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Cervical cytology versus primary HPV testing as screening tests for cervical dysplasia

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Cervical Cytology Versus Primary HPV Testing as Screening Tests for Cervical Dysplasia

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Abstract:

Background Cervical cancer is the fourth most common cancer among women, accounting for 10% of all female cancers and 7.5% of all cancer deaths worldwide. [1,2] Human papillomavirus (HPV) has been detected in up to 99.7% of cervical cancers, making it the primary risk factor for the development of cervical cancer.[3] Current guidelines recommend a screening test which includes a combination of cervical cytology as well as HPV co-testing in women over the age of 30, with HPV testing not recommended in women younger than the age of 30.[11] The Food and Drug Administration has approved primary HPV testing as a screening test for cervical cancer, however this practice has not yet been adopted by the United States. Continuing to use both methods for screening purposes may lead to discrepant results which can be confusing for both the patient and the provider.[12]

Objective To determine the diagnostic efficacy of cervical cytology versus primary HPV testing as screening tests for cervical dysplasia

Methods A Scopus and Pubmed search was conducted using the following search terms and filters: “HPV screening versus cytology NOT home,” “within 5 years,” “full test article” and “English.” Articles were screened and assessed for eligibility based on study design, sample size, year of publication, participant characteristics, and study objectives. Three articles were chosen for review.

Conclusion

Primary HPV testing may increase accuracy of referral for colposcopy and therefore cervical intraepithelial neoplasia (CIN2+) diagnoses as compared to cytology. However, these conclusions are based on diagnosis rates after colposcopy referral and therefore do not address possible false-negative screening results in patients who have non-HPV related lesions at the time of screening. Currently, there are also no clinical or pathologic features that may guide clinicians in determining which patients may present with false-negative screening results. Further study can focus on addressing this group of patients that may present with false-negative HPV results at the time of screening.

Abbreviations and Acronyms:

HPV	human papillomavirus
HR-HPV	high risk-human papillomavirus
CIN	cervical intraepithelial neoplasia
Pap	papanicolaou
LBC	liquid based cytology
HC2	hybrid capture 2
PCR	polymerase chain reaction
OB	obstetrics
ASCUS	atypical squamous cells of undetermined significance
LSIL	low-grade squamous intraepithelial lesions
LA PCR	linear chain polymerase chain reactions
HSIL	high-grade squamous intraepithelial lesions
OHR	other ‘high risk’
DS	dual stained
NILM	negative for intraepithelial lesion or malignancy

Introduction:

Cervical cancer is the fourth most common cancer among women, accounting for 10% of all female cancers and 7.5% of all cancer deaths worldwide. [1,2] Human papillomavirus (HPV) has been detected in up to 99.7% of cervical cancers, making it the primary risk factor for the development of cervical cancer.[3] HPV infection is very common with an estimated life-time risk of 80% in the general population. The highest incidence of infection occurs in young women, at the mean age of 25 years old. The prevalence of high-risk HPV infection (HR-HPV) in this age group can be as high as 60%.[4] Approximately 70-90% of HPV infections remain asymptomatic and resolve within 1-2 years without intervention.[5] Those who cannot resolve the infection spontaneously may go on to develop high-grade cervical neoplasia due to persistent active infection which may occur decades after initial exposure. Due to this active infection, there may be continued cell activation along with loss of p53 tumor suppressor gene. The loss of p-53 mediated DNA repair may lead to mutations in the normal genome which can progress to cancer.[4]

The progression from high grade cervical dysplasia within the epithelial cells of the uterine cervix to cancer is a long process. Cervical cancer can be divided into two different histotypes: squamous cell carcinoma which is responsible for 70% of cervical cancers and adenocarcinoma which is responsible for 15-20% of cervical cancers. Squamous cell carcinoma develops through precursor cells called cervical intraepithelial neoplasia (CIN), with the neoplastic cells being classified as 1-3, with 1 being mild and 3 being severe dysplasia. There is less known information regarding any precursor cells for adenocarcinoma.[6]

There are 200 different genotypes of HPV and the International Agency for Research on Cancer states there are 12 genotypes that are carcinogenic to humans, with genotypes 16 and 18 being having the highest association with high grade cervical intraepithelial neoplasia and the two most frequent genotypes leading to cancer. [5] A HPV vaccine was implemented in 2006 as a bivalent vaccine to cover 2 genotypes, 16 and 18, and has since improved to cover 9 different genotypes. Although efficacy of the vaccine has proven to be up to 90-100%, there are still other carcinogenic genotypes that are not covered by the vaccine. For this reason, screening tests for cervical cancer are still necessary.[7]

Cervical cancer screening has greatly reduced the mortality related to cervical cancer as it allows for identification of cervical dysplasia/precursor lesions before cancerous cells develop.[8] The two different types of tests involved in cervical cancer screening are the Papanicolaou smear (Pap smear), which is a cytologic test and HPV DNA testing. The Pap smear, which was introduced to use in cervical cancer screening in the 1950s, may involve conventional cytology or liquid-based automated cytology. Conventional cytology involves transferring the collected uterine cervical sample to a slide and interpreted by a cytotechnologist. Liquid-based

EPITHELIAL CELL ABNORMALITIES

SQUAMOUS CELL

- Atypical squamous cells
  - of undetermined significance (ASC-US)
  - cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL)
  (*encompassing: HPV/mild dysplasia/CIN 1*)
- High-grade squamous intraepithelial lesion (HSIL)
  (*encompassing: moderate and severe dysplasia, CIS; CIN 2 and CIN 3*)
  - with features suspicious for invasion (*if invasion is suspected*)
- Squamous cell carcinoma

GLANDULAR CELL

- Atypical
  - endocervical cells (NOS *or specify in comments*)
  - endometrial cells (NOS *or specify in comments*)
  - glandular cells (NOS *or specify in comments*)
- Atypical
  - endocervical cells, favor neoplastic
  - glandular cells, favor neoplastic
- Endocervical adenocarcinoma in situ
- Adenocarcinoma
  - endocervical
  - endometrial
  - extrauterine
  - not otherwise specified (NOS)

OTHER MALIGNANT NEOPLASMS: *(specify)*

Figure 1: Bethesda System Nomenclature[10]

cytology (LBC) involves placing the cervical sample into a liquid to make a suspension which will later be interpreted in a laboratory by an automated computer system.[9] Cytologic abnormalities are typically described and classified according to the Bethesda System nomenclature as described in figure 1.[10] The HPV test uses molecular technology to detect the genome of the uterine cervical cell sample. The test can be either non-amplified or amplified, which is usually used in clinical research and includes the hybrid capture 2 (HC2) and polymer chain reactions (PCR).[2]

Current guidelines recommend a screening test which includes a combination of cervical cytology as well as HPV co-testing in women over the age of 30, with HPV testing not recommended in women younger than the age of 30.[11] The Food and Drug Administration has approved primary HPV testing as a screening test for cervical cancer, however this practice has not yet been adopted by the United States, possibly due to the fear of false negative results or false positive results leading to more unnecessary tests. However, continuing to use both methods for screening purposes may lead to discrepant results which can be confusing for both the patient and the provider.[12] Examining the efficacy of primary HPV testing in the detection of cervical dysplasia will also lead to decreased costs if only one test is needed for screening purposes.

PICO

Population: females ages 21-65
Intervention: HPV screening
Comparison: screening cytology on Pap smear
Outcome: increased efficacy of diagnosis of cervical intraepithelial neoplasia

Question: Among females ages 21-65, does the use of HPV screening as compared to cytology screening increase the efficacy of diagnosing cervical intraepithelial neoplasia?

Methods:

In September 2017, an initial Pubmed and Scopus database search was conducted using the key terms "HPV screening versus cytology NOT home," with other criteria including "within 5 years," "full test article" and "English." 202 articles were found and 154 articles remained after duplicates were excluded. Twelve of these articles were closely screened and three were excluded due to having little relevance to this current study, two being simulation studies and one was a cost analysis. Nine full-text articles were assessed for eligibility. Six of these articles were excluded due to the following reasons: studies determining efficacy of HPV cotesting rather than primary HPV screening, literature reviews, and determining obstetrics outcomes related to screening techniques. One retrospective cohort and two randomized control studies were included in this study due to the large sample sizes, participants similar to this study population, and these studies had objectives to determine the efficacy of HPV testing as primary screening as compared to cytology. This process is displayed in figure 2.

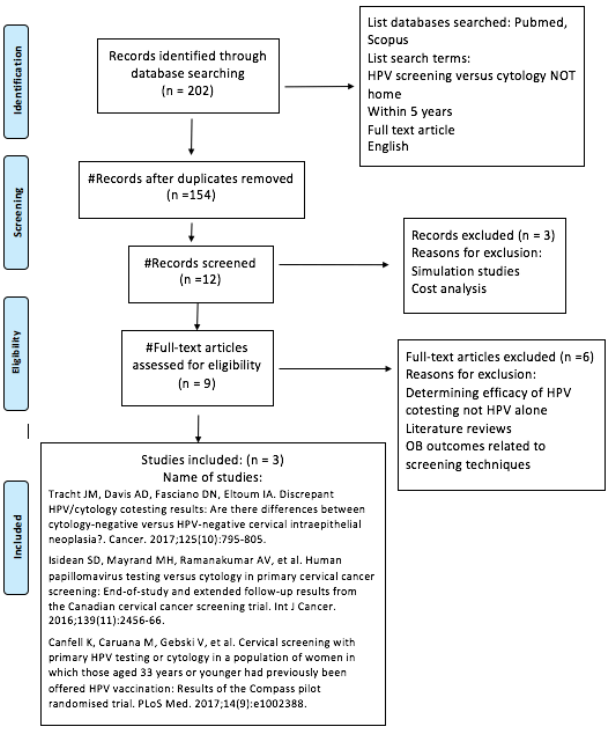

Figure 2: PRISMA Flow Chart[13]
Abbreviation key: HPV- human papilloma virus, OB: obstetric

Results:

Study 1

Human papillomavirus testing versus cytology in primary cervical cancer screening: End-of-study and extended follow-up results from the Canadian cervical cancer screening trial.[1]

Objective**:**

Cervical cancer screening has mostly been based on cervical cytology. However, given the causative relationship between HPV infection and cervical carcinogenesis, this study considers the diagnostic accuracy of HPV testing. The accuracy of detection of CIN2+ was compared for HPV testing and cytologic testing in women participating in primary cervical cancer screening.

Study Design:

The study is a randomized controlled trial that was conducted during the period of 2002 and 2005. Women aged 30–69 years who sought routine cervical cancer screening in any of the 30 participating clinics in the greater Montreal area or St. John's were invited to participate. In total *n* = 10,154 women were willing and eligible to enroll in the trial as

described by the inclusion and exclusion criteria in table 1. The women were then randomized into either HPV testing or Pap cytology.

Table 1: HPV testing vs Cytology in primary cervical cancer screening

Inclusion Criteria	Exclusion Criteria
▪ Women aged 30–69 years who sought routine cervical cancer screening, between 2002 and 2005, in any of 30 participating clinics in the greater Montreal area or St. John's in Canada.	Women who: ▪ were under evaluation, treatment, or follow-up of a cervical lesion; ▪ were without a cervix; ▪ were pregnant; ▪ had a prior history of invasive cervical cancer; ▪ were unable to provide informed consent; or ▪ had received a Pap test within 12 months.

Both study groups received both screening tests for ethical reasons, however the order in which the test were collected was randomized. This allowed maintenance of the standard of care for the participants which allowing the study team to assess the performance of the tests as if performed alone. The study had a blinding mechanism at three different levels. The participating women were not aware of the study group allocation. The cytotechnologists and cytopathologists evaluating Pap smears were unaware of inclusion of women into the study. Comparably, colposcopists and pathologists evaluating biopsy specimens were blinded to initial screening test results. Each study arm had no access to test results of each other.

Pap smears were obtained and interpreted by cytotechnologist or cytopathologists and reported according to the Bethesda System nomenclature (Figure 1). HR-HPV testing was performed using the HC2 test. Positive HR-HPV tests were then further examined using the Linear Array HPV Genotyping Assay. Authors reported HR-HPV genotypes as HPV+.

In the initial screening process, women who tested positive either on Pap smear or HPV test, underwent a colposcopic exam at participating clinics. If colposcopy biopsy specimens revealed a histologic diagnosis of CIN2+, the women were withdrawn from the study and managed appropriately. If colposcopy revealed CIN1 or no lesion, these patients received a repeat colposcopy in 6 months. In order to avoid verification bias, which is a bias in testing that may occur when participants are chosen because they have previously undergone the test of interest that is being evaluated and agree to subsequently undergo the reference standard test, a total of 30% of patients who had negative initial testing were selected for colposcopy.[14] Women not chosen for colposcopy were invited to receive repeat Pap smear testing at 12-18 months. This allowed for detection of prevalent lesions as well as lesions missed at initial enrollment. During the study, women received annual cytology as per cervical cancer screening guidelines. Women with atypical squamous cells of undetermined significance (ASCUS) Pap smear results received repeat Pap smear before colposcopy whereas women with low-grade squamous intraepithelial lesion (LSIL) Pap smear result were referred immediately for colposcopy. In 2008, HPV testing was introduced for patients over the age of 30 and therefore guidelines changed to recommend cytologic testing every 3 years after 3 consecutive normal Pap smear results. Colposcopy referral changed to include ASCUS HPV+ results while patients with HPV negative results were allowed to return to routine screening protocol.

Participants from St. John's were followed up for extended period from study enrollment date until December 31, 2013. The data on cervical cancer screening-related procedures and their outcomes were retrieved from the provincial database for the 5,754 women in the area who participated in the trial. The reasons for extended follow up study was the availability of well organized and comprehensive healthcare databases in Newfoundland.

This study compared the predictive value of the Pap smear and HPV test by using the Kaplan-Meier method which is a graphical display of survival data from a randomized controlled trial. This method uses survival probabilities to predict time to event in order to compare 2 groups, and log-rank test, which uses the p-value of a study to determine if the null hypothesis can be rejected.[14] Time-to-event was defined as time from the first visit to date of first histologic diagnosis of CIN2+. HPV status was also examined to determine predictive value of separate HPV genotypes.

Results

The average follow-up times during the protocol-defined follow-up period were 16.6 and 12.9 months for Montreal and St. John's participants, respectively. For participants of the extended follow-up, the average time was 100 months.

In general, during the initial screening, there was more probability to test positive on HPV than Pap smear in all study populations and regions. Among women screened at enrollment with a valid test, it was more common to have a positive HPV test as compared to Pap smear with 6.1% tested positive for HR-HPV and 2.9% tested Pap+ (appendix 1). Additionally, of all the women who tested HPV+ 18% of them were also Pap+.

Throughout the initial screening and follow up, there were a total of 82 cases of CIN2+ (median age = 36 years). The majority of cases were HPV+ as compared to Pap+ results (82.9% versus 44.4%). This pattern was also observed in the group of patients with discrepant test results, as there were more patients with HPV+/Pap- than HPV-/Pap+ results (43.2% and 4.9% respectively). For the St. John's extended follow-up participants, additional 30 cases of CIN2+ were diagnosed, and (54.2%) were HPV+ at enrollment, whereas only 19.3% were Pap+. The HPV genotype-specific tests were conducted using HC2 and linear array polymerase chain reaction (LA PCR). Among the 9,988 women, about 104 (1.0%) were HPV16+, 37 (0.4%) were HPV18+, 277 (2.8%) were positive for HR-HPV types other than HPV16/18, and 64 (0.6%) were positive for HR-HPV types not in HC2, but tested by LA PCR and 9,506 (95.2%) were HC2− (appendix 2).

The cumulative risks for CIN2+ detection following initial screening result of abnormal cytology or HPV testing were observed. Three year risk for CIN2+ following high-grade squamous intraepithelial lesion (HSIL) or worse result on cytology was significantly higher than risk associated with LSIL ($p=0.02$) or negative cytology ($p=<0.0001$). There was also a significant difference in associated risk of LSIL result as compared to normal cytology ($p<0.0001$). The three year risk of CIN2+ diagnosis for the HPV test showed significant difference between the HPV+ and the HPV- groups ($p<0.0001$). These results are displayed in figure 3. Risk associated with Pap+/HPV+ was significantly higher than discrepant test results (35.77% versus 8.96% HPV+/Pap-

and 2.73% HPV-/Pap+), and abnormal cytology helped to further stratify risk in those patient with HPV+ result.

During the extended follow-up period, risks, associated with results of either Pap cytology or HPV testing at initial screening had a trend similar to those of protocol-defined period. However, there were no significant differences in risks between women with HSIL versus LSIL Pap cytology. Overall, 10-year cumulative detection of CIN2+ ranged from 1.15% for HPV−/Pap− women to 26.05% for HPV+/Pap+ women (appendix 3).

Genotype specific results were also observed and showed a dramatic increased risk for patients with HPV16+ result as compared to HPV+ result with genotyping other than 16/18 (p=<0.001). There was no significant difference in HPV16+ versus HPV18+ results (p=0.19). The three year risk associated with HPV16+ was 43.84% as compared to 0.90% for patient with HC2- results. These estimates of risk were higher than those associated with Pap+ or HPV+ results. Extended follow-up results of 10 year risk associated with HPV genotyping results were again similar to the protocol-driven follow-up results, with risk associated with HC2- at 1.13% as compared to HPV16+ at 32.78%.

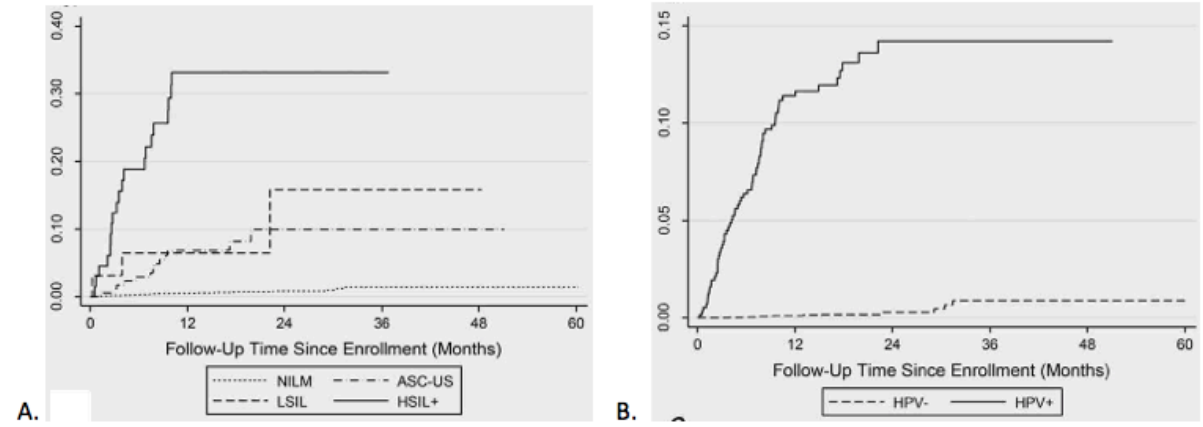

Figure 3: Risk for CIN2+ is portrayed on y-axis as percent of patients with CIN2+ diagnosis while the x-axis represents time.[1]
Figure 3a shows risk for CIN2+ diagnosis during protocol-defined follow-up period associated with results of Pap cytology. Figure 3b shows risk for CIN2+ diagnosis during protocol-defined follow-up period associated with results of HPV testing.
Abbreviations: CIN: cervical intraepithelial neoplasia, Pap: Papanicolaou

Study critique:

This study tried to address the perceived advantage of HPV testing over Pap smear in primary screening of cervical cancer, and the authors have done a good job in trying to quantify a measurable outcome. However, the validity HPV genotyping in predicting development of cervical cancer and guiding medical management of women with HR-HPV+ but normal cytology results the data is inconclusive. The reason is that, although the utilization of genotyping has the potential for identifying the high risk genotypes and improve the way we manage the patients with HR-HPV+, availability statistical data is still limited. The authors of the study recommended larger scale study.

Another concern with this study is the practicality of the testing methods they used. In the study population, the standard screening procedure is for women with HPV− and Pap− results in no further colposcopy testing is recommended. However, in the study to correct for verification bias they standardized colposcopy protocol, thereby increasing the chances of detecting CIN2+ and decrease the chances of finding cancerous lesions later in the lives of these women (because the early detection of abnormal lesions by colposcopy). Therefore, their recommendations should be taken with a little bit of caution, and the authors address this in their discussion.

Finally, the study was conducted in the Canadian population with some similarities to the US population in terms of geographical proximity and possibly the population composition. For this reason, the results of the study may be applicable to the US patients. However, it is important to note that the healthcare system is different in terms of availability and continuity between these two countries. As an example, in the above-mentioned study population, health care is government mandated and they had an ample access to health care. As a result, relatively extensive medical records were available for the study. In comparison, the availability of healthcare coverage in the US is not comparable to that of Canada's. Therefore, the conclusion of the study should be taken with some caution.

Study 2

Cervical screening with primary HPV testing or cytology in a population of women in which those aged 33 years or younger had previously been offered HPV vaccination: Results of Compass pilot trial randomised control.[15]

Objective:

Most studies about efficacy primary HPV testing for cervical screening in comparison to the traditional cytology (Pap smear) have been on populations with little or no previous access to HPV vaccination. Therefore, this study is done to assess the improved performance of primary HPV testing for cervical screening in detection of high-grade cervical intraepithelial neoplastic lesions (defined as CIN2+) and invasive cancer as compared to cytology (Pap smear) in a population previously offered HPV vaccination.

Study Design:

The Compass is an open-label randomized trial of 5-yearly HPV screening that consisted two-arms versus 2.5-yearly liquid-based cytology (LBC) screening. An open-label randomized trial means the clinical trial was conducted without an attempt to disguise the treatment/ screening options and therefore the researchers and the patients were aware of the type of screening they received. A total of 5,006 eligible women (as described in Table 2) were recruited from 29 October 2013 to 7 November 2014; of these, 22% were in the group age-eligible for vaccination that is women $\leq$30 years of age.

Table 2: Study Population selection	
Inclusion Criteria	Exclusion Criteria
▪ Australian female resident of Victoria aged 25- 64 years. ▪ Attending for routine cervical screening at participating Primary Health Care Clinics (PHCC) or sexual health clinics in Victoria (or follow-up of prior unsatisfactory smear for routine screening).	▪ Previous total hysterectomy (uterus and cervix). ▪ The presence of symptoms for which cervical cancer must be excluded. ▪ Currently undergoing treatment for cervical pre-cancer, or cancer. ▪ Attending for follow-up of a prior cervical abnormality, including repeated "test of cure" procedures in which the woman has not yet been discharged back to routine screening. ▪ Known pregnancy.

Initially, consenting women aged 25–64 years presenting for routine screening at 47 primary practices in Victoria, Australia were included in the study and a cervical sample was collected. The samples were then randomized at a central laboratory at 1 to 2 to 2 ratio allocations to the three arms of the study: (i) image-read LBC screening with HPV triage of low-grade cytology ('LBC screening'), (ii) HPV screening with those HPV16/18 positive referred to colposcopy and with LBC triage for other oncogenic or other 'high risk' (OHR) types ('HPV+LBC triage'), or (iii) HPV screening with those HPV16/18 positive referred to colposcopy and with dual-stained (DS) cytology triage for OHR types ('HPV+DS triage'). In other words for every one patient assigned to the LBC screening, two patients each were assigned to 'HPV+LBC triage', and to 'HPV+DS triage.' Study groups and management for each group are shown in figure 4.

The initial recruitment process employed two-tier blinding and randomization: the participating women and recruiting personnel were blinded to randomization assignment. Then, following the receipt of the LBC sample at the centralized laboratory, participants were randomized based on a computer-generated schedule.

The computer-generated schedule was an independent design by the Australian government National Health and Medical Research (NHMRC) Clinical Trials Centre at the University of Sydney. Furthermore, the randomization used a minimization procedure stratified by age group <30 and 30+ years; in order to try to stratify those patients who likely received HPV vaccination. This was meant to ensure a good balance between the three arms across the stratification levels, as well as overall. Laboratory personnel were only made aware of the study groups after receipt and proper logging of each sample in order to maintain blinding.

ThinPrep cytology was used for LBC and the 2 methods for HPV screening included HC2 for 22% of participants and Cobas for the initial 78% of participants. For dual-stained cytology testing, CINtec PLUS technology was used, which stains for the markers p16 and Ki67. Australian screening recommendation for cervical cancer screening were followed and included cytology every 3 years and HPV screening every 5 years. In order to correct a potential verification bias a proportion (16%) of all women not referred to colposcopy were randomly selected and invited for verification colposcopy performed at the Royal Women's Hospital, Melbourne. However, the participation of rate of the women invited was very low to be a representative of an impartial sample.

Statistical analysis of all eligible participants (minus the withdrawals) was done using Mantel–Haenszel test, a statistical significance test analysis where the null hypothesis states that no difference exists between the overall life table results for the

study and control group.[14] This test compared the colposcopy referral rates and CIN2+ detection rates in the LBC screening arm with those in the combined HPV screening arms, and between the two HPV screening arms (HPV+LBC triage, and HPV+DS triage).

Results:

Of the total number of 5,303 participants initially recruited, 297 women were ineligible and excluded from the study. 5,006 participants were randomized to the different study arms. 998 were assigned to LBC screening, with 3 withdrawing before analysis. 1,996 were assigned to arm 2 of the study and 4 participants withdrew before analysis. Finally, 2,012 participants were assigned to the third study arm, with 4 participants withdrawing before analysis. Therefore, data was collected on a total of 4,995 participants. The randomization of the study participants is described in Table 3.

Table 3: Case numbers and rates of detected CIN2+

Group= LBC Screening	N=	CIN2+	Group= HPV + LBC triage Screening	N=	CIN2+	Group= HPV + DS triage Screening	N=	CIN2+
Age-eligible for HPV vaccination	211		Age-eligible for HPV vaccination	418		Age-eligible for HPV vaccination	449	
Rate of detection		2	Rate of detection		20	Rate of detection		22
Percent		0.9	Percent		4.8	Percent		4.9
Not age-eligible for HPV vaccination	784		Not age-eligible for HPV vaccination	1574		Not age-eligible for HPV vaccination	1559	
Rate of detection		0	Rate of detection		13	Rate of detection		19
Percent		0	Percent		0.8	Percent		1.2
Total Screened	995		Total Screened	1992		Total Screened	2008	
Rate of detection		2	Rate of detection		33	Rate of detection		41
Percent		0.2	Percent		1.7	Percent		2

The final results were classified by the initial observed high-grade cytology rate, colposcopy referral rate, overall confirmed CIN2+ and CIN3+ rates in each group, and the overall colposcopy referral rate and detected CIN2+ rate (including 12-month follow-up) in young women previously age-eligible for vaccination, and older women (age-ineligible for vaccination).

As the numbers of detections increased so did the numbers of colposcopy referral rate. The referral rates were classified into those referred based on the primary screening test, a positive triage test, or those detected at 12-month follow-up. Overall 181 women were referred for colposcopy and histologic outcomes were available for 177 of these participants. However, there was no clinical significance found between referral rates of LBC screened participants as compared to all HPV screened participants (referral rates displayed in figure 4). Also, after 12 months there was no clinical significance in referral rate of the 2 different kinds of HPV testing. There was, however, clinical significance when compared to historical data of colposcopy referral rates in Victoria in 2013. LBC referral rates were significantly lower than this reference rate ($p=0.02$) and the HPV+DS referral rate was significantly higher ($p=<0.001$).

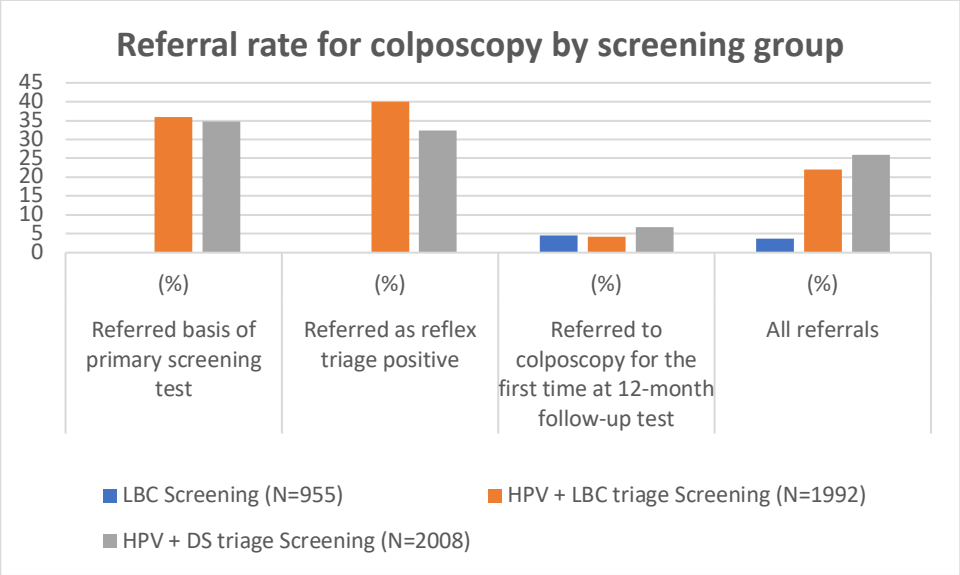

Figure 4: Referral rate for colposcopy in Study 2
y-axis: percent of patients in each screening group referred for colposcopy; x-axis: different basis for referral. Abbreviations: HPV: Human Papillomavirus, LBC: liquid based cytology; DS: dual-stained

In the initial screening, the observed percentage of high-grade cytology was 0.1% in LBC screening group. The rate of detection then jumps by 13x to 1.3% of participants were HPV16/18 positive in the HPV+LBC triage group. The overall confirmed CIN2+ rates in each group, LBC screening, HPV+LBC triage, and HPV+DS triage groups, were 0.2%, 1.7%, and 2%, respectively. This shows that it is 10x more likely to detect CIN2+ rates by HPV-screened women combined than the LBC-screened, but no significant difference was identified between overall CIN2+ rates in the 2 HPV-screened groups after adjusting for HPV vaccination eligibility. The overall colposcopy referral rate and detected CIN2+ rate (including 12-month follow-up) is higher in the HPV-screened women than in the LBC screened.

The overall CIN2+ and CIN3+ rates in each colposcopy referred group was observed to determine the accuracy of referral for each test. There was a significant difference identified between the CIN2+ rates in the LBC-screened versus all HPV-screened patients (p=0.003). On the other hand, there was no clinical significance found in CIN2+ diagnosis in the 2 HPV-screened groups. These results are displayed in figure 5 below.

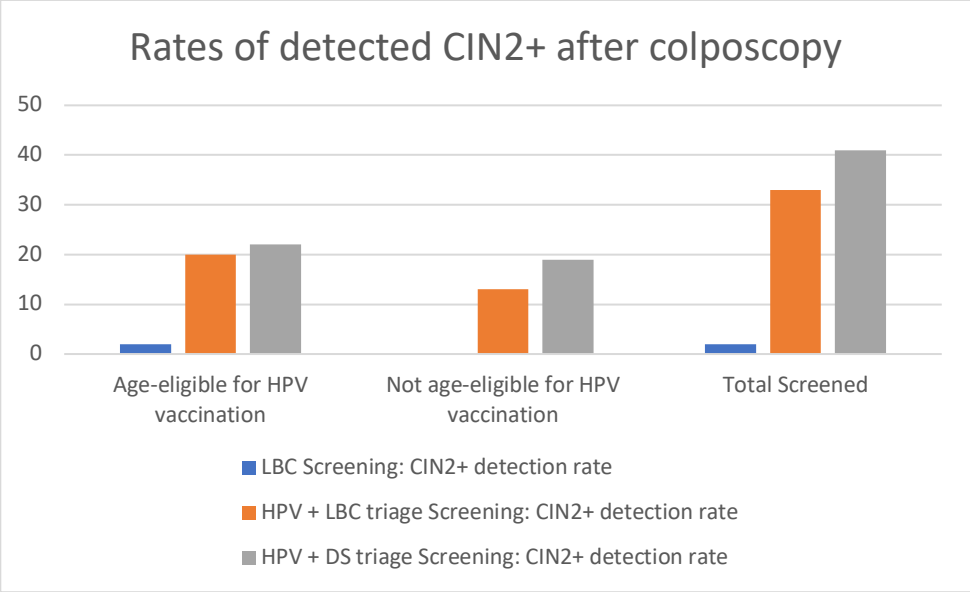

Figure 5: CIN2+ result rates after colposcopy in Study 2
y-axis: percent of patients with positive CIN2+ after colposcopy referral. x-axis: different study groups as defined as possible HPV vaccination or no HPV vaccination and total CIN2+ result. Abbreviations: HPV: human papillomavirus, LBC: liquid based cytology; CIN: cervical intraepithelial neoplasia DS: dual-stained

During the follow up for adverse events a total of four deaths were reported, two deaths from each HPV+LBC, and HPV+DS triage groups. These events were reviewed by the trial Independent Data and Safety Monitoring Committee (IDSMC), and ruled out as unrelated to the trial. Additional two adverse events, one each from LBC screening group & the HPV+LBC triage group. These were miscommunication issues involving screening results, rather than related to clinical findings.

Study critique:

This study is very applicable as one of the first studies examining efficacy of different cervical cancer screening methods to include a highly HPV vaccinated population. The study tried to incorporate a population with similar demographics to those presenting for cervical cancer screening, however it was noted that this study contained a smaller percent of patients in the 25-29 age group as compared to reported screening demographics for Australia. This may be important when considering the clinical application of these results as this age group may represent a large number of women who have received the HPV vaccine.

Researchers also tried to consider the possibility of HPV vaccination during the randomization into the different arms of the study as it included similar percentage of women offered HPV vaccination into each arm. This allowed for the study to also determine accuracy rates in patients offered vaccination. However, the study could only determine possibility of vaccination based on the patient's age since vaccination status of each patient was not provided.

A low rate of CIN was detected by LBC as compared to previously reported rates in 2013. This prompted researchers to re-read each result through an outside laboratory to make sure there were no errors in diagnosis. There was also a significantly high rate of CIN in the HPV+DS group. Researchers recommended caution when comparing

these rates to the historical data as this study did not include any women who are under surveillance for previous abnormal results but rather includes a population of well-screened women. Therefore, this may limit the application to the general population.

Finally, this study focused on positive predictive value of initial tests after the referral to colposcopy. Positive predictive value meaning the proportion of individuals with a positive test who actually have the disease as measured by the reference standard. This is the probability of having an abnormal diagnostic test if the screening test is positive.[14] This only included 181 patients and does not address the accuracy of the screening test in terms of false-negative results that would therefore not be referred to colposcopy.

Study 3

Discrepant HPV/Cytology Cotesting Results: Are There Differences Between Cytology-Negative Versus HPV-Negative Cervical Intraepithelial Neoplasia?[12]

Study Objective

To compare characteristics of HSIL (specifically categorized as CIN3+) after negative cytology but positive HR-HPV (negative for intraepithelial lesion or malignancy [NILM]/HPV-positive) with characteristics of HSILs after negative HR-HPV but positive cytology (ASCUS positive/HPV negative). The reason for these discrepancies was explored to further characterize and understand the occurrence of these false positive tests.

Study Design

This is a retrospective cohort study in which results were retrieved through a computer-based search using Cerner Millennium health information system (Kansas City, Kansas) for women who underwent both LBC screening and HPV testing from January 2010 through December 2013. This included 15,173 women ages 25-99. ThinPrep Pap test specimens were prepared and initially interpreted by cytotechnologists and pathologist and then independent retrospective review was performed by 2 pathologists who were blinded to both the initial cytologic diagnosis as well as the surgical pathological diagnosis. HPV testing included reflex HPV testing, HPV cotesting for women over 30 years old or HPV tests that were requested for patients under 30 years old for unknown reasons. This testing was performed in residual PreservCyt vials using the Cobas 4800 system which tests for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. The results of this HPV test is reported as either positive or negative with further characterization of the specific subtypes in test that are HR-HPV positive.

Histopathologic diagnosis of HSIL, specifically categorized as CIN3+, was made on hematoxylin and eosin-stained histopathologic specimens from endocervical curettage, biopsy, loop electrosurgical excision of the uterine cervix and hysterectomies. Again, an independent retrospective review by 2 pathologists was used to confirm the original diagnosis. Immunohistochemical analysis evaluated for the expression of p16 in formalin-fixed, paraffin-embedded cervical biopsy specimens using CINtec cyclin-dependent kinase inhibitor 4A histology kit and an automated Ventana BenchMark

ULTRA system. Results were defined as positive with the presence of both cytoplasmic and nuclear staining.

Statistical analysis was performed using the XLSTAT and SPSS software by analyzing both variance and chi-square tests for clinical and pathologic features and were considered significant at $P<0.05$.

Results

Of the 15,173 women screened with both Pap smear and HR-HPV, 2,944 women had a cytologic finding of ASCUS or greater. Within this group of women with abnormal cytology, 2,200 women tested positive for one of the HR-HPV subtypes. 1,184 of these women had histopathologic follow up and a total of 84 of these women had CIN3 positive results. 55 patients tested ASCUS-positive/HPV-positive, 11 tested NILM/HPV+, 10 tested ASCUS-positive/HPV-, 3 tested NILM/HPV-, and 5 tested unsatisfactory.

Table 4: Clinical parameters measured and results for Study 3

	Positive cytology/HPV-	NILM/HPV+	NILM/HPV-
Total number of patients	10 (3 later excluded)	11	3 (1 later excluded)
Average Age	51; 36 years*	42	59 years; 58 years*
Age range	25-74; 25-56*	25-64	52-64; 52-64*
Average time to event	3.7 months; 4.6 months*	8.6 months	14.3; 21.5 months*
Average lesion size	2.5mm*	2.5mm	2.3mm; 2mm*
Race: African American	6; 4*	3	2; 1*
Race: Caucasian	4; 3*	8	0; 0*
Race: Other	0; 0*	0	1; 1*
positive p16 on staining	7*	9	2; 2*
Previous abnormal cytology	5; 5*	4	1; 1*

*calculated after exclusion

Clinical and pathologic parameters were investigated for the 24 patients who had discrepant cytology/HPV results as displayed in table 4. These parameters included patient age, patient race, history of screening, time to diagnosis, lesion size and presence of viral cytopathic changes. Each of these parameters were examined for each group individually and then compared overall.

Cytology-positive/HPV-

Ten patients with CIN3+ histology had positive cytology with negative HPV screening. The clinical parameters observed are shown in table 4. On review of pathology, one patient was excluded due to unavailability of the initial biopsy for review. Two women from this result group were diagnosed endometrial cancers extending into the cervix and therefore were excluded from further discussion. The remaining 7 patients were examined and the average age, lesion size and time to occurrence were documented as provided in table 4.

NILM/HPV+

Eleven patients had normal cytology with positive HPV co-testing. The clinical parameters for these patients are displayed in table 4. Upon review of cytology, six of

the screening cytology results were reclassified as ASCUS, and this was attributed to interpreter error. The five other patient results remained classified as NILM and were attributed to cytologic sampling error.

NILM/HPV-

Three women had CIN3+ on histology after NILM/HPV- screening. Clinical parameters for these patients are discussed in table. After pathological review, there was no reclassification of cytology or pathologic results. One patient, who presented initially with postmenopausal bleeding, was diagnosed with endometrial adenocarcinoma.

There was no significance detected in the clinical or pathologic features in the patients diagnosed with CIN3+ who had NILM/HPV+ or Cytology+ /HPV- Pap smear results.

Study Critique

This study is a retrospective cohort study. This is an observational study design in which participants in the study are enrolled and studied after the outcome which is being observed has already occurred.[14] For this study specifically, investigators reviewed the medical records of patients that met criteria for the study after the Pap smears and HPV testing were already performed. They reviewed the results of these tests after the outcome already occurred. Confounding variables, which are differences in the different study groups that could potentially affect the outcome, were minimized by having specific inclusion and exclusion criteria for the study participants. However, not all confounding variables can be addressed in these studying which remains a disadvantage of this study design. Since the outcomes of this study has already occurred there may be some confounding variables of which the researchers were not aware at the time they were reviewing these patient records.

This type of study is beneficial for the objectives of the study because it is an observational study, meaning researchers are observing data rather than using any interventions in the study groups. The objective of this study, to compare accuracy of screening tests, does not need any type of specific intervention as investigators are observing the results of the two screening tests and the accuracy in predicting abnormal diagnostic testing. This type of study is beneficial in terms of the study's objectives because researchers are able to look at previous records and record incidence of abnormal Pap smear results or HPV test result after patients had an abnormal follow up diagnostic test.

Although this study initially began with a very large sample size of 15,173 women receiving cervical cancer screening, only 24 of those patients had discrepant cytology and HPV screening results and therefore the sample size discussed in this study was very small. The authors do address this issue during the discussion and state that this small sample size may be responsible for their results being different than previous studies, for example other studies that noted age as a possible explanation for discrepancy in screening test results. The inclusion and exclusion criteria for this study was not clearly discussed or displayed in the study, which may lead to confusion regarding the non-squamous cell cancer results that were later excluded from the study.

There is also one patient that was discussed in the NILM/HPV- group that had the Pap smear with HPV co-testing performed as part of her workup for postmenopausal bleeding. This means that the testing is no longer considered a screening test and should not have been included in a study examining the use of HPV as a screening test.

The discussion of the study did address possible explanations for discrepant results by referring to conclusion from other studies, including low residual volume of the sample for the HPV testing as well as the possibility of the specific subtype of HPV not being screened in those discrepant cases. This enhances the analysis of this study and may give direction to future studies that may address these issues. The authors also addressed other limitations to their study, including possible bias regarding cytologic and pathologic review since the interpreters were aware of the CIN3+ end point. The authors also noted that only by including CIN3+ results in the study may miss many other discrepant results for patients with CIN2 and CIN1 results. Acknowledging these limitations may allow for better future studies regarding these screening tests.

Discussion

Three studies were chosen for review in order to examine the efficacy of primary HPV testing as compared to cytologic testing as screening tests for cervical intraepithelial neoplasia. Overviews of each of these studies are displayed in table 5. The first two studies were applicable to our clinical question as they examined the efficacy of HPV testing as compared to cytology in different populations. The third study explored possible clinical or pathologic features that may contribute to discrepant screening results. Better understanding of possible etiologies of these discrepant results in screening tests may allow for more individualized screening methods in certain populations. More accurate screening tests may lead to increased efficiency of screening protocols and will limit the extent of screening and diagnostic tests necessary for diagnosis of precancerous and cancerous lesions.

Table 5: Overview of Studies

	Study 1 **Isidean et. al**	**Study 2** **Canfell et. al**	**Study 3** **Tracht et. al**
Objective	To compare the performance of HPV testing and Pap cytology in detecting CIN2+ for patients following routine cervical cancer screening in Canada	To estimate the test positivity rate (colposcopy referral rate) and CIN2+ detection rates for HPV-screened versus cytology-screened women in Australia's HPV-vaccinated population	To compare characteristics of CIN3 diagnosed after negative cytology but positive HR-HPV with characteristics of CIN3 diagnosed after negative HR-HPV but positive cytology
Study Design	Randomized control trial	Randomized control trial	Retrospective cohort
Patients (n)	10,154	5,000	15,173
Population age	30-69	25-64	25-99
Follow up period	12-16 months; extended follow up of 100 months	13 months	36 months
Outcome	CIN2+	CIN2+	CIN3

Conclusion	HPV-based cervical screening may allow for greater disease detection than cytology based screening	Primary HPV testing provided significantly increased detection of high grade precancerous lesion as compared to cytology in patients with high rates of HPV vaccination	There are no significant clinical or pathologic differences between discrepant cytology and HPV testing results and HPV-/cytology+ results may be missed with primary HPV screening
Critique	Increased rates of CIN2+ detection by using a verification protocol to include some of screening group who may not have been referred to colposcopy based on screening protocol	Vaccinations status of participants not recorded, assessed possibility of vaccination based on age	Large sample size initially but discrepant results from that sample only included 24 patients

The studies show the superior specificity of HPV testing as compared to Pap smear in detecting CIN2+ or worse after colposcopy referral. The first study also showed that the precision of predicting 5-year risks for HPV− women was higher than 3-year risks for Pap- women, and was comparable to 5-year risks for co-test negative women. Therefore, if primary HPV testing is incorporated into cervical screening protocols then there may be increased accuracy of the screening tests which will allow for more favorable screening intervals and reduced cost. However, referral to colposcopy for this study was based on the co-test result and therefore may not be representative of primary HPV colposcopy referrals. The inclusion of normal screening participants in the colposcopy group may have lead to a greater detection rate of CIN2+ lesions that would have been missed if primary HPV testing or cytology testing were performed alone.

With increased rates of HPV vaccination in the United States, the second study may be very applicable to determine the efficacy of HPV screening as compared to cytology in this population. This study showed more accurate rates of diagnosing CIN2+ in those patients that have been referred for colposcopy which may allow for earlier treatment and prevention of advancement of the lesion. As stated in the study, this has been examined in previous studies however this is the first study to include a highly HPV vaccinated population. This study also examined predictive value of HPV genotype testing which may be used in initial screening in order to recommend colposcopy initially rather than requiring cytology follow up. As compared to previous studies, this study found lower rates of high risk HPV infection in this HPV vaccinated population which also supports the use of HPV vaccination to prevent not only cervical cancer but also unnecessary colposcopy testing.

The final study looked to investigate possible clinical or pathologic features for discrepant cytologic and HPV testing results with later diagnosis of CIN. This study concluded that the most common causes of discrepant results were interpretation error, sampling error, HPV infection with subtype not currently screened or neoplasia due to non-HPV related carcinoma. The study found no clinical significance in the clinical characteristics of the patients or pathologic parameters of the diagnosis to distinguish which patients may present with these discrepant results. In this study, cytology-positive/HPV-negative accounts for 29% of discrepant results in screening tests. Since

there were no additional clinical parameters to help determine which patients may have discrepant HPV or cytology results, using HPV testing as a primary screening test may lead to missed diagnoses of these neoplasms that are either non-HPV related or subtypes that are not specifically tested.

Conclusion

Primary HPV testing may increase accuracy of referral for colposcopy and therefore CIN2+ diagnoses as compared to cytology. However, these conclusions are based on diagnosis rates after colposcopy referral and therefore do not address possible false-negative screening results in patients who have non-HPV related lesions at the time of screening. Currently, there are also no clinical or pathologic features that may guide clinicians in determining which patients may present with false-negative screening results. Due to the lack of identified risk factors that may help predict false-negative results, the authors of this study do not recommend the use of HPV testing alone. Further study can focus on addressing this group of patients that may present with false-negative HPV results at the time of screening.

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Appendix

Appendix 1: Case numbers and rates for detected CIN2+ and CIN3+, by age eligibility for vaccination.

Group	CIN2+	CIN3+
LBC screening (N = 995)		
Age-eligible for HPV vaccination (*N* = 211)		
Rate	1	1
Percent	0.5%	0.5%
95% CI	0.0%–2.6%	0.0%–2.6%
Not age-eligible for HPV vaccination (*N* = 784)		
Rate	0	0
Percent	0.0%	0.0%
95% CI	0.0%–0.5%	0.0%–0.5%
Total (*N* = 995)		
Rate	1	1
Percent	0.1%	0.1%
95% CI	0.0%–0.6%	0.0%–0.6%
HPV+LBC triage screening (N = 1,992)		
Age-eligible for HPV vaccination (*N* = 418)		
Rate	11	9
Percent	2.6%	2.2%
95% CI	1.3%–4.7%	1.0%–4.0%
Not age-eligible for HPV vaccination (*N* = 1,574)		
Rate	9	4
Percent	0.6%	0.3%
95% CI	0.3%–1.1%	0.1%–0.6%
Total (*N* = 1,992)		
Rate	20	13
Percent	1.0%	0.7%
95% CI	0.6%–1.5%	0.3%–1.1%
HPV+DS triage screening (N = 2,008)		
Age-eligible for HPV vaccination (*N* = 449)		
Rate	13	9
Percent	2.9%	2.0%
95% CI	1.6%–4.9%	0.9%–3.8%
Not age-eligible for HPV vaccination (*N* = 1,559)		
Rate	11	8
Percent	0.7%	0.5%
95% CI	0.4%–1.3%	0.2%–1.0%
Total (*N* = 2,008)		
Rate	24	17
Percent	1.2%	0.8%
95% CI	0.8%–1.8%	0.5%–1.4%

CIN, cervical intraepithelial neoplasia; DS, dual-stained; HPV, human papillomavirus; LBC, liquid-based cytology.

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Canfell K, Caruana M, Gebski V, et al. Cervical screening with primary HPV testing or cytology in a population of women in which those aged 33 years or younger had previously been offered HPV vaccination: Results of the Compass pilot randomised trial. PLoS Med. 2017;14(9):e1002388.

Appendix 2: Positive predictive values for CIN2+ and CIN3+, by allocation group and referral pathway.

CIN grade and group	Referral pathway			
	Referred on basis of primary screening test*	Referred as reflex triage positive*	Referred to colposcopy for the first time at 12-month follow-up test	All referrals
CIN2+				
LBC screening (*N* = 995)				
PPV	0/2	0/3	1/22	1/27
Percent	0.0%	0.0%	4.5%	3.7%
95% CI	0.0%–84.2%	0.0%–70.8%	0.1%–22.8%	0.0%–19.0%
HPV+LBC triage screening (*N* = 1,992)				
PPV	10/25	8/15	2/35	20/75
Percent	40.0%	53.3%	5.7%	26.7%
95% CI	21.1%–61.3%	26.6%–78.7%	0.7%–19.2%	17.1%–38.1%
HPV+DS triage screening (*N* = 2,008)				
PPV	9/23	13/34	2/22	24/79
Percent	39.1%	38.2%	9.1%	30.4%
95% CI	19.7%–61.5%	22.2%–56.4%	1.1%–29.2%	20.5%–41.8%
CIN3+				
LBC screening (*N* = 995)				
PPV	0/2	0/3	1/22	1/27
Percent	0.0%	0.0%	4.5%	3.7%
95% CI	0.0%–84.2%	0.0%–70.8%	0.1%–22.8%	0.0%–19.0%
HPV+LBC triage screening (*N* = 1,992)				
PPV	8/25	4/15	1/35	13/75
Percent	32.0%	26.7%	2.9%	17.3%
95% CI	14.9%–53.5%	7.8%–55.1%	0.0%–14.9%	9.6%–27.8%
HPV+DS triage screening (*N* = 2,008)				
PPV	7/23	9/34	1/22	17/79
Percent	30.4%	26.5%	4.5%	21.5%
95% CI	13.2%–52.9%	12.9%–44.4%	0.1%–22.8%	13.1%–32.2%

Overall, 181 women were referred to colposcopy; histological outcomes could be ascertained for 177 (98%), which are reported here; those for whom no histology result was available were assumed to have not received biopsy and were thus considered as screen positive but without detected CIN2+ in this intention-to-treat analysis.

*Each of these groups includes 2 women referred to colposcopy initially who had a histological result of a grade lower than CIN2 and were then referred for follow-up at 12 months, and in whom CIN2+ was then detected at the 12-month colposcopy. See protocol (S1 Text).

CIN, cervical intraepithelial neoplasia; DS, dual-stained; HPV, human papillomavirus; LBC, liquid-based cytology; PPV, positive predictive value.

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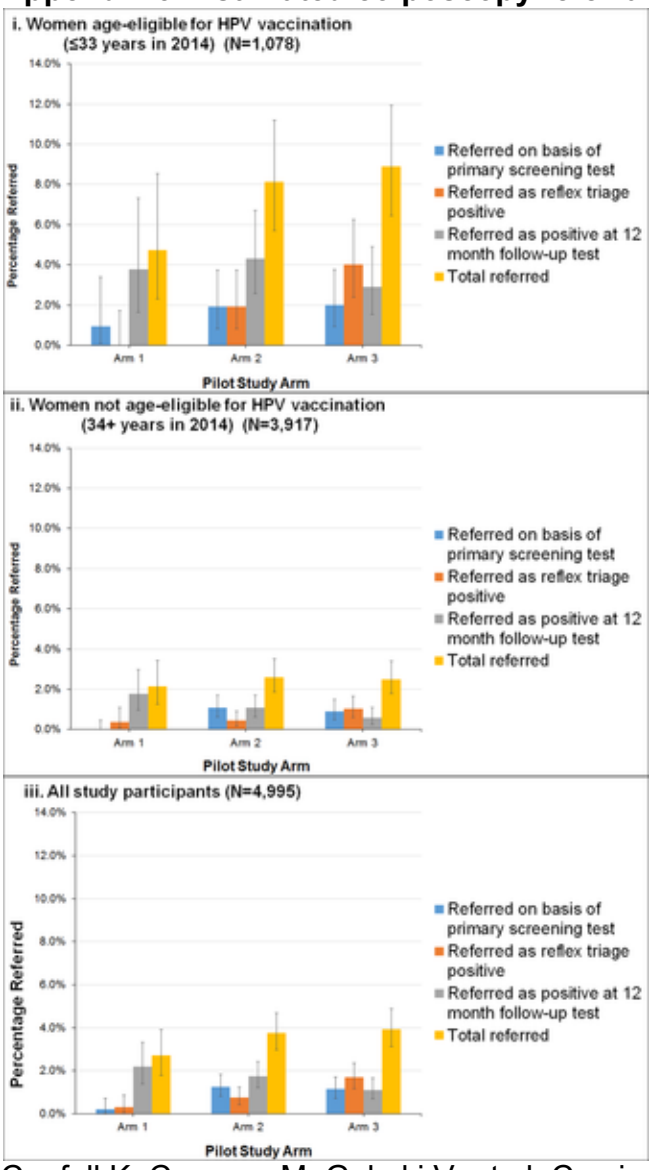
Canfell K, Caruana M, Gebski V, et al. Cervical screening with primary HPV testing or cytology in a population of women in which those aged 33 years or younger had previously been offered HPV vaccination: Results of the Compass pilot randomised trial. PLoS Med. 2017;14(9):e1002388.

Appendix 3: Estimated colposcopy referral rates by study group.



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