ if diagnostic for CIN'* (diff 12.3%, 95% CI 5.6 – 18.8%, p=0.0003) and *'the risk of cervical stenosis'* (diff 40.5%, 95% CI 34.5 – 46%, p<0.001). Of note only 33.3% (17/51) colposcopists who chose ≤10mm depth when the family was complete gave a reason compared to 79.7% (110/138) when the family was incomplete.

Of those colposcopists who preferred ≥11mm LLETZ depth (n=149), the primary reason was *'a deeper LLETZ excises an endocervical TZ'* (n=98, 65.7%). In comparison, *'the majority have high grade CIN'* (n=81, 54.2%; diff 11.5%, 95% CI 4.5 – 18.3%, p=0.001) and *'fertility in no longer an issue'* (n=50, 33.7%; diff 32%, 95% CI 24.9 – 38.5%, p<0.001) were less likely to influence this decision. When compared to factors affecting choice with low grade cytology, colposcopists were less concerned about reproductive function and more concerned that high disease would be missed with a shallower depth (p=0.02).

6.3.8 Repeating the referral cytology

Of 205 participants, 144 (70.2%) would not repeat the referral cytology at the first colposcopy appointment. Reasons given included confidence in the screening test result;

> **ID 49:** *'no need to repeat if routinely HPV tested*'
>
> **ID 131:** '*Women are seen very soon after referral. I would only repeat the cytology if it is >3 months as it would risk false negative results*.'

Sixty (29.3%) would repeat the cytology, irrespective of grade. Of these colposcopists, 31 (51.7%) did so because they believed the Cervex-Brush may not have adequately sampled the TZ;

> **ID 170:** '*Reporting the presence of endocervical cells used to make management decisions much easier*.'

If the smear quality was poor 21/60 (35%) repeated the cytology and 39 (65%) to provide reassurance that LLETZ was the correct management option in young women. There was no evidence of an association between gender (p=0.7) or years of experience (p=0.13) and repeating the cytology. When compared to gynaecology consultants, nurses were 88% less likely to repeat the cytology (OR 0.12, 95% CI 0.03 - 0.53, p=0.001).

6.4 Strengths

To the best of my knowledge this is the first nationwide study to assess how UK colposcopist's manage women with a TZ3. This survey was supported by the BSCCP, which denotes the relevance of this work, and the information provided will contribute to guidance development and direct future research. Areas of consensus had narrow confidence intervals and areas of discordance were, in most scenarios, not affected by colposcopists' demographics indicating true areas of clinical uncertainty.

Sampling was national and this will improve the generalizability of the findings. As colposcopy training (knowledge) and revalidation in the UK is standardized, variation in practice between the geographical units should be minimised. The outcomes and population demographics from this study triangulated with the regional focus group study[101] which validates the findings from Chapter 4 (see Discussion – Chapter 8). The online nature of the survey maintained data protection laws and reduced missing responses.

6.5 Limitations

In comparison to BSCCP endorsed published surveys[211, 212] the response rate was half of what I expected and this may be due to timing of release, during a national holiday. Thirty one responses were received in the first week. The remaining responses were received after the second follow-up email. Regardless, the potential for selection bias needs to be considered as colposcopists' motivation to participate will have led to a self-selected sample. This may limit the findings from the study but the participant demographics and study outcomes were comparable to the focus groups (source population). Nevertheless, colposcopists may believe this is not an area of ambiguity or conversely, due to clinical uncertainty, may have felt unable to participate. To investigate this, it would have been useful to include an item which assessed if colposcopists felt this was a topical area and / or area of clinical uncertainty.

A small proportion of colposcopists did not answer all item stems and although this would have improved the confidence intervals for some of the analysis, online surveys which

require an answer to all questions can reduce overall responses rates in areas of uncertainty.

Correlation of experience with management decisions may be valuable for guideline development but measuring these variables can be problematic. Questions which assessed the volume of patients reviewed in a six month period could have been included but some clinicians who review a lower volume of women may manage higher complexity patients. Personal experiences can affect management outcomes in areas of clinical uncertainty, such as a previous poor outcome leading to a more aggressive selection for treatment, but most areas of discordance were not affected by gender, job title or experience indicating true areas of clinical uncertainty.

6.6 Conclusions

This study was designed to help guide a national consensus strategy and, in this regard, it identified clear areas of consensus. With low grade cytology and a TZ3, young women with low risk factors for CIN progression (non-smokers, reliable attendees and normal age of sexual debut) and older women with low parity and low risk factors could be offered cytological follow-up, dependent upon patient wishes. Until population specific information is available it would appear safest to offer excision to all women with high grade cytology and a TZ3.

Pending studies which assess the diagnostic accuracy of a Cervex-Brush alone and the impact of reporting the presence of endocervical cells, colposcopists should not repeat the referral cytology. NHS CSP guidelines for depth of LLETZ should be adhered to until studies which adjust for age and parity when assessing the distal margin of a TZ3 are completed.

To reduce heterogeneity of care, patient preferences for the management of a TZ3 should be assessed, including the use of oestrogen. To inform the clinical setting, interval and total length of follow-up prior to offering a LLETZ, prospective studies are needed to assess the optimal cytological collection device and the progression rate of CIN in women with low grade cytology and a TZ3. The contribution of biomarkers and HPV genotyping to diagnostic accuracy should also be evaluated.

Chapter 7 The use of biomarkers and HPV genotyping to improve diagnostic accuracy in women with a TZ3

7.1 Introduction

In women with high grade cytology and high risk HPV the PPV of detecting CIN2+ is 86.2 - 94% but with low grade cytology it is only 16%[156]. As reported in Chapters 4 and 6, when the PPV of the screening test cannot be improved by colposcopic assessment due to the presence of a TZ3, anxiety deters long-term conservative follow-up and increases LLETZ rates. As identified in Chapter 3, women who have a LLETZ for a TZ3 have a 10-fold increased chance that the excision histology will be normal, when compared to women where the TZ is visible, and a higher treatment related morbidity due to the recommended depth of LLETZ (15 - 25mm)[85]. These data suggest novel methods which improve the diagnostic accuracy of the screening test are needed in this cohort.

As discussed in section 1.6.2, endocervical canal curettage (ECC) can be used to obtain fragments of squamous epithelium from inside the cervical canal but this is not routine practice in the UK as inadequacy rates are high[123, 126] and the inter-observer agreement moderate. The use of surrogate biomarkers for HPV infection, p16 and Ki-67, (section 1.6.3) have been shown to improve the diagnostic accuracy of cervical punch biopsies and low grade cytology samples when the TZ is visible. Their use in combination with techniques which sample an endocervical transformation zone may improve diagnostic accuracy and decision-making in women with a TZ3.

Currently, HPV DNA testing in the UK gives a pooled high-risk result but studies have evaluated individual genotypes and shown increased HPV persistence, and a higher risk of integration, with HPV 16/18 and associated subtypes (HPV 31, 33, 35, 52 and 58)[157, 213] - indicating that genotyping for these subtypes may also increase diagnostic accuracy (section 1.6.4).

The PPV of the screening test can be affected by the method of cytological collection but of the studies evaluating liquid based cytology devices[79, 81, 83, 214], none have correlated their findings with topographical position of the TZ nor adjusted for age or parity which may affect the mean cytological cell count (section 1.4.1.2).

The primary aim of this study was to assess the predictability of diagnosis of CIN2+ by p16/Ki-67 dual-stained cytology, 24 high risk genotypes and dual-stained endocervical curettings in women with a TZ3. The secondary aim was comparison of the Cervex-Brush alone to a Cervex-Brush in combination with a cytobrush to determine the optimal cytological collection device in women with a TZ3.

7.2 Methodology

A prospective diagnostic accuracy study was conducted over 18 months in a single NHS Trust. Women booked for LLETZ with any squamous cell cytological abnormalities, high risk HPV and a TZ3 were recruited. The exclusion criteria were glandular cytology, immunocompromise and pregnancy. Index tests were taken immediately prior to LLETZ; a Cervex-Brush and cytobrush sample was processed for routine cytology, p16/Ki-67 dual-stain and 24 high risk HPV genotypes. Endocervical curettings were taken and H&E, p16 and Ki67 stained. Predictability of diagnosis of CIN2+ was by blind standardised histological reporting of the reference (LLETZ) biopsy.

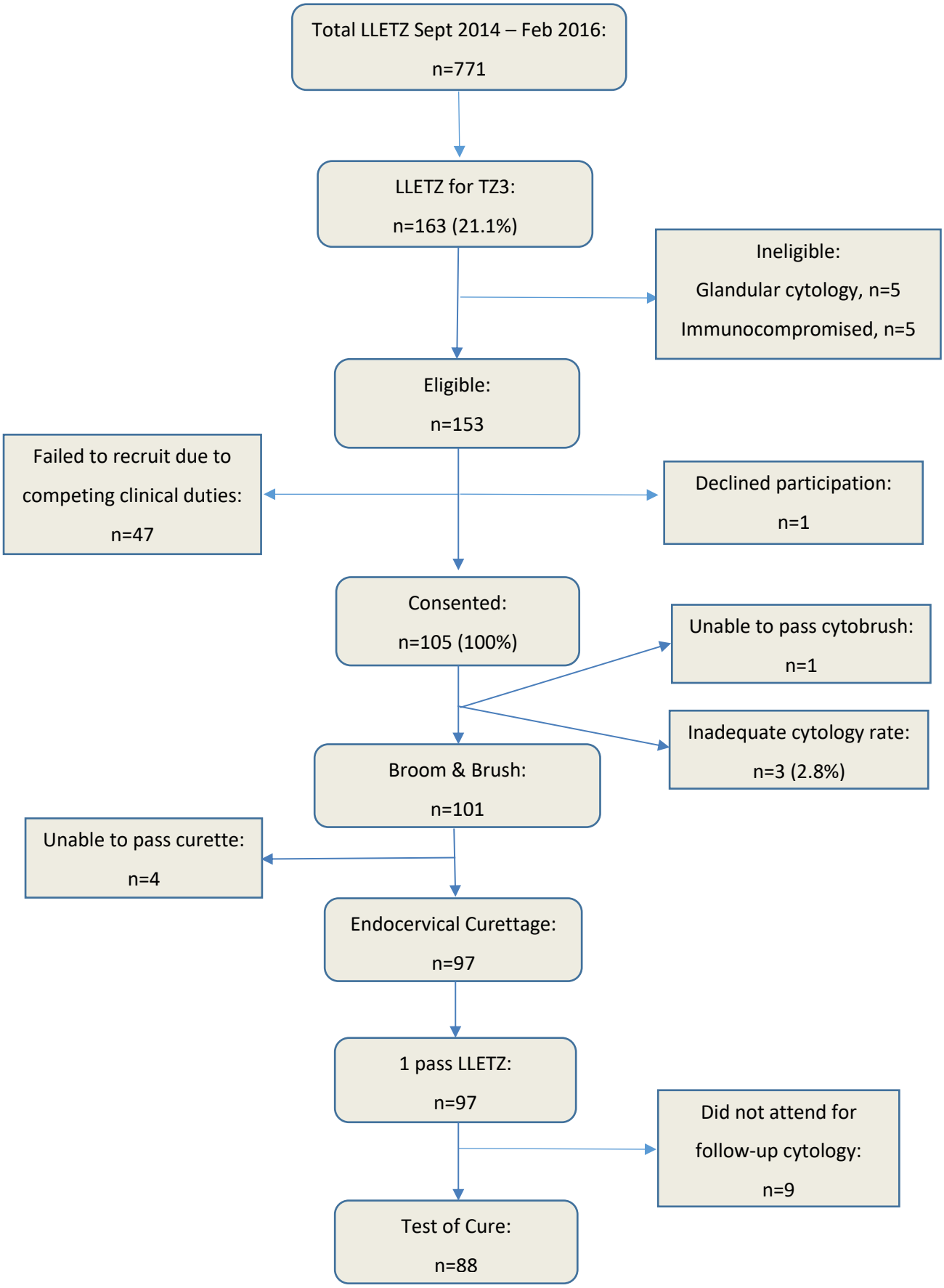
7.3 Results

7.3.1 Recruitment

Figure 7.1 outlines the study recruitment. Of the total LLETZ undertaken during the recruitment period (n=771), 163 (21.1%) had a TZ3. Ten women were ineligible as they met the exclusion criteria. As I was the only person recruiting, 47 eligible women were missed due to my competing clinical duties and one woman declined participation due to anxiety. This meant 105 of 153 (68.6%) eligible participants were recruited and agreed to participate. A broom and broom sample was taken from 105 of 101 had adequate samples; the os was stenosed in one and of the three with inadequate cytology (2.8%) all had extensive atrophy and a narrow os.

Of the 101 women with adequate cytology, I was unable to pass a curette in four (all were post-menopausal with low grade cytology and two had had a previous LLETZ). This resulted in a total of 97 matched samples.

Figure 7.1: Flowchart of study recruitment



7.3.2 Inter-rater reliability

Table 7.1 reports the inter-rater reliability of the index tests. Overall agreement was good for the index cytology and the dual-stained cytology slides. For the H&E stained curettings, the *CIN2+ vs <CIN2* diagnostic category showed better agreement than *CIN vs Normal histology*. For individual p16 and Ki-67 stained curettings agreement, was good if *strong vs not strong* and *full thickness vs less than full thickness* staining categories were respectively used. For the dual-stained curettings the agreement was very good for *<CIN2 vs CIN2+* when the most predictive staining categories for p16 & Ki67 were used.

Table 7.1: Inter-rater reliability of the index tests

Diagnostic Test	Staining Categories	Diagnostic Categories	Kappa Value	95% CI
Index cytology (n=101)	-	(i) Low Grade vs High Grade Cytology	0.65	0.52 - 0.78
Dual-stained cytology (n=101)	(i) Positive vs Negative	-	0.79	0.65 - 0.92
H&E stained curettings (n=97)	-	(i) CIN (any grade) vs Normal (ii) <CIN2 vs CIN2+	0.56 0.76	0.42 - 0.75 0.62 - 0.89
p16 stained curettings (n=97)	(i) Strong vs <Strong (ii) Strong vs Weak vs None	-	0.66 0.47	0.51 - 0.81 0.34 - 0.61
Ki-67 stained curettings (n=97)	(i) Full thickness vs <Full thickness (ii) None vs Basal vs Full thickness	-	0.73 0.53	0.60 - 0.87 0.39 - 0.59
p16/Ki-67 dual- stained curettings[(a)] *(n=97)*	-	(i) CIN (any grade) vs Normal (ii) <CIN2 vs CIN2+	0.69 0.80	0.57 - 0.82 0.68 - 0.93

(a) Staining patterns with the best reliability (*strong vs <strong* for p16 and *full thickness vs <full thickness* for Ki67) were used to assess the reliability of the dual-stained curettings

7.3.3 Study population

Table 7.2 outlines the study sample characteristics. All had high risk HPV as part of the Sentinel Sites primary screening or through HPV triage of a low grade cytology result. Sixty four had low grade cytology (35 BNC and 29 mild) and 37 high grade cytology. The median age was 39 years (IQR 34 – 51). Fifty five (54.5%) were of prime reproductive age (25 - 39 years) and 70.2% were parous. Over 40% (95% CI 32 – 52%) of the pre-menopausal women took no hormonal contraceptive.

Table 7.2: Description of the sample (n=101)

Characteristic	N (%)
Age:	
25 - 39 years	55 (54.5%)
40 - 52 years	30 (29.7%)
53+	16 (15.8%)
Parity:	
None	30 (29.7%)
1	14 (13.8%)
2+	57 (56.4%)
Smoking:	
None	64 (63.4%)
Yes	37 (36.6%)
Hormonal State:	
None	42 (41.6%)
Oestrogen (HRT or COCP)	8 (7.9%)
Progesterone (inc post-menopausal)	51 (50.4%)
Referral Cytology:	
Low Grade	64 (63.3%)
High Grade	37 (36.6%)
LLETZ result:	
<CIN2	57 (56.3%)
CIN2+	44 (43.5%)
• *CIN2* • *CIN3* • *FIGO Cervical Cancer Stage 1a1*	*19* *24* *1*

The TZ was identified in all LLETZ histology, the median time between the referral cytology and LLETZ was 8 weeks (IQR 5 - 11.5) and the median depth was 15mm (IQR 12 - 16.5). Histological limiting factors were assessed; five LLETZ samples showed denudation but the histology for four of these corresponded with the curettings and all follow-up cytology was negative. The fifth sample was reported as HPV on the LLETZ and CIN2 on the curettings but the test of cure (TOC) result was negative.

7.3.4 Outcome histology with a TZ3

Table 7.3 cross-tabulates the LLETZ outcome with the referral and index test results. Where a mismatch between the referral cytology and LLETZ occurred, all cases were reviewed and the outcome from the MDT was reported as the final result. The majority of women (72.7%, 24/33) with high grade dyskaryosis on the index cytology had CIN2+. Of the 11 women with high-grade cytology and <CIN2 at LLETZ, two had CIN2+ identified in the curettings and the remainder had a negative TOC. This indicates 78.7% (26/33) of women with high grade cytology and a TZ3 have CIN2+. A third (n=20) of the women with low grade cytology had CIN2+ at LLETZ and a further 5 had CIN2+ identified in the curettings but <CIN2 in the LLETZ histology. This indicates 36.7% (25/68) of women with low grade cytology and a TZ3 may have CIN2+; 77.8% were non-smokers, the median age was 36 (1QR 30-46), and 65.2% (15) had BNC.

There is no evidence that the proportion of women with high grade cytology and a TZ3 who have CIN2+ at LLETZ differ to national screening statistics[87] (of whom 80% will have a TZ 1-2); 78.7% vs 84.6%, p=0.13. However, there is evidence that more women with low grade cytology and a TZ3 have CIN2+; 36.7% vs 16.1% p<0.001. The one case of squamous cell cancer within the study population had BNC reported on the referral and index cytology.

Of note, 17 women were referred with high risk HPV but genotyping reported 12 were high risk HPV negative and 5 had low risk HPV. Of these 17 women none had CIN2+, the median interval from cytology to LLETZ was 9 weeks (range 4 - 17) and the median age was 43 (range 25 - 54). To evaluate the effect of genotyping on diagnostic accuracy, the sample size for this test was now 84.

Table 7.3: Cross tabulation of the diagnostic test results with the LLETZ histology

	LLETZ RESULT			
INDEX TEST	Negative (n=36)	CIN1 (n=21)	CIN2+ (n=44)	TOTAL
Referral Screening Test (n=101)				
• HR HPV & Low-grade Cytology	29 (45.3%)	17 (26.5%)	18 (28.1%)	64
• HR HPV & High-grade Cytology	7 (18.9%)	4 (10.8%)	26 (70.3%)	37
Index Cytology (n=101)				
• Low-grade	30 (44.1%)	18 (26.4%)	20 (29.4%)	68
• High-grade	6 (18.2%)	3 (9.1%)	24 (72.7%)	33
Pooled HPV status (n=101)				
• Negative	10 (83.3%)	2 (16.6%)	0	12
• Low Risk	4 (80%)	1 (20%)	0	5
• High Risk	22 (26.1%)	18 (21.4%)	44 (52.3%)	84
Dual-stained cytology (n=101)				
• Positive	7 (10.9%)	13 (20.3%)	44 (68.7%)	64
• Negative	29 (78.3%)	8 (21.6%)	0	37
High Risk HPV Genotype (n=84)				
• 16	3 (11.1%)	3 (11.1%)	22 (81.5%)	28
• 18	1 (16.6%)	2 (33.3%)	3 (50%)	6
• 31	0	4	7	11
• 33	2	0	4	6
• 35	0	0	1	1
• 39	1	0	0	1
• 44	4	0	0	4
• 45	0	3	0	3
• 51	2	0	0	2
• 52	2	1	2	5
• 56	1	0	1	2
• 58	1	2	2	5
• 59	1	2	1	4
• 66	4	0	0	4
• 68	2	0	1	3
ECC H&E (n=97)				
• Negative	28 (53.8%)	11 (21.1%)	13 (25%)	52
• CIN1	4 (23.5%)	6 (35.3%)	7 (41.2%)	17
• CIN2	2 (7.1%)	2 (7.1%)	24 (85.7%)	28
ECC p16 (n=97)				
• Negative	26 (78.7%)	4 (12.1%)	3 (9%)	33
• Weak	5 (29.4%)	9 (52.9%)	3 (17.6%)	17
• Strong	3 (6.3%)	6 (12.7%)	38 (80.8%)	47
ECC Ki67 (n=97)				
• Negative	21 (75%)	6 (21.4%)	1 (3.5%)	28
• Basal	10 (37%)	10 (37%)	7 (25.9%)	27
• Full thickness	3 (7.1%)	3 (7.1%)	36 (85.7%)	42
ECC H&E with p16 & Ki67 (n=97)				
• Negative	29 (80.5%)	5 (13.8%)	2 (5.5%)	36
• CIN1	1 (5.8%)	11 (64.7%)	5 (29.4%)	17
• CIN2	4 (9.1%)	3 (6.8%)	37 (84.1%)	44

Of the 37 women referred with high grade cytology, 2 were negative for high risk HPV, 17 (45.9%) had HPV 16, three (16.6%) HPV 18, fifteen (40.5%) had a variety of other high risk subtypes and eighteen (48.6%) were infected with multiple subtypes. Of the 64 women referred with low grade cytology, 15 (23.4%) were negative for high risk HPV, 11 had HPV 16, HPV 18 was detected in three women and non-16/18 HR subtypes in 35. Of these women with low grade cytology and high risk HPV, 16 were positive for multiple subtypes.

7.3.5 Diagnostic accuracy of the individual tests

Table 7.4 reports the accuracy and performance of the referral and index tests in predicting CIN2+ when the most reliable staining categories from Table 7.1 were used. The PPV of a pooled HPV test was poor (53%) but the NPV was excellent (100%).

7.3.5.1 Contribution of a cytobrush to improving diagnostic accuracy

There was no evidence that the addition of a cytobrush to a Cervex-Brush improved the predictability of diagnosing CIN2+ when compared to the Cervex-Brush alone, irrespective of the cytological grade (high grade p=0.73, low grade p=0.23). The impact of TZ sampling was evaluated; 22 (20.9%) of the referral cytology samples contained squamous but no endocervical cells whereas only one (0.9%) of the Cytobrush & Cervex-Brush samples lacked endocervical cells (p<0.001). There was no evidence of an association between the presence of endocervical cells and a diagnosis of CIN (p=0.21), but the sample size was 22.

When adjusted for age there was no difference in diagnostic accuracy between the two sampling methods for women with low grade or high grade cytology; in women aged 25 - 39 p=0.99 respectively and in women >40 p=0.68 and 0.98 respectively. There was no difference observed in predictability of CIN2+ between nulliparous or parous women (p=0.99 and 0.62 respectively) between the different sampling methods.

7.3.5.2 Dual-stained cytology and HPV genotyping

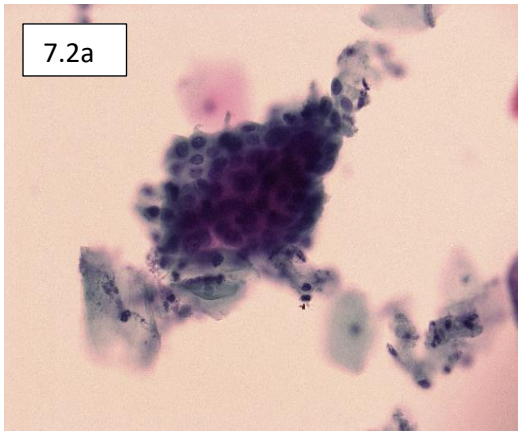
The sensitivity of dual-stained cytology for CIN2+ was excellent but the specificity moderate. Dual-staining was of most help when assessing fragmented epithelium or scattered dysplastic cells (Figure 7.2). All dual-stain positive women (n=64) had high risk HPV but 24.1% (20/83) women with high risk HPV were dual-stain negative. Sensitivity and specificity of HPV 16 for CIN2+ were moderate and not affected by the addition of HPV 18 (p=0.09), although it should be noted that only six women were positive for HPV 18.

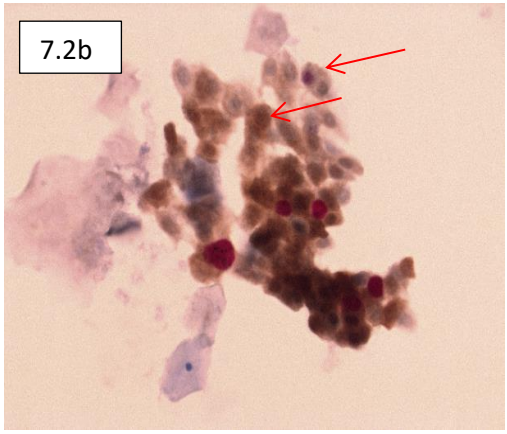
Table 7.4: Predictability of CIN2+ by the referral and index tests

DIAGNOSTIC TEST	**Sensitivity** n/N, (%, 95% CI)	**Specificity** n/N, (%, 95% CI)	**PPV** n/N, (%, 95% CI)	**NPV** n/N, (%, 95% CI)
Referral Screening Test				
• HR HPV + Low Grade Cytology	18/44 (40.9%, 27.6–55.5)	11/57 (19.3%, 11.1–31.3)	18/64 (28.1%, 18.6-40.1)	11/37 (29.7%, 17.4-45.8)
• HR HPV + High Grade Cytology	26/44 (59.1%, 44.1-72.3)	46/57 (80.7%, 68.6-88.8)	26/37 (70.3%, 54.2-82.5)	46/64 (71.9%, 59.8-81.4)
Index Cytology				
• Low Grade	20/44 (45.4%, 31.7-59.9)	9/57 (15.7%, 7.9-28.3)	20/68 (29.4%, 19.3-41.8)	9/33 (27.3%, 15.0-44.2)
• High Grade	24/44 (54.5%, 40-68.3)	45/57 (84.2%, 71.6-92.0)	24/33 (72.7%, 55.7-84.9)	45/68 (70.5%, 58.1-80.7)
HR HPV vs <HR HPV	44/44 (100%, 91.9-100)	18/57 (31.6%, 21.0-44.8)	44/83 (53.0%, 42.3-63.3)	18/18 (100%, 82.4-100)
CINtec *(p16/Ki67 cytology)*	44/44 (100%, 91.9-100)	37/57 (64.9%, 51.9-76)	44/64 (68.8%, 56.1-78.7)	37/37 (100%, 90.5-100)
HPV 16 vs other HR subtypes	22/44 (50%, 35.8-64.1)	33/39 (84.6%, 70.3-92.7)	22/28 (78.6%, 60.4-89.7)	33/55 (60%, 46.8-71.8)
16 & 18 HR HPV	25/44 (56.8%, 42.3-70.3)	30/39 (76.9%, 61.6-87.3)	25/34 (73.5%, 56.8-85.4)	30/49 (61.2%, 47.2-73.5)
ECC H&E				
• CIN2+ vs <CIN2	24/28 (85.7%, 68.5-94.3)	49/69 (71%, 59.4-80.3)	24/44 (54.5%, 40-68.3)	49/53 (92.4%, 82.1-97)
• CIN vs normal histology	31/45 (70.4%, 55.7-81.8)	39/52 (73.6%, 60.4-83.5)	31/44 (68.9%, 54.3-80.4)	39/53 (75%, 61.7-84.7)
ECC p16				
• Strong staining vs <strong stain	38/47 (80.8%, 67.4-89.5)	44/50 (88%, 76.2-94.3)	38/44 (86.4%, 73.2-93.6)	44/53 (83%, 70.7-90.8)
• Any staining vs no stain	41/64 (64%, 51.8-74.7)	30/53 (56.6%, 43.2-69)	41/44 (93.2%, 81.7-97.6)	30/33 (90.9%, 76.4-96.8)
ECC Ki67				
• Full thickness staining	36/42 (85.7%, 72.1-93.3)	47/55 (85.5%, 73.8-92.4)	36/44 (81.8%, 68-90.4)	47/53 (88.7%, 77.4-94.7)
• Full thickness & basal vs none	43/69 (62.3%, 50.5-72.8)	27/28 (96.4%, 82.2-99.3)	43/44 (97.6%, 88.2-99.6)	27/53 (50.9%, 37.8-63.8)
ECC H&E with p16 and Ki67				
• CIN2+ vs <CIN2	37/44 (84.1%, 70.6-92.7)	46/53 (86.8%, 75.1-93.4)	37/44 (84.1%, 70.6-92.7)	46/53 (86.8%, 75.1-93.4)
• CIN vs normal histology	42/61 (68.8%, 56.4-79)	34/36 (94.4%, 81.8-98.4)	42/44 (95.4%, 84.8-98.7)	34/53 (64.1%, 50.6-75.7)

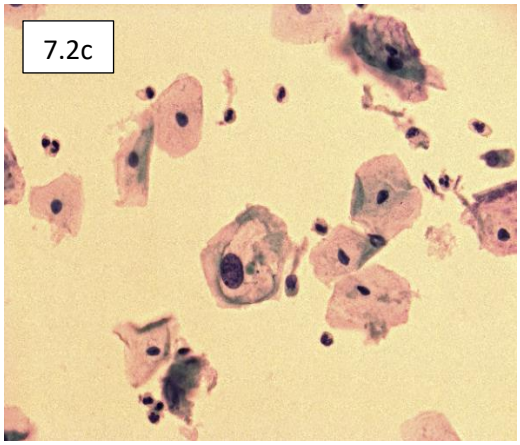
Figure 7.2: How dual-stained cytology can improve accuracy of the screening test

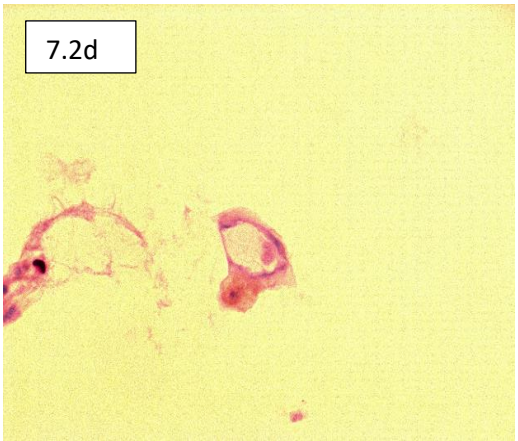
ACORN ID 66. The referral cytology was reported as low grade dyskaryosis and the index cytology as low grade (BNC) change. The BNC category was a result of some cells exhibiting a slightly increased nuclei:cytoplasmic ratio but clumping of the cells (Figure 7.2a) reduced differentiation of reactive cells from dyskaryotic. HPV 16 and 33 were identified on genotyping. Dual-staining was reported as positive (Figure 7.2b) and CIN2 identified in the LLETZ histology. This case illustrates how dual-stained cytology can improve false negative screening (NPV) as many colposcopists would be reassured by the cytology result and recommend cytological follow-up.





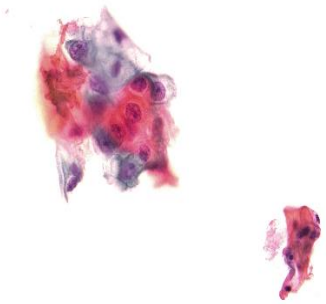
ACORN ID 71. The referral cytology was reported as BNC and the index cytology as mild dyskaryosis with koilocytosis (Figure 7.2c). Genotyping identified HPV 16, 53 and 70. Dual-staining was negative (Figure 7.2d) and the LLETZ report stated HPV only. This is an example of how dual-staining can improve the PPV of screening. It also illustrates that infection with high-risk HPV, even HPV 16, does not always equate to CIN2+.



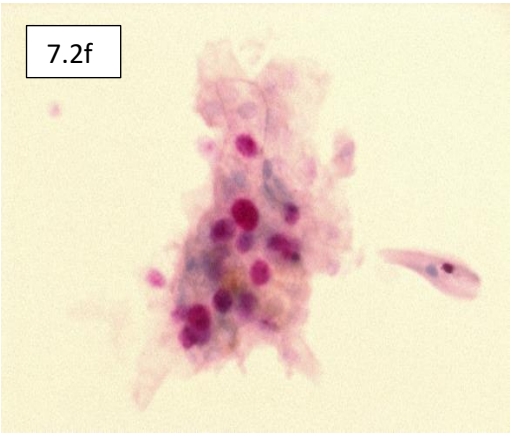


ACORN ID 68: The referral and index cytology were reported as mild dyskaryosis (Figure 7.2e). Genotyping identified HPV 58 and 73. Dual-stained cytology was negative (Figure 7.2f) and the LLETZ report stated HPV only. This is another example of how dual-staining can decrease false positive screening (improve the PPV) as Chapters 4 & 6 has illustrated that colposcopists will offer a LLETZ to women with a TZ3 who have this cytology result for 12 months.

7.2e



7.2f



7.3.5.3 Scoring Protocols for the immunostained endocervical curettings

The predictability of CIN2+ by different immunostaining and diagnostic categories was evaluated (Table 7.4).

- *H&E slides;* If *'<CIN2 and CIN2+'* were used as the diagnostic categories the sensitivity and specificity were moderate. If *'CIN (any grade) vs Normal histology'* was used, there was no evidence of a difference in the sensitivity (p=0.10) or the specificity (p=0.63). The contribution to the diagnostic yield of a deeper level was evaluated; one case was upgraded from metaplasia to CIN1 but this was also detected by p16 and Ki-67.
- *p16 slides:* If *'any staining vs no staining'* was used as the scoring category the specificity for CIN2+ was poor but the sensitivity very good. If *'strong vs <strong'* staining category was used the specificity improved (p=0.04) but the sensitivity was equivocal (p=0.68).
- *Ki-67 slides: 'Full thickness vs <full thickness'* staining category improved the specificity for CIN2+ when compared to *'no staining vs basal vs full thickness'* (p=0.008). However there was no evidence of a difference in the sensitivity (p=0.13).

7.3.5.4 Accuracy of the H&E, p16, Ki-67 & dual-stained endocervical curettings

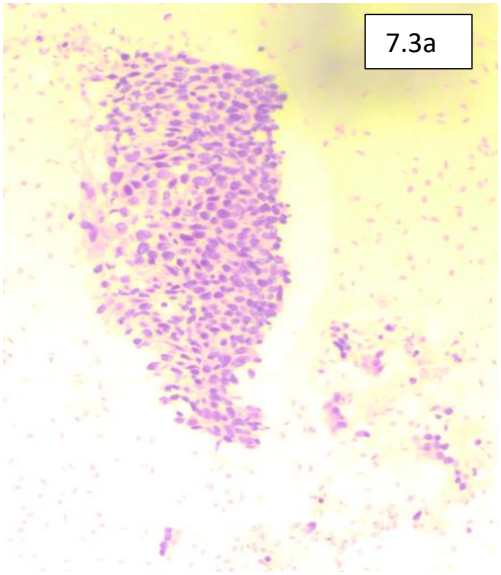
The most accurate staining and diagnostic categories as outlined in 7.3.4.3 were used to assess the diagnostic accuracy of the H&E and immunohistochemistry slides (Table 7.4). Sensitivity and specificity for the H&E slides were moderate and good for the individually stained p16 and Ki-67 slides. Diagnostic accuracy of the H&E, p16, Ki67 and dual-stained curettings for CIN2+ were compared; sensitivity was improved with p16 and Ki-67 when compared to the H&E stained slides ($p<0.001$ respectively). Specificity was improved with Ki-67 ($p=0.03$) but this association was not observed with p16 ($p=0.06$). There was no evidence of a difference in sensitivity between Ki-67 and p16 stained slides ($p=0.5$).

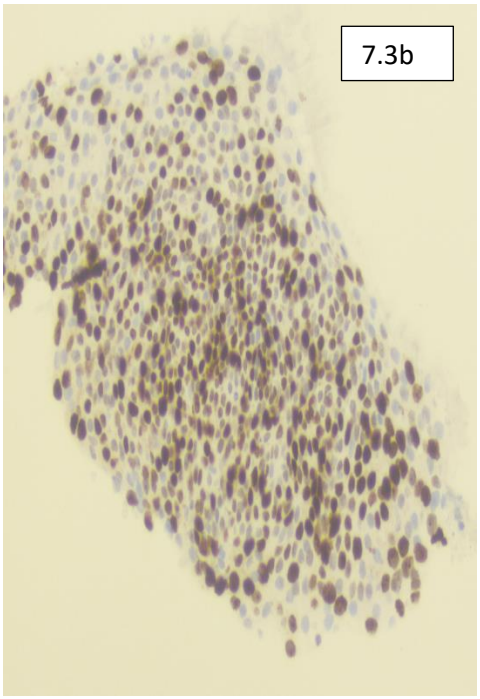
Diagnostic accuracy of the dual-stained slides was compared to p16 and Ki-67 alone; there was no evidence of a difference in sensitivity when compared to p16 ($p=0.99$) or specificity when compared to Ki-67 ($p=0.99$). However, the inter-rater reliability was better for dual-stained slides and four slides which stained strongly positive for p16 had basal staining only on the corresponding Ki-67 slides; the result was both Pathologist's downgrading the final diagnosis to HPV infection, which was also reported on all four LLETZ histology. For these reasons dual-stained histology, rather than p16 or Ki-67 alone, was used in the regression models and treatment algorithms. The slides in Figure 7.3 illustrate how the use of p16 & Ki67 can improve predictability of CIN2+ in fragmented endocervical curettings.

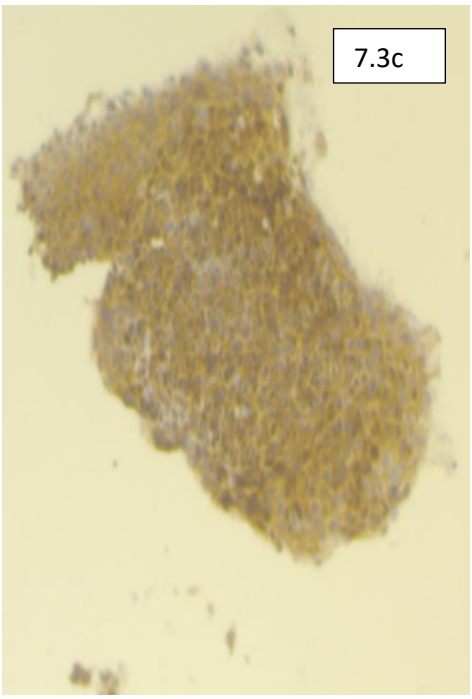
Figure 7.3: How p16 & Ki-67 immunostains can improve the predictability of CIN2+ in endocervical curettings

ACORN ID 26:

- Referral cytology BNC ?high grade
- Index Cytology BNC
- HPV 33 & 35 on genotyping
- Curettings fragmented. CIN ?grade identified on the H&E slide (7.3a), full thickness Ki67 staining (7.3b) and strong p16 staining (7.3c). The final diagnosis was CIN2+
- LLETZ histology identified CIN3.







7.3.5.5 Prediction models

To stratify which women would most benefit from LLETZ, I was interested to assess which patient variables may increase prediction of CIN2+. Univariable models (Table 7.5) identified that cytological grade, age and interval from cytology to LLETZ were predictors of CIN2+. As a negative dual-stained cytology result perfectly predicted a negative LLETZ, there was no variability on which to calculate a regression model. In the bivariable models, which examined each predictor after adjusting for diagnosis of CIN2+ by immunostained curettings (Table 7.6), there was evidence that women aged 25 - 39, independent of their immunostained curetting result, had higher odds of CIN2+. In bivariable models which examined each predictor after adjusting for HPV 16/18 (Table 7.6), there was evidence that women aged 25 - 39 and those with high grade cytology, independent of their HPV 16 or 18 result, had a higher odds of CIN2+.

Table 7.5: Predictors of CIN2+ in women with a TZ3 – univariable analysis

Variable	Odds Ratio	95% confidence interval	P-value	AUC
p16/Ki67 curettings	33.7	10.8 - 85.1	<0.001	0.85
HPV 16/18 positive	7.8	2.9 - 20.5	<0.001	0.71
CINtec	*	*	*	*
Smoking	0.97	0.42 – 2.24	0.95	0.50
Cytological grade	5.97	2.38 - 14.9	<0.001	0.69
Parity	0.61	0.25 – 1.44	0.25	0.55
Contraceptive	0.96	0.43 – 2.17	0.94	0.50
Age	3.08	1.33 – 7.11	0.008	0.63
Cytology to LLETZ interval	0.30	0.10 – 0.83	0.03	0.58

**Unable to calculate as no variation. All women with CIN2+ were dual-stain positive*

Table 7.6: Predictors of CIN2+ in women with a TZ3 – bivariable analysis

Variable	p16/Ki67 stained curettings				HPV 16/18 Genotype		
	OR, *95% CI*	p value	AUC		OR, *95% CI*	p value	AUC
Smoking	0.58, *0.17 – 1.97*	0.38	0.86		0.90, *0.35 – 2.31*	0.84	0.71
High grade cytology	1.78, *0.50 – 6.26*	0.36	0.86		3.73, *1.37 – 10.1*	0.01	0.77
Parity	0.74, *0.21 – 2.52*	0.63	0.74		0.51, *0.19 – 1.36*	0.18	0.74
Contraceptive	0.59, *0.18 – 1.95*	0.34	0.86		0.74, *0.29 – 1.87*	0.53	0.72
Age	4.14, *1.19 – 14.3*	0.02	0.89		2.59, *1.02 – 6.51*	0.04	0.76
Cytology to LLETZ interval	0.11, *0.01 – 1.38*	0.08	0.87		0.20, *0.02 – 1.74*	0.14	0.74

In the final models (Table 7.7) the direction of risk for variables which predicted CIN2+ were evaluated. In those women who tested positive for HPV 16/18, women aged 25-39 were 3 times more likely to have CIN2+ when compared to women ≥40 and those with high grade cytology were four times more likely to have CIN2+ when compared to women with low grade cytology. When CIN2+ was reported on the immunostained curettings, these women were four times more likely to be aged 25 - 39 than ≥40.

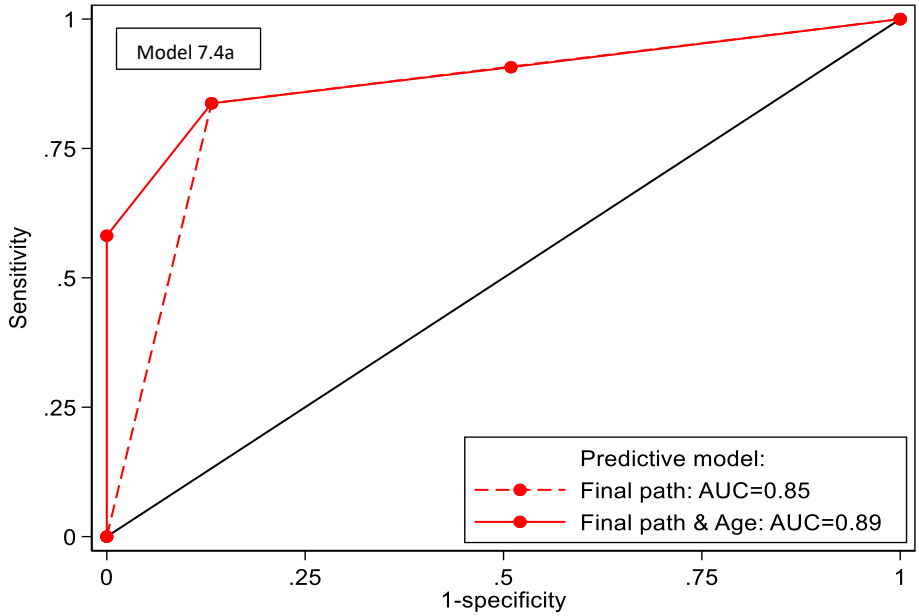
Table 7.7: Predictive model for HPV16/18 Genotype and dual-stained curettings

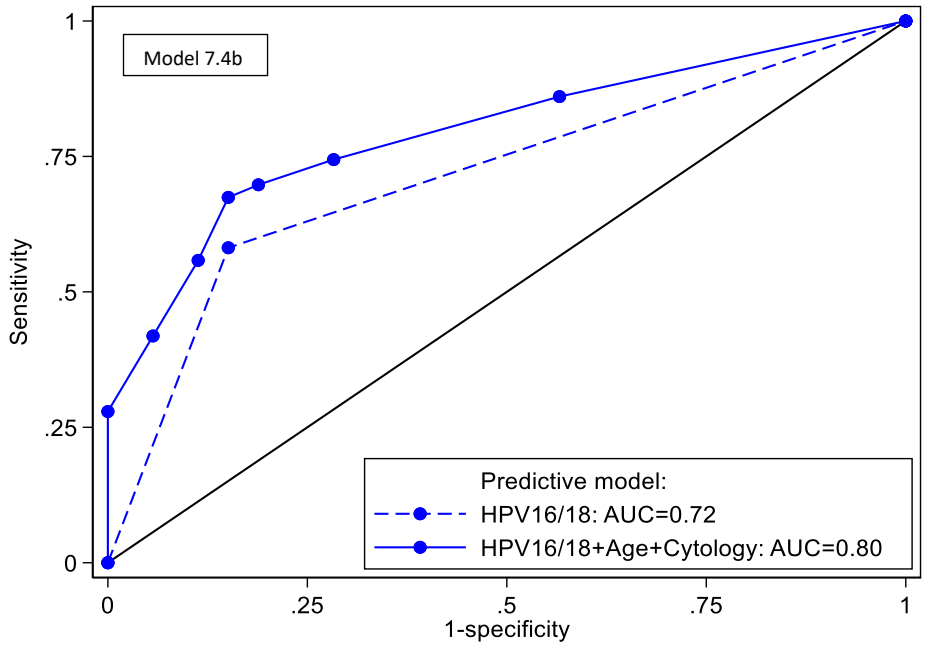
	Odds Ratio	95% Confidence Interval	p value	AUC
p16/Ki67 curettings				
Histology:				
<CIN2	Ref			
CIN2+	38.8	11.2 – 84.8	<0.001	0.86
Age:				
≥40	Ref			
25-39	4.14	1.19 – 14.3	0.02	0.89
HR HPV Genotype				
HPV 16 / 18:				
No	Ref			
Yes	4.54	1.58 – 12.9	0.005	0.71
Cytology grade:				
Low	Ref			
High	4.07	1.44 – 11.5	0.008	0.79
Age:				
≥40	Ref			
25-39	2.88	1.08 – 7.65	0.03	0.76

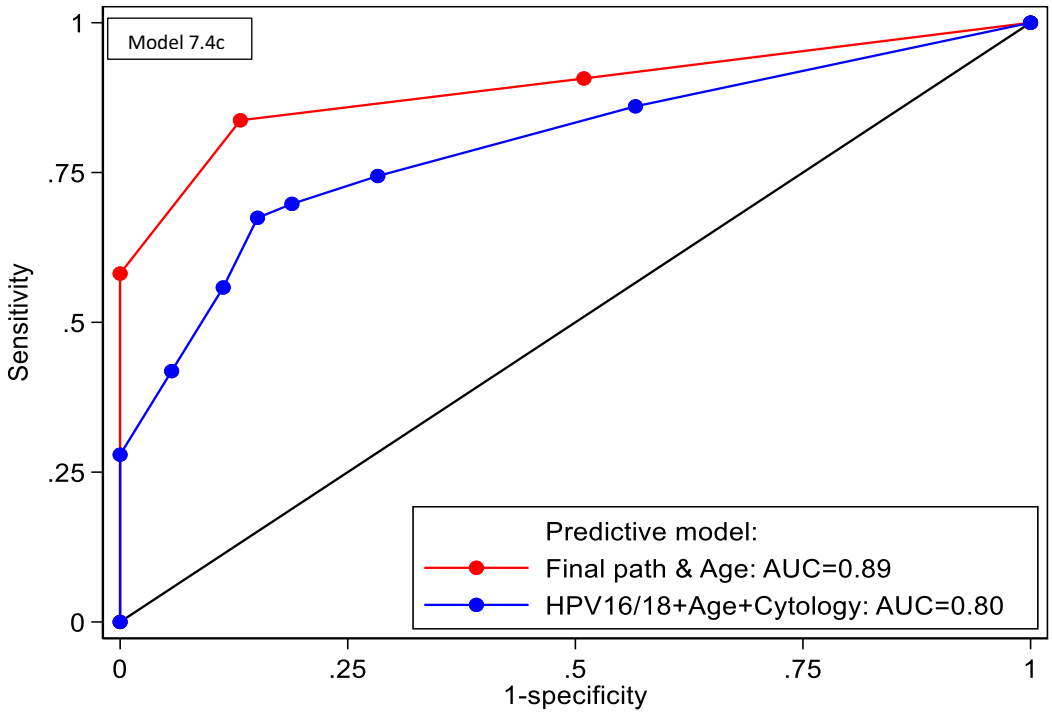
The predictive ability (area under the curve or AUC) of HPV 16/18 genotyping and p16/Ki67 stained curettings were compared. There is some evidence that immunostained curettings improve the predictability of CIN2+ when compared to HPV 16/18 genotyping: AUC 0.86 vs 0.71, p=0.01 (Table 7.7). The receiver operator curves and area under the curve for these tests individually and in the highest risk subgroups can be viewed in Figure 7.4.

Figure 7.4: Receiver operator curves

ROC curves and AUC for (i) immunostained curettings (Final path – Model 7.4a) and (ii) HPV 16/18 genotyping, individually and in combination with their highest risk subgroups (Model 7.4b). Comparison of the area under the curve for immunostained curettings and HPV 16/18 genotyping can be viewed in Model 7.4c.







7.3.6 Accuracy of the most predictive tests in combination with the screening test result

7.3.6.1 Low grade cytology and high risk HPV

My data suggests women with low grade dyskaryosis would benefit from dual-stained cytology (Table 7.8); CIN2+ would have been identified in 89.5 - 100% of my sample (NPV) and, in comparison to standard screening, an unnecessary LLETZ would have been prevented in 71.7% (33/46) of the women who had <CIN2 (Figure 6.5b). The use of dual-stained endocervical curettings would have detected 82.6% (38/46) women with <CIN2 prior to LLETZ (Figure 7.5c) but only 11/18 (61.1%) of the women with low grade screening who had CIN2+ would have been correctly identified (Table 7.8).

The specificity of the endocervical curettings in women with low grade cytology was similar to that of the dual-stained cytology (Table 7.8) but the sensitivity was poorer; 7/18 (38.8%) of women with CIN2+ would potentially have been missed (Figure 7.5c). The PLR for routine cervical screening was poor (7.5% probability that CIN2+ will be detected) but improved when combined with dual-stained cytology and dual-stained curettings (PLR 22.5% and 30% respectively). Indeed, the PLR for both dual-stained cytology and curettings triangulate with the incidence of CIN2+ that was observed in women with low grade cytology and a TZ3 (see section 7.3.3 and Table 7.3).

Table 7.8: Predictability of CIN2+ for the most accurate tests in women with low grade and high grade cytology

	Sensitivity (%, 95% CI)	**Specificity** (%, 95% CI)	**PPV** (%, 95% CI)	**NPV** (%, 95% CI)	**PLR**	**NLR**
LOW GRADE CYTOLOGY						
1. Referral screening test	40.9, 26.3 – 56.7	19.3, 10.1 - 31.9	28.1, 18.6 - 40.1	29.7, 17.4 - 45.8	0.51, 0.35 – 0.74	3.06, 1.7 – 5.5
2. Referral test & CINtec* (n=64)	100, 81.4 - 100	71.7, 56.5 - 84.1	58.1, 40.7 - 73.5	100, 89.5 - 100	3.54, 2.23 - 5.61	**
3. Referral test & p16/Ki67 curettings (n=64)	61.1, 35.7 - 82.7	82.6, 69.3 - 90.9	68.7, 44.4 - 85.8	84.4, 71.2 - 92.2	5.26, 2.13 - 12.9	0.44, 0.24 - 0.79
Difference (%, CI, p-value)						
1 vs 2	59.1, 48.6–68.1, <0.001	52.4, 37.5-63.9, <0.001	30, 8.9-48.2, 0.004	70.3, 51-82.4, <0.001	N/A	N/A
1 vs 3	20.2, 4.5-34.3, 0.01	63.3, 47.3-73.8, <0.001	40.6, 13.4-60, 0.002	54.7, 22.8-69.2, <0.001		
2 vs 3	38.9, 26.5-51.1, <0.001	10.9, -6.3-27.4, 0.21	10.6, -18.2-34.9, 0.4	15.6, 2.4-28.7, 0.01		
HIGH GRADE CYTOLOGY						
1. Referral screening test (n=101)	59.1, 43.2 - 73.6	80.7, 68.1 - 89.9	70.2, 54.2 – 82.5	80.7, 68.6 – 88.8	3.06, 1.71 - 5.50	0.51, 0.35 - 0.74
2. Referral test & CINtec* (n=37)	100, 86.7 - 100	36.4, 10.9 - 69.2	78.8, 62.2 – 89.3	100, 51.1 - 100	1.57, 1.01 - 2.46	**
3. Referral test and p16/Ki67 Curettings (n=37)	100, 86.7 - 100	81.8, 48.2 - 97.7	92.8, 77.4 – 98.0	100, 67.5 – 100	5.50, 1.57 - 19.2	**
Difference (%, CI, p-value)						
1 vs 2	40.9, 27.8-50.6, <0.001	44.3, 25.9-59.3, <0.001	8.5, -12.1 – 27.6, 0.4	19.3, -30.4-31.2, 0.3	N/A	N/A
1 vs 3	40.9, 27.8-50.6, <0.001	1.1, -15.4-13.8, 0.88	22.5, 2.7-39.4, 0.02	19.3, -14.2-31.2, 0.1		
2 vs 3	N/A	45.4, 23.2-61.7, <0.001	14, -4.7-31.3, 0.1	0, -32.4-48.9, 1		

** CINtec is p16/Ki67 cytology*

*** could not calculate as the sensitivity was 100%*

The NLR for standard cervical screening in women with low grade cytology and a TZ3 is very poor; a result greater than 0 implies that the screening test is not very useful in identifying the absence of CIN2+, which triangulates with the poor NPV and sensitivity. As a negative dual-stained cytology result perfectly predicted the absence of CIN2+ it was not possible to calculate a NLR, denoting the utility of this test for identifying the absence of CIN2+.

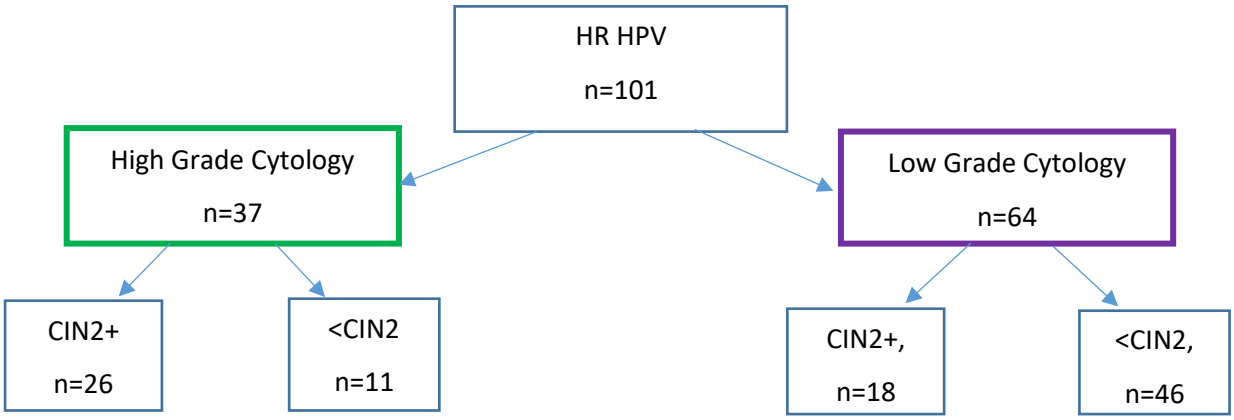
7.3.6.2 High Grade Cytology

In women with high grade cytology, the PPV and NPV of the screening test were improved by both dual-stained cytology and curettings. Indeed the NLR could not be calculated as a negative result, for both tests, perfectly predicted the absence of disease (Table 7.8). Of 11 women with <CIN2 who had a LLETZ for high grade cytology and a TZ3, dual-stained cytology would have identified (prevented over-treatment) in 36.3% (4/11) women (Figure 7.5b) and dual-stained curettings 72.7% (8/11) women (Figure 7.5c).

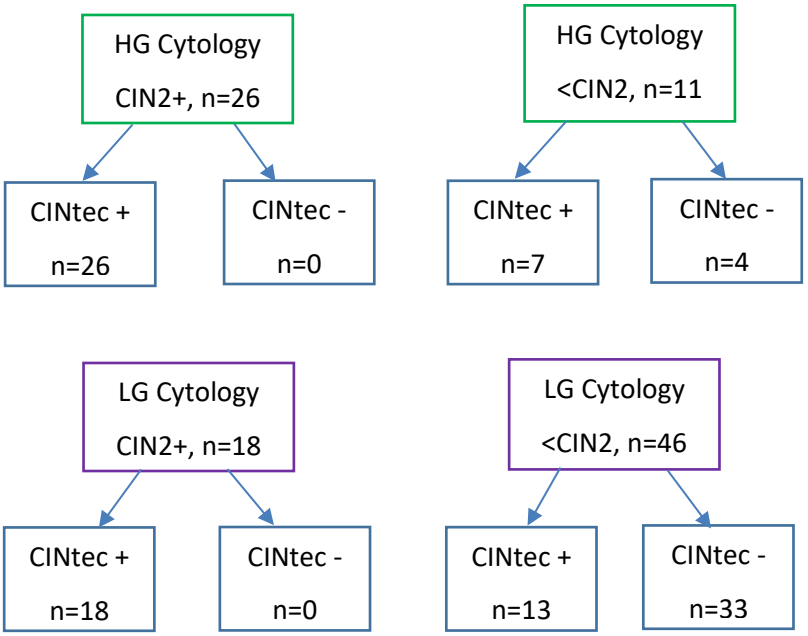
The PLR for high grade cytology predicting CIN2+ was not improved by the addition of immunostained curettings and reduced, by 15%, following the addition of dual-stained cytology. These results triangulate with the poorer PPV / specificity of dual-stained cytology for CIN2+ in women with high grade cytology and a TZ3 and the reduced need for adjuncts in these women, when compared to women with low grade cytology.

Figure 7.5: Visual representation of false positive and false negative screening by the most predictive tests when compared to the current referral screening test.

a.) Current Cervical Screening Test Accuracy (n=101)



b.) Accuracy of the screening test in combination with dual-stained cytology (CINtec) (n=101)



c.) Accuracy of the screening test in combination with dual-stained curettings (n=97)

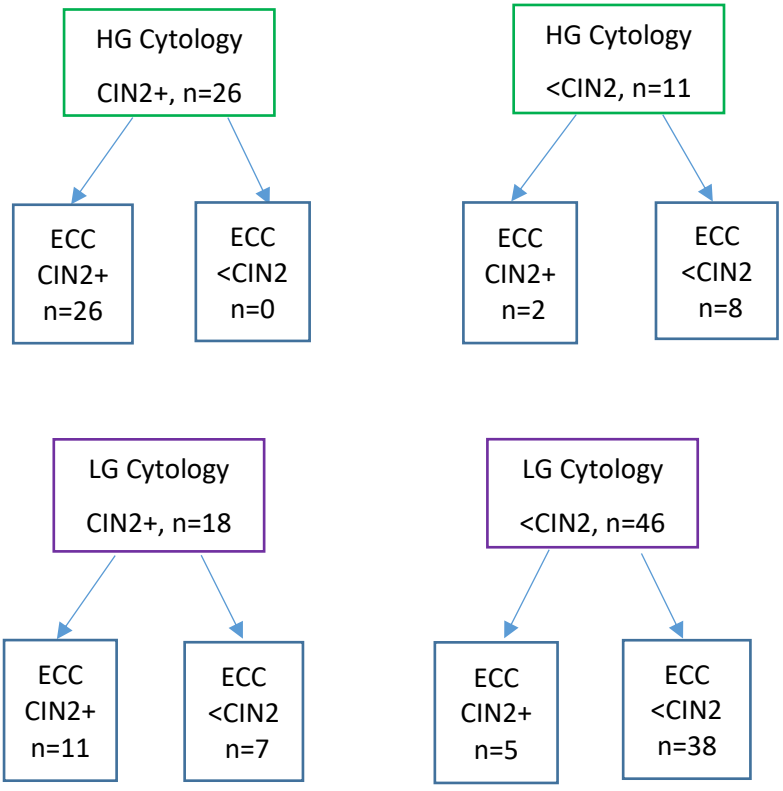


Figure 7.6: Treatment Algorithm A – potential management of women with low grade cytology and a TZ3

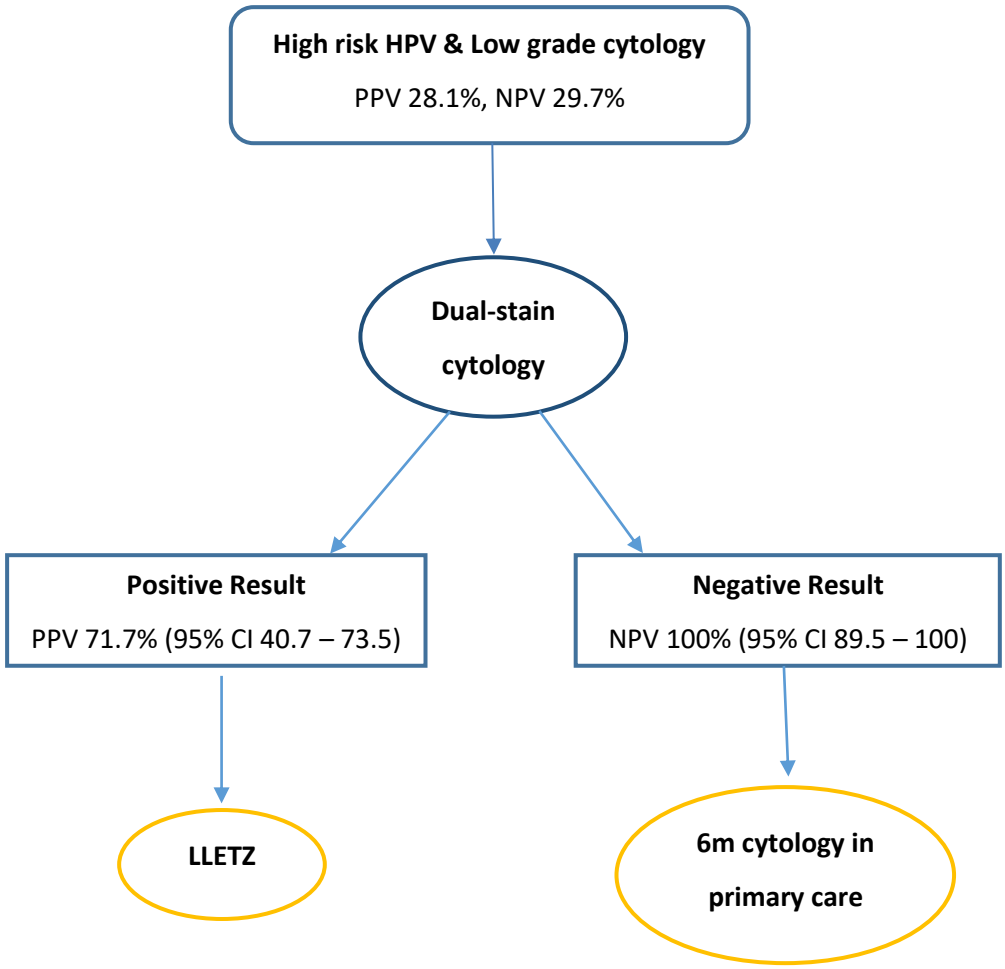
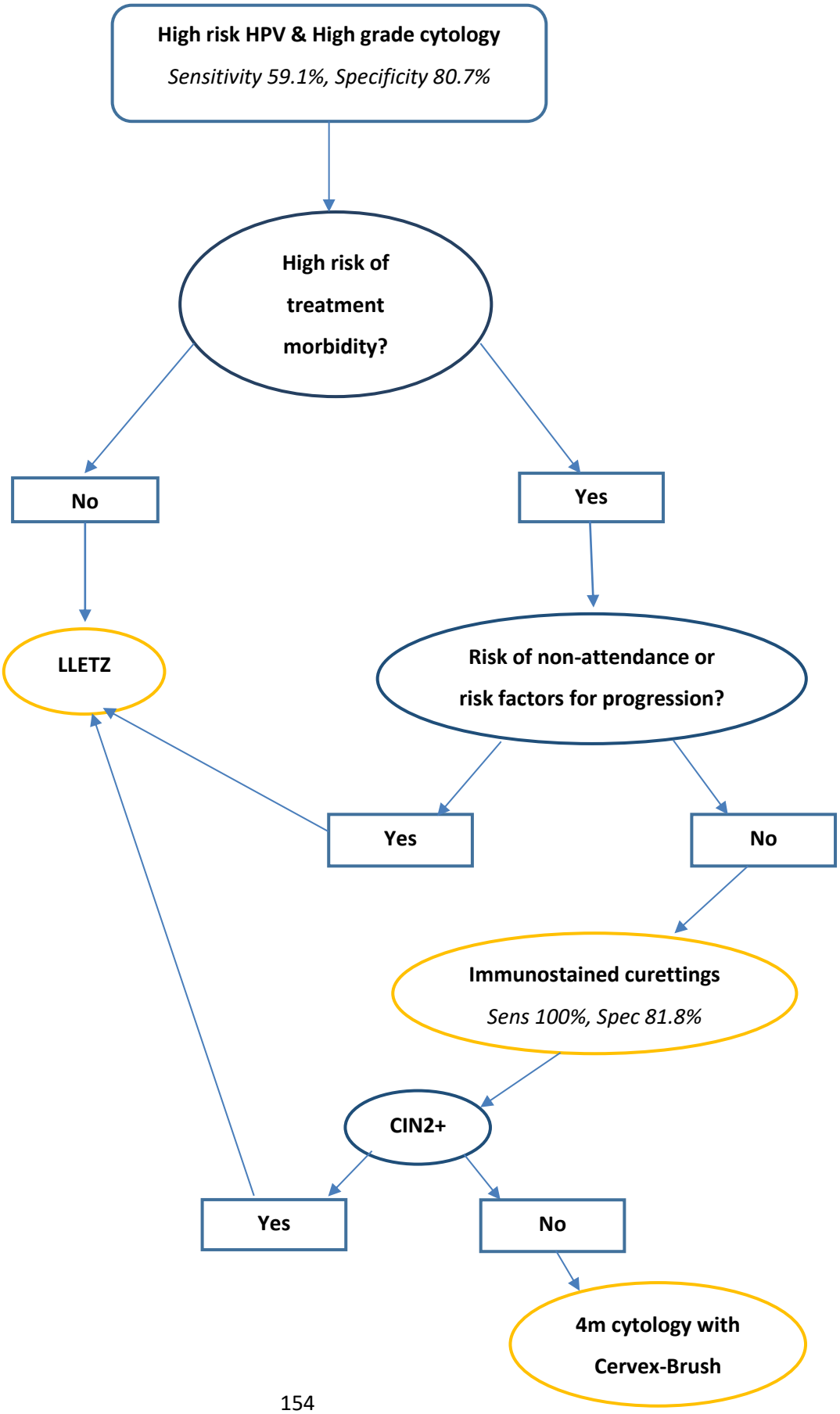


Figure 7.7: Treatment Algorithm B – potential management of women with high grade cytology and a TZ3



7.4 Strengths

Involvement (and endorsement) of a patient participation group whilst I was writing the study protocol was extremely useful in ensuring clarity during discussions with the ethics committee; this meeting focused on the recruitment process and the potential contribution of the study to women's health care. Of 153 eligible women, 105 were recruited. Only one woman declined when approached and indeed women who had seen the recruitment posters made participation enquiries, suggesting the value women attribute to this research. As the same sample was used for both the index and reference tests, the patient demographics were balanced between cohorts.

Further strengths included whole sample verification using the reference standard and a short period of time between the index and reference tests to reduce the risk of CIN progression or regression. Furthermore, unlike a retrospective review, which seeks women known to have high grade disease, the prospective nature of this study promotes a realistic assessment of biopsy confirmed high-grade CIN; incorporation (work-up) bias was avoided as the outcome of the index test was not known prior to the reference standard.

Double data entry removed bias caused by erroneous and missing data. A sample size calculation, which was achieved, helped ensure sufficient power. Reproducibility was very good as shown by the inter-rater reliability of the most predictive tests. Blinding of the cytologists and pathologists interpreting the index and reference samples will reduce expectation bias. ThinPrep liquid based cytology media was used as dual-stained slides processed from samples stored in a SurePath medium have a lower sensitivity[215]. When there was a mismatch between the referral cytology and LLETZ histology all slides were double reviewed and the diagnosis confirmed or refuted. Finally, the clinical data that was used to interpret these test results will be available in 2019 when primary HPV screening is introduced in the UK making these results relevant to clinical practice.

7.5 Limitations

Selection bias needs to be considered. Randomisation is the most effective method but with a small target population this may sometimes not be practical. The sample needs to be large enough to detect an effect size and randomisation, in this case, may have reduced

the final sample size. Whole cohort sampling would ensure an accurate representation of the target population and potentially allow the achievement of the sample size calculation. This was my aim. Of the 47 women who were not recruited, competing clinical duties rather than declining participation, were the cause. This was likely to be random in nature, as my duties occurred on different days in the month. This suggests the study sample may be representative of the target population but if future studies aim to corroborate my results, the use of multiple centres which will allow for randomisation of recruitment would reduce selection bias.

The logistics of consent, taking the samples, completing the LLETZ and trial paperwork (labelling the samples, completing the GP letter, photocopying the consent for the medical records and participant, registering the samples on the national database and completing the baseline proforma), took approximately one hour per participant, which the colposcopists were unable to complete during a busy clinic. Although this standardized sample taking and reduced missing data, as I did all recruitment and sample taking, future studies should assess outcomes based on multiple sample takers. Furthermore, of four women who had adequate dual-stained cytology samples a curette could not be passed; to prevent crushing of the epithelium I did not dilate the cervix, but this could be assessed in future studies.

Repeating the cytology between 5 - 11.5 weeks of the referral test risks false negative results. However, due to limited storage capacity the referral cytology is disposed of by the first colposcopy appointment, TZ sampling was present on 99.1% of the index slides, the inadequate rates were equivocal to national cytology standards[216] and taking the index tests on the same day as the reference test reduces mismatch due to immuno-clearance. The small number of smokers in the study sample will also reduce mismatch between the referral and index cytology but this may affect the incidence of CIN2+ in populations which have higher rates of smoking.

The median depth of LLETZ was 15mm (the minimum recommended by the BSCCP) which could have been secondary to shrinkage or human error, however the TZ was reported to be present in all samples. Seven of the endocervical curettings (five women with low grade cytology and two women with high grade cytology) were reported as CIN2+ but the corresponding LLETZ report stated <CIN2; removal of focal CIN2+ with the curette could account for this. Sub-analysis identified no difference in specificity if these LLETZ were

upgraded to CIN2+, but future large studies should assess the accuracy of the dual-stained cytology and curettings in women who have one index test to reduce the impact of sample taking on their individual accuracy. I stored the index cytobrush and Cervex-Brush samples in the same pot but future studies could store these separately to assess the contribution of the cytobrush sample alone.

Finally, the sample size for the genotyping was smaller than calculated; if the effect size was small I may not have detected a difference, accepting the null hypothesis when it may in fact be false.

7.6 Conclusions

The introduction of primary HPV testing will improve the NPV of cervical screening but the PPV is still poor in women with low grade cytology and this is compounded in women with a TZ3 where histological selection for treatment cannot be undertaken.

Irrespective of TZ type, the majority of women with high grade cytology (80%) will have CIN2+. In those women at high risk of treatment morbidity (young and nulliparous), dual-stained curettings could be used to detect false positive screening in 72.7%. In all other women, my results suggest excision should be offered.

Women with low grade cytology, high risk HPV and a TZ3 have a two-fold increased risk of CIN2+ (36.7%) when compared to women where the TZ is visible. However, excision should not be the primary management as the majority of these women (63%) have <CIN2 at LLETZ. My results suggest the use of dual-stained cytology will prevent overtreatment in 58.1% (PPV) of women with <CIN2 but more importantly detect 89.5 - 100% of women with CIN2+ (NPV). This will improve the accuracy of routine screening for CIN2+ which has a PPV of 28.1% and a NPV of 29.7% in women with low grade cytology and a TZ3.

In women with a TZ3 the cytobrush + Cervex-Brush increased the cytological yield of endocervical cells when compared to the Cervex-Brush alone but there was no difference in predictability of CIN2+ in women with low grade or high grade cytology between sampling methods. This suggests women who will reliably attend for cytological follow-up can be safely referred to primary care for a Cervex-Brush alone.

Chapter 8 **Discussion**

In this thesis I have explored the impact of HPV testing on the specificity of cervical screening in women with a TZ3. I evaluated colposcopists' decision-making when managing women with a TZ3 and provided recommendations for a national consensus strategy. I used HPV genotyping and surrogate biomarkers for HPV, in conjunction with cervical cytology and histology, to evaluate the sensitivity and specificity of these investigations individually and in combination when compared to routine screening. I have considered the effect of different immunostaining and diagnostic categories on the accuracy of these tests and proposed new scoring protocols. Finally, I have examined the contribution that a cytobrush adds to the diagnostic accuracy of a Cervex-Brush in women with a TZ3 to guide colposcopists in the optimal technique and clinical setting for cytological follow-up.

In this chapter I will reflect on the main findings, how these can be interpreted in line with the current literature and explore potential reasons for the differences between my results and those reported from other studies. I will also discuss how I aim to progress with my investigations in view of the limitations of my studies and my own personal development during the course of this thesis.

8.1 The impact of HPV testing on negative LLETZ histology

This cohort study of 802 women provides contemporaneous information on the impact that HPV testing will have on false positive screening and suggestions for where future improvements in the screening programme should be targeted. Negative LLETZ is an important performance indicator in colposcopy and quality management of a cervical screening programme. This study has provided evidence, for the first time, that the incidence of negative LLETZ has decreased following the introduction of HPV testing but the prevalence of false positives is still high at 13.4%. Risk factors for negative LLETZ in the HPV testing cohort were a TZ3, low grade cytology and parity greater than two. Women with both low grade cytology and a TZ3 were most at risk (absolute risk 40%).

8.1.1 Potential confounders

The reported incidence of negative LLETZ in women with biopsy confirmed CIN2+ varies from 5.9% to 41%[105, 160-164]. The rates in my study fell within this range. To determine whether the differences in the incidence of negative LLETZ can be attributed to HPV testing, rather than confounders, variables which can affect the histological outcome were compared between the pre- and post-HPV testing cohorts. A negative LLETZ can occur when CIN is not removed with the initial treatment or missed during interpretation of the histology, but there was no evidence of a difference in the incidence of post-LLETZ dysplasia between cohorts. Variables such as referral cytology, limiting histological factors and inclusion of the transformation zone in the LLETZ sample were equivocal. The interval between the referral cytology and LLETZ were also similar, reducing the impact of immuno-clearance on the outcome histology. Furthermore, the routine practice of cutting extra levels and double reporting the LLETZ histology when it is initially identified as normal, standardizes reporting across the cohorts.

Focal lesions which may have been removed after punch biopsy can affect outcomes but a strict selection criteria for treatment in both cohorts mandated that confirmatory biopsies were needed prior to treatment if a significant change in lesion size and / or grading occurred. This policy does not account for patient choice such as women who prefer excision to repeat biopsy, but this should be documented in the medical records. Changes in national policy could affect the incidence of negative LLETZ between cohorts but the following NHS CSP recommendations were no different pre- and post-HPV testing:

(a) *'Treatment should be offered with a histological diagnosis of CIN2+'*

(b) *'If a TZ1 - 2 is present and CIN1 is detected, offer cytological follow up for 24 months'*[35].

The difficulty, as illustrated by my results, is when women present with a TZ3.

8.1.2 The impact of HPV testing

HPV testing is a more sensitive cervical screening test than cytology alone for the detection of squamous cell lesions and my results suggest the incidence of false positive screening has decreased since its introduction. Recent UK cervical cancer screening statistics support my findings[87]; they have reported an increase in the proportion of women reviewed in colposcopy with CIN following the introduction of HPV triage of low grade cytology i.e. improved detection of dysplasia which reduces the incidence of

negative LLETZ. It is not the rate of CIN that is increasing but rather the proportion of women referred to colposcopy with no dysplasia that is decreasing. This has important clinical ramifications; as seen in 4.3.3.1, unless colposcopists are updated on this new information they may perceive women who have had HPV testing to be at increased risk of dysplasia which will increase anxiety and the rates of negative LLETZ.

8.1.3 Predictors of negative LLETZ

My findings support the importance of colposcopic assessment and confirmatory biopsies[105, 163] – which reduced the incidence of negative LLETZ by 75% if CIN2 was detected. In the presence of CIN3, this protective effect was not apparent and may be secondary to the removal of focal dysplasia or a post biopsy inflammatory response. Although confirmatory biopsies should be taken if a change in lesion has occurred prior to LLETZ, in the presence of biopsy confirmed CIN3, the colposcopist or woman may be anxious and prefer LLETZ irrespective. It is essential that colposcopists explain the potential benefits to the woman of confirmatory histology if a significant change in the lesion has occurred. The importance of adhering to national guidance[35] and routinely incorporating vaginal assessment in the colposcopic examination seems clear as 1% of women with a negative LLETZ will have VAIN and an unnecessary LLETZ if not completed.

The use of the COCP reduced the risk of negative histology by 60% when compared to those who did not use any contraceptive and this may be a reflection of the higher rates of a TZ1-2 seen in this cohort. Despite the improved sensitivity of HPV screening, my results indicate that women with a TZ3 and low grade cytology are 10 times more likely to have a negative LLETZ when compared to women where the TZ is visible. In women with false positive cytology and a TZ3, colposcopic assessment or a reassuring biopsy cannot be undertaken and this may account for the increased incidence of negative LLETZ observed in this cohort. There are currently no UK recommendations to guide the management of a TZ3 in the presence of low grade cytology[35].

The American Society for Lower Genital Tract Disorders recommends that women with low grade cytology should not be treated unless high grade CIN is detected on biopsy[97]. As endocervical curettage is not routine practise in the UK, it is difficult to implement this policy and provide histological confirmation in the presence of a TZ3 without offering a

LLETZ. Colposcopists are reliant on the diagnostic value of the screening test, patient preferences and their own experience of managing these women to determine who requires treatment. Assessment of how and why colposcopists manage women with a TZ3 may be of use in reducing negative LLETZ histology by providing evidence to guide a national consensus strategy.

8.2 Colposcopists' decision-making in women with a TZ3

The exploration of factors which influence decision-making may enable targeted guidance; in women with a TZ3 this may aid in the reduction of negative LLETZ histology. As such, this study targeted an important issue – the ways in which medical practitioners, in this case colposcopists, make decisions under conditions of uncertainty. A qualitative approach sheds a useful light on the process of decision-making and to the best of my knowledge this is the first study which has addressed this issue.

Where rational judgement, cognition and affect could be applied, areas of consensus were identified; a multidisciplinary team decision, patient preference, a high-risk screening result or a low-risk result in combination with patient risk factors such as poor compliance, smoking, high parity, older age or persistent high risk HPV resulted in recommendations for excisional treatment. In areas of clinical uncertainty colposcopist's experience, knowledge, perception and affect influenced decision-making. When faced with an inability to provide colposcopic assessment or diagnostic histology the psychological stress of missing a cancer, even in women with low grade screening, deterred prolonged or community based cytological follow-up. Anxiety of treatment-morbidity influenced excision depth, with the majority of colposcopists deviating from national recommendations and reporting a preference for 7-10mm excisions - the depth recommended when the transformation zone is visible. A paucity of guidance[35, 97, 98] and patient anxiety further compounded decision-making and led to heterogeneity in care.

8.2.1 The effect of clinical uncertainty on decision-making

Decision-making is a complex process which incorporates knowledge, risk assessment, analytical skills, prior experience and affect[217]. Decision-making can be challenging in

areas of clinical uncertainty where guidance is sparse[218, 219], when an adverse outcome such as a cancer may occur as a result of the decision[220] or if a large number of variables need to be contemplated when making a decision[218].

These themes were illustrated in this qualitative study when participants, particularly gynaecological oncologists, suggested that the possibility of removing high grade disease outweighed the risk of treatment-related morbidity if women had significant risk factors for dysplasia. Whilst conscious of the risk of over-treatment, particularly in younger women, participants were more concerned about missing a developing cancer. This finding is supported by studies which have shown that in areas of uncertainty, decisions are made faster and more easily by relying on emotion[221]. Furthermore, when an emotive thought induces anxiety, this can lead individuals to place more weight on the negative outcomes than the positive[222, 223]. Once distracted by a negative stimulus it is then difficult to divert attention from these negative thoughts[224]. Anxiety has been associated with increased amygdala and reduced pre-frontal activity[225]; this suggests that in areas of uncertainty the affective components of decision-making may take precedence over rational cognitive elements[226, 227]. Clinicians strive to balance the risks and benefits of intervention but perception of risk differs dependent upon experience and personality. The value of guidance seems clear when rational thought is superseded by affect.

8.2.2 The influence of a high risk HPV result on decision-making

Uncertainty of decision-making in women with low grade cytology was reduced by the perceived increase in risk that a persistent HPV result conferred. However, recent evidence has shown that the proportion of women with a low grade screening result and subsequent grades of CIN 1, 2 or 3 are no different following the introduction of HPV testing[87]. What has fallen is the number of women referred to colposcopy with inadequate results and normal colposcopy[87] which, as alluded to in section 8.1.2, could be falsely viewed as an increase in individual risk, leading to a more aggressive management approach when histological selection for treatment is not possible. Most people are naturally risk adverse and look to avoid poor outcomes by selecting the least risky option[228].

Uncertainty of outcome (inability to visualise the transformation zone) heightens anxiety and compounds this risk aversion. When it is not clear whether the alternative decision may result in further risk or benefit, willingness to take a risk, in this case prolonged cytological follow-up, is avoided[229]. National guidance on the risk conferred by a high risk HPV result in women with low grade screening and a TZ3 may reduce the dominant role of affect and strengthen the cognitive component of decision-making (see section 8.4.1).

8.2.3 Shared care model

Patient choice was cited as a major influence affecting decision-making. The majority (81%) of referrals to colposcopy are for low grade screening results[87] but patients report the same level of anxiety irrespective of the cytological grade[103]. This anxiety is driven by fear of cancer, worries that subsequent cytology will be abnormal and future fertility concerns[103, 230]. There is a plethora of literature assessing women's preferences for the management of low grade cytology when colposcopy is satisfactory, with the majority of studies showing a preference for colposcopic review over cytological surveillance[231, 232]. Furthermore, if cytological follow-up is chosen, women have cited a preference for 'regular' screening[233]. When the clinical outcome is uncertain and condition specific information is sparse (as with a TZ3), patients can either be influenced by health care professional preferences for treatment or their anxiety can prevent attendance for follow-up[103, 210].

Until such time as one outcome is shown to be superior to another or patient preferences for management of a TZ3 have been assessed, it could be argued that colposcopists should advocate the more cost-effective approach of cytological follow-up. However, in a shared care model, determining patient preferences will improve patient satisfaction and outcomes[234] – even if this involves, as shown in our study, young women with low grade screening and low risk factors for CIN progression choosing excisional treatments over cytological follow-up. This illustrates the importance of assessing LLETZ outcomes in women with a TZ3 and improving the specificity of screening as the provision of a targeted diagnostic test and information may reduce health care provider and patient anxiety, in turn reducing negative LLETZ histology and non-attendance rates.

To reduce the emotional burden of decision-making, health care providers will defer management decisions[235]. In my study, the majority of participants expressed a reduction in emotional burden following an MDT decision to offer excision, particularly in young and/or nulliparous women. This finding is supported by studies which have shown a reduction in colposcopy overtreatment following an MDT review[236]. Although, with a paucity of evidence to guide this expert body's management, homogeneity of care may be achieved within departments or regions but may not occur at a national level.

8.2.4 Paucity of evidence affects decision-making

In areas that lack evidence prior expertise can form the basis of decision-making[237] and this can lead to heterogeneity of care. This was evident when participants recommended different collection methods and clinical settings for cytological follow-up. Colposcopy nurses preferred community follow-up and this may be a reflection of the higher volume of patients they see. In contrast, the majority of doctors favoured colposcopy follow-up, and this attitude may be influenced by the higher proportion of women with cervical cancer they manage. Although current evidence suggests an increased cytological yield when using a cytobrush in combination with a Cervex-Brush[238], there is a paucity of evidence correlating this yield of cells with improved detection of dysplasia in women with a TZ3[239]. This lack of knowledge and the inability of community services to offer a cytobrush compounded decision-making, particularly for doctors. Studies which improve knowledge in this area may aid rational judgement.

Prior experience and lack of evidence influenced the depth of excision that colposcopists recommended. They offered a LLETZ to aid diagnosis of CIN but they were anxious about the treatment-related morbidity and preferred a 7 - 10mm LLETZ rather than the 15 - 25mm advocated in recent national guidance[35]. Although there is a paucity of evidence which adjusts for age and parity when assessing the endocervical position of the TZ, the disadvantage of shallower treatments is relying on patients to return for a second treatment if CIN is diagnosed or false negative histology if dysplasia is positioned distally in the endocervical canal.

8.2.5 The quandary of evidenced-based guidelines

Colposcopists are independent practitioners and it could be argued that guidance may not be necessary in scenarios which lack consensus of opinion. Furthermore, it is clear that not all clinical scenarios can conform to guidance and removing all uncertainty from the medical profession may hinder adaptability, critical analysis, maturity of thought and patient choice. Evidence has suggested that experts are *'wise risk takers'*[240], their knowledge reduces anxiety and uncertainty allowing them to make decisions which deviate from set guidance to individualise care[241]. Despite this, part of a clinicians' duty is to reduce patient anxiety and optimise clinical outcomes - but how can this be achieved if the clinician themselves is plagued by anxiety? In situations where there is a lack of clear evidence, affect may compromise rational judgements. Homogeneity of care improves service provision and clinical outcomes through consistent use of evidenced based interventions[242]. Guidelines improve decision-making in areas of ambiguity, recognize shortfalls in the literature, provide assurance that clinicians are advocating appropriate treatments and promote under-recognised and neglected patient cohorts.

8.2.6 Summary

The focus groups provided depth of information, assessing not just how colposcopists manage women with a TZ3 but why. This part of my research may have been affected by geographical bias and to address this, the national survey that I developed aimed to ratify my guideline recommendations (Section 8.6) by evaluating the frequency of the opinions identified in the focus groups.

8.3 Current management of a TZ3: A UK survey

This is the first study which has developed a reliable and valid questionnaire to assess how UK colposcopists' manage women with a TZ3. The information provided will add to the literature by focusing training needs, clarifying guidance and directing future research.

There were areas with a clear consensus that supported the findings from the focus groups and these included; offering excision to all women with high grade cytology and women with low grade cytology who have risk factors for disease progression such as

smokers, non-attenders and parous women. In women with low grade cytology and an incomplete family, there is a strong preference for colposcopy follow-up. In postmenopausal women the majority of colposcopists offer topical oestrogen to improve the adequacy of the examination but no other adjuncts, such as biomarkers or genotyping, are routinely used. Areas of discordance, which are affected by paucity of evidence, include the interval between follow-up appointments, total length of follow-up before recommending a LLETZ and a preference for a shallower excision (7-10mm) than currently recommended by the NHS CSP.

8.3.1 Initial management of low grade cytology

The majority of colposcopists, irrespective of experience, job title or gender, reported a strong preference for cytological follow-up. The patient's parity and age decreased the strength of this association. As outlined in section 1.2.4.3, existing evidence would support stratification of patient risk factors as it has been estimated that smokers are 1.6 times more likely to develop squamous cell cervical cancer when compared to never smokers[40] and parity, greater than four, increases the odds of a squamous cell cancer four fold when compared to nulliparous women[60].

Evidence is contradictory regarding the association of older age with HPV clearance. The 5 year risk of CIN3+ has been reported as comparable in women with low grade cytology who are aged 25 - 29 years and 30 - 64 years (5% and 5.2% respectively)[243] - this finding is supported by other large studies which have adjusted for parity and lifestyle choices[213]. Many countries, particularly the USA, recommend that women under the age of 30 should not have HPV screening as at least 50% will have a transient HPV infection and this reduces the PPV of screening for CIN2+[92] .

In the UK, 25% of girls have their first sexual contact before the age of 16 and this proportion, plus risky sexual behaviour, has been increasing over the last decade[244]. Integration of HPV following early age of first intercourse may be mediated by a large, metaplastic ectocervical TZ or an immature immune response[38] and this makes younger women more vulnerable to acquiring, retaining and transforming a HPV infection. This evidence and UK cancer statistics which reported that cervical cancer is the most common cancer in women under 35[3] suggest not only a need for HPV screening but a need for

methods which can improve the PPV of screening in young women, particularly when assessment of the TZ is not possible.

8.3.2 Cytological follow-up with low grade cytology

8.3.2.1 Clinical setting

Similar to the focus groups, the data suggest colposcopists have a strong preference for colposcopy clinic follow-up to facilitate the use of a Cytobrush in combination with a Cervex-Brush. Colposcopists' rely on the accuracy of the referral cytology to aid decision-making in women with a TZ3 and they assume, although it is not proven, that TZ sampling is required to optimise this result[101]. Three inadequate samples with a Cervex-Brush alone are required in the community before a colposcopy referral is instituted which can delay assessment of women with potential dysplasia.

Of the studies evaluating liquid based cytology devices[79, 81, 83, 214], none have correlated their findings with topographical position of the TZ. Decision-making in women with a TZ3 would be enhanced by population specific studies which adjust for topographical position of the TZ, age and parity when evaluating cytological collection devices (Section 8.4.2).

8.3.2.2 Frequency and total length of follow-up before offering a LLETZ

UK guidance denotes that women with low grade cytology should be reviewed twice at 12 monthly intervals, prior to offering a LLETZ, if CIN1 or less is identified at colposcopy [35]. Of those women who test positive for high risk HPV, 61% can have a positive test six months later whereas only 35% are positive at 12 months indicating the utility of waiting at least six months before retesting [158]. In women with a TZ3 there is a preference for six monthly follow-up. As identified in the focus groups, there is a preference to offer LLETZ if dyskaryosis is persistent 12 months after the referral cytology in women ≥40 or when the family is complete. In women aged 25 – 39 there was no consensus on the total length of follow-up before recommending a LLETZ.

In the absence of colposcopic assessment, or confirmatory histology, my findings suggest colposcopists' feel a responsibility to ensure these women are reviewed regularly to prevent loss to follow-up and to make a diagnosis. Studies which assess the LLETZ outcomes and progression rate of CIN in women with low grade cytology, high risk HPV

and a TZ3 are needed to improve homogeneity of care, to optimize outcomes and improve service efficiency.

Whilst not a reported concern in the survey, focus group participants emphasized the impact that a high risk HPV result had on their decision to offer a LLETZ, if still persistent 6 to 12 months after the referral screening test. The involvement of two of the focus group units in the Sentinel Sites Study, with the increased exposure to outcomes in women with high risk HPV, may have contributed to the perceived increase in risk and preference for treatment in women with a 12 month history of high risk HPV (Section 8.2.2). This finding illustrates the relevance of improving diagnostic accuracy and providing targeted guidance in women with a TZ3 before the introduction of primary HPV testing in 2019 similarly influences decision-making nationally.

8.3.3 Repetition of the referral cytology

In line with national UK recommendations[35], 70.2% of colposcopists do not repeat the referral cytology at the first colposcopy appointment. Of note, 29.3% *do* repeat and the main reason reported is the belief that a Cervex-Brush alone may not adequately sample a TZ3. Recent UK guidance recommends that the presence of endocervical cells should only be reported in women with previous cGIN[78] (Section 1.4.1.1) but this has clearly impacted on colposcopist's management of women with a TZ3, increasing the potential for false negative screening on the repeat cytology. Until studies have assessed the diagnostic accuracy of a Cervex-Brush alone in this cohort, it would seem safest to adhere to national recommendations and rely on the referral screening test result. During construct validity testing 91.7% did not repeat the cytology; this increased adherence to national guidelines may be a consequence of completing the form in front of me.

8.3.4 The use of adjuncts to improve decision-making

To aid decision-making the majority of participants use oestrogen, a fifth HPV genotyping, 10% endocervical curettage and 3% surrogate biomarkers for HPV. Use of these adjuncts may be affected by cost, access to these investigations - as only specific laboratories undertake HPV genotyping - training in endocervical curettage and experience of processing and interpreting immuno-stained cytology and histology. This part of my

research would have been improved by the addition of items within the questionnaire which assessed colposcopist's access to and acceptability of these adjuncts.

The majority of colposcopists (89.7%) recommend topical oestrogen in postmenopausal women to improve the visibility of the TZ or the quality of the repeat cytology. Gynaecological oncologists are less likely to advocate this practice and this may be a result of the larger proportion of oestrogen driven cancers that they manage. Studies have shown TZ eversion success rates of 64% and 70% with six weeks of topical[119] and four weeks of systemic oestrogen[117], but most of these studies were based on small sample sizes and the use of oestrogen relies on patient acceptability or compliance (which has not been assessed). Post-menopausal women, who would benefit most from oestrogen, are least at risk of treatment morbidity but potentially at higher risk of disease progression due to their increased parity.

In pre-menopausal women with atrophic changes, only 45% of colposcopists offer topical oestrogen and only 9% the combined oral contraceptive pill. Factors which could influence this choice include an inability to prescribe, health care professionals are medico-legally obliged to do a lengthy 'pill teach' as it is a contraceptive, patient choice and compliance, concerns of loss to follow-up and the side-effect profile – all of which need to be assessed during a busy clinic.

A TZ3 may be more prevalent in postmenopausal women[101] but there are no cross-sectional studies which corroborate this, particularly in view of the high proportion of young women who do not use the COCP. Where oestrogen is not acceptable, applicable or does not evert the TZ, the use of HPV genotyping or surrogate biomarkers for HPV may be of benefit and their utility are discussed in Section 8.4.

8.3.5 Initial management of high grade cytology

LLETZ is the primary management choice in all patient demographics but age and parity affect the likelihood of this choice. In women whose family is incomplete a higher than anticipated proportion of colposcopists, particularly nurses, refer women for MDT review; 30% of respondents refer women aged 25 - 39 and 20% refer women aged ≥40. Of note, 10% recommend three month cytological follow-up in women aged 25 - 39.

An American study reported that the risk of high grade CIN and cancer is increased in women aged 30 - 64 with high grade cytology when compared to women aged 25 - 29 (47% vs 28%, p=0.04 and 7.3% vs 2%, p=0.004)[182]. UK screening statistics, although not adjusting for age or parity, report an 84.5% chance of CIN2+ and a 2.6% chance of cancer with high grade cytology[5]. Although neither of these studies adjusted for topographical position of the TZ, until population specific evidence is available, particularly in view of the young age of sexual debut in the UK, it would seem safest to recommend a LLETZ in all women with high grade cytology and a TZ3. A policy of delayed excision does not account for patient choice or reliability. Furthermore, patient anxiety, which arises from uncertainty of histological diagnosis, is reduced when women with high grade cytology are offered treatment at the first appointment[245].

8.3.6 Recommended depth of LLETZ

The NHS cervical screening programme recommends a depth of 15-25mm for a TZ3 and 7-10mm for a TZ1[35]. Irrespective of cytological grade, and as identified in the focus groups, colposcopists' report a strong preference for 7-10mm in women with a TZ3 when the family is incomplete. A depth ≤6mm was included as a negative control for the construct validity but 10% of respondents reported a preference for this depth in young women with low grade cytology. In all women with high grade cytology and in women with low grade cytology and a complete family, half of the respondents recommend 11-15mm. This discrepancy suggests colposcopists are aware of UK recommendations but anxiety for future fertility and the ability to offer a second, more targeted treatment if the first is diagnostic for CIN2+ appears to supersede this knowledge.

Although consideration needs to be applied to the risks of preterm labour, the oncological consequences of incomplete margins or false negative histology, particularly in women with high grade cytology, also needs to be contemplated. As does the risks of non-attendance for follow-up or repeat LLETZ. It is importance to emphasize that recurrence of CIN2+ has a prevalence of 18% with incomplete margins vs 3% if complete[106].

In Section 1.5.4 a review of the available literature illustrated that knowledge of the maximum depth of the epithelial crypts is important to reduce recurrence of CIN and to inform depth of LLETZ. It is also important to know the mean topographical position of

the transformation zone to estimate where the crypts may begin. A study which adjusted for age but not TZ type reported that total volume of crypt involvement and the mean depth of the proximal margin was smaller in women aged 20 - 40 when compared to women over 50 (61.5% and 12.5mm vs 88.3% and 16.4mm)[108]. Furthermore, the mean depth and the total volume of crypt involvement is reported to be increased with parous women when compared to nulliparous (13.5mm and 71.1% vs 12.6mm and 51.8%)[108, 246].

Although the evidence that UK guidance[35] is based upon did not correlate excision depth with TZ type, age or parity, the findings above suggest colposcopists' may be undertreating this cohort of women. This has not been reported before and, although it was not an initial aim of my thesis, suggests that colposcopists require evidence that the benefits of a deeper excision outweigh the risks. Methods which improve the diagnosis of CIN2+ in women with a TZ3 and studies which adjust for age and parity when determining the mean topographical position of a TZ3 will aid this decision-making.

8.4 Improving diagnostic accuracy in women with a TZ3

As far as I am aware, this is the first study following the introduction of HPV testing which has been specifically designed to improve the accuracy of screening in women with a TZ3.

8.4.1 Study population

The majority of the study population were pre-menopausal women and half were of prime reproductive age. These results suggest a TZ3 is not a condition that is solely attributed to poorly oestrogenised older women who are at low risk of treatment-related morbidity. In chapter 3[247] I reported an association between a TZ3 and age >50 but the population these data were based upon also included women in the non-HPV testing cohort. Half of the study population in Chapter 7 were not oestrogen deficient (using progesterone contraceptive or post-menopausal) and this finding was supported by the outcomes in chapter 3[197]. This indicates that the use of oestrogen, even if acceptable to the patient, may not be of benefit in everting the TZ. Indeed, in pre-menopausal women the focus group and national survey participants did not routinely use oestrogen even if atrophic changes were identified[101, 248] – suggesting a need for other adjuncts to improve diagnostic accuracy.

In comparison to national screening statistics (where 80% of the women assessed will have a TZ1-2) there was no difference in the proportion of women reviewed with low grade cytology (Mild or BNC)[87] but in my study sample these women had a two-fold increased risk of CIN2+. The strength of this association was highest in women with BNC suggesting that sampling of a TZ3 may make the cytology trickier to interpret. Furthermore, women aged 25 - 39 were four times more likely to have CIN2+ than women ≥40. This is a significant and relevant finding which has not been reported before. Most of the studies assessing age related risks are from the Americas whose populations, including age of sexual debut and HPV subtype prevalence, are different from the UK.

Decision-making, as suggested by the focus group and national survey data, is influenced by the referral cytology. In the survey and focus groups, the majority of colposcopists offered 25 - 39 year olds 6 to 12 month cytological follow-up. Outcome data from Chapter 7 suggests women with low grade cytology and a TZ3 would benefit from adjuncts which improve the diagnostic accuracy of the screening test before discharging to the community, as a substantial proportion (36.7%) are at risk of CIN2+. As discussed in 8.3.1, risk factors for acquiring and transforming a HPV infection can differ between countries and the young age of sexual debut in the UK may account for these findings and ratify the need for population specific guidance.

8.4.2 The contribution of a cytobrush in detecting cervical dysplasia

My findings suggest that the addition of a cytobrush, irrespective of age or parity, does not improve diagnostic accuracy in women with a TZ3 when compared to a Cervex-Brush alone. Despite a median interval of eight weeks between the referral and index cytology, the yield of endocervical cells was increased with the addition of a cytobrush. However, there was no evidence of an association between the presence of endocervical cells and increased predictability of CIN2+, nor was there a difference in diagnostic accuracy between the referral and index cytology, irrespective of the cytological grade. These findings are relevant to clinical practice as they may affect service provision and the current management of women with a TZ3.

This increased cytological yield of endocervical cells when the cytobrush is used in combination with the Cervex-Brush has previously been reported[239] but studies which

have evaluated the contribution of endocervical cells in predicting high grade disease are contradictory. Studies prior to the introduction of LBC promote their importance in reducing false negatives[80, 81] whilst other studies do not support this benefit[82]. Zhao *et al*[249] reported an increased detection of low-grade dysplasia with a Cervex-Brush LBC in combination with a cytobrush resulting in a US recommendation that cytological follow-up should occur 12 months, rather than 3 years, after a negative smear that lacks TZ sampling[216].

The UK has not adopted this US recommendation due to the controversy surrounding the association of endocervical cells with the detection of dysplasia. Furthermore, as discussed in 8.3.6, studies have shown that the topographical position of the TZ is more proximal to the ectocervix in younger women[108] which may influence sampling. In my study, cytological outcomes were not affected by age or parity, but half of the population were aged 25 – 39. To validate my findings larger studies, if the effect size is small, are needed to determine the relevance of the cytobrush in older women – particularly in view of their potential for a deeper position of the transformation zone.

In the focus groups and national survey (Chapters 4 and 6 respectively), colposcopists report that diagnostic accuracy may be optimised by the presence of TZ sampling[101, 248]. This results in;

a. The majority of colposcopists recommending the colposcopy clinic as the setting for cytological follow-up, as cytobrush sampling is not currently offered in primary care
b. A quarter of colposcopists repeating the referral cytology at the first colposcopy appointment.

These management decisions have economic as well as outcome implications. The cost of a follow-up appointment in the colposcopy clinic is £180 whilst a community smear is £40. Filling clinic lists with follow-ups who could be managed in primary care increases waiting times for women with abnormal screening and places increased burden on an already stretched tertiary care system. My data suggests that offering these women cytological follow-up in primary care with a Cervex-Brush alone is safe and may consequentially improve service efficiency.

8.4.3 Pooled high risk HPV testing

My data supports the plethora of robust methodological studies which have assessed the use of primary HPV screening and reported a negligible risk of CIN2+ (very high NPV) in women who are negative for high risk HPV[35]. The difficulty for colposcopists is that their decision-making is based upon the poor PPV of HPV testing – they will not review women who are HPV negative. Indeed, seventeen (16.8%) of the women in this study who were referred with high risk HPV had either low risk HPV or a negative result at genotyping. None of these 17 women had CIN2+ and potential reasons could be immuno-clearance (although the median interval between the referral and index tests was nine weeks) or sensitivity of the Papillocheck array. Either way, this illustrates the importance of not offering excision based on a pooled high risk HPV result, as was seen in the focus groups, but rather the need for improved diagnostic accuracy in women who test positive for high risk HPV. This is particularly relevant in view of the imminent implementation of national HPV screening.

8.4.4 HPV genotyping

Irrespective of cytological grade, the predictability of HPV 16/18 genotyping for CIN2+ was lower than observed with dual-stained curettings or dual-stained cytology. Although a limitation of this part of my study was the sample size of 84, (17 women who were referred with high risk HPV had a negative or low risk result at genotyping), a recent large multicentre prospective study which grouped TZ types, and was published during the period I was recruiting for this study, corroborated this finding[250].

A potential reason for the reduced specificity of 16/18 genotyping for CIN2+ may be the variety of high-risk subtypes that were detected in women with high grade cytology. Women aged <30 years of age, when compared to women ≥30 years, can have higher rates of CIN3+ with non-HPV 16 and 18 high risk subtypes[251] and a quarter of my study population were <30. My findings suggest that women who are 16 and / or 18 negative but positive to other high risk subtypes still have a significant risk of CIN2+ and should not be discharged to primary care without further assessment. This result is supported by other recent studies[252, 253] and may be of greater significance than previously reported as women vaccinated against HPV 16 and 18 are now eligible for screening. Modelling studies have also reported that subtypes not covered by the vaccination programme may 'replace' those that are[254] (Section 1.3.1).

As outlined in 1.3.1, there is no denying that the introduction of a HPV vaccine will reduce the prevalence of cervical cancer but until a vaccine is developed which provides broad spectrum immunity, to boys and girls, there will continue to be a need for cervical screening and colposcopic assessment. Furthermore, the effects of the vaccine on the PPV of screening[74] mean that the development of methods which improve the diagnostic accuracy of the screening test, such as biomarkers, will be of increasing relevance.

Vaccination status was not adjusted for in Chapter 7 and it would be of interest for future studies to assess this. Moreover, viral load may improve the specificity of screening as women who are high risk HPV positive may have a low-viral load secondary to immuno-clearance [255]. The presence of HPV 16 or 18 does not mean integration into the host's genome, nor the presence of CIN2+, which may account for the reduced predictability of genotyping. Studies which had assessed viral load were evaluated when I was writing the protocol for the study described in Chapter 7, but the range that constituted a 'significant' viral load differed in each of the papers that reported this finding and this range varied with different subtypes. Standardization of testing is currently difficult but may become more robust.

Patient factors also need to be considered when assessing the impact of genotyping; HPV 16 infection does not always equate to CIN3 but if women are informed of their positivity to this subtype they may request a LLETZ irrespective of the underlying pathology.

8.4.5 Histological scoring protocol for dual-stained curettings

Unlike my study in Chapter 7, to the best of my knowledge previous studies which have assessed the utility of endocervical curettings alone, have not evaluated their diagnostic accuracy in conjunction with both p16 and Ki67, corroborated all outcomes with definitive histology, been prospective or correlated the variables which led to increased diagnostic yield with topographical position of the TZ[120, 256]. Previously reported staining patterns were in studies where the epithelium was intact (not fragmented) and their criteria for negative expression included focal and sporadic staining in isolated cells and small clusters[137, 257]

To improve diagnostic accuracy I evaluated the inter-rater reliability and predictability of diagnosing CIN2+ by different immunostaining and diagnostic categories. As reported in other studies[257], CIN2 has poor reproducibility when LLETZ and punch biopsy H&E slides are interpreted but the addition of p16 and Ki67 immunostains improved diagnostic accuracy and inter-rater reliability.

Due to the nature of the endocervical curettings the epithelium was fragmented and scattered dysplastic cells with focal staining were common. The scoring protocols were adapted to record positive expression if scattered cells showed morphological changes or dual-staining was seen in fragmented strips of epithelium. Full thickness Ki67 staining and strong staining of p16 had better predictability for CIN2+ than 'any grade' of staining. P16 in conjunction with Ki67 improved the inter-rater reliability when compared to outcomes with the individual stains and was of use when interpreting scanty or fragmented samples. For example, strong p16 staining was present when HPV alone was reported at LLETZ and in these cases the addition of Ki67 was particularly useful in illustrating the nuclei's morphology and possible pleomorphism. To validate these findings a bigger sample size is required to assess the impact of p16 and Ki67 alone and in conjunction on the specificity of endocervical curettings.

8.4.6 Management of women with low grade cytology and a TZ3

My findings suggest that dual-stained cytology (CINtec) could improve the diagnostic accuracy of a low grade screening result. Women negative to dual-stained cytology did not have CIN2+ at LLETZ indicating that a negative result can reassure women and colposcopists that cytological follow-up is a safe management option. In women who are dual-stain positive, offering a LLETZ would seem reasonable considering the significant risk of high grade CIN observed in women with borderline nuclear change and a TZ3. Although the specificity of dual-stained cytology is moderate (30% of women would have a negative LLETZ), this is a substantial improvement when compared to the referral test specificity (~20%) - on which colposcopists currently base their decision-making[258].

A recent multicentre prospective trial conducted in five European countries[215] compared dual-stained cytology to HPV testing in women with ASCUS and LSIL. Although they reported a higher PPV for dual-staining, when compared to routine screening, in women

of all ages with both ASCUS (16.3 vs 10%) and LSIL (26.5 vs 18.6%), these values were less than half of what I observed (58.1%). Potential reasons for this difference could include their inclusion of women with all TZ types with their lower rates of CIN2+ (4.2% for ASCUS and 16.4% for LSIL), differences in dual-stain tests and population variances. Half of my study sample were aged 25 - 39 and if the TZ is positioned more proximally to the ectocervix in younger women[108], cellular adequacy may have been affected by this.

All women with dual-stain positive cytology had high risk HPV but the converse was not true. This suggests that integration of HPV, rather than infection, may influence the outcome of the test. Despite this, reasons for the moderate PPV need to be explored and a potential reason may be failure of dual-staining to account for women who have current HPV integration but will go on to clear the virus. Further studies which assess the progression rate of CIN in women who are dual-stain positive but initially have normal histology would be useful. Although some studies have promoted the improved inter-rater reliability of dual-staining when compared to cytology alone[259], I did not observe this. A potential reason may be the extensive experience of the cytologists who were interpreting these slides.

Processing limitations can contribute to the specificity of dual-stained cytology. The brown DAB staining can be trapped within mucus or cell debris increasing the intensity and distribution of background staining. The FastRed reaction can 'bleed' into the cytoplasm and neutrophils leading to false positive staining patterns or slides which are difficult to interpret. With clusters, individual cells are difficult to assess in the same plane of focus. The fast red stain can fade if it is exposed to alcohol, cracking artefact can arise with incomplete drying of the aqueous mounting media (Figure S7.1 in appendix 7) and 'cornflaking' of the cells can occur if there is air drying before the aqueous mounting media is applied (Figure S7.2 in appendix 7).

The relevance for clinical practice needs to be considered. The cost of a liquid based cytology test and dual-stained cytology slide are approximately £40 each. The addition of dual-staining to the referral cytology doubles the laboratory costs but in comparison a full genotyping array costs £120 and a LLETZ treatment is approximately £650. Given that pre-term births significantly increase neonatal mortality and cost the British economy £939 million a year[260], reducing iatrogenic factors will have considerable economic as well as patient outcome implications.

My data also supports current literature which reports a 20% incidence of a TZ3 [4, 197], illustrating this is a significant proportion of women who will be reviewed, potentially accounting for more than 25,000 of the 127,171 women seen annually in the UK with low grade cytology [87]. Although half of these women will be >40, these patients may choose improved diagnostic accuracy in preference to an unnecessary treatment and efforts should be made to assess patient preferences.

Currently, the availability of dual-stained cytology within NHS trusts is for research purposes only. For UK wide access, the NHS CSP would need to endorse the utility of this test and larger studies are needed to corroborate these findings and assess the cost-benefit ratio.

8.4.7 Management of women with high grade cytology and a TZ3

In my study, women with high grade cytology and a TZ3 had a 75.6% risk of CIN2+. Data from the survey (Chapter 6) showed that in women aged 25 - 39, 10% of colposcopists recommend three month cytological follow-up and 30% will refer to the MDT. This is of concern considering my data from Chapter 7 showed younger women with a TZ3 are three times more likely to have CIN2+ at LLETZ than women >40. Based on these data, and national screening statistics, it seems safest to offer women with high grade cytology a LLETZ. The caveat is reliable attenders who are at high-risk of treatment morbidity; in these women immunostained curettings could be considered as no-one with CIN2+ was missed, false positive screening was detected in 72% and inter-rater reliability was very good. Furthermore, the use of these immunostains is relatively cheap (~£30).

The sensitivity of the curettings was improved in women with high grade cytology when compared to low grade cytology and a potential reason is the sampling method; to allow correlation with the LLETZ histology quadrants were sampled rather than circumferential stripping. This technique may have missed focal areas of CIN2+ in women with low grade cytology. Furthermore, scattered dysplastic cells (as discussed in 2.4.5.3), could have been 'lost' during processing, which may account for the poorer sensitivity.

If multi-centre studies do corroborate the diagnostic value of p16/Ki67 stained curettings in women with high grade cytology, education in the use of endocervical curettage would

also need to be promoted as data from the focus groups and national survey suggest this is rarely offered by colposcopists due to their inexperience with the technique.

Dual-stained cytology improved the sensitivity but not the specificity of routine screening in women with high grade cytology. Recent studies assessing the use of dual-staining in women with high grade cytology support this finding[148, 261]. There is no current evidence which explains the potential reason for this but in the study sample in Chapter 7, more women with high grade cytology, when compared to women with low grade cytology, had multiple high risk HPV subtypes and high risk subtypes which have a lower prevalence. The prevalence of a disease, as discussed in 2.4.7, can affect the PPV of a diagnostic test.

Finally, the utility of the immunostained curettings needs to be tempered with the practical aspects of the test; these include patient choice, patient reliability to attend follow-up, the invasiveness of the procedure and the proportion of women this will affect in practice. A TZ3 could account for 6377 of the 31,886 women reviewed annually with high grade cytology[87] but half of these women may have completed their family or prefer immediate treatment in view of the cytological grade and inability to visualize the TZ.

8.5 Future Work

8.5.1 Shared care modelling

The management preferences of patients when the cytology is low grade and the TZ is visible have been reported[232, 262]. Patient preferences in the presence of a TZ3 have not been assessed and this, in conjunction with their acceptability of adjuncts, needs to be determined. The use of oestrogen, particularly in view of the high proportion of young women who use progesterone-only contraceptive, would be useful in evaluating the relevance of diagnostic adjuncts and for the provision of guideline recommendations.

8.5.2 Availability and acceptability of adjuncts within the NHS

Colposcopists' knowledge, access, training and acceptability of adjuncts, including an economic assessment comparing these tests to standard practice, would be of benefit when planning a national consensus strategy.

8.5.3 Relevance to practice

The assessment of outcomes in women with high risk HPV, a TZ3 and negative or persistent inadequate cytology would be of benefit, particularly in view of the imminent introduction of national HPV screening.

8.5.4 Cytological follow-up in women with a TZ3

To inform interval lengths in women who are managed cytologically, prospective studies which assess the progression rate of CIN in women with a TZ3 who a.) have low grade cytology and b.) are dual-stain cytology negative would be of use.

To validate my findings that cytological follow-up using a Cervex-Brush in the community provides equivalent accuracy to the cytobrush and Cervex-Brush combined, large prospective studies which adjust for age and parity, and include efficacy of primary care sampling, are needed.

8.5.5 Depth of LLETZ

Studies which adjust for age and parity when assessing the mean distal and proximal margins of the TZ in women with a TZ3 would be of use to guide treatment recommendations. Proximal margins would be of use if a diagnostic LLETZ, rather than a treatment excision, is recommended.

8.5.6 Further evaluation of adjuncts

The impact of vaccination status on the diagnostic accuracy of the biomarkers would be of use, as would the assessment of viral load and the use of mRNA testing as better assays become available. Multicentre studies which adjust for age, parity and cytological grade (dividing low grade cytology into BNC and mild dyskaryosis) and studies which evaluate outcomes based on multiple sample takers are needed to corroborate the benefits of dual-stained cytology in women with low grade cytology and a TZ3.

The evaluation of dual-staining in women with high grade cytology may provide better understanding of the biological activity of multiple HPV infections and subtypes with a lower prevalence. Studies which assess the progression rate of CIN in women who are dual-stain cytology positive but initially have normal histology would help determine if dual-stain positivity predicts future CIN or fails to recognize immuno-clearance.

Although the sensitivity of the immunostained curettings was too low to be used in women with low grade cytology, outcomes based on circumferential stripping may be of use but this will be difficult to validate if the reference standard has limited epithelium to base a diagnosis upon.

8.6 Conclusions

A TZ3 is a common condition occurring in 20% of the women reviewed in colposcopy, potentially accounting for more than 25,000 women who are reviewed with low grade cytology and 6000 women with high grade cytology in the UK each year. Epidemiological data reported in this thesis suggests a TZ3 is not a condition restricted to poorly oestrogenised older women who are at low-risk of treatment morbidity. More than half of the women assessed will be of reproductive age; these women are at highest risk of treatment morbidity, have four times the risk of CIN2+ when compared to women >40 and are also the demographic cohort in whom the use of oestrogen may not be applicable or acceptable. The introduction of primary HPV testing in 2019 will improve the sensitivity of cervical screening but the specificity will still be poor in women with low grade cytology and this is compounded in women with a TZ3 where histological selection for treatment cannot be undertaken.

My analysis of the focus groups and national survey identified that anxiety of missing a cancer when the TZ cannot be visualised is compounded by a lack of guidance in this cohort. This anxiety deters colposcopists from recommending long-term cytological follow-up in women with low grade cytology and a TZ3. Although HPV triage of low grade cytology has decreased the incidence of negative LLETZ by 25%, through its very high NPV[197], this does not benefit the decision-making of colposcopists as they will only review women who test positive for high risk HPV. Without the reassurance of diagnostic colposcopy, differentiation of HPV integration from infection is impossible and, as identified in Chapters 3, 4 and 6, colposcopists are risk adverse, resulting in higher than anticipated treatment rates in women with a TZ3 and low grade cytology[101].

A positive high risk HPV result is not an indicator of high grade disease, as illustrated by the poor PPV in women with low grade screening results (16%)[91], but women with a TZ3 have double the risk of CIN2+ when compared to women where the TZ is visible. Population data from my diagnostic study (Chapter 7) indicates that this risk is highest in women aged 25 – 39. My findings are not strong enough to support a 'see and treat' for all women with a TZ3 and low grade cytology but they do suggest that adjuncts which improve diagnostic accuracy are required prior to offering long term cytological follow-up.

In women with high grade cytology, HPV genotyping did not improve the sensitivity or specificity of the screening test result to an acceptable level. The effect of the vaccination programme on the prevalence of high risk subtypes may account for this lower than expected accuracy and illustrates that women who are positive to other high risk subtypes still have a significant risk of CIN2+. The specificity of dual-stained cytology for CIN2+ in women with high grade cytology was half of that observed in women with routine screening; potential reasons for the difference in diagnostic accuracy between low grade and high grade cytology when dual-staining is used is not currently clear.

In order for experts to analyse information they need to utilise cognition as well as the emotional impact from their past experiences. However, in areas of clinical uncertainty when decisions are dominated by emotive factors, clinical guidance can reduce the difficulty and anxiety of decision-making. The studies outlined in this thesis were designed to help guide a national consensus strategy and, as such, I have proposed areas of guidance to aid in areas of uncertainty:

- **High grade cytology and a TZ3:**
    - There is an 80% risk of high grade CIN in these women and those aged 25 - 39 are four times more likely, than women aged >40, to have CIN2+ at LLETZ. Until population specific information is available it would seem safest to offer a see and treat LLETZ to these women.
    - The caveat is reliable attenders who are at high-risk of treatment morbidity; in these women the use of immunostained curettings improves the sensitivity of screening from 59.1% to 100% and has an equivocal specificity (80%).

- **Low grade cytology and a TZ3:**
    - *Initial Management:* Women with low grade cytology, high risk HPV and a TZ3 have a 36% risk of CIN2+. Prior to offering long-term cytological follow-up, the use of dual-stained cytology could provide an excellent sensitivity and improve the specificity of screening (negative LLETZ) from 19.3% to 71.7%.
    - *Clinical setting and collection method for cytological follow-up:* The use of the Cervex-Brush alone, which could be offered in a primary care setting, provides equivocal sensitivity and specificity to the Cytobrush used in conjunction with the Cervex-Brush.

- **Repetition of the referral cytology:**

  To reduce the risk of false negative screening, the referral cytology should not be repeated at the first colposcopy appointment unless the interval between is greater than three months. The absence of endocervical cells does not appear to reduce detection of dysplasia.

- **Depth of LLETZ:**

  To reduce false negative histology, and until studies adjust for age and parity when assessing the distal margins of a TZ3, national excision guidance (15-25mm) should be adhered to.

Clinicians strive for improvement in patient outcomes in their day-to-day life. Continued exploration of the themes identified within my work may help in the continuing global efforts to diagnose and prevent progression of cervical intraepithelial neoplasia and improve outcomes in women with a TZ3. My data confirms the 20% incidence of a TZ3 and suggests, for the first time, that in the presence of low grade cytology, these women have double the risk of CIN2+ when compared to women where the TZ is visible, establishing the relevance and utility of adjuncts which improve diagnostic accuracy in this cohort.

With the imminent introduction of primary HPV screening my findings are of increasing importance for the provision of management guidance and for the introduction of new technologies which may improve outcomes in women with a TZ3 and optimise service provision within primary care and the colposcopy service.

APPENDIX 1: Grant Approvals

Above +Beyond
For Patients. For Health. For Bristol.

University Hospitals Bristol NHS
NHS Foundation Trust

[redacted]

Thank you for your application to the Research Funding Committee. I am pleased to confirm that it has been agreed to fund your study as follows:

Amount Requested	**£14,214.60**
Awarded	**£14,214.60**
Start and end dates:	**01/04/2015 - 31/07/2016**
Cost centre:	58199-9922

General comments:

- It was noted that this study would complement an ongoing brush cytology study, and that it should generate pilot data for a future grant application, although there were some concerns about the timescale as represented in the Gantt chart.
- A subcontract would be needed between UHBristol and Manchester if the work went ahead for the HPV testing to be performed at Manchester.

Outcome
Awarded in full.

Congratulations in the award of this funding and good luck with your project. Instructions for accessing funds and conditions of award are found below.

Yours sincerely

[redacted]

cc: Above and Beyond Charities

This grant provided funds for the p16/Ki67 immunostains, the laboratory technicians' supervision, the qualitative software package Nvivo and sustenance for the focus group participants.

DAVID TELLING CHARITABLE TRUST

[redacted]

16 October 2013

[redacted]

Project 330 - The use of endocervical curettage and P16 ^{INK4a} staining in reducing unnecessary LLETZ by improving diagnostic accuracy in women with unsatisfactory colposcopy

Thank you for submitting the above application to the David Telling Charitable Trust. I am pleased to inform you that the Trust has agreed to support your project in full to the sum of £9,280 subject to receipt of a copy of your letter of approval from the Research Ethics Committee and R&D department. Please note that the Charity is not VAT exempt and I would be grateful if you could advise the supplying companies to include VAT on their invoices. Funding will be provided against actual expenditure on the project rather than as a block grant. Please send original invoices to Mr. Marshall Thomas at the address below for settlement. The Trust requires a written report on the progress of your research, detailing any publications, abstracts, presentations, major grants awarded as a result of this work and any other indicators of productivity, by the 31 October 2014. Funding will be available until 31 October 2015. Continued funding after this date will be at the discretion of the Advisory Board after considering your report. Please let us know of any specific reasons if you wish to defer funding into the next financial year. Funding will be withdrawn without discussion if no report is received. The Trust will also require a final report once the project has been completed. Many congratulations and I hope your project is completed successfully.

With best wishes

Dictated but not signed

[redacted]

APPENDIX 2: Ethics and Research & Development (R&D) approvals

University Hospitals Bristol NHS
NHS Foundation Trust

[redacted]

03/09/2014

NHS Permission for Research has been granted for the study detailed below at University Hospitals Bristol NHS Foundation Trust (UH Bristol). Permission is subject to any conditions and is effective from 03/09/2014 until 12/11/2014

Dear Dr Manley

RE: TITLE: Has HPV cervical LLETZ before and after HPV testing as a primary cervical screening tool A comparative cohort study — R&D Number: OG/2014/4626

NHS permission for the above research has been granted on the basis of the application submitted and a favourable opinion from an authorised REC.

Permission is granted on the understanding that the study is conducted in accordance with the Research Governance Framework, Good Clinical Practice, and NHS Trust policies and procedures. As Principal Investigator it is your responsibility to ensure you and your team are familiar with relevant research related policies and procedures; these can be found at http://www.uhbristol.nhs.uk/media/2097744/research_policy_final_v0_7_21_02_14.pdf

It is also a condition of NHS Permission at this site that local recruitment data is uploaded to the EDGE system and the study record is kept up-to-date. Please contact the Research Management Office if you are unsure how to do this.

The following conditions must be met prior to recruitment commencing:

- A site file is set-up and delegation log established

UH Bristol is required to monitor research to ensure compliance with the Research Governance Framework and other legal and regulatory requirements. For further details about monitoring arrangements please contact the Research Management Office. The Research Management Office will monitor recruitment on an on-going basis and can provide support and advice if you are experiencing problems in meeting your targets within the agreed time frame.

The Research Management Office should be notified of any urgent safety measure taken in order to protect research participants against any immediate hazard to their health or safety. This should be

University Hospitals Bristol NHS
NHS Foundation Trust

within the same time frame as notification to the REC and any other regulatory bodies and should include the reasons why the measures were taken and any plan for further action.

NHS indemnity is provided for the period of permission given above. Requests for changes to the period of permission (e.g. an extension of the study) must be made to the Research Management Office before permission ceases with an explanation as to why the change is being sought.

All amendments (including changes to the local research team) need to be submitted in accordance with regulatory and national requirements which can be found on IRAS. Please note if we are sponsoring this study separate notification of an amendment already authorised by us as sponsor for submission to the regulatory bodies is not required, the sponsor authorisation will cover R&D acknowledgement of the amendment at this trust. The Research Management Office also needs to be notified if there are any changes to the study status.

We wish you every success with this study.

Yours sincerely,

[redacted]

Copy to:

[redacted]

SPONSOR University of Bristol

30 May 2014

Dear Dr Manley

Study title: The significance of negative LLETZ before and after HPV testing as a primary cervical screening tool: A comparative cohort study.

REC reference:
Protocol number:
IRAS project ID: 

Thank you for your letter of 27 May 2014. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 19 May 2014

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Letter confirming compliance with conditions of Favourable opinion letter		27 May 2014

Approved documents

Document	*Version*	*Date*
Covering letter on headed paper		08 May 2014
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		01 May 2014
Other [CV for Prof Lopez-Bernal]		
REC Application Form		08 May 2014
Research protocol or project proposal	1.4	05 May 2014
Summary CV for Chief Investigator (CI)		
Letter confirming compliance with conditions of Favourable opinion letter		27 May 2014

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

14/SW/0127	**Please quote this number on all correspondence**

Yours sincerely

[redacted]

University Hospitals Bristol NHS
NHS Foundation Trust

[redacted]

07/04/2014

NHS Permission for Research has been granted for the study detailed below at University Hospitals Bristol NHS Foundation Trust (UH Bristol). Permission is subject to any conditions and is effective from 07/04/2014 until 31/07/2015.

Dear Dr Manley

RE: Endocervical Curettage & p16 to diagnose pre-cancerous cervical change - R&D Number: OG/2013/4461

NHS permission for the above research has been granted on the basis of the application submitted and a favourable opinion from an authorised REC.

Permission is granted on the understanding that the study is conducted in accordance with the Research Governance Framework, Good Clinical Practice, and NHS Trust policies and procedures available at http://www.uhbristol.nhs.uk/research-innovation/are-you-a-researcher/information-for-researchers/post-approval/ As Principal Investigator it is your responsibility to ensure you and your team are familiar with relevant research related policies and procedures; these can be found at http://www.uhbristol.nhs.uk/research-innovation/research-and-innovation-department-at-uh-bristol/

It is also a condition of NHS Permission at this site that local recruitment data is uploaded to the EDGE system and the study record is kept up-to-date. Please contact the Research Management Office if you are unsure how to do this.

The following conditions must be met prior to recruitment commencing:

- A site file is set-up and delegation log established

UH Bristol is required to monitor research to ensure compliance with the Research Governance Framework and other legal and regulatory requirements. For further details about monitoring arrangements please contact the Research Management Office. The Research Management Office will monitor recruitment on an on-going basis and can provide support and advice if you are experiencing problems in meeting your targets within the agreed time frame.

Approval Non-IMP Study v4 20082013

University Hospitals Bristol NHS
NHS Foundation Trust

The Research Management Office should be notified of any urgent safety measure taken in order to protect research participants against any immediate hazard to their health or safety. This should be within the same time frame as notification to the REC and any other regulatory bodies and should include the reasons why the measures were taken and any plan for further action.

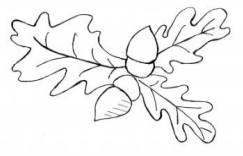
NHS indemnity is provided for the period of permission given above. Requests for changes to the period of permission (e.g. an extension of the study) must be made to the Research Management Office before permission ceases with an explanation as to why the change is being sought.

All amendments (including changes to the local research team) need to be submitted in accordance with regulatory and national requirements which can be found on IRAS. The Research Management Office also needs to be notified if there are any changes to the study status.

We wish you every success with this study.

APPENDIX 3: Patient Information Sheets and Consent Forms

ACORN STUDY INFORMATION SHEET: Discussion Groups



Endocervical Curettage & Cytology with p16/Ki-67 to diagnose pre-cancerous cervical change in women with a TZ3

- You are being invited to take part in the ACORN study discussion group.
- Before you decide to take part, we would like to explain the reason for this research and what is involved.
- Please feel free to use the contact details below if you would like more information.

[redacted] [redacted]

Introduction – What are we trying to find out?

There is a paucity of guidance to aid colposcopists' decision-making in women with a TZ3. We would like to investigate colposcopists' opinions on the different management options for women with a TZ3 (unsatisfactory colposcopy). As part of a University of Bristol project, we would like to hear colposcopist's views on current practices in this cohort of women. We aim provide recommendations for management of a TZ3, which may guide a consensus opinion.

Why have I been asked to take part?

We are asking you to help because you manage women with a TZ3 and may institute management protocols that are different to policies at University Hospitals Bristol NHS Trust.

Do I have to take part?

It is entirely your decision to take part. If you do agree to join the ACORN Study Discussion Group, we will seek your written consent. You are free to change your mind and withdraw from the study at any time and you do not need to give your reasons.

What will taking part involve?

Taking part will involve joining one focus group, which will take about an hour. We would like a minimum of four colposcopists to take part. It will occur in a location of your convenience. Questions will focus on your experiences and views of the management of women with a TZ3. The discussions will be audio recorded and transcribed. We welcome your honest answers and views, both positive and negative.

Are there any possible benefits?

We aim to improve care by providing recommendations for management of a TZ3, which may guide a consensus opinion.

Version 1.6: 25/02/2014

Is there any possible harm?

We do not envisage any harm coming to you as a consequence of taking part in the focus (discussion) group. If any problems arise during the discussion group, the research team will be happy to facilitate referral to other members of the research team for further guidance.

If you have any concerns or other questions about this study or the way it has been carried out you should contact the Patient Support & Complaints Team, Trust Headquarters, University Hospitals Bristol, Marlborough Street, Bristol, BS1 3NU.
Tel No: 0117 342 3604 *email:* pals@uhbristol.nhs.uk

Will the information I give in this study be kept confidential?

All the data collected from you during the course of the study will be strictly confidential and anonymity will be preserved. All audio data will be stored securely and confidentially on password-protected computers in a locked research office. Files will be destroyed after 5 years in line with NHS regulations. Identifiable information will not be included in the transcription of the audio files.

Anonymous quotes from the interview discussions may be used in medical articles or presentations. Quotes will be free of personal identifiable information. In the event that you lose the capacity to consent during the study, the research team will retain your previous contributions for use in the study. Anonymity and confidentiality will be maintained.

Who has reviewed the study?

The NRES Committee South West, Frenchay Research Ethics Committee has reviewed and agreed to this study.

What do I do now?

If you have read this information sheet and want to take part, please contact one of the research team members using the contact details provided. One of the project team will then contact you to arrange a convenient time.

Thank you

ACORN STUDY DISCUSSION GROUP CONSENT FORM

Endocervical Curettage & Cytology with p16/Ki-67 to diagnose pre-cancerous cervical change in women with a TZ3

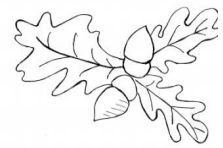
Please initial the boxes below:

1. I confirm that I have read and understand the information sheet (v1.6) dated 25.02.14 for the above study. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. ☐
3. I understand that the focus group discussion will be audio recorded. ☐
4. I agree that anything I say may be published as quotes in written publications, other academic and quality improvement work and reports, after identifying information is removed so that I cannot be recognised. ☐
5. I agree to take part in the above study and I understand that relevant sections of data collected during the study may be looked at by individuals from the research team, from regulatory authorities, or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to the data. ☐

……	……	……
Name of participant (Block capitals)	***Date***	***Signature***
……	……	……
Person taking Consent	***Date***	***Signature***
……	……	……
Researcher	***Date***	***Signature***

ACORN STUDY INFORMATION SHEET: Survey Validity

Endocervical Curettage & Cytology with p16/Ki-67 to diagnose pre-cancerous cervical change in women with a TZ3



You are being invited to take part in the ACORN Survey Validity Study.

Before you decide to take part, we would like to explain the reason for this research and what is involved.

Please feel free to use the contact details below if you would like more information.

[redacted] [redacted]

Introduction – What are we trying to find out?

There is a paucity of guidance to aid colposcopists' decision-making in women with a TZ3. We would like to investigate colposcopists' opinions on the different management options for women with a transformation zone type 3 (unsatisfactory colposcopy). As part of a University of Bristol project, we have developed a national survey to evaluate current UK practice, but need to assess the psychometric properties of the survey before it is released to BSCCP members. If you have any questions please do not hesitate to contact the research team using the contact details at the end of this information sheet.

Why have I been asked to take part?

We are asking you to help because you manage women with a TZ3 and may institute management protocols that are different to policies at University Hospitals Bristol NHS Trust.

Do I have to take part?

It is entirely your decision to take part. If you do agree to participate, we will seek your written consent. You are free to change your mind and withdraw from the study at any time and you do not need to give your reasons.

What will taking part involve?

In order to achieve our goal, we need to work out if colposcopists read the questions in the same way. We would like you to complete the survey in front of a researcher and then discuss what you think each question is asking, discuss whether you feel all the areas of interest relating to a TZ3 have been included and evaluate whether the design of the questionnaire is user friendly.

Are there any possible benefits?

We aim to improve care by providing recommendations for management of a TZ3.

Is there any possible harm?

We do not envisage any harm coming to you as a consequence of taking part in this study. If any problems arise during the discussion, the research team will be happy to facilitate referral to other members of the research team for further guidance.

If you have any concerns or other questions about this study or the way it has been carried out you should contact the Patient Support & Complaints Team, Trust Headquarters, University Hospitals Bristol, Marlborough Street, Bristol, BS1 3NU.
Tel No: 0117 342 3604 *email:* pals@uhbristol.nhs.uk

Will the information I give in this study be kept confidential?

All the data collected from you during the course of the study will be strictly confidential and anonymity will be preserved. All data will be stored securely and confidentially on password-protected computers in a locked research office. Files will be destroyed after 5 years in line with NHS regulations.

Anonymous quotes from the interviews may be used in medical articles or presentations. Quotes will be free of personal identifiable information. In the event that you lose the capacity to consent during the study, the research team will retain your previous contributions for use in the study. Anonymity and confidentiality will be maintained.

Who has reviewed the study?

The NRES Committee South West, Frenchay, Research Ethics Committee has reviewed and agreed to this study.

What do I do now?

If you have read this information sheet and want to take part, please contact one of the research team members using the contact details provided. One of the project team will then contact you to arrange a convenient time.

Thank you

Participant ID: ……..

ACORN STUDY *Survey Validity* CONSENT FORM

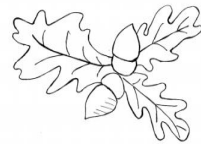
Endocervical Curettage & Cytology with p16/Ki-67 to diagnose pre-cancerous cervical change in women with a TZ3

Please initial the boxes below:

1. I confirm that I have read and understand the information sheet (v1.2) dated 17.04.2014 for the above study. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. ☐
3. I agree to allow the researcher to use anonymous direct quotes from my responses in the questionnaire and interview in articles or presentations. ☐
4. I agree to take part in the above study. ☐

……	……	……
Name of participant (Block capitals)	***Date***	***Signature***
……	……	……
Person taking Consent	***Date***	***Signature***
……	……	……
Researcher	***Date***	***Signature***

ACORN STUDY INFORMATION SHEET: Survey Reliability



Endocervical Curettage & Cytology with p16/Ki-67 to diagnose pre-cancerous cervical change in women with a TZ3

- You are being invited to take part in the ACORN Survey Validity Study.
- Before you decide to take part, we would like to explain the reason for this research and what is involved.
- Please feel free to use the contact details below if you would like more information.

[redacted]

Introduction – What are we trying to find out?

There is a paucity of guidance to aid colposcopists' decision-making in women with a transformation zone type 3 (TZ3). We would like to investigate colposcopists' opinions on the different management options for women with a TZ3 (unsatisfactory colposcopy). As part of a University of Bristol project, we have developed a national survey to evaluate current UK practice, but need to assess the psychometric properties of the survey before it is released to BSCCP members. In order to achieve our goal, we need to work out if the results of the test are consistent i.e. if a colposcopist completed the survey weeks apart would they still answer the questions in the same way. If you have any questions please do not hesitate to contact the research team using the contact details at the end of this information sheet.

Do I have to take part?

It is entirely your decision to take part. If you do agree to participate, we will seek your written consent. You are free to change your mind and withdraw from the study at any time and you do not need to give your reasons.

What will taking part involve?

We would like you to complete the survey at a convenient time for you and then complete the survey two weeks later. The link to this survey can either be emailed to you or a hard copy can be provided, dependent on your preferences. There are 15 questions overall and it should take no more than 15 minutes to complete. Some background questions ask for simple facts and will be answerable easily. Other questions may take more time as they invite you to indicate your management preferences in areas that currently have little guidance.

Are there any possible benefits?

We aim to improve care by providing recommendations for management of a TZ3.

Is there any possible harm?

We do not envisage any harm coming to you as a consequence of taking part in this study. If any problems arise during the discussion, the research team will be happy to facilitate referral to other members of the research team for further guidance.

If you have any concerns or other questions about this study or the way it has been carried out you should contact the Patient Support & Complaints Team, Trust Headquarters, University Hospitals Bristol, Marlborough Street, Bristol, BS1 3NU.

Tel No: 0117 342 3604 *email:* pals@uhbristol.nhs.uk

Will the information I give in this study be kept confidential?

All the data collected from you during the course of the study will be strictly confidential and anonymity will be preserved. All data will be stored securely and confidentially on password-protected computers in a locked research office. Files will be destroyed after 5 years in line with NHS regulations.

Anonymous quotes from the survey may be used in medical articles or presentations. Quotes will be free of personal identifiable information. In the event that you lose the capacity to consent during the study, the research team will retain your previous contributions for use in the study. Anonymity and confidentiality will be maintained.

Who has reviewed the study?

The NRES Committee South West, Frenchay Research Ethics Committee has reviewed and agreed to this study.

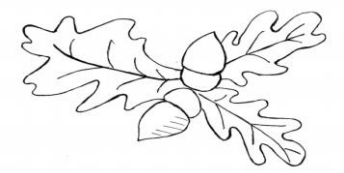
What do I do now?

If you have read this information sheet and want to take part, please contact one of the research team members using the contact details provided. One of the project team will then contact you to arrange a convenient time.

Thank you

ACORN STUDY *Survey Reliability* CONSENT FORM

Endocervical Curettage & Cytology with p16/Ki-67 to diagnose pre-cancerous cervical change in women with a TZ3

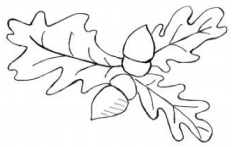


Please initial the boxes below:

1. I confirm that I have read and understand the information sheet (v1.1) dated 21.04.2014 for the above study. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. ☐
3. I agree to allow the researcher to use anonymous direct quotes from my responses in the survey in articles or presentations. ☐
4. I agree to take part in the above study. ☐

…….	…….	…….
Name of participant (Block capitals)	***Date***	***Signature***
…….	…….	…….
Person taking Consent	***Date***	***Signature***
…….	…….	…….
Researcher	***Date***	***Signature***

ACORN STUDY INFORMATION SHEET:



Endocervical Curettage & Cytology with p16/Ki-67 to diagnose pre-cancerous cervical change in women with a TZ3

You are being invited to take part in a University of Bristol research study. Before deciding whether you wish to take part, it is important you understand why the research is being done and what it involves. The aim of the study is to use a new test to improve the diagnosis of pre-cancerous change in the cervix. We are asking you because you are attending the colposcopy clinic after the results of your smear test.

What is the purpose of the study?

After the results of your smear test, assessment at a colposcopy clinic (viewing the skin of the cervix and taking a tissue sample) helps to decide who needs treatment. In women whose cells are tucked inside the cervix, normal and abnormal cells cannot be directly seen and a LLETZ (removing a core of tissue) may be recommended. In 7 out of 10 women with mildly abnormal smears these results will be reassuring but the LLETZ can put them at risk of pre-term labour (2 out of 20 women) or make future smear assessments more difficult (3 out of 100 women). This project aims to compare the results of a new test to the LLETZ you may already be having, to improve the diagnosis of pre-cancerous cervical change and reduce the side effects of treatment.

What will happen if I take part?

If you are having a LLETZ because the cells of interest are tucked inside your cervix, you will be able to take part in this study.

1. As part of your routine treatment, a smear will be taken from inside the cervix. We would like to do an additional laboratory test on this sample – this does not involve extra tests to you.
2. After the local anaesthetic for your LLETZ we would like to take up to 4 small scrapings of tissue (1mm) from the cervix. This will take about 20 seconds. After the scrapings you will have your LLETZ as planned. A new laboratory test will be used on the scrapings and these will be compared to your LLETZ result.
3. If the new test shows the same results as the LLETZ, we will invite you to participate in a discussion group 6 months after your LLETZ. This will focus on your experiences and views of the available treatments. It will take up to an hour and will take place at St. Michael's Hospital. If you would like to attend, with or without having the new investigation, please indicate on the consent form and further information will be sent to you.

We will inform your GP that you are helping with the study.

Do I have to take part?

It is entirely your decision to take part. You are free to change your mind and withdraw from the study at any time and you do not need to give your reasons. It will not affect your care if you do not take part.

Are there any possible benefits to me?
Your samples from the new test may help us to improve the diagnosis of pre-cancerous change within the cervix. It is possible that the new test may find pre-cancerous cells further up your cervix than would be found by the LLETZ - identifying potential problem cells earlier. If this happens, a full review of your case will be undertaken and any changes to your treatment will be discussed with you.

Is there any possible harm to me?
The new test does not cause more side effects than the LLETZ procedure you will already be having. Side effects of the LLETZ include mild to moderate bleeding and mild period cramps for up to 6 weeks. Possible side effects from the new test include mild bleeding or mild period cramps for up to 5 days.
If you have any concerns about this study or the way it has been carried out you should contact the Patient Support & Complaints Team, Trust Headquarters, University Hospitals Bristol, Marlborough Street, Bristol, BS1 3NU.

Tel No: 0117 342 3604 ***email:*** pals@uhbristol.nhs.uk

What happens to the tissue samples / data?
The tissue samples will be given a laboratory number and anonymised (your name and other personal details will not be used). After the study, tissue slides are stored for at least 5 years as part of your medical records, as they may help in your future treatment. There will be no left over tissue as we are taking very small samples. All information you give will be confidential and kept for 5 years in line with NHS regulations. Destruction of this information will be compliant with the Data Protection Act 1998.

Will I be informed of the study results?
The results will be presented in medical journals and conferences to increase our understanding of the diagnosis of cervical cancer. Your name and other personal details will not be published.

Who has agreed to this study?
The National Research Ethics Committee South West (Frenchay REC) has reviewed and agreed to this study.

What do I do now?
If you are happy to participate in this study and would like further information, please use the contact details provided or ask to speak to a research associate on booking into the colposcopy clinic.

[redacted]

[redacted]

[redacted] (please leave a message and a research associate will return the call).

Participant ID: ……..

ACORN STUDY CONSENT FORM

Endocervical Curettage & Cytology with p16/Ki-67 to diagnose pre-cancerous cervical change in women with a TZ3

Please initial the boxes below:

1. I confirm that I have read and understand the information sheet (v1.7) dated 26.02.14 for the above study. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time. If I withdraw consent, samples already taken will be disposed of under NHS Trust guidelines. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I consent to the removal, storage and use of my tissue samples for the above study in line with the Human Tissue Act 2004. ☐
5. I consent to my GP being informed of my participation in this study. ☐
6. I understand my responses may be published anonymously in articles or presentations. ☐
7. I agree to allow data to be retained in anonymised form for five years after the completion of this study in line with NHS regulations. ☐
8. I agree to take part in the above study. ☐

……	……	……
Name of Patient (Block Capitals)	***Date***	***Signature***
……	……	……
Name of person taking consent	***Date***	***Signature***
……	……	……
Researcher	***Date***	***Signature***

Appendix 4: Methodology

Table S4.1: Negative LLETZ study variable and code definitions for ExCel

VARIABLE	CODE
Age	Raw data
Smoking	Per/day
Contraceptive	0 – none 1 – COCP 2 – Progesterone (POP, depo-provera, nexplanon, mirena coil, postmenopausal)
Parity	Raw data
Referral Smear	0 – negative 1 – low grade cytology 2 – high grade cytology 3 – no result
Cytology to colposcopy interval	In weeks
TZ type	0 – unsatisfactory (TZ3) 1 – Satisfactory (TZ1 or 2)
Colposcopy to LLETZ interval	In weeks
Reason for LLETZ	0 - HG cytology / TZ3 1 - HG cytology & HG colp 2 - CIN2 on biopsy 3 - CIN3 on biopsy 4 - CIN1 >24m 5 - LG cytology / TZ3 6 – cGIN 7 - other
See and Treat	0 – Yes 1 - No
Date of LLETZ	DD/MM/YY
LLETZ result	0 – Normal / HPV 1 – CIN1 2 – CIN2 3 – CIN3 4 – cGIN 5 - Invasive
Excision Depth	millimetres
TZ in LLETZ	0 – Yes 1 - No
Limiting Factors	0 – metaplasia 1 – TEM 2 – fragmentation 3 – denudation 4 – diathermy artefact
Total follow-up period	In months
Screening result post LLETZ	0 – Negative 1 – HPV positive, negative cytology 2 – low grade cytology 3 – high grade cytology 4 – no result

Histology post LLETZ	0 – Normal 1 – HPV 2 – CIN1 3 – CIN2 4 – CIN3 5 – cGIN 6 – invasion 7 - VAIN

Figure S4.1: Focus Group Cover Letter to Colposcopists

[redacted]

Dear Colposcopists,

I am a Research Fellow in Colposcopy at St Michaels Hospital, Bristol. The focus of my thesis is the diagnosis and management of CIN in women with a TZ3. There is currently a paucity of guidance to aid Colposcopists' decision-making in these women. I aim to evaluate practice within the UK to assess areas of consensus and uncertainty.

I am hoping to recruit Colposcopists from different NHS trusts to participate in discussion (focus) groups at their own hospitals. I have attached a study information sheet and sample consent form.

If you would like to participate or require further information please use the contact details enclosed.

Yours Sincerely,

Dr Kristyn Manley MB BS, DFSRH, FHEA

Clinical Research Fellow (Colposcopy)

Figure S4.2: HPV Primary Screening Management Protocol 2012

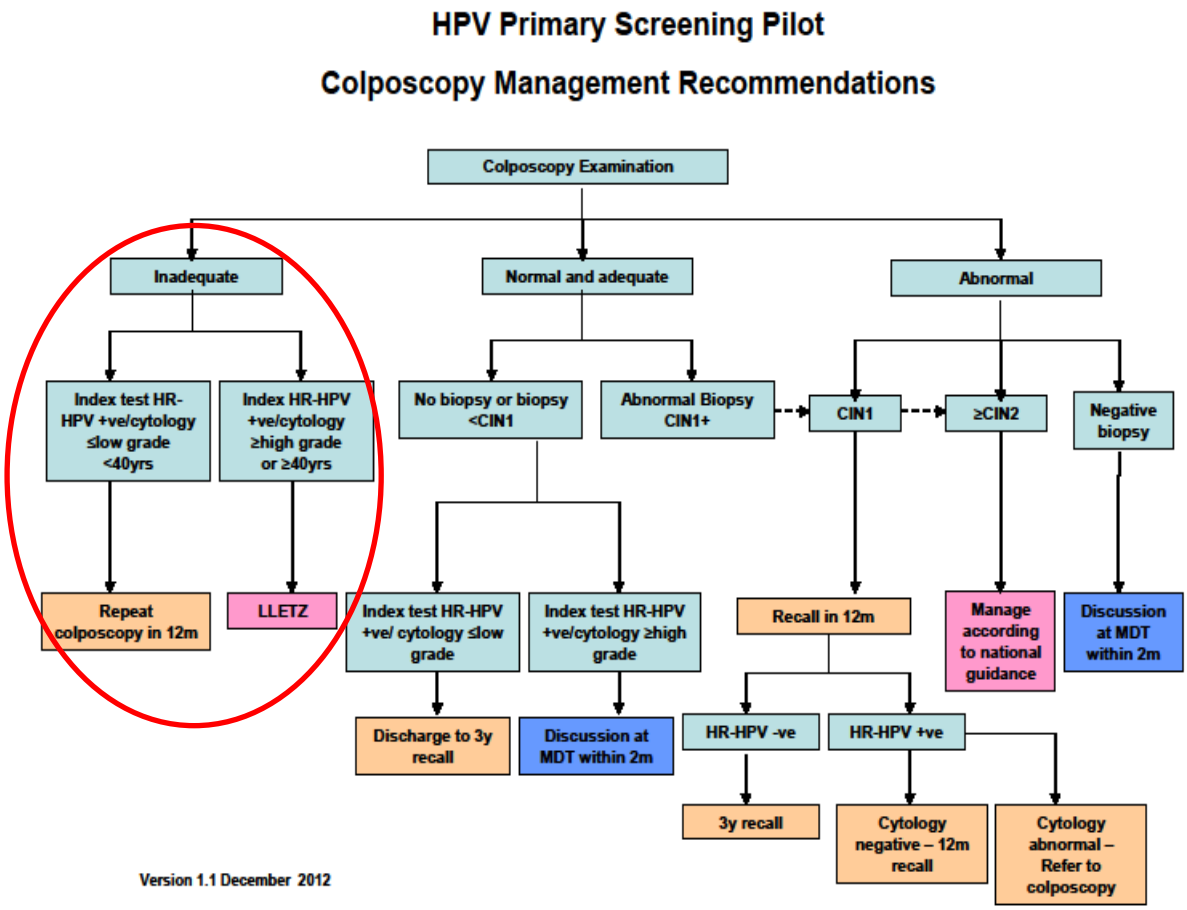


Figure S4.3: Survey Cover Letter to Colposcopists

Colposcopist's experience in managing unsatisfactory colposcopy (a TZ3): The Acorn Study

Dear BSCCP Member,

Currently, there is limited guidance on the management and follow up of women who present with a transformation zone type 3 (unsatisfactory colposcopy). This survey will explore colposcopist's experience of managing these women and specifically evaluate the management choices for:

1. Women who attend their first appointment with low or high grade cytology, with an interest in assessing if age or parity influences decision-making
2. Length, technique and clinical setting of cytological follow-up for women with low grade cytology and a TZ3
3. Average depth of LLETZ in women with a TZ3 and whether choice is influenced by age, parity or cytological grade

If you have any questions about the survey please contact Dr Kristyn Manley, Research Fellow (Colposcopy), University Hospitals Bristol NHS Trust:

[redacted]

The survey will take no more than 15 minutes, and the deadline for completion is Friday 29th April 2019. We appreciate your time and views in helping to complete this.

Please use the link below to start the survey:

https://www.surveymonkey.co.uk/r/unsatisfactorycolposcopy

Figure S4.4: ACORN Advertising Poster

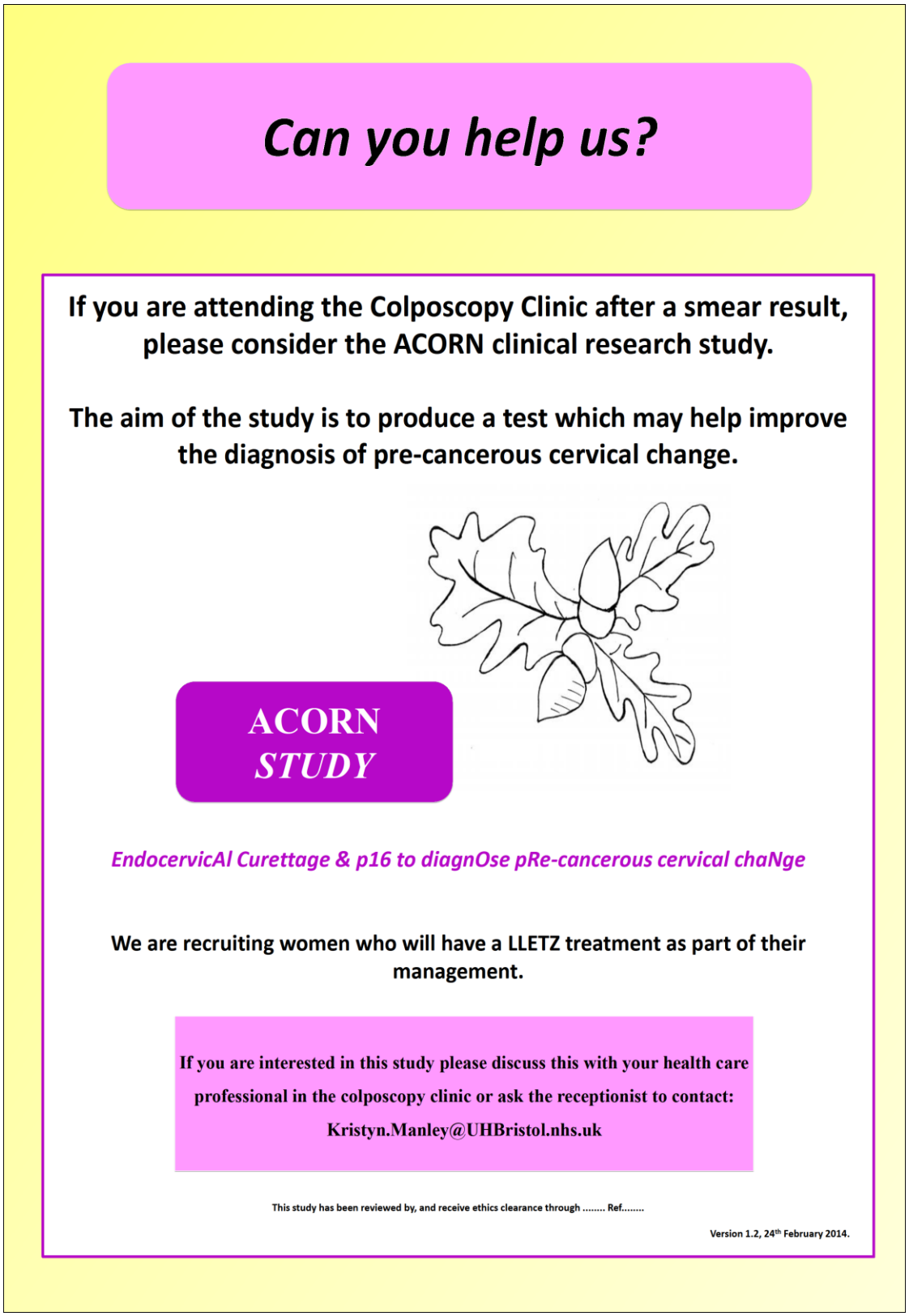


Figure S4.5: ACORN patient demographic proforma

Participant ID:…….

ACORN Study Baseline Information Proforma

1. Age……

2. Parity……

3. Smoking status…….

4. Contraceptive……

5. Referral screening test result (circle)

BNC Mild Moderate Severe Invasive

HR HPV result……

6. Biopsy prior to LLETZ?

No Yes Result……

7. Previous LLETZ? Yes No

Result…….

Margins……

Timescale prior to recruitment (months) ……..

8. Able to pass the cytobrush YES NO

9. Able to pass the curette YES NO

Figure S4.6: ACORN treatment checklist

Participant ID:…….

ACORN STUDY: Treatment Checklist

Endocervical Curettage & Cytology with p16/Ki-67 to diagnose pre-cancerous cervical change in women with a TZ3

1. Confirm consent and file in notes ☐

2. Confirm study eligibility (TZ3, squamous cytology, not pregnant) ☐

3. Broom and brush sample ☐

a. Put ACORN study sticker on pot

b. Trial Participant number ONLY on pot

c. Mark for Kath Hunt (NBT)

d. Complete cytology form with trial sticker and ID only

4. Inject citonest as per standard LLETZ treatment ☐

5. 4 quadrant ECC (1 scrape each at depth of 2.0 cm) ☐

6. Place curettage in separate histology pot to LLETZ ☐

a. Put ACORN study sticker (with participant ID only) on pot

b. Mark for Joya Pawade (UH Bristol)

7. Complete curettings histology form (separate to LLETZ form) ☐

a. Put ACORN study sticker (with participant ID only) on form

b. Mark for Joya Pawade (UH Bristol)

8. Continue with standard LLETZ – label pot and complete form as normal ☐

9. Give the patient the emergency contacts letter ☐

10. Complete the patient demographic information sheet ☐

Figure S4.7: ACORN patient emergency contact letter

University of BRISTOL

NHS
University Hospitals Bristol
NHS Foundation Trust

[redacted]

[redacted])

Thank you for participating in the ACORN study.

Please do not hesitate to use the contact details above in the event of a query about the study or if you wish to discuss any symptoms you may have after the treatment.

Yours Sincerely,

Dr Kristyn Manley MBBS, DFSRH, FHEA

Research Fellow (Colposcopy), St Michael's Hospital.

Version 1.1: 06.01.2014

Figure S4.8: ACORN GP letter

University of BRISTOL

NHS
University Hospitals Bristol
NHS Foundation Trust

[redacted]

Date……

Dear Dr ……..

R.E. DOB

The above named patient has consented to enrolment in the ACORN Study. The aim of this project is to use a cytobrush and endocervical curettage in conjunction with surrogate biomarkers (p16 and Ki67) for Human Papillomavirus infection. These tests are taken immediately prior to the LLETZ they are already scheduled to have.

Side effects of this procedure are no different than those of a standard LLETZ – mild per vaginam bleeding for up to 6 weeks and mild period pain. All participants have been given a colposcopy helpline and a research office contact (see above) in the event of a query or an adverse reaction. Follow up will be as per national protocols i.e. cytology 6 months post LLETZ.

This study has been sanctioned by the Research and Development Department at University Hospitals Bristol NHS Trust and by NRES South West – Frenchay Research Ethics Committee.

Please use the contact details above if you have any queries regarding this study.

Yours Sincerely,

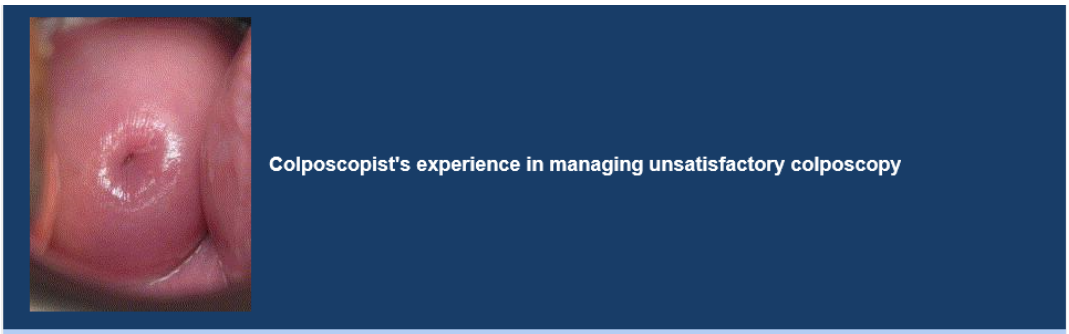
Dr Kristyn Manley MBBS, DFSRH, FHEA

Research Fellow (Colposcopy), St Michael's Hospital.

Version 1.2: 06.01.2014

APPENDIX 5: DEVELOPMENT OF THE NATIONAL SURVEY

Figure S5.1: The Final Version of the Online Survey



Currently, there is limited guidance on the management and follow up of women who present with a transformation zone type 3 (unsatisfactory colposcopy). We are conducting a survey to explore colposcopist's experience of managing these women. It will take no more than 15 minutes and includes a maximum of 14 questions.

We appreciate your time and views in helping to complete this.

1. How would you manage women at their FIRST colposcopy appointment who have been referred with a low grade cytology, HPV positive report? The appointment is within 6 weeks of the referral cytology, they are a non-smoker and your examination shows a transformation zone type 3 (unsatisfactory colposcopy) with a normal ectocervix and vagina. Choose ONE answer for each row:

	Initial Management:
25-39 years old, no children	a. Perform a LLETZ b. Recommend follow up in the colposcopy clinic in 6 months c. Recommend follow up in the colposcopy clinic in 12 months d. Recommend community cytological follow up in 6 months e. Recommend community cytological follow up in 12 months f. Discuss at the MDT g. Other (please explain in 'Other / Comments' section)
25-39 years old, family not complete	
More than 40 years old, family not complete	
Completed family, any age.	

Other / Comments:

2. If you recommended follow up in the colposcopy clinic for one of the women in question 1 please give your reason/s for this. ***Please move onto question 3 if you did not recommend follow up in the colposcopy clinic in question 1.***

	Not important	Slightly important	Moderately important	Extremely important
a. To obtain an ecto- and endocervical sample (broom & brush)	○	○	○	○
b. So they do not get lost to follow up	○	○	○	○
c. To perform a colposcopy	○	○	○	○
d. They are HPV positive	○	○	○	○

Other reasons / Comments:

3. If the transformation zone cannot be visualised with Kogan's / Kuri-Hari forceps or with other routine measures, what techniques do you use to help obtain a diagnosis in women with unsatisfactory colposcopy? You can choose more than one option.

	Yes
a. None	○
b. HPV genotyping	○
c. Endocervical curettage	○
d. Biomarker use with the cytology	○
e. Topical oestrogen if atrophic and postmenopausal	○
f. Topical oestrogen if atrophic and pre-menopausal	○
g. Systemic oestrogen (HRT) if atrophic and postmenopausal	○
h. Systemic oestrogen (COCP) if atrophic and pre-menopausal	○

Other methods / Comments:

4. If you use oestrogen in women with unsatisfactory colposcopy, what is the MAIN reason for this?

- ◯ To improve the cytology
- ◯ To improve the adequacy of the colposcopy (make the TZ visible)
- ◯ To make the examination more comfortable
- ◯ Other:

5. Following the FIRST colposcopy appointment, after how many months of *persistent* low grade HPV positive cytology and unsatisfactory colposcopy would you recommend performing a LLETZ?

	6 months	12 months	18 months	24 months		None of the previous (please comment)
25-39 years old, no children	◯	◯	◯	◯	◯	◯
25-39 years old, not completed family	◯	◯	◯	◯	◯	◯
More than 40 years old, not completed family	◯	◯	◯	◯	◯	◯
Completed family	◯	◯	◯	◯	◯	◯

Other / Comments:

6. What depth of LLETZ do you aim for in women with low grade, HPV positive cytology and unsatisfactory colposcopy?

	≤6mm	7-10mm	11-14mm	≥15mm
25-39 years old, no children	☐	☐	☐	☐
25-39 years old, not completed family	☐	☐	☐	☐
More than 40 years old, not completed family	☐	☐	☐	☐
Completed family	☐	☐	☐	☐

7. Please give your reason/s for depth of LLETZ in question 6.

	25-39 yo, no children	25-39 yo, family not complete	>40 years, family not complete	Family complete
a. The LLETZ can be repeated if it is diagnostic of CIN	☐	☐	☐	☐
b. A deeper LLETZ will excise a high endocervical transformation zone	☐	☐	☐	☐
c. The risk of cervical stenosis	☐	☐	☐	☐
d. Reproductive function is NOT a consideration	☐	☐	☐	☐
e. Reproductive function IS a consideration	☐	☐	☐	☐
f. Most of these women have reassuring changes at histology	☐	☐	☐	☐
g. She is HPV positive	☐	☐	☐	☐
h. None of the above	☐	☐	☐	☐

8. A woman presents to her first colposcopy appointment with a HIGH grade cytology report. The appointment is within 2 weeks of the referral cytology. Your examination shows a TZ type 3 (unsatisfactory colposcopy) with a normal ectocervix and vagina. Choose ONE management option from the list below for the described patients:

	Initial Management
25-39 year old, no children	
25-39 year old, family not complete	
More than 40 years old, family not complete	
Completed family	

a. Perform a LLETZ
b. Recommend follow up in the colposcopy clinic in 3 months
c. Recommend follow up in the colposcopy clinic in 6 months
d. Recommend follow up in the colposcopy clinic in 12 months
e. Recommend cytological follow up in the community in 3 months
f. Recommend cytological follow up in the community in 6 months
g. Recommend cytological follow up in the community in 12 months
h. Discuss at the MDT
i. Other (please explain in 'Other / Comments' section)

Other / Comments:

9. When you perform a LLETZ on a woman with high grade cytology and unsatisfactory colposcopy, what is the depth that you aim for? Please choose one option for each described patient.

	≤6mm	7-10mm	11-14mm	≥15mm
25-39 years old, no children	☐	☐	☐	☐
25-39 years old, not completed family	☐	☐	☐	☐
More than 40 years old, family not complete	☐	☐	☐	☐
Completed family	☐	☐	☐	☐

Other:

10. Please give your reason/s for depth of LLETZ in question 9. If there is more than one reason please rank them in order of importance.

	25-39 yo, no children	25-39 yo, family not complete	>40 years old, family not complete	Completed family
a. The LLETZ can be repeated if it is diagnostic for high grade CIN	☐	☐	☐	☐
b. A deeper LLETZ will excise a high endocervical TZ	☐	☐	☐	☐
c. Reproductive function is NOT a consideration	☐	☐	☐	☐
d. Reproductive function IS a consideration	☐	☐	☐	☐
e. Most of these women have high grade CIN at histology	☐	☐	☐	☐
f. The risk of cervical stenosis	☐	☐	☐	☐
g. None of the above.	☐	☐	☐	☐

Other reasons:

11. The NHSCSP document no 20 recommends that a cytology sample should not be repeated at the first colposcopy appointment following a routine referral. Do you believe there is a role for repeating the cytology at the first appointment in women with unsatisfactory colposcopy?

- [ ] No, this is not recommended practice.
- [ ] Yes, the transformation zone may not have been adequately sampled
- [ ] Yes, if the smear quality is poor
- [ ] Yes, to avoid LLETZ in young women if the repeat cytology is negative

Other reasons / comments:

12. If in question 11 there was a scenario in which you would repeat the cytology at the first colposcopy appointment, please give your reason/s for how a repeat test would affect your clinical practice. ***If you never repeat the cytology please move into question 13.***

- [ ] I would discuss both cytology samples at the MDT
- [ ] I would offer a LLETZ if the repeat cytology confirmed low grade dyskaryosis in younger women
- [ ] I would offer a LLETZ if the repeat cytology confirmed low grade dyskaryosis in older women
- [ ] I would offer a LLETZ if the women was young and the repeat cytology confirmed high grade dyskaryosis
- [ ] I would offer cytological follow up in the community if the repeat cytology confirmed low grade dyskaryosis
- [ ] I would offer follow up in colposcopy if the referral and repeat cytology confirmed low grade dyskaryosis
- [ ] I would offer follow up in colposcopy if the repeat cytology was negative
- [ ] I would offer cytological follow up in the community if the repeat sample was negative
- [ ] It wouldn't affect my management decisions

Other reasons / comments:

13. Demographics:

	Job Position	Experience of colposcopy in the UK	Gender
.	Nurse Colposcopist Gynaecological Oncologist Gynaecology Consultant O&G Trainee Associate Specialist		

Other (ple

TABLE S5.1 Face validity - raw data of items completed by participants

PARTICIPANT	Items and Stems (N (n))												
	1 (4)	**2*** (4)	**3** (1)	**4** (1)	**5** (4)	**6** (4)	**7** (4)	**8** (4)	**9** (4)	**10** (4)	**11** (1)	**12*** (1)	**13 - 15** (3)
1	4	N/A	1	1	4	4	4	4	4	4	1	N/A	3
2	4	4	1	1	4	4	4	4	4	4	1	N/A	3
3	4	N/A	1	1	4	4	4	4	4	4	1	N/A	3
4	4	N/A	1	1	4	4	4	4	4	4	1	N/A	2
5	4	N/A	1	1	4	4	4	4	4	4	1	N/A	3
6	4	4	1	1	4	4	4	4	4	4	1	N/A	3
7	4	N/A	1	1	4	4	4	4	4	4	1	1	3
8	4	4	1	1	4	4	4	4	4	4	1	N/A	3
9	4	4	1	1	4	4	3	4	4	4	1	N/A	3
10	4	4	1	1	4	4	4	4	4	4	1	N/A	3
11	4	N/A	1	1	4	4	4	4	4	4	1	N/A	3
12	4	N/A	1	1	4	4	4	4	4	4	1	N/A	3
Missing item	0%	0%	0%	0%	0%	0%	2.1%	0%	0%	0%	0%	0%	2.8%

**Items 2 and 12 were gated questions*

APPENDIX 6: National Survey Supplementary Information

Table S6.1: Association between respondents' years of experience, job title and gender

	YEARS OF EXPERIENCE N (%), *95% CI*				TOTAL
	0-2 years	***3-4 years***	***5-10 years***	***>11 years***	
Nurse	2 (6.3%) *0.8 - 20.8%*	2 (6.3%) *0.8 - 20.8%*	16 (50%) *31.9 - 68.1%*	12 (37.5%) *21.1 - 56.3%*	32 *15.7%*
Gynaecological Oncologist	0 *0 - 12.8%*	0 *0 - 12.8%*	2 (7.4%) *0.9 - 24.3%*	25 (92.6%) *75.7 - 99.1%*	27 *13.3%*
Gynaecology Consultant	5 (4.5%) *1.5 - 10.1%*	6 (5.4%) *1.9 - 11.3%*	22 (19.6%) *12.7 – 28.2%*	79 (70.5%) *61.2 – 78.8%*	112 *55.2%*
Registrar	3 (37.5%) *8.5 - 75.5%*	1 (12.5%) *3.2 - 52.7%*	4 (50%) *15.7 - 84.3%*	0 *0 - 36.9%*	8 *3.9%*
Associate Specialist	1 (4.2%) *0.1 - 21.1%*	2 (8.3%) *1 - 27%*	4 (16.7%) *4.7 - 37.4%*	17 (70.8%) *48.9 - 87.4%*	24 *11.8%*
TOTAL	11 (5.4%)	11 (5.4%)	48 (23.5%)	133 (65.2%)	
FEMALE	6 (4.7%)	11 (8.7%)	39 (30.7%)	71 (55.9%)	127 *63.5%*
MALE	5 (6.8%)	0	9 (12.3%)	59 (80.8%)	73 *36.5%*

Table S6.2: Association between respondents' job title and gender

	GENDER	
	Male (n=73)	**Female (n=127)**
Job Title:		
Nurse Colposcopist	0	32 (25.2%)
Registrar	1 (1.4%)	7 (5.6%)
Gynaecological Oncologist	18 (24.7%)	7 (5.6%)
Gynaecology Consultant	48 (65.8%)	63 (49.6%)
Associate Specialist	6 (8.2%)	18 (14.2%)

Table S6.3: Association between respondents' years of experience in colposcopy and their use of adjuncts to improve diagnosis

Method to improve diagnosis with a TZ3	Years of experience				Total	p
	0-2	3-4	5-10	>11		
None						
Yes	0	1	0	12	13	0.12
No	11 (100%)	10 (100%)	48 (100%)	122 (91%)	191	
HPV Genotyping						
Yes	1 (9%)	5 (45.5%)	8 (16.7%)	21 (15.7%)	35	0.07
No	10 (90.9%)	6 (54.5%)	40 (83%)	113 (84%)	169	
Curettage						
Yes	2 (18.2%)	2 (18.2%)	3 (6.3%)	17 (12.7%)	24	0.49
No	9 (81.8%)	9 (81.9%)	45 (93.8%)	117 (87.3%)	180	
Biomarkers with cytology						
Yes	0	0	1 (2.1%)	6 (4.5%)	7	0.68
No	11 (100%)	11 (100%)	47 (97.9%)	128 (95.5%)	197	
Topical oestrogen and postmenopausal						
Yes	11 (100%)	9 (81.8%)	46 (95.8%)	116 (86.6%)	182	0.16
No	0	2 (18.2%)	2 (41.7%)	18 (13.4%)	22	
Topical oestrogen and premenopausal						
Yes	5 (45.5%)	2 (18.2%)	19 (39.6%)	67 (50%)	93	0.16
No	6 (54.5%)	9 (81.8%)	29 (60.4%)	67 (50%)	111	
Systemic oestrogen and postmenopausal						
Yes	0	0	4 (8.3%)	12 (8.9%)	16	0.54
No	11 (100%)	11 (100%)	44 (91.7%)	122 (91%)	188	
Systemic oestrogen and premenopausal						
Yes	0	2 (18.2%)	6 (12.5%)	10 (7.5%)	18	0.33
No	11 (100%)	9 (81.8%)	42 (87.5%)	124 (92.5%)	186	
Other:						
Yes	1 (9.1%)	1 (9.1%)	4 (8.3%)	6 (4.5%)	12	0.70
No	10 (90.9%)	10 (90.9%)	44 (91.7%)	128 (95.5%)	192	
TOTAL	11	11	48	134	204	

Table S6.4: Association between respondents' job title and their use of adjuncts to improve diagnosis

Method	Job title					Total	p
	Nurse	Registrar	Gynae Oncologist	Gynae Consultant	Associate Specialist		
None							
Yes	0	0	4 (14.8%)	8 (7.1%)	1 (4.2%)	13	0.18
No	32 (100%)	8 (100%)	23 (85.2%)	104 (92.9%)	23 (95.8%)	190	
HPV Genotyping							
Yes	3 (9.4%)	1 (12.5%)	4 (14.8%)	24 (21.4%)	2 (8.3%)	34	0.35
No	29 (90.6%)	7 (87.5%)	23 (85.2%)	88 (78.6%)	22 (91.7%)	169	
Curettage							
Yes	1 (3.1%)	1 (12.5%)	2 (7.4%)	16 (14.3%)	3 (12.5%)	23	0.46
No	31 (96.9%)	7 (87.5%)	25 (92.6%)	96 (85.7%)	21 (87.5%)	180	
Biomarkers with cytology							
Yes	1 (3.1%)	0	0	4 (3.6%)	2 (8.3%)	7	0.56
No	31 (96.9%)	8 (100%)	27 (100%)	108 (96.4%)	22 (91.7%)	196	
Topical E2 and postmenopausal							
Yes	32 (100%)	8 (100%)	18 (66.7%)	101 (90.2%)	22 (91.7%)	181	<0.01
No	0	0	9 (33.3%)	11 (9.8%)	2 (8.3%)	22	
Topical E2 and premenopausal							
Yes	8 (25%)	2 (25%)	12 (44.4%)	55 (49.1%)	15 (62.5%)	92	0.03
No	24 (75%)	6 (75%)	15 (55.6%)	57 (50.9%)	9 (37.5%)	111	
Systemic E2 and postmenopausal							
Yes	2 (6.3%)	0	2 (7.4%)	11 (9.8%)	1 (4.2%)	16	0.76
No	30 (93.8%)	8 (100%)	25 (92.6%)	101 (90.2%)	23 (95.8%)	187	
Systemic E2 and premenopausal							
Yes	3 (9.4%)	0	2 (7.4%)	12 (12%)	1 (4.2%)	18	0.73
No	29 (90.6%)	8 (100%)	25 (92.6%)	100 (88%)	23 (95.8%)	185	
Other:							
Yes	1 (3.1%)	0	2 (7.4%)	8 (7.1%)	1 (4.2%)	12	0.82
No	31 (96.9%)	8 (100%)	25 (92.6%)	104 (92.9%)	23 (95.8%)	191	
TOTAL	32	8	27	112	24	203	

Table S6.5: Colposcopists' management choices for women with high grade cytology and a TZ3

	LLETZ	3m colposcopy follow up	6 month colposcopy follow up	12 month colposcopy follow up	3 month community follow-up	6 month community follow up	12 month community follow up	MDT	Other	TOTAL
	N (%) ***95% CI***									
25 - 39, nulliparous	96 (46.8%) *39.8 - 53.9*	24 (11.1%) *7.8 - 17.1*	8 (3.9%) *1.8 - 7.8*	1 (0.5%) *0.03 - 3.1*	0	0	0	71 (34.6%) *28.2 - 41.6*	5 (2.4%) *0.9 - 5.9*	205
25 - 39, family incomplete	118 (57.6%) *50.5 - 64.4*	18 (8.8%) *5.4 - 13.7*	7 (3.4%) *1.5 - 7.2*	0	0	0	0	58 (28.3%) *22.3 - 35.1*	4 (1.9%) *0.6 - 5.3*	205
>40, family incomplete	142 (69.3%) *62.4 - 75.4*	14 (6.8%) *3.9 - 11.4*	3 (1.5%) *0.4 - 4.6*	0	0	0	0	42 (20.5%) *15.3 - 26.8*	4 (1.9%) *0.6 - 5.3*	205
Family complete, any age	179 (87.3%) *81.8 - 91.4*	4 (1.9%) *0.6 - 5.3*	0	0	0	1 (0.5%) *0.3 - 3.1*	0	18 (8.8%) *5.4 - 13.7*	3 (1.4%) *0.4 - 4.6*	205

Table S6.6: Colposcopists' reasons for choice of LLETZ in women with low grade cytology and a TZ3

1. The LLETZ can be repeated if diagnostic for CIN (n=99, 48.3%, CI 41.3-55.3%)					
	25 - 39 nullip, n=82	**25 - 39, family incomplete, n=78**	**>40, family incomplete, n=62**	**Family complete, n=35**	***P value***
≤6	n=17 (20.7%)	n=13 (16.7%)	n=8 (12.9%)	n=1 (2.8%)	0.09
7-10	n=58 (70.7%)	n=58 (74.4%)	n=45 (72.6%)	n=19 (54.3%)	0.17
11-14	n=7 (8.5%)	n=7 (8.9%)	n=7 (11.3%)	n=11 (31.4%)	0.003
≥15	n=0	n=0	n=0	n=4 (11.4%)	<0.001
P value	<0.0001	<0.0001	<0.0001	<0.0001	-
2. A deeper LLETZ will excise an endocervical TZ (n=123, 60%, CI 53.1-66.4%)					
	25 - 39 nullip, n=25	**25 - 39, family incomplete, n=31**	**>40, family incomplete, n=65**	**Family complete n=103**	***P value***
≤6	n=0	n=0	n=0	n=0	-
7-10	n=0	n=1 (3.2%)	n=0	n=0	0.1
11-14	n=23 (92%)	n=27 (87.1%)	n=53 (81.5%)	n=74 (71.8%)	0.06
≥15	n=2 (0.8%)	n=3 (9.7%)	n=12 (18.5%)	n=29 (28.2%)	0.03
P value	<0.0001	<0.0001	<0.0001	<0.0001	-
3. The risk of cervical stenosis (n=60, 29.3%, CI 23.2-36.1%)					
	25 - 39 nullip, n=47	**25 - 39, family incomplete, n=43**	**>40, family incomplete, n=35**	**Family complete, n=17**	***P value***
≤6	n=11 (23.4%)	n=9 (20.9%)	n=5 (14.3%)	n=1 (5.8%)	0.36
7-10	n=31 (65.9%)	n=28 (65.1%)	n=19 (54.3%)	n=8 (47.1%)	0.42
11-14	n=4 (8.5%)	n=4 (9.3%)	n=8 (22.9%)	n=5 (29.4%)	0.08
≥15	n=0	n=0	n=0	n=0	-
P value	<0.001	<0.001	<0.001	0.002	-
4. Fertility IS an issue (n=164, 84.1%, CI 74.4-87.2%)					
	25 - 39 nullip, n=141	**25 - 39, family incomplete, n=134**	**>40, family incomplete, n=101**	**Family complete, n=0**	***P value***
≤6	n=17 (12.1%)	n=14 (10.4%)	n=7 (6.9%)	n=0	<0.001
7-10	n=102 (82.3%)	n=99 (73.9%)	n=70 (69.3%)	n=0	<0.001
11-14	n=22 (15.6%)	n=21 (15.7%)	n=24 (23.7%)	n=0	<0.001
≥15	n=0	n=0	n=0	n=0	-
P value	<0.001	<0.001	<0.001	-	-
5. Fertility is not an issue (n=123, 60%, CI 52.9 – 66.7%)					
	25 - 39 nullip, n=2	**25 - 39, family incomplete, n=9**	**>40, family incomplete, n=26**	**Family complete n=119**	***P value***
≤6	0	0	0	0	-
7-10	1 (50%)	4 (44.4%)	10 (38.5%)	26 (21.8%)	0.15
11-14	1 (50%)	5 (55.6%)	12 (46.2%)	72 (60.5%)	0.59
≥15	0	0	4 (15.3%)	20 (16.8%)	0.67
P value	0.44	0.006	<0.001	<0.001	
6. Most of these women have reassuring histology (n=80, 39%, CI 32.4 - 46.1%)					
	25 - 39 nullip, n=75	**25 - 39, family incomplete, n=67**	**>40, family incomplete, n=52**	**Family complete, n=38**	***P value***
≤6	n=14 (18.7%)	n=11 (16.4%)	n=6 (11.5%)	n=1 (2.6%)	0.10
7-10	n=50 (66.7%)	n=47 (70.1%)	n=32 (61.5%)	n=17 (44.7%)	0.05
11-14	n=11 (14.7%)	n=9 (13.4%)	n=13 (25%)	n=19 (50%)	<0.001
≥15	n=0	n=0	n=1 (1.9%)	n=1 (2.6%)	0.34
P value	<0.001	<0.001	<0.001	<0.001	-

7. They are HPV positive (n=44, 21.4%, CI 16.8 – 27.8%)					
	25 - 39 nullip, n=32	**25 - 39, family incomplete, n=32**	**>40, family incomplete, n=40**	**Family complete, n=31**	***P value***
≤6	n=7 (21.8%)	n=5 (15.6%)	n=4 (10%)	n=1 (3.1%)	0.13
7-10	n=22 (68.7%)	n=22 (68.7%)	n=22 (55%)	n=10 (32.2%)	0.01
11-14	n=3 (9.3%)	n=4 (12.5%)	n=13 (32.5%)	n=18 (58%)	<0.001
≥15	n=0	n=1	n=1 (2.5%)	n=2 (6.4%)	0.5
P value	<0.001	<0.001	<0.001	<0.001	

Table S6.7: Colposcopists' reasons for choice of LLETZ in women with high grade cytology and a TZ3

1. LLETZ can be repeated if diagnostic for CIN (n=96, 46.8%, CI 39.8 – 53.9%)					
	25 - 39 nullip, n=84	**25 - 39, family incomplete, n=76**	**>40, family incomplete, n=55**	**Family complete, n=32**	***P value***
≤6	n=8 (9.5%)	n=5 (6.6%)	n=2 (3.6%)	n=0	0.21
7-10	n=58 (69%)	n=55 (72.3%)	n=39 (70.9%)	n=19 (59.4%)	0.59
11-14	n=18 (21.4%)	n=16 (21%)	n=14 (25.4%)	n=13 (40.6%)	0.14
≥15	n=0	n=0	n=0	n=0	-
P value	<0.001	<0.001	<0.001	<0.001	-
2. A deeper LLETZ will excise an endocervical TZ (n=140, 68.3%, CI 61.3 – 74.5%)					
	25 - 39 nullip, n=49	**25 - 39, family incomplete, n=52**	**>40, family incomplete, n=88**	**Family complete n=122**	***P value***
≤6	n=0	n=0	n=0	n=0	-
7-10	n=15 (30.6%)	n=15 (28.8%)	n=21 (23.9%)	n=16 (13.1%)	0.02
11-14	n=28 (57.1%)	n=40 (76.9%)	n=55 (62.5%)	n=79 (64.7%)	0.18
≥15	n=6 (12.2%)	n=7 (13.5%)	n=12 (13.6%)	n=27 (22.1%)	0.23
P value	<0.001	<0.001	<0.001	<0.001	-
3. Fertility is NOT an issue (n=125, 60.9%, CI 53.9 – 67.6%)					
	25 - 39 nullip, n=5	**25 - 39, family incomplete, n=5**	**>40, family incomplete, n=25**	**Family complete, n=130**	***P value***
≤6	n=0	n=0	n=0	n=0	-
7-10	n=0	n=0	n=0	n=0	-
11-14	n=3 (60%)	n=3 (60%)	n=20 (80%)	n=103 (79.2%)	0.65
≥15	n=2 (40%)	n=2 (40%)	n=5 (20%)	n=27 (20.8%)	0.55
P value	0.07	0.07	<0.001	<0.001	-
4. Fertility IS an issue (n=132, 64.4%, CI 57.3 – 70.8%)					
	25 - 39 nullip, n=126	**25 - 39, family incomplete, n=119**	**>40, family incomplete, n=97**	**Family complete, n=0**	***P value***
≤6	n=6 (4.7%)	n=4 (3.4%)	n=2 (2%)	n=0	0.75
7-10	n=85 (67.4%)	n=83 (69.7%)	n=56 (57.7%)	n=0	0.28
11-14	n=35 (27.7%)	n=32 (26.8%)	n=35 (36%)	n=0	0.37
≥15	n=0	n=0	n=4 (4.1%)	n=0	0.22
P value	<0.001	<0.001	<0.001	-	-
5. The risk of cervical stenosis (n=42, 20.4%, CI 15.3 – 26.8%)					
	25 - 39 nullip, n=31	**25 - 39, family incomplete, n=30**	**>40, family incomplete, n=31**	**Family complete n=17**	***P value***
≤6	n=0	n=0	n=0	n=0	-
7-10	n=23 (74.2%)	n=23 (76.7%)	n=14 (45.2%)	n=4 (23.5%)	<0.001
11-14	n=8 (25.8%)	n=7 (23.3%)	n=17 (54.8%)	n=13 (76.5%)	<0.001
≥15	n=0	n=0	n=0	n=0	-
P value	<0.001	<0.001	<0.001	<0.001	
6. Most of these high grade histology (n=114, 55.6%, CI 48.5 – 62.8%)					
	25 - 39 nullip, n=91	**25 - 39, family incomplete, n=94**	**>40, family incomplete, n=105**	**Family complete, n=17**	***P value***
≤6	n=0	n=0	n=0	n=0	-
7-10	n=61 (67%)	n=62 (65.9%)	n=48 (45.7%)	n=4 (4%)	<0.001
11-14	n=27 (29.7%)	n=29 (30.8%)	n=49 (46.7%)	n=13 (13%)	<0.001
≥15	n=3 (3.3%)	n=3 (3.2%)	n=8 (7.6%)	n=0	0.03
P value	<0.001	<0.001	<0.001	<0.001	-

APPENDIX 7: ACORN Study Supplementary Material

Figure S7.1: Cracking artefact on a dual stain cytology slide secondary to incomplete drying of the aqueous media before slide application

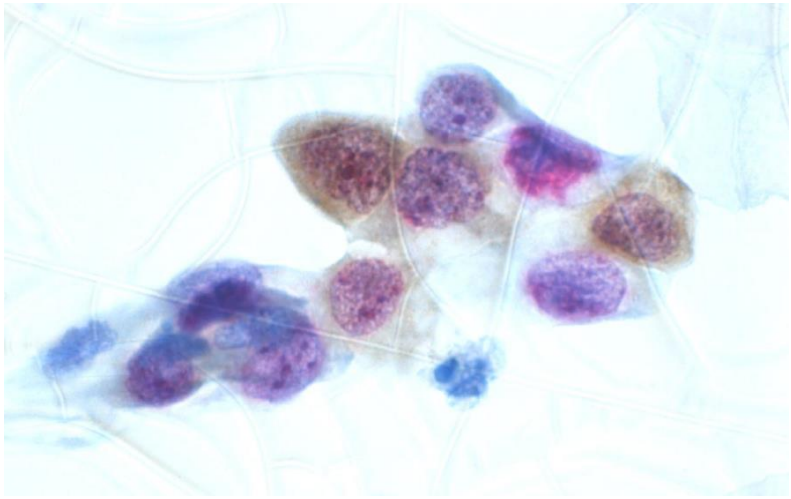
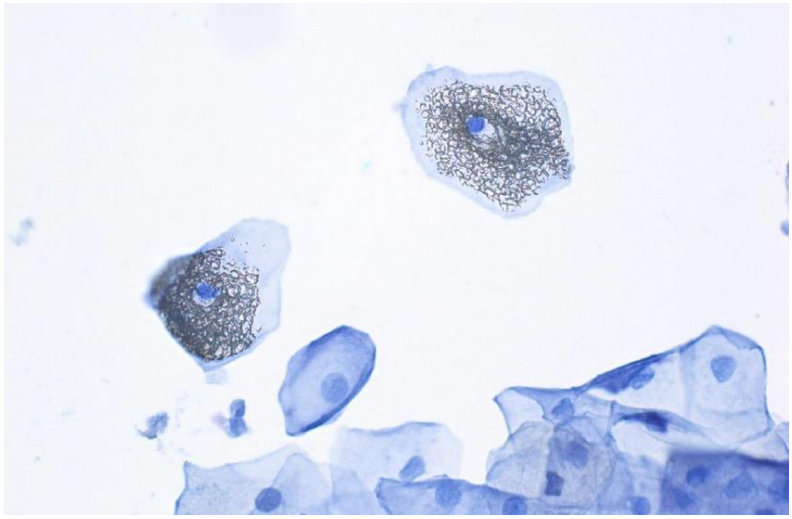


Figure S7.2: 'Cornflaking' artefact of cells on a dual-stain cytology slide secondary to air drying rather than oven baking before the aqueous media is applied.



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