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FC08-09 HPV TESTING USING XPERT HPV ON SELF-COLLECTED VAGINAL SWABS VS. CLINICIAN-COLLECTED CERVICAL SAMPLES ........................................................................ 421
Background / Objectives

Identify socio-economic and demographic determinants for non-attendance in cervical screening

Methods

Design: Population-based case-control study

Setting: Sweden

Population: Source population was all women eligible for screening. Based on complete screening records, two groups of women aged 30 - 60 were compared. One group (N=266,706) attended within 90 days of invitation. The other group (N=314,302) had no smear registered for 6-8 years.

Main outcome measures: Risk of non-attendance by 9 groups of socioeconomic and demographic variables

Methods: Unadjusted odds ratios (OR) and OR after adjustment for all variables in logistic regression models were calculated.

Results

Women with low disposable family income (OR 2.06; 95% confidence interval (CI) 2.01-2.11), with low education (OR 1.77; CI 1.73-1.81) and not cohabiting (OR 1.47; CI 1.45-1.50) were less likely to attend cervical screening. Other important factors for non-attendance were being outside the labour force and receiving welfare benefits.
Swedish counties are responsible for running screening programs; adjusted OR for non-participation in counties ranged from OR 4.21 (CI 4.06-4.35) to OR 0.54 (CI 0.52-0.57), compared to the reference county. Being born outside Sweden was a risk factor for non-attendance in the unadjusted analysis but this disappeared in certain large groups after adjustment for socioeconomic factors.

Conclusion

Low income and low education were associated with increased probability of non-attendance. Low attendance among large groups of immigrant women might be explained by socio-economic factors. Residing in particular Swedish counties was also a strong independent factor. As counties are responsible for effectuating the screening program this indicates considerable potential for improvement of cervical screening attendance in several areas if best practice of routines is adopted.
FC 01-02
SCREENING HISTORY IN CERVICAL CANCER PATIENTS ≥ 55 YEARS DIAGNOSED DURING 1990-2013 IN DENMARK

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Background / Objectives

The incidence of cervical cancer has declined significantly in developed countries following the implementation of cervical cancer screening. However, previous studies have reported that ~50% of cervical cancer patients have not attended screening and that the fraction of unscreened women increases by age. This study aimed to describe the temporal pattern of screening in cervical cancer patients ≥55 years diagnosed during 1990 – 2013 at Aarhus University Hospital, Denmark

Methods

This hospital based cohort study included women ≥ 55 years diagnosed with cervical cancer at the Department of Pathology, Aarhus University Hospital, Denmark during 1990-2013 (n=515). Information on their previous history of cervical cancer screening was obtained from the Danish Pathology Databank.

Results

Overall, 47.0% (95% CI 42.6 – 51.4) had never been screened prior to cervical cancer diagnosis. The fraction of never screened cases declined over calendar time from 69.8% (95% CI 61.4 – 77.3) in 1990 – 1994 to 20.0% (95% CI 12.7 – 29.2) in 2010 – 2013, reflecting a period effect of screening. Conversely, the fraction of never screened cases increased by age from 22.5% (95% CI 14.6 – 32.0) in women aged 55 – 59 years to 63.2% (95% CI 49.3 – 75.6) in women ≥ 80 years. Noteworthy, the vast majority of never screened (90.9% ; 95% CI 86.6 – 94.2) lived in a period where screening was not available, whereas only 9.1% (95% CI 5.8 – 13.4) had not been screened although screening was available. Among women who had been screened 5 years or 5-10 years prior to cervical cancer diagnosis, 84.6% (95% CI 77.1 – 92.2) and 85.9% (95% CI 75.0 – 93.4) had a normal cytology result, respectively.

Conclusion

Cervical cancer in older women may partly be attributed to a lack of screening or due to a failure in screening. However, older women were in general less screened because they either lived in a period where screening was not available or they were too old to be screened when screening was implemented. Furthermore, since
previous studies have shown that older women are at high risk of cervical cancer\textsuperscript{1,2}, more information on how to best screen older women is needed.

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INVITING WOMEN TO CERVICAL CANCER SCREENING AT THE AGE OF 65

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Background / Objectives

In Finland the organized cervical cancer screening program invites women for routine screening up to the age of 60. Some municipalities also invite 65-year-olds. The aim is to study whether screening at the age of 65 reduces cervical cancer mortality.

Methods

Screening records for women aged 55 and above were collected from the mass screening registry in 1991—2014 (612,622 women born in 1926—1946). Cervical cancer deaths (N=265) were linked from the cancer registry for women aged 65 and above.

Results

Of all women, 383,411 were invited of whom 85% attended to screening at least once during the follow-up. Of all invited women, 77,479 (13%) received an invitation to routine screening at the age of 65. The risk of death due to cervical cancer was reduced for women who were screened at the age of 65 (RR = 0.57 (95% CI = 0.32–0.95)).

Conclusion

Mortality was reduced for women screened at the age of 65. However, a more detailed examination of the effect of previous screening history is still needed, e.g. taking into account participation to screening at younger ages and previously detected abnormalities. The results will help to assess until what age the whole target population should be invited to screening.
BACKGROUND / OBJECTIVES

An organised population-based cervical cancer screening was set up in 2013 in the Flemish Region for women aged 25 to 64, based on a call-recall system. Cytology is used as primary screening test and HPV detection as triage for atypical cells. The Belgian Cancer Registry (BCR) calculates yearly quality indicators to monitor this program on demand of the Agency for Care and Health of the Flemish Ministry of Welfare, Public Health and Family.

METHODS

Besides new cancer diagnoses, the BCR collects all anatomo-pathological results of cervical samples in a central cyto-histopathology registry, which is completed with administrative data from health insurance companies. BCR plays a crucial role in the cost-effective organisation and the quality assurance of the screening program due to the centralisation of all these data and due to the possibility of linking at the personal level using a unique patient identifier. By linking these databases with a Flemish population registry, BCR calculated for 2013 several quality indicators.

RESULTS

In 2013, 64% of the Flemish female population between 25 and 64 years old was covered by the screening program. 7% of the eligible women had an abnormal screening. 27% of the women with an abnormal screening had no follow-up within one year. 236 new invasive tumours were diagnosed within the target population. Analysis of the screening history revealed that 111 of these tumours were diagnosed in women that were not screened within 5 years before. About 40% of the tumours in these non-screened women are stage I. In contrast, more than 70% of the women who had at least one screening in the last 5 years had a stage I tumour. 82 of the 236 women with an invasive tumour were tested for HPV in the past 5 years, whereof 10 with a negative HPV test result.

CONCLUSION

Quality indicators reveal the weaknesses in the screening program. They can be directly translated into policy decisions to increase the program coverage, to improve the follow-up rate after abnormal screening and to investigate whether or not these
cancers are truly HPV negative. Centralisation of databases and the possibility of individual linking are crucial to a successful screening program.

References

Acknowledgements

The Agency for Care and Health, part of the Flemish Ministry of Welfare, Public Health and Family, finances the Flemish cervical cancer screening program. It subsidizes the Center for Cancer Detection to carry out the program and the BCR to support the organization and evaluation of the program.
Cervical Screening in Sweden in 2015

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Background / Objectives

To collect data to enable calculation of key quality indicators and basic statistics on cervical screening in Sweden.

Methods

We collected individual level data on all (both organized and non-organized) cervical cytologies and histopathologies in Sweden in 2015, as well as individual level data on all invitations for cervical screening that were sent.

Results

There were 723,500 cervical smears (662,350 tests were cytologies and the remainder were HPV tests). Organized smears (smears resulting from an invitation) constituted 69% of smears. The screening test coverage of the target population was calculated by linkage with the population registry (all resident women aged 23–60) and found to be 81%. The coverage has been similar for >10 years, but varied greatly between counties (from 71% to 91%) and over time. The incidence of the disease screened for (cervical cancer) was also stable over time at the national level, but varied between counties. There were 7,982 women with HSIL+/AIS in cytology in 2013. Of these, 181 women had not been followed up with biopsy by 2014-12-31.

Conclusion

Straightforward collection of all screening data in the country enables reliable reporting of screening quality indicators which are the basis for evidence-based optimization and innovation of the program.
NORDSCREEN – AN INTERACTIVE TOOL FOR PRESENTING CERVICAL CANCER SCREENING INDICATORS IN NORDIC COUNTRIES

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Background / Objectives

Quality assurance and improvement of cancer screening programs require up-to-date monitoring systems and evidence-based indicators. The Nordscreen database [1] is planned to include performance and outcome indicators for cervical cancer screening programs in the Nordic countries and Estonia. The tool will be publicly available and facilitate comparison of cancer screening programs over time and between the Nordic countries.

Methods

The screening data originates from population-based mass screening registries in each of the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden) and Estonia. Comparability between countries is ensured by using uniform data structure. The developed indicators are based on the European guidelines for cervical cancer screening and other ongoing research projects. [2] Fact sheets summarising the cancer screening policies and programs in place in all the Nordic countries and Estonia will be created to provide context for the indicators.

Results

Currently cervical cancer screening test coverage data is available from Norway (years 1992 - 2015) and Finland (1991 - 2014) with other Nordic countries and Estonia to be included soon. The test coverage within screening interval of 3 years in age group 30-59 was 75.0% in Norway in 2014 (60.4% in Finland). Test coverage in 2014 increased to 90.4% in 10-year follow-up in Norway (82.2% in Finland). The application can be present data in graphical or table form based on user specifications such as follow-up time, calendar year and age group.

Conclusion

Lower test coverage in Finland can be explained by policy of 5-year screening interval and that mass screening registry in Finland only includes tests that are provided within the organized screening process whereas Norwegian data also includes opportunistic screening tests. Despite some limitations, the performance and outcome indicators are likely to be relevant to many stakeholders such as researchers, policy makers and journalists. In the future, the database may also be expanded to on-going screening programs for breast cancer and colorectal cancer.
References


TEN YEARS EXPERIENCE IN 541.000 CASES: LIQUID BASED CYTOLOGY AND COMPUTER-ASSISTANCE COMPARED TO CONVENTIONAL CYTOLOGY

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Background / Objectives

Major studies showed inconsistent results for the comparison of liquid-based cytology (LBC) with conventional cytology (CC). However, some trials found a significantly higher sensitivity for HSIL (high grade intraepithelial lesions) with the computer-assisted ThinPrep-Imaging-System (TIS) compared to conventional cytology (CC) and even manually read LBC. Here we report the performance of TIS compared with CC in women who participate in the German cervical cancer screening program.

Methods

At Cytomol, a commercial lab specialized in cervical cancer prevention, since 2007 all LBC specimens have been processed by TIS. In Germany LBC is reserved to privately insured and self-paying patients while public healthcare only reimburses CC. To avoid bias we split this analysis between privately insured and self-paying patients. Finding rates of cytologic abnormalities with TIS and CC were compared. Cytologic diagnoses originally reported in the Munich Nomenclature II (MN; with the use of the unofficial Pap IIW category) until 30.6.2014, from then in the MN III (which is still the reporting standard in Germany) were translated to TBS (The Bethesda System).

Results

From 2007 to 2016 463.966 slides of privately insured patients have been analyzed among them 320.416 by TIS and 143.550 with CC. Except of extremely bloody and very cell-rich probes 97.4% of the smears were accepted for analysis by TIS. TIS had a rate of LSIL (low grade intraepithelial lesions; MN III: Pap IIID1) of 2.03% compared to 0.54% for CC, an increase of 276%. HSIL (MN III: Pap IIID2 + Pap IVa/b) was found in 1.10% with TIS vs 0.33% with CC (+233%). The ASC-US rate (MN III: Pap II/p/g + III-p/g) was 2.54% with TIS and 1.21% with CC, an increase of 110% which is much lower than the rise in LSIL and HSIL. This points to a higher sensitivity of TIS without decreasing specificity. Among 77.282 self-paying patient cases (all TIS) we found almost the same rates for ASC-US and LSIL but 51% more for HSIL compared to private patients. All these results remained stable over the 10 years analyzed. With TIS 20.4 slides/h were screened, compared to 12.2 for manually read TPs and 8.0 with CC. However, the technical expenditure for TIS was much higher.

Conclusion
In long-time routine use of a commercial lab computer-assisted LBC with the ThinPrep-Imaging-System provided higher sensitivity and higher productivity without lower specificity at the cost of higher technical expenditure.
The value of “diagnostic cytology” with p16/Ki-67 dual-staining

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Background / Objectives

When implemented appropriately, cervical cancer screening has the power to save lives. Its effectiveness, however, rests on the sensitivity and specificity of the diagnostics themselves—namely, pap cytology and HPV DNA testing. The introduction of p16/Ki-67 dual-stain cytology as a triage test for abnormal pap results offers the ability to improve detection of precancer in equivocal and abnormal cervical cells. This study seeks to evaluate the performance of dual-stain “diagnostic cytology” in Belgium (women age 25-65) through a systematic literature review; this includes a component of meta-analysis in order to draw conclusions about the increasingly complex, quantitative body of existing knowledge available.

Methods

A literature search was conducted of published studies from 1 May 2016 according to CRD, PRIMSA, and NICE guidelines. Studies needed to report diagnostic performance with key metrics: sensitivity, specificity, detection rate, odds ratio, PPV, NPV, true and false positives, and true and false negatives. These outcomes were chosen since they are the most significant and widely used outcomes to compare performance, and can be calculated from one another using established formulas. Sensitivity and specificity were chosen as outcome measures in the final meta-analysis based on guidance from the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy. The meta-analysis of dual-staining performance focused on sensitivity and specificity. Results of multiple studies collected during the literature review were combined into a single, standardized metric for comparison across different tests.

Unlike meta-analyses of treatment intervention effects, meta-analyses of diagnostic test accuracy must allow for the trade-off between sensitivity and specificity that occurs between studies whose threshold values for test positives and negatives vary. Hierarchical models (in contrast to many classical regression models) allow for correlation between sensitivity and specificity, in addition to the aforementioned trade-off. Therefore, the bivariate random effects model was used in this meta-analysis because of its ability to jointly evaluate sensitivity and specificity, thus providing an estimate of the diagnostic accuracy of dual-staining.

Conclusion
Dual-stain “diagnostic cytology” with p16/Ki-67 is an attractive biomarker for triage in cervical cancer screening. In a Belgian screening population (age 25-65 years), dual-stain cytology offered significant gains in sensitivity with minimal reduction in specificity. This could lower the number of cases of missed disease and reduce morbidity from additional interventions such as colposcopy and biopsy.
CERVICAL CANCER TUMOR HISTOPATHOLOGY CLASSIFICATION - IN THE SWEDISH NATIONAL AUDIT OF CASES FROM 2002-2011

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Background / Objectives

The Swedish cervical screening program is changing from cytology to HPV based screening in 2017. To document the performance of the screening program and to provide a basis for evaluating the effect of changes in the program we performed a nationwide audit of the cervical cancer cases from 2002 to 2011. Our aim with this analysis is to compare different sources of information for the tumor histological classification, and to determine how robust this classification is for subsequent analyses of data.

Methods

Data on all 4254 cases of cervical cancer or unspecified uterine cancer diagnosed between 2002 and 2011 was identified from the Swedish National Cancer Registry. Tumor histopathology is provided as a SNOMED classification code by the local pathologist and the clinician at the initial diagnosis. For the Audit an experienced gynecologist reviewed all medical records to identify cervical cancer cases with primary, invasive, epithelial tumors of cervical origin and to extract relevant data on e.g. treatment, mode of detection and histological classification of the tumor. Additionally we collected diagnostic slides and tissue blocks: an external review of the tumor was performed by a senior pathologist and HPV-genotyping was done on the tissue material. The Swedish National Cervical Screening Registry (NKCx) provided data on screening for all cases and matched population controls.

Results

One of 26 different SNOMED codes was reported for all except 6 cases. The systematic external review on 86% of the cases classified the tumors into following groups: Squamous Cell Carcinoma, Adenocarcinoma, Adenosquamous carcinoma and rare histological types (i.e. neuroendocrine, small cell, undifferentiated cancer). The medical record provides information on histology in various levels of detail and in a non-systematic form, so we could only extract this information for 69% of cases, in the same categories as above. There is a high concordance (87%) between all three sources of histology classification. For 9.8% of cases with discrepancies in the histology classification or missing data we decided for a final classification based on:
1) external histopathology review of the sample (in cases of good quality samples), 2) medical record and 3) initial SNOMED diagnosis (this was changed only in 7.5% of cases) for subsequent analyses of data.

Conclusion

Thorough nationwide ascertainment from multiple sources found limited ambiguity regarding tumor histology. For most uses, any one of the sources can be used, as the concordance between different sources of data is high.
HPV testing in routine cervical screening in rural Malawi – prevalence, link to clinical findings and challenges

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Background / Objectives

Developed countries are moving fast to replace cytology-based cervical screening with HPV primary testing. Our primary objective was to establish feasibility of HPV testing for primary screening in Malawi and to identify/address the major challenges to implementation, including outcomes of different collection devices and media. We also aimed to determine prevalence of HPV genotypes in the Nkhoma region.

Methods

Specimens were obtained from women attending routine VIA (Visual Inspection with Acetic acid) clinics in Nkhoma Hospital catchment area. VIA assessments were carried out by competent providers. HR-HPV prevalence was established using samples collected in Preservcyt® and tested by Xpert®HPV according to manufacturer’s instructions. Modifications were also tried, including reduction in collection volume, change of collection medium, use of self-collected samples and different collection devices. A sensitive multiplex PCR based assay (Papilloplex AnyHPV) was used for genotyping on a subset of samples selected according to the VIA result (139 VIA -; 156 VIA+; 42 VIA suspicious cancer).

Results

HR-HPV positivity using Xpert HPV was ~20%(n=750). Multiple infections were common and HR-HPV prevalence in HIV+ women was 43%. For HR-HPV, concordance was good between Xpert and Papilloplex HPV tests (k=0.68). In women with suspicious cancers HPV16,18 and 45 predominated (22.7%, 11.4% and 11.4%). The most frequently detected HPV type in VIA+ women was HPV16 but Xpert P3 group (HPV52>35>31>33>58) dominated. A number of VIA+ women were HPV negative, most were not due to LR-HPV presence. HPV+ results were frequently reported in VIA- women, with HPV16 the most frequent individual type, while P3 predominated and LR-HPV was detected in ~20%. Reduction of collection medium to 5ml, alternative media and self-collected samples gave comparable HPV results.

Conclusion
HPV16 was the commonest individual type detected in women who are VIA+ /suspicious cancer and in VIA- women. HPV31 and related types (Xpert P3) is the most commonly detected subgroup in VIA+ and VIA- women but not in those with suspicious cancers.

Xpert HPV showed high concordance with Papilloplex for HR-HPV. The latter test is more sensitive and detected LR-HPV, but is less suitable for LMIC4. Xpert® HPV is straightforward, has rapid turnaround and should be validated with low cost collection systems.

There was high correlation between VIA suspicious cancers and HR-HPV positivity but disagreement between HPV positives and VIA positives requires further analysis before considering HPV testing for primary screening.

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Cervical cancer screening in the remote island of Principe

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Background / Objectives

The island of Principe, part of the archipelago of São Tomé and Príncipe, in the Gulf of Guinea, suffers the problems inherent to its double insularism. Its 7,000 inhabitants have very limited access to health care. In 2014, the privately funded NGO Ascedere started a cervical cancer screening program. A previous report has shown that this island has a unique HPV genotype distribution: HPV prevalence is high (36.7%), but HPV16 and 18 are very rare (0 and 2%, respectively) 1.

Methods

Retrospective evaluation of the records of the women screened between January 2014 and September 2016.

Results

During this time period 972 women were screened, with a mean age of 35.5±10.04 years old (21-83 years old). Out of these, 968 (99.6%) had a satisfactory Pap test; 150 (15.5%) had an abnormal Pap test: ASC-US 58 (6.0%), LSIL 56 (5.8%), LSIL-H 2 (0.2%), ASC-H 9 (0.9%), HSIL 23 (2.4%), carcinoma/adenocarcinoma 2 (0.2%). Colposcopy was indicated in 111 (11.4%) women; 31 (27.9%) of them did not show up for evaluation. An histologic diagnosis of HSIL (CIN2 p16+/CIN3) was made in 35 women (3.6%) and of invasive carcinoma in 2 (0.2%). Of the women with HSIL, 33 (94.3%) had an excision of the transformation zone.

The mean age of women with a final histological diagnosis of HSIL/invasion was similar to those without evidence of it (36.5±11.59 vs. 35.5±9.97 years, p=0.542). Only one case was detected in a woman younger than 25 years old. There were no differences in contraception use, parity, use of alcohol, menopause status, existence of co-infections (C. trachomatis, N. gonorrhoea and T. vaginalis), age of sexual debut, menarche, age of first delivery, or number of sexual partners in women with and without HSIL/invasion. There were no differences in HIV status or smoking, but the occurrence of both risk factors is very rare in this setting.

Conclusion
Strategies must be implemented to reduce the number of women lost to follow-up. Despite the low prevalence of HPV16 and 18, still there is an elevated risk of HSIL, which justifies the maintenance of a cervical cancer screening program and the introduction of the nonavalent vaccine.

References

Background / Objectives

The incidence of cervical cancer (CC) varies greatly with a large difference between developing and developed countries, where CC cases have been significantly reduced since the implementation of effective screening programmes. Exfoliative cervicovaginal cytology has been regarded as the gold standard for cervical cancer screening programs. Limitations are incorrect and inadequate sampling in 5-10% of cases. Manual Liquid Based Cytology (MLBC) is a technique that enables cells to be suspended in a monolayer and thus improves detection of precursor lesions and specimen adequacy. The residual sample can be used for other tests like detection of HPV, DNA and immunocytochemistry. Cell blocks can be prepared from all types of cytological specimens, with advantage of cell blocks is that many slides can be prepared for extensive panels of immunostains. Overexpression of p16INK4a in almost all cervical precancer has been shown to be directly linked to the transforming activity of E7 oncoprotein, which is produced by HPV. Cellular accumulation of p16INK4a has been shown to be directly linked to the transforming activity of E7 oncoprotein, which is produced by HPV. Cellular accumulation of p16INK4a has been shown to be directly linked to the transforming activity of E7 oncoprotein, which is produced by HPV. Our current PCR set-up gives rapid, type-specific HPV detection with a turnaround time of less than 24 hrs and cost-effectiveness compared to commercial available alternatives.

Methods

Samples from examined patients were collected using the direct to vial technique from 75 patients in the age group of 20 to 60. Cervex brush was used to scrape the cervix. were done for collecting samples for HPV testing and cell block processing in separate vials containing 5ml of the fixative and centrifuged at 2000 rpm for 5 minutes. The supernatant was decanted and 1-2 ml of polymer solution was added. The cell blocks studied were lesser than the liquid based cytology cases, using equal amount of cell block fixative (10% formalin and 95% alcohol) and to compare it with conventional pap smears. In the present study p16 markers were done on cell block
preparation. DNA was extracted from 50 LBC samples using the manual Phenol IsoChloroform method.

Results

conventional pap smear (CPS) vs cell block (CB) is 75 cases VS 50

NILM vs Chronic cervicitis (47/36), LSIL (3/1), HSIL (2/1), SCC (3/3), infections (7/7), Koilocytic atypia (1/1), AGUS (1/1), Unsatisfactory on CPS vs No deposit on CB (5+6/25).

Histopathological correlation was available for 25 cases. Out of which 22 being chronic cervicitis and 3 neoplastic which correlated with cell block diagnosis.

The results for HPV DNA testing done for 50 cases showed 5 cases positive for HPV. 4 cases were low risk HPV while one case was high risk HPV DNA 16.

Conclusion

MLBC is an useful costeffective method for early detection of cervical cancer in resource poor settings.

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PREVALENCE OF SEXUALLY TRANSMITTED INFECTIONS AMONG 2000 WOMEN IN RURAL GHANA - THE ACCESSING STUDY


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Background / Objectives

To determine the prevalence of 18 sexually transmitted infections (STI) among 18-65 year old women living in the rural North Tongu District in Ghana.

Methods

This population-based study included 2000 women who were representatively selected by geographical distribution and invited to self-collected vaginal samples (Evalyn brush, Rovers). Extracted DNA was tested for 18 STIs by multiplex PCR followed by Luminex bead-based hybridization (STIP Assay, Schmitt et al., 2014, J Infection 69:123).

Results

1937/2000 samples collected during the ACCESSING study had sufficient DNA quality and were eligible for STI analyses. The median age of the study population was 30 years. The most prevalent infectious agents were Gardnerella vaginalis (53.7%), Atopobium vaginae (49.1%) and Mycoplasma hominis (33.7%), all known to be associated with bacterial vaginosis (BV). Using a scoring system (according to Schmitt et al. 2014), 24.4% (472/1937) of the women showed a strong or very strong indication for BV. Ureaplasma parvum and Ureaplasma urealyticum were detected in 50.1% (971/1937) and 30.5% (591/1937) of the samples, respectively. Chlamydia trachomatis, causing pelvic inflammatory disease, was detected in 4.9% (94/1937; 95% CI: 4.0% to 5.9%) of the women and Neisseria gonorrhoeae in 2.5% (48/1937). Trichomonas vaginalis showed a prevalence of 4.1% (79/1937) and Treponema pallidum was detected in one sample only.

Conclusion

Data for BV is very rare in the literature and therefore the prevalence of the infectious agents causing BV along with its scoring system provide a first insight into the
estimated prevalence in Ghana. Prevalence reported for WHO African Region for Neisseria gonorrhoeae with 2.3% is similar to what we found in our study population. On the contrary WHO reports a prevalence of 2.6% for Chlamydia trachomatis, i.e. 2.3% lower than our data. This difference is statistically significant (p-value < 0.0001). This could possibly be due to the relatively young age group with a median age of 30 years in our study. Treponema pallidum with only one case is below the WHO reported prevalence of 3.5%. This differences could be due to the acutely infected vaginal sample used for our analysis, compared to anamnestic serum samples used for the detection of Treponema pallidum in many studies.

The rare estimates of STI prevalence in developing countries published in the literature represent a contrast to the high burden of disease associated with STIs. Therefore, this data is of great importance. At the same time it highlights the urgent need for further research in this field, due to varying prevalence rates seen, to guide future public health policy.
The Study of Folate Receptor-Mediated Staining Solution (FRD™) Used for Detecting High Grade Cervical Lesions and Invasive Cancer

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Background / Objectives

To evaluate the sensitivity and specificity of Folate Receptor-Mediated Staining Solution (FRD™) used in detecting high grade cervical lesions and invasive cancer.

Methods

The FRD™ is designed for rapid visualization of CIN2+. Results are determined immediately (within 30 sec) after staining of the entire cervical epithelia. Patients who visited the outpatient clinic were recruited for this study. HR-HPV and cytology tests were performed before FRD™ testing. During the FRD™ testing both the cervix and cervical canal were stained. Colposcopy and biopsy was performed on the patients with either ≥ASC-US cytology test, positive HPV test, or positive FRD™ test. An ECC was completed on patients if the result was positive for the FRD™ test in the cervical canal, cytology result was AGC, or after a colposcopy the transformation zone of cervix was type II, III.

Results

This study involved 404 women. CIN 2+ was found in 65 patients (16.1%) including 9 patients with cervical invasive cancer. CIN 1 and inflammation accounted for 7.4% (30/404) and 76.5% (309/404), respectively. Cytology results included: NILM: 140 (34.7%), ASC-US: 119 (29.5%), ASC-H: 5 (1.2%), LSIL: 81 (20.0%), HSIL: 59 (14.6%). The HPV positive rate was 93.6% (378/404). Positive FRD™ test was determined in 53.2% (215/404). The sensitivity of cytology, HPV, and FRD™ in detecting CIN 2+ lesions was 90.8%, 96.9%, and 80.0%, respectively. The specificity was 39.5%, 7.1%, and 51.9%, respectively.

Conclusion

The specificity of the FRD™ is the highest, comparing with cytology and HPV test, and the sensitivity is compatible. The FRD™ is suitable for detecting high grade cervical lesions and invasive cancer. Test results are determined immediately (within 30 sec) after staining of the entire cervical epithelia for detecting abnormal cervical lesions (CIN2+). Also, the FRD™ is easy to operate, since operators do not need professional training or professional equipment. Therefore, the FRD™ testing is an accessible and inexpensive method, especially for less-developed countries, and can be used as alternative cervical cancer detecting method.
COMPARISON OF THREE HPV ASSAYS IN DETECTION OF CERVICAL CANCER


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Background / Objectives

Without HPV vaccination, the cervical cancer incidence rate is still increasing in China. Screening remains the primary way to prevent cervical cancer, and there were so many methods that can be used for HPV detecting. This study was to describe the dominated HPV types in squamous cell cancer (SCC) and adenocarcinoma (ADC) in China, and to compare the clinical performance of three assays detecting HPV E6/E7 DNA, E6/E7 mRNA and E6 oncoprotein in cervical cancer.

Methods

487 women were recruited from 4 central hospitals in China, among whom 448 were diagnosed with SCC and 39 with ADC. Specimens of exfoliated cervical cells from these participants were tested by 3 assays: HPV E6/E7 DNA-based detection (BD onclarity) which could test 6 individual HPV genotypes (HPV16, 18, 31, 45, 51 and 52) and 3 groups of hrHPVs (HPV33/35, 35/39/68 and 56/59/66), HPV E6/E7 mRNA detection of 14 HR HPV types (APTIMA) and HPV16/18 E6 oncoprotein strip test.

Results

SCC mainly occurred in women at 45-49 years old, and ADC was most frequently observed in women at 40-44 and 60-64 years old in China. HPV16, HPV18 and HPV33/58 were dominated in SCC, as to ADC, the dominated genotypes were HPV 16, HPV18 and HPV39/68/35. HPV 16/18 accounted for 85.4% cervical cancer in China. HPV DNA test and HPV mRNA test showed the same sensitivity in detection for cervical cancer (94.9% vs. 94.0%, P>0.05). When focused on HPV 16/18, we noticed that HPV 16/18 DNA detection found more cases than HPV 16/18 E6 oncoprotein testing (85.4% vs. 79.3%, P<0.05). And the positivity rate of HPV 16 DNA was significantly higher than that of HPV 16 oncoprotein (77.6% vs. 69.6%, P<0.05), while the positivity rate of HPV 18 DNA was the same as HPV 18 oncoprotein (11.9% vs. 13.6%, P>0.05).

Conclusion
HPV16 and HPV 18 were dominated in SCC and ADC in China, thus HPV16/18 vaccine would protect about 80% Chinese women from cervical cancer if the vaccination was implemented. HPV DNA and HPV mRNA testing showed almost the same sensitivity in detection of cervical cancer, both of which were better than HPV 16/18 E6 oncoprotein testing.
FC 02-07
COMPARISON OF VIA with molecular testing using HPV-DNA and the biomarker p16INK4a/Ki-67 for cervical cancer screening in a high-prevalent cervical cancer setting

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Background / Objectives

Cervical cancer is the most common cause of cancer death among women in Kenya. Visual inspection with acetic acid (VIA) has become the standard screening method. However, diagnostic accuracy of VIA is limited due to observer subjectivity and participation in VIA screening program is very low. We therefore compared VIA with molecular screening methods i.e. HPV testing and the p16INK4a/Ki-67 immunocytochemistry, a biomarker specific for HPV-transformed cells.

Methods

In two rural clinics belonging to the catchment area of Moi Teaching and Referral Hospital women who participated in the VIA program offered by the regional health service were invited to also participate in the molecular screening study. Consenting women had a liquid-based cytology sample taken before conducting VIA according to the manual on visual screening for cervical neoplasia published by IARC,2003. Liquid-based cytology (LBC) samples were used (i) to prepare a microscopic slide using Thinprep® 2000 processor (Hologic®) and (ii) performing HR-HPV DNA (HC2, Qiagen®) assay on the remaining sample. Slides were sent to Heidelberg, Germany, where p16INK4a /Ki-67 immunostaining using Roche® CINtec PLUS® kits was performed.

Results

576 women have been recruited so far. The analysis is done on a preliminary dataset of 321 women. 158 (49.2%) women were 30+ years old, 37 (6.2%) were multipara, 33 (10.7%) of 308 women reported to be HIV-infected. The positivity rate of HPV DNA, VIA and p16INK4a /Ki-67 were 30.8%, 4.98%, and 2.5%. 8.1% of all HPV DNA samples were p16INK4a /Ki-67 positive. All p16INK4a /Ki-67 positive sample were
also HPV DNA positive. Of all VIA positive samples 31.3% were HPVDNA positive and none p16INK4a /Ki-67 positive.

**Conclusion**

The VIA results correlated poorly with both the HPV DNA status and the biomarker p16INK4a /Ki-67. Colposcopic follow-up of all HPV positive women will be done. The use of p16INK4a /Ki-67 as a triage test for HPV DNA positive women will be studied in future.
VULVAR CANCER: TWO PATHWAYS WITH DIFFERENT LOCALIZATION AND PROGNOSIS

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Background / Objectives

There are two etiologic pathways for vulvar squamous cell carcinoma (SCC). The first occurs in a background of lichen sclerosus and/or differentiated vulvar intraepithelial neoplasia (dVIN). The second is related to high-risk human papillomavirus (HPV) infection with its precursor lesion high grade squamous intraepithelial lesion (HSIL). The aim of this study was to investigate the predilection site and survival of HPV-related compared to non HPV-related vulvar SCCs.

Methods

Data of all consecutive patients with primary vulvar SCC treated at the Department of Gynaecologic Oncology at the Radboud university medical center are prospectively stored in a database: data of patients who have been treated between March 1988 and January 2015 were analyzed. All available histological specimens were tested for HPV with the SPF10/DEIA/LiPA25 system assay and p16INK4a immunohistochemical staining was performed using CINtec® histology kit. Vulvar SCCs were considered HPV-related in case of either >25% p16INK4a expression and HPV positive or >25% p16INK4a expression, and HSIL next to the tumour. The tumour localization, disease specific survival (DSS), disease free survival (DFS) and overall survival (OS) of patients with HPV-related and non HPV-related vulvar SCC were compared.

Results

In total 318 patients were included: 55 (17%) patients had an HPV-related vulvar SCC (Group 1) and 263 (83%) patients had a non HPV-related vulvar SCC (Group 2). The tumours in Group 1 were significantly more often located at the perineum compared to Group 2, 30% and 14%, respectively (p = 0.001). The DSS, DFS and OS were significantly better in the HPV-related than in the non HPV-related vulvar SCC patients.

Conclusion

HPV-related vulvar SCCs are more frequently located at the perineum and have a favourable prognosis compared to non HPV-related vulvar SCCs. Both localization of the tumour and the HPV-related pathway could explain the favourable prognosis. HPV-related vulvar malignancies seem to be a separate entity within vulvar SCC.
FC 03-02
The role of the antileukoprotease secretory leukocyte protease inhibitor (SLPI) in squamous cell carcinoma of the vulva in relation to HPV-infection and smoking habit of the patients

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Background / Objectives

It was previously shown that protein and mRNA expression of the antileukoproteinase SLPI was significantly inverse correlated with HPV-infection in HNSCC and led to the suggestion that elevated expression of SLPI protects against HPV-infection in HNSCC. In addition we could show that SLPI expression was upregulated in HNSCC patients reporting a smoking habit. Here we investigate whether this inverse correlation between HPV-infection smoking habit and SLPI expression could also be found in other HPV-driven cancers, namely vulvar squamous cell carcinoma (VSCC).

Methods

FFPE samples of 116 VSCC were analyzed by PCR and RT-qPCR for HPV-DNA-, and SLPI mRNA-expression and data were correlated. Data correlating HPV- and SLPI-expression with smoking habit are so far preliminary since at present only 68 patients files are complete. The same holds true for Kaplan-Meyer-analysis correlating HPV-status and SLPI-expression with overall (OS) and progression free survival (PFS).

Results

Of the analyzed 116 VSCC 10 (8.6%) are HPV-DNA positive (Genotyping by Sanger sequencing is ongoing, followed by HPV-RNA-analysis). Of the 68 patients with complete files 24 (35.3%) reported a smoking habit and of these patients 3 (12.5%) were HPV-positive. So far 3 further HPV-positive patients were identified as non-smokers and for the remaining 4 at present no data are available. SLPI-expression however, was independent of the smoking habit, 4.0-fold lower in HPV-positive than HPV-negative patients. Smoking on the other hand resulted, independent of the HPV-status of the patients, in 5.6-fold higher SLPI expression. HPV-positivity and low SLPI-expression are associated with better PSF (analysis of OS data is ongoing).

Conclusion

The data presented here indicate that SLPI plays a pivotal role in HPV-infection not only in HNSCC but also in VSCC and possibly also in other HPV-driven cancers.
This however, needs to be analyzed in future studies. Furthermore these data lead to the hypothesis that the smoking induced SLPI-increase is systemic rather than local, as assumed based on the HNSCC data.
FC 03-03
DNA COPY NUMBER ABERRATIONS ASSOCIATED WITH HPV-DEPENDENT AND -INDEPENDENT VULVAR CARCINOGENESIS

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Background / Objectives

Vulvar squamous cell carcinoma (VSCC) can develop through HPV-dependent (25%) and HPV-independent pathways, indicating a heterogeneous disease. High-grade vulvar intraepithelial neoplasia (VIN) is the precancerous state of VSCC but only a minority of VINs progress to cancer. Current clinical and histological classifications are insufficient to predict the cancer risk. Consequently, affected women are treated similarly with mutilating interventions. Hence there is a clinical need for objective biomarkers reflecting the cancer risk. Here we analysed copy number alterations (CNA) to assess the significance of molecular heterogeneity of vulvar lesions in relation to HPV status and cancer risk.

Methods

25 VSCC and 42 VIN, including VIN of women with associated VSCC (VIN with VSCC) and VIN of women who did not develop VSCC during > 10 year follow-up (VIN without VSCC) were analysed for HPV-status by means of p16INK4a immunohistochemistry and HPV testing. CNA were determined by whole-genome next-generation shallow sequencing and CGHcall, CGHregion and CGHtest analysis.

Results

HPV-positive VSCC (n=11) and HPV-negative VSCC (n=14) showed a partially overlapping pattern of recurrent CNA, including frequent gains of 3q and 8q. HPV-negative VIN (n=11) had significantly less CNA (P = 0.010), mainly consisting of 8q gains and 8p losses. Amplification of 11q13/cyclinD1 was exclusively found in 46% of HPV-negative lesions. In HPV-positive lesions no difference in CNA frequency was found between VIN with VSCC (n=17) and VSCC (P = 0.48), though CNA were less frequent in VIN without VSCC (n=14; P = 0.058). Interestingly, almost all (88%) HPV-positive VIN with VSCC had chromosome 1 gain, whereas this alteration was infrequent (21%) in VIN without VSCC.
Conclusion

HPV-dependent and independent vulvar carcinogenesis is characterized by frequent alterations of chromosome 3 and 8, as well as distinct CNA such 11q13 amplification in HPV-independent lesions. The extent of CNA in HPV-positive VIN was found to reflect the cancer progression risk. In particular, gain of chromosome 1 was strongly associated with cancer progression.
DOES HPV GENOTYPE AFFECTS THE GRADE AND THE RISK OF RECURRENCE OF VAGINAL INTRAEPITHELIAL NEOPLASIA?

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Background / Objectives

Vaginal intraepithelial neoplasia (VAIN) is a rare pre-malignant lesion of the female genital tract. Like cervical squamous intraepithelial lesions, Human Papillomavirus (HPV) infection represents the main risk factor and three grades of VAIN can be identified, according to the depth of epithelial involvement. However, there is still controversial data about HPV detection rate in VAIN and prognostic factors of recurrence and progression to malignancy. The aim of this study was to investigate any correlation between HPV genotype and grade of VAIN and between pre-treatment HPV genotype and risk of recurrence and/or progression.

Methods

Women attending the European Institute of Oncology, Milan, and the IRCCS Fondazione Policlinico San Matteo, Pavia, from January 2000 to December 2016, were enrolled in a multicentre retrospective study. Clinical and histological characteristics of all patients were recorded. Only patients in which HPV testing was performed at the moment of diagnosis were selected. The presence of HPV DNA was evaluated with the Cobas HPV assay in Milan and with the INNO-LiPA genotyping system in Pavia. For our analysis, results of both HPV tests were classified as HPV 16-18, other pooled High Risk (HR)-HPV and negative HR-HPV. The χ2-test was used to evaluate associations and a P-value <0.05 was considered statistically significant.

Results

Among 266 patients enrolled, only 167 diagnosed with VAIN1 (59), VAIN2 (42) and VAIN3 (66) were suitable for this analysis. The median follow-up time was of 23.6 months (95% CI: 18.8 – 30.1). HPV 16-18 was detected in 12 (20.3%) VAIN1, 13 (30.9%) VAIN2 and 37 (56.1%) VAIN3, whereas other HR-HPV in 36 (61.0%) VAIN1, 24 (57.1%) VAIN2 and 21 (31.8%) VAIN3 (p<0.001). Overall, 48 (28.7%) recurrences/progressions occurred, with a median time of progression free survival of 93.4 months. No statistical difference was found in number of relapses between pre-treatment HPV 16-18 (22) and other HR-HPV (21) (p=0.26).

Conclusion
HPV 16-18 represent the main genotypes associated with the development of high-grade VAIN, whereas other HR-HPV are more frequently related to low-grade VAIN. Nevertheless, pre-treatment HPV genotype does not affect the risk of recurrence and/or progression of vaginal dysplasia.
DISTRIBUTION OF HIGH-RISK HPV TYPES IN WOMEN WITH INVASIVE CERVICAL CARCINOMA IN KAZAKHSTAN

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Background / Objectives

In Kazakhstan, cervical carcinoma (CC) is the most common cancer in women aged 15 to 44 years, ranking second as the leading cause of female cancers. According to the National Cancer Registry, the incidence rate of CC in Kazakhstani women is very high. In 2012, the estimated crude incidence rate of CC was 22.8 per 100,000 women with a mortality rate of 9.8 per 100,000 women. Unfortunately, data regarding the prevalence and distribution of high-risk HPV (hrHPV) types in CC in Kazakhstan are scarce. Hence, the aim of our study was to evaluate the distribution of hrHPV types in invasive CC samples obtained from Kazakhstani women.

Methods

A total of 99 archival formalin-fixed paraffin-embedded (FFPE) tissue samples, obtained from the same number of Kazakhstani women with histologically confirmed invasive CC, were included in the study. Total DNA was extracted from three 10 μm tissue sections of each FFPE block using a DNA Mini Kit (Qiagen, Hilden, Germany), following our in-house protocol for DNA extraction from FFPE tissues. Detection of hrHPV types was performed using a RealTime High Risk HPV Test (Abbott, Wiesbaden, Germany), which enables concurrent separate genotyping of HPV16 and HPV18 and pooled detection of 12 other hrHPV types: HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Up to 200 ng of each DNA isolate was used per PCR reaction to allow efficient PCR amplification.

Results

Out of 99 samples tested, two (2.0%) were excluded from the analysis due to invalid results for amplification of beta-globin. In total, 77/97 (79.4%) samples tested positive for the presence of hrHPV types. HPV16, HPV18, and other hrHPV types were present in 69/77 (89.6%), 3/77 (3.9%), and 6/77 (7.8%) samples, respectively. An infection with a single hrHPV type was detected in the majority of samples (98.7%).

Conclusion

To the best of our knowledge, this is the first study to evaluate the distribution of hrHPV types among Kazakhstani women with invasive CC. The prevalence of hrHPV types in FFPE CC samples was slightly lower compared to previous studies, most likely due to...
the fixation process and/or storage conditions. However, approximately 80% of samples tested positive for the presence of hrHPV types, of which HPV16 was detected in almost 90% of cases. Nevertheless, further studies evaluating the distribution of hrHPV types in fresh tissue samples are needed to confirm our observations. Our data suggest that the implementation of HPV vaccination could have an enormous impact on the incidence rate of CC in Kazakhstan.
FC 03-06
PHYSICAL ACTIVITY, OBESITY AND CERVICAL CANCER IN GERMANY

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Background / Objectives

The incidence of cervical cancer in Germany remains high in relation to other countries in Western Europe. Germany currently does not have an organized cervical cancer screening (CCS) program and screening efforts with the Pap smear remain opportunistic. Recent studies have shown levels of physical activity and sport to be associated with cancer prevention, although data on the association with cervical cancer remain limited. The TeQaZ study is a case-control study investigating participation in CCS. Associations between cervical cancer and other risk factors, such as physical activity and obesity, were investigated.

Methods

Incident cases of cervical cancer, diagnosed between 2012 and 2016 in different regions in Germany, were recruited. Cases were matched with three population-based controls, recruited via population registries, based on age and region of residence. Gynecologists were asked to report frequency of CCS participation during the past ten years. Socio-demographic and other risk factors were assessed in cases and controls via telephone interviews. Physical activity was defined as engaging in any form of movement such as walking stairs and doing housework. Additionally, sport activity was documented and Body Mass Index (BMI) calculated. Conditional logistic regression analyses were performed.

Results

A total of 218 cases and 654 controls were included in the analysis. 94.5% of cases engaged in any kind of physical activity at least 30 minutes a day compared to 92.2% of controls. 21.2% of cases and 21.1% of controls participated in sport at least three times a week. With regards to obesity, 20.6% of cases versus 12.2% of controls had a BMI over 30. When adjusting for additional factors, participating in sport at least three times a week and engaging in any physical activity at least 30 minutes a day did not show any preventive effect. However, BMI >=30 and participation in CCS less frequently than every three years were strong risk factors for cervical cancer. However, only 52.1% of women with a BMI over 30 participated in CCS at least every three years, compared to 71.6% of women who were not overweight (p<0.05). Results of the conditional logistic regression will be presented.

Conclusion
Initial findings suggest that physical activity and sport are not associated with developing cervical cancer. An association between BMI and cervical cancer was found, although this may be due to decreased participation in CCS among overweight women.
Background / Objectives

To study the impact of Human Papillomavirus (HPV) screening plus cytology (co-testing) in the detection of invasive cervical carcinomas in a low incidence area in the north of Spain, adapting the Spanish Society of Gynaecology and Obstetrics (SEGO) scientific protocols.

Methods

Area served by Hospital of Barbastro: A target population of 27,490 women between 25 to 65 years. Period of time: From January 1st 2006 to December 31st 2010 (G1) screening was performed with cytology and from January 1st 2011 to December 31st 2015 (G2) the screening was with cytology and HPV test. The detection of HPV has been through Hybrid Capture (HC2) until the end of 2011 and PCR hrHPV-DNA with Cobas 4800® later. The follow up was until April 30th 2017. The patients came from Primary Care (PC) screening and from gynaecology service screened according to SEGO 2006 protocol (only cytology) for G1 and to 2010 protocol (co-testing) for G2. Demographic, pathological and clinical characteristics were studied.

Results

A total of 30 invasive cases were detected. In G1, 22,888 cytologies (69.6% in PC) and 1,877 HPV tests (43.0% in PC) were performed. The mean age was 45.9 years. 12 (40%) invasive cases were detected in this group. 4 patients (33.3%) died with a survival average of 37.9 months. In G2, 22,740 cytologies were performed (83.5% coming from PC) and 17,209 HPV tests (80.5% in PC). The mean age was 48.6 years. 18 invasive cases were detected, 4 patients died (22.2%) and the survival average was 7.6 months. In G1 the histological type was squamous carcinoma in all
except 1 neuroendocrine carcinoma. 3 (25%) cases were A1 stage. In G2, 7 adenocarcinomas and 11 squamous carcinomas were diagnosed. 6 (33.3%) cases were A1 stage.

Conclusion

In G2 period more microinvasive cases and adenocarcinomas were detected. This increase is related mainly to screening based in Primary Care and co-testing. Mortality has not changed despite the detection of a higher number of cases due to early stages at the moment of diagnosis. 5 years are not enough to know the impact of early detection in mortality.
What is the impact of the HPV vaccination program on the natural history of high grade squamous intraepithelial cervical lesions in New Zealand?

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Background / Objectives

A free quadravalent HPV vaccination program for young women commenced in 2008, publicly funded vaccinations are registered in the NZ vaccination registry. Current recommendations for cervical screening include cervical cytology tests every 3 years for all women over the age of 20. Subsequently we have seen a modest reduction of high grade abnormalities in young vaccine eligible women. However, as vaccination protects against only 2 oncogenic HPV types, has this resulted in a change in type distribution of HPV among women with high grade abnormalities? If this is so, what if any, are the clinical implications of this change?

Methods

To explore this question we will present data from 2 studies. The first is a matching of data from the NZ vaccination register and the National Cervical screening register. The second is a large multicenter study of over 600 women under the age of 25 undergoing observational management in young women with CIN2 ref 1.

Results

In New Zealand approximately 60% of women aged between 20 and 25 have received at least 2 doses of Gardasil ©. Annually in NZ approximately 53,000 cytology samples are taken in women under the age of 25, 16% of which are reported as abnormal. Approximately 1000 high grade biopsies were reported in women under the age of 25 annually. By matching data from these registries we are able to determine that women who were vaccinated had a lower rate of high grade histological abnormalities and there was an overall trend to a lowering rate of high grade histology.

In the population of young women with high grade histological abnormalities women taking part in our observational study the proportion of women with HPV 16/18 related lesions has fallen from 40-12%. This fall has been most marked in non vaccinated women.
Conclusion

While HPV vaccination has resulted in only a modest decrease in high grade cytological abnormalities in young women there is evidence to suggest there has been a rapid decrease in the proportion of these abnormalities caused by HPV 16 and 18. There is evidence to suggest that non 16/18 lesions have a more benign course. We suggest therefore this has implications for screening and the treatment of high grade abnormalities in young women.

References


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Background / Objectives

Infants born preterm are at increased risk of morbidity and mortality. Conization for the treatment of cervical dysplasia has been associated with an increased risk of preterm delivery. However, valid comparable data for Germany are still missing. Our study aimed to investigate the association between conization and perinatal outcomes in subsequent pregnancies, using data from a German population database.

Methods

A retrospective cohort study was performed on data from the German nationwide performance measurement program in healthcare quality. The survey routinely collects parameters of women who give birth in a German hospital. Approximately 98,5% of all births in Germany are covered within the data collection, comprising a total of 4.002.503 births between 2009 and 2014. Women with history of conization prior to pregnancy were compared to a control group of women without. To control for multiple pregnancies the cohort was limited to singleton deliveries and to avoid double-counting, only primipara were included. Main outcome measures are gestational age at birth, birth weight, neonatal morbidity and perinatal mortality. Data were analyzed using univariate and multivariate statistical methods.

Results

A total of 1.573.200 cases were eligible for inclusion. There were 14.337 women with history of conization and 1.328.057 women without. Women with history of conization were more likely to be single, (self-) employed, older, had a lower body mass index and delivered infants with lower birth weight [mean (SD), 3.240g (± 603g) vs. 3.307g (±545g), p < 0.0001]. The preterm birth rate was significantly higher in the conization cohort compared to the non-exposed cohort (12,2% vs. 7,5%; Chi²<0,0001).
Conization was a significant risk factor for preterm birth (odds ratio, OR 1.7; 95% CI: 1.65-1.83). There was no significant difference in stillbirth and death after 7 days of birth between both groups (OR 0.9, 95% CI: 0.66-1.25; OR 1.6, 95% CI: 0.92-2.65).

Conclusion

Pregnancies complicated by conization are at a greater risk of preterm delivery. There was no increase in perinatal mortality. Further research of the data set will investigate whether preterm delivery after conization affects the perinatal morbidity.
FC 03-10
Correlation of isotope count with sentinel node positivity in vulvar cancer

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Background / Objectives

Sentinel node biopsy (SNB) has become standard of care in early stage vulvar cancer. As the correlation of isotope count with the presence of metastases remains unclear, often several active nodes are excised per groin. This can result in increased morbidity in node-negative disease despite of SNB. In the current analysis we assess, whether resection of the hottest node could be sufficient to detect sentinel lymph node (SNL) metastasis.

Methods

All patients with primary vulvar cancer receiving a SNB with radioactive tracer at the University Medical Center Hamburg-Eppendorf between 2008 and 2015 were evaluated. The day before surgery, patients received four peritumoral intradermal deposits at 3, 6, 9 and 12 o’clock with an overall mean dosage of 85±12MBq99mTc–nanocolloid. Planar lymphscintigraphy was performed one hour after injection. Intraoperatively, a handheld gamma counter was used to identify the SNL.

Results

145 patients with SNB were included; thereof 144 underwent bilateral SNB, resulting in 289 analyzed groins. A median of 2 (range 1-7) SNL per groin were removed. From 94/289 (32.5%) groins more than 2 SNL were excised. Median overall SNL isotope count was 1400. In 50 groins, a positive SNL was detected (unilateral in 38 patients, bilateral in 6). The median number of positive SNL per groin was 1 (range 1-4). The SNL with the highest isotope count carried metastases in 36/46 groins (78.3%; in 4 cases the highest count was unknown). In 10/46 (21.7%) positive groins, the SNL with the highest count was not the metastatic SNL (9/10 second highest count). Median count of these 12 SNL was 60% of the highest count with a range from 11.0% to 74.0%.

Conclusion

The highest isotope count does not reliably detect the positive SNL in vulvar cancer. To prevent mostly fatal groin recurrences, surgeons should continue to remove all SNL accumulating relevant radioactive tracer over minimal background activity.
PREVALENT AND INCIDENT CANCERS IN HPV NEGATIVE WOMEN

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Background / Objectives

HPV testing is better than cytology in primary screening because it reduces subsequent cancer incidence. Moreover, the long-term CIN3+ risk is lower after a negative HPV test than after normal cytology, justifying a longer screening interval in older women who are less likely to become infected. The main concern with a screening interval of up to 10 years in HPV negative women aged over 40 is their invasive cancer risk, which is about 1 in 1,000 over 10 years.

Methods

In the ARTISTIC Trial 24,510 women attending for routine cervical screening in 2001-2003 had cytology and HPV testing at entry and 3-yearly until 2009. We have followed the cohort to 2015 through national cancer and CIN3 registration.

Results

Respective numbers of CIN3s and cervical cancers by age at diagnosis were 208 and none aged 20-29, 192 and 7 aged 30-39, and 83 and 15 aged 40-65. Six of the 22 women with cancer were HPV negative by HC2 at entry, but on retesting their stored entry samples by PCR 5 were HR-HPV positive. 2 of these 6 cancers were diagnosed within 5 years of the negative HC2 test and were probably present at entry. At entry one was cytologically abnormal and both had HPV16 detectable by PCR.

Conclusion

The important measure of efficacy with a long screening interval is the cancer risk, not the CIN3 risk. Most cancers caused by subsequent HPV infection will develop towards the end of the interval due to the lag from infection and CIN3 development to malignancy. These are likely to be diagnosed at an early stage even with a 10-year interval. However, cancers present at the time of the negative HPV test would be at high risk of being advanced or metastatic 10 years later. The results of ARTISTIC and other large studies suggest that most of these prevalent cancers would be detected by more sensitive HPV testing and/or cytology co-testing, but the numbers are small even in very large studies. Collaborative analyses focussed on this issue are needed to provide evidence on the effects on early and advanced cancer incidence of enhanced testing procedures with screening intervals of up to 10 years at different ages, and particularly at a woman’s final HPV test. Modern HPV tests
may already be sensitive enough to prevent most of the small but serious hazard of undetected invasive cancer.
REINVESTIGATION OF A PROPORTION OF HPV-NEGATIVE TUMORS IN A SWEDISH COHORT OF CERVICAL CANCER

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Background / Objectives

Most cervical cancer develops as a result of a permanent infection with human papillomavirus virus (HPV). Despite the common perception that HPV is a requirement for the development of cervical cancer, a smaller proportion of HPV negative cervical cancer is often found in larger studies.

The aim of this study was to reinvestigate a proportion of HPV-negative tumors in a Swedish cohort (n=209) of patients diagnosed with cervical cancer, previously analyzed for detection of HPV where 14.4 % of the tumors were found to be negative.

Methods

Cervical cancer tumors with a HPV negative or invalid result from genotyping with AnyplexTM II HPV28 (Seegene) were included (n=37). This real time PCR method targets 28 genotypes using the viral L1 gene together with the human gene HBB. Second approach included an in house real time PCR protocol instead targeting the viral oncogenes E6 or E7 for 12 high-risk and two low-risk genotypes.

Samples with HPV negative results with both real-time PCR methods were assessed by pathologist and tumors with lacking amount and quality were excluded. Remaining HPV-negative samples were investigated with immunohistochemistry (p16, Vim, ER, PR, CD10, CEA, CK5, P63 and MUC2) to exclude the inclusion of tumors of non-cervical origin.

Results

The initial results showed a proportion of 14.4 % negativity. With repetitive analysis (Anyplex) and second approach with alternative genotyping method the HPV-negativity was 9.6 %. After assessment of tumor material together with immunohistochemistry, five samples were excluded due to lack of tumor material or suspicion of other than cervical origin. This resulted in a HPV-negative proportion of 7.2 % in this Swedish cohort of cervical cancer. HPV-negativity was significantly
(Pearson chi-square test; p < 0.0001) associated with adenocarcinoma (AC) histology and worse cancer-specific survival rate at 5 years (log-rank test; p = 0.010).

Conclusion

Reinvestigation of HPV-negative samples led to a drop of the total proportion of HPV negative tumors from 14.4 % to 7 %. HPV negativity in this group was associated with poor prognosis.
HUMAN PAPILLOMA VIRUS NEGATIVITY: WORSE PROGNOSIS IN INVASIVE CERVICAL CANCER

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Background / Objectives

HPV-negativity has been reported to be associated with worse prognosis for some HPV-associated cancers. Whether detectability of HPV is related to prognosis of invasive cervical cancer is more controversial and would need very large studies to be clearly answered.

Methods

We identified all cervical cancers diagnosed in Sweden during a 10 year period (2002-2011; 4254 confirmed cases), requested the archival blocks and subjected them to HPV genotyping using general primers targeting the L1 region, followed by typing with Luminex for 14 high risk types including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 and non-high risk types including 6, 11, 26, 30, 40, 42, 43, 53, 54, 61, 67, 69, 70, 73, 74, 81, 82, 83, 86, 87, 89, 90, and 91). Blocks from 2848 cases were retrieved and analyzed, and were prospectively followed up from date of cancer diagnosis to 31 December, 2015, migration from Sweden, or death, whichever occurred first. Five year relative survival ratios were calculated and excess hazard ratios (EHRs) with 95% confidence intervals (CIs) were estimated by a Poisson model, adjusted for age at cancer diagnosis, FIGO (International Federation of Gynecology and Obstetrics) stage and education.

Results

The HPV L1 region was detected among 2368 (83.1%) of all cases. For HPV L1-negative women, the 5-year relative survival ratio was 0.54 (95% CI, 0.49-0.59) and for women with HPV L1-positive tumours 0.74 (95% CI, 0.72-0.76), yielding a crude EHR of 0.45 (95% CI, 0.38-0.53) and adjusted EHR of 0.53 (95% CI, 0.45-0.62). The 5-year age-specific adjusted EHRs for women with HPV L1-positive tumor were 0.53 (95% CI, 0.32-0.88) at age 30-44, 0.69 (95% CI, 0.48-0.99) at age 45-59, 0.59 (95% CI, 0.44-0.79) at age 60-74 and 0.40 (95% CI, 0.30-0.52) at age 74 and above respectively compared to women with negative tumours. Compared to negative
tumours in each stage, the adjusted EHRs of HPV L1-positive tumours were IA: 0.67 (95% CI, 0.08-5.69), IB: 0.61 (95% CI, 0.41-0.91), II: 0.47 (95% CI, 0.34-0.65) and III+: 0.53 (95% CI, 0.43-0.66). For squamous cell carcinoma the adjusted EHR of HPV L1 positive tumours was 0.57 (95% CI, 0.46-0.70), while for adenocarcinoma it was 0.52 (95% CI, 0.36-0.74).

**Conclusion**

Women with tumors negative for HPV L1 have much worse prognosis than women with HPV L1-positive tumours, irrespective of age, clinical stage and histological tumour type.
THE RELATION BETWEEN HRHPV-NEGATIVE HIGH-GRADE CYTOLOGICAL LESIONS AND HISTOLOGY: A SYSTEMATIC REVIEW.

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Background / Objectives

There is a consensus that a persisting infection with human papillomavirus (HPV) is the main causative agent for the development of a cervical carcinoma. Testing for HPV therefore plays an important role in cervical cancer screening. Suggestions have been made to introduce the HPV test as the primary screening method, replacing cytology as the current screening technique. However, there is discussion on the value of HPV-negative high-grade cytological lesions.

It is of vital importance to establish whether using an HPV test as the primary screening method for cervical cancer will result in missing high-grade cytological lesions, which are the precursors of cervical carcinomas. This systematic review gives an overview of the percentage of women with high-grade lesions on cytology (ASC-H and HSIL) and a negative HPV test, who present with moderate or severe dysplasia or with a malignancy on histology.

Methods

A comprehensive literature search was carried out, including MEDLINE (PubMed, 1 January 2001 until 25 November 2016) and the Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library). 925 titles and abstracts were screened. 40 eligible studies included women presenting with a cervical cytology result of ASC-H or HSIL and an HPV-negative test result, who were subsequently subjected to reference standard verification with colposcopy and colposcopy directed biopsies for histologic verification. Two review authors independently extracted data from the selected articles and assessed the quality of the studies. Disagreements were resolved by consensus.

Results

The percentage of females who develop moderate to severe dysplasia or a malignancy (CIN2+) from HPV-negative ASC-H is 10.7%, 95%CI [5.3; 16.0]. The percentage CIN2+, originated from HPV-negative HSILs, is 35.9%, 95%CI [28.4; 43.4].

Conclusion

Histologically confirmed CIN2+ lesions out of an HPV-negative high-grade cytological population are an existing, yet insufficiently studied entity. Consequently, it might result in missing high-grade precursor lesions when HPV testing would be used as a unique
primary screening test for cervical cancer. Additional research is needed to establish the prevalence and the importance of HPV-negative high-grade cytological lesions in Belgium in order to determine the most appropriate screening method.
FC 04-05
HPV-NEGATIVE CARCINOMA OF THE UTERINE CERVIX: A DISTINCT TYPE OF CERVICAL CANCER?

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Background / Objectives

Small proportion of cervical cancers (CC) are negative for human papillomavirus (HPV), even using highly sensitive HPV tests. It has been suggested that HPV-negative CC may represent a biologically distinct subset of tumours carrying a poorer prognosis. However, the significance of HPV-negativity in CC remains unclear. We aimed to provide insight into the differential clinical, pathological, and prognostic characteristics of the unusual HPV-negative CCs.

Methods

A cohort of 215 women with CC diagnosed in the Hospital Clinic (Barcelona) from 1999 to 2014 underwent HPV testing using: 1)a highly sensitive polymerase chain reaction (PCR): SPF10PCR/DEIA/LiPA25 system for HPV-DNA detection and genotyping and 2)p16INK4a immunostaining. Clinical, histological and immunological characteristics of the women included were recorded.

Results

Twenty one out of 215 tumors (9.8%) were negative for HPV-DNA detection. Nine of them (9/21;42.9%) showed also a negative p16INK4a immunostaining result. These double negative tumors were considered as confirmed HPV-negative CC. Within the confirmed HPV-negative CC, 5 were squamouscarcinoma, 2 were adenocarcinoma and 2 were neuroendocrine. Women with confirmed HPV-negative CC were diagnosed at advanced FIGO stage and showed worse disease free survival [47.5 months (95%CI:8.7-86.22 months) vs. 129.6 months (95%CI:116.22-143.01 months); p=0.009] and overall survival [72.1 months (95%CI:25.44-118.80 months) vs. 151.4 months (95%CI:139.70-163.05 months); p=0.056] than women with HPV-positive tumours.

Conclusion

DNA-HPV negative result is an uncommon finding in women with CC, and almost half of these cases show a positive p16INK4a immunostaining. Confirmed HPV-
negative CC seems to be associated with advance FIGO stages and worse prognosis.
IMPLEMENTATION VALIDATION OF THE PAPILLOCHECK® (GREINER BIO-ONE) KIT FOR GENOTYPING HUMAN PAPILLOMA VIRUSES (HPV) IN PRESERVCYT LIQUID MEDIUM


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Background / Objectives

Persistent infection of the uterine cervix by the same type of high-risk human papillomavirus(es) (HR-HPV) is related with cervical cancer. It becomes increasingly important to know which HR-HPV type(s) is/are present in the cervical smear. The objective of this study is to evaluate the analytical performance of the PapilloCheck kit for the detection and genotyping of 18 high risk and 6 low risk HPVs.

Methods

DNA from patient samples was extracted using the MagNA Pure platform (Roche DNA I High Performance protocol): 1 ml sample was first concentrated by centrifugation (20 min, 20,000 g). 800 µl supernatant was removed and the remaining 200 µl was used for extraction.

Due to the low concentration of cell material of the Quality Control for Molecular Diagnostics (QCMD) panel, the whole sample (5 ml) was concentrated (20 min, 4000 g).

DNA was eluted in 110 µl elution buffer.

DNA of the WHO Proficiency Panel 2011 (PP) was already extracted.

5 µl DNA was used for the PCR.

The assay was checked for analytical sensitivity, specificity, accuracy and precision following the Belgian guidelines.

Results

Analytical sensitivity: a negative PreservCyt specimen was spiked with the WHO-HPV16 DNA standard to determine the limit of detection (LOD with a 95% hit rate). The lowest concentration was 13.333 international units (IU)/ml, correlating with 120 copies/PCR.

Looking at the results of the WHO PP we can assert that the PapilloCheck detects 50 genome equivalent (GE)/PCR of HPV6, HPV11, HPV33, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, HPV68. For HPV18, HPV31 and HPV35 the sensitivity was 500 GE (IU)/PCR. HPV16 was detected at 5 IU/PCR. The PP was designed for genotyping needs in HPV vaccinology. The test is not proficient for HPV18 (50 IU/PCR) but according to the manual the LOD for HPV18 is 300 IU/PCR and is in accordance with the clinical needs.
Specificity: the specificity was sufficiently documented by the manufacturer, and was not tested again.

Accuracy: 52 specimens were tested.

WHO PP2011: 5 out of 43 were false negative (lower than LOD). QCMD 2011 panel: 8 out of 9 were typed correctly. HPV45 in cc10b cells was missed. This cell line does not contain the full target used by the assay. HPV45 was 4/4 times detected in the WHO PP.

Precision: One sample positive for HPV 31 and HPV51, a second sample with a multiple infection of 3 types: HPV81, HPV33 and HPV73 were extracted in triplicate on 3 different days. All types were detected correctly. This met our validation criteria.

Conclusion

The PapilloCheck method met all our validation criteria and was implemented in our routine diagnostic laboratory.

References

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A COMPARISON OF THE PERFORMANCE OF PAPTYPE USING CYTOFLEX AND ATTUNE FLOW CYTOMETER PLATFORMS ON CERVICAL SCREENING SAMPLES COLLECTED FROM PRESERVCYT

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Background / Objectives

To compare the performance of PapType, a bead-based full genotyping DNA assay for 14 high-risk (hr) and 2 low-risk (lr) HPV types, using two flow cytometer platforms - CytoFLEX and Attune.

Methods

Residual material from the PreservCyt samples of 6000 women attending for routine cervical screening sent to the cytology laboratory at St. Mary's Hospital, London were tested using PapType HPV test kit (Genera Biosystems, Scoresby, Victoria, Australia). The samples were run on CytoFlex (Beckman Coulter) and Attune Acoustic Focusing (Thermo Fisher Scientific) flow cytometer platforms following the manufacturer’s instructions. These samples were collected for the Predictors 3 Study. The discordance between the platforms was analysed by hrHPV type. Comparisons were also made of sensitivity and specificity for CIN2+

Results

98.2% of cases gave an adequate result on Attune versus 99.1% on Cytoflex. Overall 17% of Attune cases were positive for at least 1 hrHPV type, and 21.1% of Cytoflex cases were positive for 1 or more hrHPV type. Overall agreement between Attune and Cytoflex was good for detecting hrHPV types (kappa 72.4, 95% CI (70.1, 74.6)). Cytoflex called more cases positive than Attune (significant difference for HPV types 16, 18, 45, 52, 58, 66 and 68). Attune had statistically significantly more positives for HPV 59. There was a wide range of agreement for individual HPV types (kappa 41.1 for HPV 18 to kappa 92.1 for HPV 33). Overall for CIN2+ cases only 1 case was discordant (positive for Cytoflex, negative for Attune). CIN2+ sensitivity was higher for Cytoflex (97.5 vs 95.0). Specificity was higher for Attune (<CIN2 83.5% vs 79.4%).

Conclusion
These preliminary data suggest that both Attune and CytoFlex are reliable flow cytometry platforms on which to run the PapType test. Further HPV type specific and signal strength data will be presented in the final analyses.

References

THE TRANSITION FROM HC2® TEST TO COBAS® 4800 TEST IN THE HPV PRIMARY SCREENING OF THE FLORENTINE AREA

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Background / Objectives

In cervical cancer screening, HPV test detects DNA of 12 high risk (hr) HPV types (16/18/31/33/35/39/45/51/52/56/58/59) and, depending on test, HPV with probable (68) or possible (66) risk. Among clinically validated tests, Hybrid Capture®2 (HC2®, QIAGEN®) detects 13 HPV types (hr and 68) by in solution hybridization, while Cobas®4800 (ROCHE®) detects 14 types (also 66) in Real-Time PCR. In the Florentine area, from May 2016, HC2® test has been replaced by Cobas® test.

The objective of the study is to evaluate the impact of the transition from HC2® to Cobas® on the HPV primary screening of the Florentine Area, comparing: HPV positivity, cytology triage abnormalities and histological results to immediate colposcopy.

Methods

We considered samples from women participating to HPV primary screening program of the Florentine area (34-64 years), collected in ThinPrep®(HOLOGIC®) from June 2015 to March 2017. Until May 2016, samples were analysed by HC2® on automatic instrumentation (QIASymphony®/RCS®, QIAGEN®). From June 2016, samples were analysed by Cobas®.

Results

The samples collected in ThinPrep® during the considered period were 37775, of which 17137 (45.4%) tested on HC2® and 20638 (54.6%) on Cobas®. The average age of women is similar (46.5 vs 46.1).

HPV positivity was 9.8% (1677/17137) for HC2® samples and 7.4% (1529/20368) for Cobas® samples (p<0.0001).

480 HC2® positive women (28.6%) had abnormal cytology triage and 17 (1%) inadequate; 418 Cobas® positive women (27.3%) had abnormal cytology triage and 21 (1.37%) inadequate.

Adhesion to colposcopy was 90.3% (449/497) in HC2® group and 77.4% (340/439, of which 14 are waiting for histology) in Cobas® group until now, as several women are waiting to perform colposcopy.
For the 449 women of HC2® group, compared to the 326 women of Cobas® group, at the immediate colposcopy we found: 108 vs 118 CIN2+(PPV: 24.1% vs 36.2%, p<0.0002), 145 (32.3%) vs 88 (27%) CIN1, 195 (43.4%) vs 118 (36.2%) normal colposcopies/histologies (p<0.05) and 1 (0.2%) vs 2 (0.6%) inadequate histologies.

Conclusion

The use of HC2® as primary screening test, compared to Cobas®, has registered: greater HPV positivity, lower CIN2+ PPV at the immediate colposcopy and higher frequency of normal colposcopies/histologies (all statistically significant differences). These results could be explained by the well known HC2® cross-hybridization with non-hrHPV types, unlike Cobas®, which has a higher analytical specificity. Non-hrHPV types detected by HC2® but not by Cobas® likely increase HPV positivity and abnormal cytologies, but decrease PPV at the immediate colposcopy, since they are mostly associated with no lesions or low grade lesions (CIN1), as resulted by our data.
COMPARISON OF VALIDATED MOLECULAR METHODS FOR HPV PRIMARY SCREENING TEST: HC2® TEST VS. COBAS® 4800 TEST.

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ISPO (Cancer Prevention and Research Institute) (Italy)

Background / Objectives

In the Cancer Prevention Regional Laboratory of ISPO (Florence, Italy), after the transition from Hybrid Capture® 2 test (HC2®, Qiagen®), that detects 13 HPV types (12 HR-HPV+HPV68), to Cobas® 4800 HPV test (Roche®), that detects 14 HPV types (12 HR-HPV+HPV66 and 68), a decrease in HR-HPV positivity was observed in all the Local Sanitary Areas on which the HR-HPV test was already performed by our laboratory. The aim of this study is to compare the performance of the two methods using the same set of samples.

Methods

620 routine screening samples, HC2® positive, were retested on Cobas®. The samples that resulted negative to Cobas® (discordant) were typed by a Reverse Line Blot (RLB) method (Ampliquality HPV-Type express 3.0®-AB Analitica®), that detects the presence of 40 HR and non HR-HPV types. These results were linked with cytological and histological data.

Results

419 samples (67.6%) was confirmed HR-HPV positive to re-test with Cobas®, while 201 samples (32.4%) resulted HR-HPV negative (discordant). This decrease of the positivity to HR-HPV is also confirmed in the screening samples analyzed after the introduction of Cobas®.

The discordant samples reported the following results of cytology triage: 165 (82.1%) resulted with normal cytology and 36 (17.9%) with abnormal cytology (31/36 LSIL (86.1%), 3/36 ASC-H (8.3%), 1/36 HSIL (2.8%), 1/36 AGC (2.8%)).

All discordant samples were typed using RLB. 14/201 samples (7%) resulted HR-HPV positive by typing and only one of these (HPV58+) had an abnormal cytology (LSIL), but resulted negative to colposcopic exam. The typing results of the other 187 discordant samples were: 88/187 (47%) HPV negative, of which 5 with abnormal cytology triage (2 ASC-H, 1 AGC, 2 LSIL) and none with CIN2+ lesion; 99/187 (53%) were non HR-HPV positive, of which 30 (30.3%) with abnormal cytology triage (1 ASC-H, 1 HSIL and 28 LSIL) and 2 with CIN3 lesions (HPV26 and HPV54+73+90 respectively).
Furthermore, a considerable number of discordant samples (20/201, 10%) resulted HPV68a positive by RLB (5 with abnormal cytology and none CIN2+).

**Conclusion**

Cobas® is a reliable method and is more specific than HC2® (92.5% of discordant samples are HR-HPV negative, so we would have registered a lower rate of false HR-HPV positive samples). Between Cobas® HPV negative samples, but HC2® HR positive, we found two CIN3 lesions resulted associated with LR-HPV types by PCR RLB. HPV 68 is a target type for Cobas® but it is not clear if 68a and 68b are detected at the same level, because, in our set of samples, several HPV 68a resulted negative by Cobas®.
COMPARISON OF THREE DIFFERENT SYSTEMS TO TEST FOR THE PRESENCE OF A HR-HPV INFECTION IN SYMPTOMATIC AND FOLLOW-UP PATIENTS.

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Background / Objectives

As part of the former screening policy in the Netherlands, an hrHPV test is performed on cytological material in symptomatic patients, in triage after ASCUS/LSIL cytological screening results and post-CIN follow-up. Aim of the present study was to compare 3 HPV assays for the detection of hrHPV in smears from these patient groups.

Methods

A total of 1265 residual PreservCyt cervical cytological samples taken either because of symptoms or for follow-up reasons were rendered anonymous, randomized and tested for hrHPV using three automated HPV assays on their respective platforms: QIAGEN’s digene® HC2 HPV DNA Test® (HC2, signal amplification), Roche Cobas® HPV test (DNA amplification) and Hologic Aptima® HPV Test (RNA amplification). To determine the agreement between results generated using the different assays, pair wise comparison of the systems was performed by determining kappa coefficients. Additionally, inter-assay agreement on hrHPV positive smears was determined for the 3 assays.

Results

1151 samples had valid results in all of the 3 tests. The majority of patients was between 29 and 53 years old (85.8%, n=988). Of these patients 59.7% (n=688) had normal cytology, in 23.6% (n=272) ASCUS was found, 9.3% (n=108) had LSIL and 7.0% (n=81) had HSIL or invasive cancer. Analysis of the results yielded an hrHPV prevalence with Aptima of 41.1% (n=473), with HC2 of 47.9% (n=551) and with Cobas of 44.4% (n=511). Kappa coefficients of 0.81, 0.83 and 0.77 (HC2 vs Cobas, Cobas vs Aptima and Aptima vs HC2, respectively) indicate substantial agreement between the results generated. With increasing degree of cytological abnormalities the hrHPV prevalence rose from 25.8% in normal cytology to 95.7% in HSIL. The kappa values also improved. With regard to inter-assay agreement of hrHPV positive samples, 71.6% (n=426) tested positive in all 3 assays, whereas the percentage of cases that tested positive with a single method was 13.6% (n=81) and the percentage that tested positive with two methods was 14.7% (n=88). The level of
hrHPV positivity in these groups is presently under investigation. Interestingly, preliminary results showed that with respect to age there were 2 peaks in hrHPV prevalence (respectively at 20-29 years and in the over 60’s): Final analysis of the results will include a further division based on specific clinical indications.

**Conclusion**

As expected, an high hrHPV prevalence was found in this symptomatic patient group, with rising hrHPV positivity with increasing severity of cytology. Based on kappa-values the 3 assays showed substantial agreement.
The concordance of HPV DNA and HPV oncogenes mRNA in adenocarcinoma and squamous carcinoma of cervix

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Background / Objectives

The causative role of high-risk human papillomavirus (HR-HPV) in cervical cancer development is well recognized, but HPV infection was less common in cervical adenocarcinoma (CADC) than squamous cell carcinoma (SCC) and CADC is diverse pathologically and in HPV status. Nevertheless, most studies to date have focused primarily on viral DNA rather than the viral transcription. The aim of this study was to investigate the presence of HR-HPV in cervical cancer tissues at HPV DNA level and HPV oncogenes mRNA level by polymerase chain reaction (PCR) and in situ hybridization (ISH) respectively.

Methods

We studied DNA and mRNA levels of HPV in paraffin-embedded samples from patients with CADC and SCC. 60 cases of CADC and 14 cases of SCC were included. Cases were tested for HPV using whole-tissue sections (WTS) and laser-capture microdissection (LCM). All cases were HPV-tested by L1 based broad-spectrum SPF10-DEIA-LiPA25 PCR. HR-HPV mRNA was assayed by novel RNAscope ISH. Type-specific oligonucleotide probes were used for the RNA detection of HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82.

Results

HPV DNA was detected in all 14 (100.0%) SCC and in 36 out of 60 (60.0%) CADC cases by WTS-PCR. Overall, the HR-HPV mRNAs was detected in 12 out of 14 (85.7%) SCC and 20 out of 60 (33.3%) CADC by RNAscope ISH. 20 out of 36 (55.6%) WTS-PCR HPV DNA+ CADC cases detected HR-HPV mRNAs. The remaining 24 (100%) cases of WTS-PCR HPV DNA- CADC were also HPV mRNA-.

Also, 16 out of 36 cases of WTS-PCR HPV DNA+ with multiple HPV infections were tested by LCM-PCR to determine whether one or more viruses are present in one lesion. 11 out of 16 (68.8%) multiple HPV infection cases were LCM-PCR HPV DNA- and HPV mRNA-; 4 out of 16 (25%) were LCM-PCR HPV DNA+ and HPV mRNA+; Only 1 case was LCM-PCR HPV DNA+ and HPV mRNA-. In CADC, the kappa coefficient of RNAscope and WTS-PCR was 0.500 (P < 0.001), while the kappa coefficient of RNA scope and LCM-PCR was 0.846 (P = 0.001).

<table>
<thead>
<tr>
<th>HPV DNA / HPV mRNA</th>
<th>RNAscope (HPV mRNA)</th>
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<tbody>
<tr>
<td>Concordance of HPV mRNA and DNA when using RNAscope and WTS-PCR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>-------------------</td>
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</tr>
<tr>
<td>WTS-PCR (HPV DNA)</td>
<td>20</td>
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<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Kappa coefficient</td>
<td>0.500 (P &lt; 0.001)</td>
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</tbody>
</table>

Table 2. Concordance of HPV mRNA and DNA when using RNAscope and LCM-PCR

<table>
<thead>
<tr>
<th>HPV DNA / HPV mRNA</th>
<th>Positive</th>
<th>Negative</th>
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<tr>
<td>LCM-PCR (HPV DNA)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Kappa coefficient</td>
<td>0.846 (P = 0.001)</td>
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</table>

Conclusion

Compared with WTS-PCR, using LCM-PCR for HPV DNA evaluation yielded lower prevalence of HPV DNA and better concordance of HPV mRNA and DNA when using RNAscope assay to evaluate the expression of HPV mRNA. Overall, HR-HPVs exist in CADC tissue with less active transcription, which implies that the causal role of HPV in CADC development need further study.
AN UPDATE ON THE INTERNATIONAL HPV REFERENCE CENTER

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Background / Objectives

To provide an update of ongoing activities at the International HPV Reference Center.

Methods

The center i) receives clones of potentially novel HPV types, clones them again and re-sequences them. If the confirmed sequence is found to represent a novel type, a unique HPV type number is assigned, reported to the originator and immediately posted on www.hpvcenter.se. ii) Distributes the reference clones, for academic research use, under Material Transfer Agreements agreed upon with the originator. iii) Provides a service with preliminary checking of whether new sequences may represent novel types. iv) issues international proficiency panels for HPV genotyping.

Results

Since 2013, 332 reference clones have been transferred to 55 different laboratories worldwide. Since the reference center was transferred from Heidelberg to Stockholm in 2012, 49 clones with putative new types have been submitted. Forty-six clones have been confirmed as novel types and assigned official numbers. One clone was found to be a subtype and two clones were not novel (recently established types, but not yet in GenBank). The g-genus now contains 90 HPV types, surpassing the diversity of the μ and β genera, which contain 65 and 51 HPV types, respectively.

Conclusion

Currently, the highest HPV type number awarded is HPV 216 (www.hpvcenter.se). Because 5 previously awarded HPV types have been withdrawn there are as of today (2017-04-27) 211 different HPV types.
DETECTION OF HPV mRNA AND HPV DNA UP TO 8 YEARS BEFORE DIAGNOSIS OF CIN3+

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Background / Objectives

Knowledge is sparse concerning proportions of normal cervical smears (Pap smears) that are positive for HPV mRNA and/or for HPV DNA among women who develop cervical intraepithelial neoplasia 3+ (CIN3+) or adenocarcinoma in situ (AIS). The aim is to compare proportions HPV mRNA- and HPV DNA-positivity of baseline cytology-samples before development of CIN3+ and AIS.

Methods

In Malmö of Sweden 2012 women were diagnosed with histopathology confirmed CIN3+ or AIS between 2007 through 2015, and also had a baseline biobank cytology sample at -80°C. Overall, 1204 cytology samples were eligible for HPV testing out of which 578 and 626 had normal and ASCUS+ cytology, respectively. The mean age at CIN3+ was 34 years (range 23-86) at diagnosis. The cell pellet from each LBC-sample (2mL SurePath solution) was suspended in 420 uL STM (Qiagen). Then a split sample approach was used where 100 uL was analyzed for high-risk HPV types by APTIMA (Hologic) and 100 uL by Cobas 480 (Roche).

Results

Among women with normal cytology 74% and 80% had HPV mRNA and HPV DNA, respectively. Among women with ASCUS+, 88% and 91% had HPV mRNA and HPV DNA, respectively. The overall agreement between the HPV mRNA and HPV DNA assays was 87% (K=0.64) and 92% (K=0.60) for those with normal and ASCUS+ cytology, respectively.

Among HPV-positive women with normal cytology, 8 years before diagnosis of CIN3+ 64% and 82% had detectable HPV mRNA and HPV DNA, respectively. Seven years before diagnosis: 85% vs 82%, 6 years: 69% vs 78%, 5 years: 73% vs 78%, 4 years: 73% vs 79% P=0.0108, 3 years: 78% vs 88%, 2 years: 79% vs 79%, 1 year: 58% vs 58%.

The median period between normal cytology and CIN3+ was 44 months (range 5-96) both for women with positive HPV mRNA-test and/or HPV DNA-test. Among HPV mRNA-positive (427 women) and/or HPV DNA-positive (460 women) cytologically normal women, 22% from each group had CIN3+ within 3 years.
The median period between ASCUS+ cytology and CIN3+ was 8 months (range 4 to 85) and 8 months (range 4 to 92) for women with positive HPV mRNA-test and/or HPV DNA-test, respectively.

Among 22 cases with histology of AIS, 95% (21/22) and 91% (20/22) had detectable HPV mRNA and HPV DNA in the cytology samples (all ASCUS+), respectively. The overall agreement between the HPV mRNA and HPV DNA assays was 95% (K=0.64).

**Conclusion**

High proportions of HPV positivity (74%-80%) were observed for HPV mRNA and HPV DNA assays among cytologically normal women who developed CIN3+. About a fifth of these HPV-positive cytologically normal women had diagnosis of CIN3+ within 3 years. Further studies are needed in order to predict which of HPV-infected women who will progress to high-grade dysplasia.
Background / Objectives

This study reports on the performance and acceptability of optimized HPV detection and genotyping in self-collected first-void urine (FvU) versus cervical (Cx) samples for the detection of high-risk (hr)HPV DNA and cervical precancerous lesions in a referral population in Belgium.

Methods

Women between 25-64 years old (median age: 36.00 ± 10.08 year) referred for colposcopy at the Antwerp University Hospital (UZA) collected a FvU sample (Colli-PeeTM, Novosanis) (NCT02714127) prior to their visit with the gynecologist. HPV DNA genotyping was performed on paired FvU (after in-house DNA extraction (1)) and Cervex-Brush® (Rovers Medical Devices) collected Cx samples in PreservCyt® (Hologic) with the Riatol qPCR HPV genotyping assay (Belgium) (2). Histology on biopsies (when indicated) was investigated at the pathology laboratory (UZA). Data regarding acceptability of different sampling methods were gathered through questionnaires. Statistics was performed using IBM SPSS24.

Results

HrHPV DNA was detected in 69.09 (n=76/110) and 66.36% (n=73/110) of FvU and Cx samples respectively, with HPV16 the most prevalent genotype (n=23/110 versus n=21/110). A good agreement for hrHPV DNA in FvU and Cx samples of 86.36% (Cohen's kappa: 0.688; 95% CI: 0.543-0.833) was found. On individual genotype level, excellent agreement for HPV16 (96.36%; Cohen's kappa: 0.886; 95% CI: 0.776-0.996) and HPV18 (99.09%; Cohen's kappa: 0.918; 95% CI: 0.759-1.077) was
obtained. Moreover, significant positive correlations of HPV16 and 18 DNA copies per μl DNA extract were found between both sample types (Spearman rho HPV16: 0.570, p-value: 0.009; and HPV18: 0.829, p-value: 0.042). For 33 out of 110 samples with histological reference, a relative sensitivity for CIN2+/CIN3+ and specificity for <CIN2 of HPV16/18 detection in FvU versus Cx samples of 1.00 (both CIN2+ and CIN3+) and 0.93 (<CIN2) was acquired.

Preference of using a female urination device upon FvU collection compared to use of a standard urine cup, a pap smear taken by a clinician, or use of a vaginal self-sampling device was respectively 91.75 (n=89/97), 77.36 (n=82/106), and 91.67% (n=11/12).

Conclusion

Good to excellent agreement between FvU and Cx samples on hrHPV DNA and HPV16/18 genotype level was obtained, with significant positive correlations in HPV16/18 copies per μl DNA between both samples. These results furthermore demonstrate that FvU self-sampling is highly preferred among 25-64 year old women referred to colposcopy in Belgium.

References

ANTIRETROVIRAL THERAPY, HIGH-RISK HUMAN PAPILLOMAVIRUS AND CERVICAL INTRAEPITHELIAL NEOPLASIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background / Objectives

The interactions of antiretroviral therapy (ART) with high-risk (HR) HPV and cervical lesions in women living with HIV (WLHIV) are poorly understood. We reviewed HR-HPV and cervical intraepithelial neoplasia (CIN) and squamous intraepithelial lesions (SIL) outcomes in ART-taking compared to ART-naive WLHIV.

Methods

We performed a systematic review and meta-analysis by searching Medline and Embase databases for cross-sectional or cohort studies from 1 January 1996 to 6 November 2016 that reported the association of ART with prevalence of HR-HPV or prevalence, incidence, progression or regression of CIN or SIL abnormalities. We performed random-effects meta-analyses to estimate summary statistics. Heterogeneity was examined using the I² statistic.

Results

A total of 6,441 and 8,262 WLHIV were included from 29 studies evaluating the association of ART with prevalence of HR-HPV and high-grade CIN (CIN2+) or SIL (HSIL+), respectively. ART users had lower HR-HPV prevalence than ART-naive WLHIV (adjusted Odds Ratio [aOR] =0.83, 95%CI: 0.70-0.99, I²=51%, adjusted for CD4+ count and ART duration), and was also lower among prolonged ART users (>2 years) compared to short-duration users and ART-naïve combined (crude OR=0.65, 95%CI: 0.55-0.77, I²=0.0%). There was some evidence of lower risk of CIN2+/HSIL+ among ART users (aOR=0.65, 95%CI: 0.40-1.06, I²=30%).

Sixteen studies reported the association of ART with longitudinal cervical lesions (SIL) outcomes, from a combined total of 6,664 WLHIV. ART use was associated with a lower risk of any SIL incidence (adjusted Hazard Ratio [aHR] =0.64, 95%CI: 0.47-0.86, I²=19%, adjusted for time-varying ART and CD4+ count), and progression (aHR=0.64, 95%CI: 0.54-0.75, I²=18%) and increased likelihood of SIL regression (aHR=1.58, 95%CI: 1.28-1.94, I²=18%).

Conclusion
Prolonged ART use in WLHIV can decrease the risk of HR-HPV and CIN2+/HSIL+ prevalence, SIL incidence and progression and induces regression.
INCIDENCE TRENDS IN HPV-RELATED CANCERS IN NORWAY, AND CASES PREVENTABLE BY HPV VACCINATION

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Cancer Registry of Norway (Norway)

Background / Objectives

To examine incidence trends in squamous cell cancers of the cervix, vulva, vagina, anus, penis and oropharynx, and cervical adenocarcinoma for the period 1953-2015, and to determine how many currently incident cases may be prevented by bi/quadrivalent and nona-valent HPV vaccination

Methods

We extract data from the Cancer Registry of Norway, which has complete and accurate registration of all cancer cases in Norway for the whole study period. Trends in incidence are examined by joinpoint regression and the annual percentage change statistic for each cancer site and for each sex. To estimate the preventive potential of HPV vaccination, we use previously published accounts of fractions attributable to HPV for each cancer site

Conclusion

Over the period 1953-2015, we observe significantly increasing incidences of anal, oropharyngeal, penile and vulvar cancer, and of cervical adenocarcinoma. The increase was most pronounced, with annual percentage changes exceeding 2, for anal cancer (for each sex) and for oropharyngeal cancer among men. Cervical squamous cell cancer incidence decreased after the introduction of screening, but remained stable after 2004. The incidence trends highlight the importance of primary prevention of HPV-related cancers. We show that the number of cases that can be prevented by HPV vaccination in Norway is substantial, also for non-cervical HPV-related cancers, and among men. Moreover, in comparison with the bi/quadrivalent HPV vaccines, use of the nona-valent vaccine will prevent a substantial additional number of cervical cancer cases in Norway
A COMPREHENSIVE LANDSCAPE OF 27 HPV VIRUSES’ PREVALENCE AND MULTI-INFECTION PATTERNS, HIGH CONSISTENCY BETWEEN THE HPV16/18 CO-INFECTION PREFERENCE PATTERN AND THE CROSS-PROTECTIVE EFFICACY OF HPV16/18 VACCINE AGAINST NON-VACCINE HPV TYPES.


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Background / Objectives

In China, the attribution of HPV52/58 is significantly higher than elsewhere. China’s medical resources are concentrated in tertiary hospitals, where bearing “HPV-heavy-burden”, which can provide representative samples to delineates a national comprehensive landscape of the prevalence and multi-infection patterns of HPV among gynecological outpatients (GOP), and to evaluate the cross-protective efficacy of HPV16/18 vaccine.

Methods

We recruited participants out of GOP from 8 tertiary hospitals in 7 provinces of China. Cervical exfoliated cell samples were collected for HPV genotyping using Tellgenplex™ HPV DNA Assay. Odds ratio was used for the evaluation of the preference of any two types co-infection (AB): the real infection rate of AB divided by it’s theoretical rate (the multiplication of the single infection rate of A and B).

Results

Among 137,949 samples from GOP, the total prevalence of 27 HPVs (17hr/10lr) was 23.5%. Age-specific prevalence showed a flat “U-formed” pattern. The most prevalent hrHPVs were all from α: 16(3.3%), 52 (2.3%), 58 (1.9%). The most prevalent lrHPVs were both from α: 81 (0.9%), 61 (0.9%). Overall, the prevalence of
6 and 11 were 0.6% and 0.3%, which differed by geographic region and decreased with age. Multi-infection was identified in 25.8%. The two-types-infection was predominant. We found that 15% of hrHPV infections were co-infected with lrHPV; while 40% lrHPV infections were co-infected with hrHPV. HPV16 consisted of 66.51% single infection, the highest, followed by 52(60.2%), 58(59.81%), while 26(33.3%) as the lowest. The mixed genotypes 16+58(283) and 16+52(265), 52+58(242), 16+18(195) were the most common multi-infections. The co-infection (AB) preference pattern of 13 hrHPVs to 16 was 31(3.4), 45(2.8), 33(2.7), 35(2.4), 18(2.3), 56(2.0), 59(1.8), 58(1.7), 39(1.7), 66(1.7), 51(1.6), 68(1.3), 52(1.2). The co-infection (AB) preference pattern of 13 hrHPVs to 18 was 31(3.9), 35(3.0), 56(2.7), 51(2.7), 33(2.6), 66(2.5), 59(2.4), 58(1.6), 39(2.2), 45(2.0), 52(1.7), 58(1.6), 68(0.7). This analysis revealed that HPV31 was the most involved in HPV 16/18 co-infection, while HPV52/58 had less co-infection preference to HPV16/18.

Conclusion

These co-infection (AB) preference patterns are highly consistent with cross-protective efficacy of HPV16/18 vaccine against HPV31, but almost negative vaccine efficacy against HPV52/58. On one side, this finding may explore the mechanism of cross-protection of HPV vaccines. On another side, it indicates that it is urgently needed to evaluate the efficacy of HPV vaccines and the influences to the HPV epidemic in China, where with high prevalence of HPV52/58 as the current vaccines only covering HPV16/18.

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FC 06-04
ESTIMATION OF THE OVERALL BURDEN OF CANCERS, PRECANCEROUS LESIONS, AND GENITAL WARTS ATTRIBUTABLE TO 9-VALENT HPV VACCINE TYPES IN WOMEN AND MEN IN EUROPE

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Background / Objectives

In addition to cervical cancer, human papillomavirus (HPV) is responsible for a significant proportion of cancers and precancerous lesions of the vulva, vagina, anus, penis, head and neck, as well as genital warts. We estimated the annual number of new cases of these diseases attributable to 9-valent HPV vaccine types in women and men in Europe.

Methods

The annual number of new cancers of the cervix, vulva, vagina, anus, penis, and selected head and neck sites in the population of the European Medicines Agency territory was estimated based on age-specific incidence rates extracted from Cancer Incidence in 5 Continents, Volume X and Eurostat population data for 2015. The annual number of new cancers attributable to 9-valent HPV vaccine types was estimated by applying the HPV attributable fraction from reference publications based on a large European multicenter study. For non-cervical cancers, HPV attributable fractions were based on oncogenically-active HPV infections only (i.e., detection of HPV DNA and either mRNA and/or p16 positivity). For precancerous lesions of the cervix, vulva, vagina, and anus, and for genital warts, previously published estimations were updated for the 2015 population.

Results

The annual number of new cancers attributable to 9-valent HPV vaccine types was estimated at 47,992 (95% bound: 39,785-58,511). Cervical cancer showed the highest burden (31,130 cases), followed by head and neck cancer (6,786 cases), anal cancer (6,137 cases), vulvar cancer (1,466 cases), vaginal cancer (1,360 cases), and penile cancer (1,113 cases). About 81% were estimated to occur in women and 19% in men. The annual number of new precancerous lesions (CIN2+, VIN2/3, VaIN2/3, and AIN2/3) and genital warts attributable to 9-valent HPV vaccine types was estimated at 232,103 to 442,347 and 680,344 to 844,391, respectively.
Conclusion

The burden of cancers associated with 9-valent HPV vaccine types in Europe is substantial in both sexes. Head and neck cancers constitute a heavy burden, particularly in men. Overall, about 90% of HPV-related cancers, 80% of precancerous lesions, and 90% of genital warts are expected to be attributable to 9-valent HPV vaccine types each year, demonstrating the important preventive potential of the 9-valent HPV vaccine in Europe.
Declines in genital warts diagnoses since change in 2012 to use the quadrivalent HPV vaccine in England: data to end 2016

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Background / Objectives

A national school-based HPV vaccination programme for girls aged 12-13 years old was introduced in the UK in September 2008 offering the bivalent HPV 16/18 vaccine. In 2012, the programme changed to offer the quadrivalent vaccine, additionally protecting against HPV types 6 and 11, responsible for approximately 90% of genital warts (GW). Coverage for the vaccination programme has been high, with over 85% of routine cohorts completing all doses. Previously reported data have shown modest declines in GW diagnoses, suggesting a potentially cross-protective effect of the bivalent vaccine against GW. We present the first evidence of declines in GW diagnoses following the programme change to the quadrivalent vaccine.

Methods

Data were obtained from the GUM Clinic Activity Dataset (GUMCADv2) submitted by GUM and integrated GUM/sexual and reproductive health clinics for years 2009-2016. GUMCADv2 is a mandatory reporting system, providing disaggregate records of all attendances, testing and diagnoses at GUM clinics in England and has been reported to Public Health England (PHE) since 2008, with full coverage from 2009. All records coded as first episode GW for females and males aged 15-24 years old were extracted. Diagnoses of recurrent GW were excluded.

Results

Data to end 2015 – available at abstract submission: In 2015, 254,775 and 77,584 attendances were recorded by GUMCADv2 in 15-19 year old females and males, respectively. The rate of GW diagnoses for females aged 15 to 19 years was 38.9% lower (from 685.8 to 419.2 per 100,000 population) in 2015 than in 2009, and 30.2% lower (from 274 to 191.2 per 100,000 population) for 15-19 year old males. Over the same time period, the greatest declines were observed in 15 year old females (83.2%) and 16 year old females (58.0%); around 2/3 of vaccinated 15 year olds and 1/6 of vaccinated 16 year olds would have received the quadrivalent vaccine. Reductions in the rate of GW diagnoses among same aged males were 31.6% and 32.7%. Decreases of 17.5% (from 698.9 to 576.8 per 100,000 population) and 15.5% (from 849.6 to 718.2 per 100,000 population) were seen in 20-24 year old females and males, respectively.

Conclusion
The moderate, unexpected declines in GW that we have seen since the introduction of a high coverage HPV vaccination programme using the bivalent vaccine are being followed, as expected, by much larger declines amongst females offered the quadrivalent vaccine.

These ecological observations suggest that the high coverage female-only HPV vaccination programme is affording substantial herd protection to young males.

We will present analyses including data to end 2016 (available in June 2017), with additional sub-group analyses.
CHARACTERIZATION OF GENOTYPE-SPECIFIC HPV PREVALENCE IN CUTANEOUS WART BIOPSIES

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Background / Objectives

Cutaneous warts are a common, infectious and sometimes very painful problem, with a varying worldwide prevalence of 0.84-12.9% 1. Warts are caused by infection with the human papillomavirus (HPV). The most frequently found HPV types in cutaneous warts are HPV1, 2, 3, 4, 7, 10, 27, 41, 57, 60, 63 and 65. The aim of this study was to develop a high-yield DNA extraction protocol for formalin-fixed paraffin-embedded biopsies and determine the prevalence of HPV in cutaneous warts in a Belgian population.

Methods

A total of 50 biopsies were included in this study. Before and after slicing of the sections predetermined for DNA extraction (10x5µm), additional sections were made for haematoxylin-eosin (HE) staining to ensure that these were derived from wart epithelium. The optimized protocol involved overnight Proteinase K and EDTA digestion, followed by automated extraction on the NucliSENS® easyMAG® system (bioMérieux). A newly developed wart-associated HPV qPCR assay, capable of detecting the above mentioned cutaneous types, together with the in-house HPV RiatoL genotyping assay, capable of detecting the most relevant mucosal types (HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67 and 68), were used to determine the HPV prevalence.

Results

The wart diagnosis was confirmed by HE staining. All samples tested positive for B-globin (cell control) and were considered valid. 24% [95%CI 12-36%] of the samples were negative for the above mentioned cutaneous as well as mucosal HPV types. Cutaneous HPV types 41, 60 and 63 were not detected. 8% [95%CI 0.5-16%] of the samples was infected with mucosal low-risk (HPV6 and 11) and high-risk (HPV16, 58 and 59) HPV types and 36% [95%CI 23-49%] contained multiple infections.

Conclusion
The most prevalent HPV types in the Belgian population were HPV1, 57, 4, 2, 27 and 7. Multiple HPV infections were detected in 36% of lesions, contradicting the current literature claiming that in immunocompetent patients only 0-16% of cutaneous warts exhibit multiple HPV infections\textsuperscript{2,3,4}. Considering that cutaneous warts are very inconvenient disorders that can cause not only pain, but also diminish the quality of life of the affected individuals, a more efficient management should be implemented. Since it has been suggested that HPV type can influence natural course and response to treatment in certain subsets of verrucae\textsuperscript{2}, genotype specific strategies should be considered, indicating an important role for future HPV genotyping.

References


BURDEN OF GENITAL WARTS IN PERU, ARGENTINA AND ECUADOR: AN OBSERVATIONAL STUDY

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Background / Objectives

Genital warts (GW) are mucosal or skin lesions caused by human papilloma virus. The burden of disease due to genital warts in Latin America is not well characterized. The study objectives were to estimate the burden of genital warts (GW) within the healthcare system and usual practices of GW management in Peru, Argentina and Ecuador.

Methods

We recruited a convenience sample of 250 physicians from the public sector in Peru and both the public and private sector in Ecuador and Argentina: primary care (28), gynecology (119), urology (30), dermatology (50), infectious diseases (10), proctologists (2) and other (11). Physicians completed a daily log of all patients 18-60 years of age seen over 10 days in their offices, as well as a survey collecting data on patient demographics, GW diagnosis, referral patterns, diagnosis, in-office procedures, duration of treatment and estimated number of office visits required for treatment.

Results

The 250 physicians reported seeing a total number of 31,111 patients, 77.1% were women. 1,294 males and females had a GW diagnosis, 38.02% were in men. GW overall prevalence was 4.16% (95% CI 3.94% - 4.39%); 2.28% (95% CI 2.02-2.56) in Peru, 5.51% (95%CI 5.10-5.92) in Ecuador and 5.1% (95%CI 4.54-5.58) in Argentina. The prevalence was highest among primary care providers in Peru, 4.68 (98% CI 3.86-5.59), infectious disease specialists in Ecuador 7.38 (95%CI 4.53-10.23) and urologists in Argentina 10.9% (95% CI 8.9-12.8). Of the GW cases observed, 52.7% were the first reported episode in the patient’s life, 12.5% were cases without an episode in the previous 12 months and 34.8% were existing cases. Peru reported the highest proportion of first time cases, 64.0%, with Ecuador and Argentina reporting 50.4% and 48.2% respectively. Most physicians reported seeing patients who were direct-consult.
Conclusion

GW cases are commonly seen by physicians in Peru, Ecuador and Argentina and only a slight majority (52.7%) of these was the first reported episode in the patient’s life. In Peru, cases were most often seen in primary care providers, whereas in Ecuador and Argentina cases were seen by specialists, with that being said, physicians recruited in Peru were from the public sector while those in Ecuador and Argentina were a mix of public and private providers. Our data suggests that GW may represent a substantial healthcare burden in Peru, Argentina and Ecuador.
AN OVERVIEW OF CERVICAL CANCER EPIDEMIOLOGY AND PREVENTION IN SCANDINAVIA

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Background / Objectives

In the Scandinavian countries of Denmark, Norway and Sweden, organised cervical cancer prevention programmes have contributed to reducing the cervical cancer burden. However, new technologies, such as primary human papillomavirus (HPV) DNA testing and HPV vaccination, necessitate comprehensive policy analyses to identify optimal prevention approaches. To inform future policy analyses, we aimed to provide an overview of cervical cancer epidemiology and existing prevention efforts in Scandinavia.

Methods

We compiled and summarised data on current prevention strategies, population demography, and epidemiology for each Scandinavian country by reviewing published literature and official guidelines, performing registry-based analyses using primary data, and discussions with experts in each country. We compared age-specific cervical cancer incidence for years 1960-66 and 2010-14 across the countries using Poisson regression with indicators for five-year age-groups (ages 20-84 years) and for each country. We also assessed country-specific variations in age-specific HPV prevalence using Fisher’s exact test and logistic regression.

Results

In general, nationwide organised cytology-based screening was implemented in all Scandinavian countries by 1996, but opportunistic screening occurred as early as the 1950s. Prior to implementation of widespread screening and during years 1960-1966, cervical cancer incidence was considerably higher in Denmark than in Norway and Sweden. Decades of cytology-based screening later (i.e. years 2010-14), the incidence remains the lowest in Sweden, with Norway and Denmark having an age-adjusted incidence rate ratio (95% CI) of 1.28 (1.20-1.37) and 1.36 (1.28-1.45), respectively. HPV prevalence peaks at younger ages (i.e. younger than age 24
years) and thereafter decreases by age for all genotypes in all countries, but was generally lowest in Sweden. For all countries the most prevalent HPV genotypes were HPV16, 18 and 31.

Conclusion

Scandinavian countries generally face similar cervical cancer burden and utilise similar prevention approaches; however, important differences remain as cervical incidence and HPV prevalence remains lowest in Sweden. Future policy analysis will need to evaluate whether these differences warrant differential prevention policies, or whether efforts can be streamlined across Scandinavia.
Type-specific human papillomavirus profile, absolute risk and attributable fraction to cervical cancer and precancerous lesions—a population-based study of 3,083 women in Inner Mongolia, China.

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Background / Objectives

Given the variation of region-specific HPV genotypes prevalence, knowledge about the distribution of human papillomavirus (HPV) genotypes in general population and their attribution to high-grade cervical lesions is crucial to guide the introduction of prophylactic vaccines and the implementation of cervical cancer screening. Few studies, however, comprehensively focused on the HPV genotypes profiles in such high-risk regions with ethnic minority as Inner Mongolia, in China. To analyze the HPV genotypes characteristics and the presence of multiple HPV infections among general population and estimate type-specific absolute risk and relative contributions to cervical intraepithelial neoplasia grade 2 or worse (CIN2+).

Methods

Between Jun and Aug 2016, 3,083 women aged 21–64 years were enrolled in a cervical cancer screening study in Inner Mongolia Province, China. Each participant was examined by Hybrid Capture 2 testing and liquid-based cytology. Women positive with any screening results were referred to colposcopy and biopsy was taken if necessary. All cervical cytological cells were tested using SPF10-LIPA system to discriminate 28 HPV genotypes. Absolute risk of cervical (CIN2+) for type-specific HPV was calculated and the corresponding attribution to cervical lesions was estimated using a fractional contribution approach.

Results

High-risk HPV (HR-HPV) prevalence was 17.5% and abnormal cytology rate was 14.2% in the general population. Most five common genotypes were HPV 52, 39, 16, 51, 58. Multiple-type infection rate varied by age and peaked at women with 50 years old and more (12.0%). Women infected with HPV16 were at highest absolute risk of CIN2+ at 28.7%, followed by HPVs 58, 33, 35, 18 and 52 at 12.0%, 11.8%, 9.5% and
9.3%, 8.8% respectively. Attributable fraction (AF) to CIN2+ differed by type-specific HPV and was predominated by HPV 16 with the AF of 54.9%, followed by HPV 52 (52.4%), HPV 39 (4.6%), HPV 58 (2.7%), HPV18 (1.9%).

Conclusion

Type-specific high-risk HPV-DNA-based screening tests and protocols and introduction of polyvalent HPV vaccines might give the priority to HPV types 16, 18, 52 and 58 in the high-risk region in China, as their high prevalence, high absolute risk and notable attributable fraction to high-grade cervical lesions.
SEX DIFFERENCES IN PREVALENCE, INCIDENCE AND CLEARANCE OF ANOGENITAL HUMAN PAPILLOMAVIRUS INFECTION IN CHINA: A POPULATION-BASED PROSPECTIVE STUDY

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Background / Objectives

Understanding sex differences in natural history of anogenital human papillomavirus (HPV) infection is essential for making policies to prevent and control HPV infection and related diseases. However, there is a scarcity of researches focused on both sexes, and direct comparing among different studies is difficult due to different sampling and typing methods.

Methods

From May to July 2014, a total of 2309 men and 2378 women aged 18-55 years old were enrolled from communities and universities in Liuzhou, China. Penis/glans penis/coronary sulcus and perianal/anal canal (PA) specimens of men and vaginal, vulvar and PA specimens of women were collected biannually for up to three visits and genotyped for 12 oncogenic HPV (classified as Group 1 by IARC) and 2 non-oncogenic HPV types (HPV 6 and 11) by PCR. Prevalence analysis was performed among 1937 (83.9%) men and 2344 (98.6%) women with the valid HPV typing result at baseline. Totally 1643 (71.2%) men and 1752 (73.3%) women with a median follow-up of 12.5 months (range 5.0-19.1) and 12.6 months (range 5.0-20.1), respectively, were included in incidence analysis.

Results

The prevalence of oncogenic HPV type was higher in women than that in men (18.7% vs 9.4%, P < .001), whereas the prevalence of HPV 6 and 11 infection was similar (1.4% vs 1.2%, P = .6832). Incidences of oncogenic HPV infection in men and women were 10.1 (95% confidence interval (CI) 8.6-11.5) and 9.4 (95% CI 8.2-10.6) per 1000 person-months, respectively, and no sex differences were found (P = .4659). However, men was more likely to acquire HPV 6 or 11 infection than was women (2.0 vs 1.1, P = .0223). Median duration of HPV infection was longer in women than that in men for both oncogenic (11.6 months vs 6.8 months, P < .001) and non-oncogenic (11.9 months vs 6.4 months, P < .001) types. Both prevalence and incidence of oncogenic HPV infection decreased with age in women, but did not
vary by age in men. Besides sex behavior, hygiene behavior was also associated with prevalence and incidence of HPV infection in both sexes.

Conclusion

This is the first large population-based prospective study focused on HPV prevalence, incidence and clearance in anogenital sites of both sexes. For oncogenic HPV, though newly acquired anogenital infections were comparable between men and women, the median duration of infection was shorter in men, thus women were more of a major reservoir than men. For HPV types 6 and 11, men had a higher speed to both acquire and clear infection, thus the two sexes seem to contribute similarly to the virus circulation. Our study indicated that interaction of host and virus might be different for oncogenic and non-oncogenic HPV types between sexes.
EVALUATION OF MUCOSAL AND SYSTEMIC IMMUNOGLOBULIN A/G RESPONSES ONE YEAR AFTER 3 DOSES OF THE HUMAN PAPILLOMA VIRUS-16/18 ASO4-ADJUVANTED VACCINE

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Background / Objectives

Vaccination against oncogenic human papillomavirus (HPV) types is an intervention for cervical cancer prevention. Despite there being a relatively long period of time since the beginning of clinical use of HPV vaccines no evidence-base data is available on the need of a boost vaccination. We investigated one-year post-vaccination antibody responses against HPV 16/18 by detection of IgG and IgA HPV-specific antibodies in cervical secretion samples and serum.

Methods

This study was designed to describe the course of IgG/IgA responses in cervical secretions and in serum one year after the first dose of intramuscular administration of the HPV16/18 AS04-adjuvant vaccine. Blood and cervical mucus samples were collected for immunologic assays, 7 months after the first doses and 1 year following the last boost vaccination (month 7) by enzyme linked immunosorbent assay (ELISA). The detection of IgG and IgA anti-HPV/VLP was developed for this purpose.

Results

It was observed that approximately 100% of the IgG serum samples reacted when the antigen was present in the first dilution of both collections. The positivity, however, decreases, according to the dilutions. Regarding IgA reactivity in serum, initial conversion was observed in 95% at month 7 of vaccination and 79% after 1 year. Similar results can be seen in the mucus samples with a higher positivity at month 7 and decreasing after 1 year, lower levels of IgG and IgA antibody were detected in the cervical mucus (33%) and 29%, respectively, after 1 year of vaccination. The median absorbance detected in serum samples for IgG and IgA anti-HPV-VLP antibodies was significantly higher at 7 months after vaccination, in the dilutions 1:100, 1:1.000, 1:10.000 and 1:10, 1:100, respectively, when compared to 1 year after vaccination (P<0.0001). The median absorbance detected in cervical mucus samples was significantly higher at 7 months after vaccination, in the dilutions 1:100, 1:1.000 and 1:10 for anti-HPV-VLP IgG and IgA, respectively, when compared to 1 year after, with 1:100 and 1:10, for IgG and IgA respectively (P< 0.0001).
serum and cervical mucus samples, the median absorbance was significantly higher 7 months after vaccination and it is possible to see the decrease at 1 year after, according to dilutions.

Conclusion

One year after the first dose, the immune responses induced by the HPV-16/18 AS04-adjuvant vaccine were significantly decreased in cervical secretion samples and serum when compared to seven months after the first dose. A possible vaccine booster may be necessary. However, longer follow-up studies are necessary to assess the need for booster doses after primary vaccination with 2 as well as with 3 doses.
ADVANCING HPV VACCINE DELIVERY: 12 PRIORITY RESEARCH GAPS


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Background / Objectives

Recent reviews have identified interventions for increasing HPV vaccination, but effects were small and evidence was often insufficient to identify best practices. The National HPV Vaccination Roundtable sponsored a one-day meeting in the United States in 2016 on best and promising practices in HPV vaccine delivery, in part to identify important research gaps.

Methods

Meeting participants were ~500 HPV vaccine delivery experts including scientists, clinicians, and other stakeholders, including ~400 who streamed the event online. Throughout the meeting, facilitators encouraged attendees to identify gaps that future research should address, and write them on display boards or send via email or Twitter. In-person attendees then voted for up to five gaps they believed were top priorities. Gap numbers refer to their priority ranking, with Gap 1 having received the most votes.

Results

Attendees identified 33 research gaps. Several themes emerged among the 12 prioritized gaps. One theme was social media and vaccine confidence, which included: Gap 1, how to increase HPV vaccine confidence by intervening in social media; Gap 4, how to address rumors about HPV vaccine that spread via social media; and Gap 8, how to address parents’ concerns and hesitancy about HPV vaccine. A second theme was healthcare provider interventions, which included: Gap 2, how to encourage providers to attend in-clinic quality improvement interventions; Gap 6, how to intervene with the entire medical team; and Gap 10, how to increase HPV vaccination during acute care visits. A third theme was system-level approaches, which included: Gap 3, best practices for health insurers and plans; Gap 12, the impact of quality standards; Gap 11, effective system-level changes in large health systems and hospitals; and Gap 5, the impact of connecting immunization information systems to electronic health records (EHRs) and exchanging data bi-directionally. Two other prioritized gaps that did not fit these themes were Gap 7,
determining what interventions work in rural areas; and Gap 9, the impact of survivor testimonials.

**Conclusion**

Experts identified and prioritized research gaps that may have promise for increasing HPV vaccination in the US and internationally. It is critical to develop and evaluate interventions in each of these areas to close existing gaps and identify best practices for increasing HPV vaccination. Grant support: US Centers for Disease Control and Prevention (1H23IP000931-01, Saslow, PI).
TRENDS IN PREVALENCE OF HUMAN PAPILLOMAVIRUS TYPES AND THE IMPACT OF NONAVALENT VACCINATION: ANALYSIS ON 13,665 PATIENTS OVER A 18-YEAR STUDY PERIOD

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Background / Objectives

Human Papillomavirus, HPV, vaccination significantly reduced the incidence of cancerous/precancerous condition of the genital tract. The quadrivalent vaccine type 6,11,16,18 was licensed in 2006; more recently, the Food and Drugs Administration (FDA) approved the nonavalent vaccination against HPV. Here, we aimed to test the theoretical utility of the incorporation of nonavalent vaccination into a clinical setting.

Methods

Data of consecutive patients undergoing sampling for HPV DNA testing from 1998 to 2015 were retrospectively searched in order to identify changes in HPV prevalence during three study periods (T1, 1998-2003; T2, 2004-2009; and T3, 2010-2015).

Results

We enrolled 13,665 patients: 1361, 5130, 7174 patients, in T1, T2 and T3, respectively. Potentially, the quadrivalent vaccine protected against HPV infection in 71.5%, 46.5% and 26.5% of patients tested in T1, T2 and T3, respectively (p-trend<.001). While, the nonavalent vaccine protected against HPV infection in 92.5%, 72.3% and 58.1% of patients tested in T1, T2 and T3, respectively (p-trend<.001). The proportion of patients with genital dysplasia grade2+, not related to HPV genotypes covered by quadrivalent vaccine (13% in T1, 21% in T2 and 34% in T3) and nonavalent vaccine (3% in T1, 12% in T2 and 19% in T3) increased over the time (p-for-trend<.001). For all study period the nonavalent vaccine was superior that quadrivalent vaccine in protect against HPV infection (p<.001).

Conclusion

Our data suggested that potentially the introduction of the nonavalent vaccine would improve protection against HPV infections and HPV-related genital dysplasia2+. Moreover, we can speculate that cross protection of nonavalent vaccine will be related to a highest coverage against other HPV types.
FC 07-04
DESIGN, BASELINE FINDINGS AND HPV GENOTYPES FROM A RANDOMIZED CONTROLLED TRIAL WITH THE QUADRIVALENT HPV VACCINE COMPARING A 2-DOSE (0, 6 MONTHS) TO AN EXTENDED (0, 6, 60 MONTHS) SCHEDULE: ICI-VPH STUDY

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Background / Objectives

In Quebec (Canada), the HPV vaccination program targets 9-10 year-old girls who receive 2 doses of the quadrivalent HPV vaccine (Q-HPV) at 0, 6 months, through a school-based program. The main objectives of ICI-VPH are to evaluate whether a 2-dose schedule (0, 6 months) is non-inferior to a 3-dose schedule (0, 6, 60 months) to prevent persistent HPV 16 and 18 infections and to compare HPV6-11-16-18 GMTs between the 2 study groups, 10 years after the first dose. Here, we present the design of the trial, the baseline characteristics of the participants, and the HPV genotypes identified at recruitment.

Methods

We recruited teenage girls who had been vaccinated with 2 doses of Q-HPV in fourth grade, 5 years earlier. The participants were randomized to receive a 3rd dose or not. Participants self-collected a vaginal sample for HPV genotyping at baseline and will do so every 6 months for 5 years. Participants also provided health and behaviour data at baseline and will continue to do so every year for 5 years. We collected a blood sample (first 500 participants) at baseline for HPV serology testing, and other samples will be collected after 2.5 years and 5 years. Self-collected vaginal swabs are tested for HPV by a generic HPV PCR assay, and if positive, tested with the Linear Array for the detection of 36 genotypes.

Results

Between 2013 and 2016, we randomized 3364 participants 13-16 years of age, in the province of Quebec, Canada. The 2 study groups were comparable at recruitment:
92.2% were born in Canada, 85.4% identified as French Canadians, 91.0% were non-smokers, 16.0% were sexually active and 21.2% had used hormonal contraception. Among those reporting sexual activity, 80.5% had had intercourse. Genotyping results at recruitment were available for 216 participants who reported having had intercourse. Among them, HPV prevalence was 8.3% (n=18). Single infections (4.2%) were as frequent as multiple infections (4.2%). The most prevalent types were: HPV 51 and HPV 84 (each 2.8%, n=5); HPV 62 (1.9%, n=4); HPV 53, HPV 66, HPV 73, HPV 89 (each 1.4%, n=3); HPV 33, HPV 56 (each 0.9%, n=2); HPV 31, HPV 35, HPV 39, HPV 40, HPV 42, HPV 58, HPV 59, HPV 67 (each 0.5%, n=1). HPV types 6/11/16/18 were not detected.

Conclusion

Vaccine targeted types were not detected in this cohort of 13-16 year-old, 5 years after vaccination with a 2-dose schedule of Q-HPV. To our knowledge, this ongoing study is the first to assess the role of an HPV booster dose for the Q-HPV vaccine within a high-coverage vaccination program.
Efficacy of HPV Vaccine in Young Women in Colombia After Five Years of Its Introduction.


Background / Objectives

Reduction in the prevalence of vaccine type HPV infection in young women offers the opportunity to monitor early effects of vaccination program. In Colombia, HPV vaccination was introduced in 2012 by National Immunization Program as primary strategy for the prevention of cervical cancer. We evaluated the program’s impact on genotype-specific HPV infection prevalence and the distribution in a group of non-vaccinated vs vaccinated young women aged 18-25 years old from Colombian

Methods

Young women aged 18–25 years from Manizales were invited to participate to this study through different communications strategies established at local health centers and Technological and higher education institutes. Cervical samples were tested for type-specific HPV DNA using a Linear Array genotyping test. HPV prevalence infection among 807 women in the post-vaccine group (2016–2017) were compared with prevalence of 951 women non-vaccinated (2015).

Results

The prevalence of vaccine HPV genotypes (16, and 18) was significantly lower in vaccinated sample than in the non-vaccinated sample: 4.8 % (39/807) vs 15.5% (147/951); P < .001). Moreover, this reduction was higher in women vaccinated before starting sexual activity compared to after 2.4% (8/329) vs 6.5% (31/478) or women who received 3 doses (0%) or at least 2 doses (2.4%) of vaccine compared who received 1 doses 8.5% (28/329). We found evidence of cross-protection for HPV31, HPV 45 after vaccine introduction but it was no significant. Besides, we found slight increases in 4 nonvaccine high-risk HPV types (HPV39, HPV 51, HPV52 and 59). Interestingly, to HPV6/11 infection although the frequency was low in non-vaccinated group (3.3%), after vaccination it was 0.2% to HPV6 and no infection were observed to HPV 11. Finally, although there is a reduction of HPV vaccine type,
the prevalence of other HR-HPV remain high, even a slight increase in the vaccinated population is observed (36.6% non-vaccinated Vs 39.7% vaccinated).

**Conclusion**

Five years after the introduction of the Colombian HPV vaccination program, a decrease in vaccine-targeted genotypes is evident. Variables such as age, number of doses and application of the vaccine before sexual debut increase the effectiveness of the vaccine. In addition, knowing the actual state of the HPV infection in Colombia allows to evaluate the current vaccination schemes and raises the possibility of inclusion of the nonvalent vaccine in our population.
Quantifying the impact of HPV vaccination of 12 year old girls on cervical disease and cytology performance

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Background / Objectives

Routine school-based HPV immunisation was offered to girls aged 12-13 in Scotland from 2008, with a catch-up programme over 3 years for 14-18 year old girls. To date, we have shown that vaccination of catch-up cohorts is associated with a significant decrease in prevalence of HPV 16/18/31/33/45, in cervical intraepithelial neoplasia of all grades and in cytology performance, particularly the predictive value of low-grade cytology. This reduction is achieved despite probable exposure to HPV before immunisation, particularly of the older catch-up girls.

The first cohort immunised at age 12-13 entered the Scottish Cervical Screening Programme in September 2015 at age 20. A further decline in HPV 16/18/31/33/45 prevalence was observed in these women when compared to the catch-up population. Evidence of herd immunity in the unvaccinated population has also emerged, including for cross-protective types.

To complement the work on viral outcomes we will present data on (1) the level of CIN (2) the performance of cytology in the routinely immunised cohort.

Methods

The Scottish Cervical Call Recall System - the national IT system containing all screening records and also vaccination status - will be interrogated. By June 2017 we will have a minimum of 12 months follow-up on all 31,000 routinely immunised women invited for screening. Linked data on degree of immunisation, on cytological abnormalities and on histological diagnosis following referral for colposcopy will be used to calculate odds ratios by immunisation status for cytological abnormality and histological diagnosis. The cytological abnormalities will be correlated with histological diagnosis to determine performance of cytology as a screening test.

Results

Approximately 31,000 women aged 20 were invited between September 2015 and June 2016. Over 23,000 of these had received 3 doses of vaccine. Initial evidence suggests that high grade disease (CIN2+/HSIL+) is virtually absent routinely.
immunised females. Before immunisation, CIN 2+ was confirmed in 2.84% of women screened at age 20. In the 1995 cohort (immunised at age 12-13), CIN 2+ is present in 0.33% of women. Comprehensive data on disease prevalence and on the performance of cytology as a screening test will be presented.

**Conclusion**

This will be the first population-based demonstration of the clinical effect of bivalent HPV immunisation of girls who are likely to have been HPV-naive at the time of vaccination. The information presented will be of great importance for the design and delivery of screening programmes in all highly vaccinated populations, and for planning cancer prevention strategies in resource-poor countries.
CELLULAR IMMUNE RESPONSES SIX YEARS FOLLOWING REDUCED-DOSE QUADRIVALENT HPV VACCINE IN ADOLESCENT FIJIAN GIRLS


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Background / Objectives

The World Health Organization has recommended 2-dose HPV vaccine schedule separated by 6 months to girls <15 years old as an alternative to the current 3-dose schedules. However, the long-term protection following reduced-dose schedules is unknown. This study examined long-term immunity by comparing the cellular immune responses in girls previously given 3 doses of 4vHPV (Gardasil®, Merck Inc.) 6-7 years ago with reduced doses (1 or 2 doses).

Methods

A prospective cohort study was undertaken in 200 Fijian girls (15-19 years old) who previously received 0, 1, 2 or 3 doses of 4vHPV 6-7 years ago (N=50/group). Blood was taken pre- and 28 days following a single dose of 2vHPV (Cervarix®, GSK), and cellular immune responses in terms of IFNγ producing cells and cytokines production (IFNγ, IL-2, TNFα, IL-10 and IL-5) were measured against HPV16 and 18 using the IFNγ-ELISPOT assay and Multiplex Bead Array assay, respectively.

Results

Six years following the last dose of 4vHPV, girls who received 2 doses of 4vHPV (p=0.008) had significantly lower HPV18-specific IFNγ producing cells compared with girls who received 3 doses. Significantly lower cytokine responses (IFNγ: p=0.002;
IL-2: p=0.022; TNFα: p=0.016; IL-10: p=0.018) against HPV18 were also observed in girls who received 2 doses of 4vHPV when compared with girls who received 3 doses. These differences were no longer significant 1 month following a dose of 2vHPV. There was no significant differences in cellular immunity against HPV16 between the 2- and 3-dose groups six years after the last dose of 4vHPV and 1 month after a dose of 2vHPV. Interim analysis for the comparison of cellular responses between 1- and 3-dose groups are ongoing, which showed a similar trend (significant lower responses against HPV18 but not HPV16) as the 2-dose group.

Conclusion

Lower HPV18-specific IFNγ producing cells and cytokines were observed in the 2-dose group when compared with the 3-dose group after 6 years. These data suggest that cellular immunity against HPV18 following reduced-dose schedules may not persist as long as a 3-dose schedule, although the clinical significance is unknown. Despite the lower cellular immune responses, the neutralising antibody responses between the 2- and 3-dose groups were similar as shown previously, although lower antibody responses against HPV18 than HPV16 were observed (Toh et al., Clin. Infect. Dis, 2016). Longer follow-up studies on reduced-dose schedules are needed to determine whether cellular immune responses has a role in the long-term protection against HPV18.

References

The efficacy of vaccine prophylaxis of HPV-associated diseases in the Moscow region

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Background / Objectives

From 2007 to 2015 the absolute number of newly diagnosed cases of cervical cancer (CC) in the Moscow region (MR) increased from 594 to 785; the incidence rate per 100,000 in MR for the last 14 years increased from 7.9 in 2002 to 19.5 in 2015, and mortality rate during the 1st year of follow-up exceeds 12%. The program of vaccine prophylaxis of HPV-associated oncological diseases with quadrivalent vaccine in 12 to 13-year-old girls with a 0-2-6 month regimen has been conducted in MO (18 municipal districts) since 2008.

To conduct the efficacy analysis of the vaccine prophylaxis program.

Methods

The incidence rate of HPV-associated diseases in girls and women in 2009 to 2015 and the vaccination safety with the use of the specially designed register were studied.

Results

20,000 female adolescents were vaccinated during the 9-year program. The decrease in the incidence rate of anogenital condylomas (AC) was registered during the study period: the general incidence rate decreased from 127.2 to 24.7, the incidence rate per 100,000 children – from 63.3 to 11.9, and the incidence rate per 100,000 girls who reside in districts covered with vaccine prophylaxis - from 14.2 to 6.1 per 100,000; the decrease of the incidence rate of AC for the study period in the whole female population from 56.7 to 20.2 (per 100,000) was also reported. A positive trend in the decrease of CC case detection in the 15-24 age group from 0.3 to 0.1 (the rate of the detected CC cases among women of all ages) was registered in districts covered by vaccination; no CC cases in young women were registered. According to the register data, no serious adverse events were reported for the vaccination period; some vaccinated patients became pregnant and delivered healthy children.

Conclusion

The results of the vaccine prophylaxis program of HPV-associated diseases conducted in MR have demonstrated the safety and efficacy of vaccine prophylaxis
using a quadrivalent vaccine in decreasing the incidence rates of anogenital condylomas in girls and cervical cancer in young women.
Hazard of complex regional pain syndrome (CRPS) following HPV vaccination among adolescents in the United States

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Background / Objectives

We estimated the association between adolescent vaccination and incident complex regional pain syndrome (CRPS) in adolescent girls in the U.S.

Methods

We used insurance claims to identify claims for CRPS and adolescent vaccination, including HPV, tetanus-diphtheria-acellular pertussis (Tdap) and meningococcal conjugate vaccine (MenACWY), using diagnosis and procedure codes. We identified 11-year-old girls between 2006-2014 without a prior history of adolescent vaccination or CRPS, and followed them from the 11th birthday until CRPS diagnosis, loss to follow-up, or December 31, 2014. Time-dependent Cox models estimated hazard ratios (HRs) comparing CRPS hazard following recent vaccination (≤ 30 days) to CRPS hazard following prior (> 30 days) or no vaccination. HRs were adjusted for history of physical trauma in the year preceding follow-up or an instance of trauma during follow-up. We then estimated time-dependent HRs for CRPS following recent co-administration of common vaccine combinations. Finally, we identified common health diagnoses received by girls in the sample over follow-up, and estimated time-dependent HRs for CRPS comparing girls with each diagnosis to girls without.

Results

We identified 563 CRPS cases among 1,232,572 girls (incidence rate: 20.6/100,000 person-years). CRPS hazard was not significantly elevated following recent HPV (adjusted HR [aHR]: 1.41, 95% CI: 0.83, 2.40), Tdap (aHR: 1.20, 95% CI: 0.64, 2.59), or MenACWY (aHR: 1.21, 95% CI: 0.57, 2.55) vaccination. Comparing recent or non-recent vaccination to no vaccination, Tdap (HR: 1.59, 95% CI: 1.34, 1.89) and MenACWY (HR: 1.70, 95% CI: 1.43, 2.02) vaccination were associated with CRPS in crude analysis, but were not associated after adjusting for trauma (Tdap - aHR:1.09, 95% CI: 0.91, 1.29; MenACWY - aHR: 1.19, 95% CI: 1.00, 1.42). CRPS hazard comparing concomitant HPV vaccination to HPV vaccination alone was not significantly elevated (aHR: 2.30, 95% CI: 0.80, 6.56). Girls with lower limb injuries had the greatest CRPS hazard compared to girls without (HR: 12.4, 95% CI: 10.4, 14.7). Girls with anxiety disorders had a threefold hazard of CRPS compared to girls without (HR: 3.12, 95% CI: 2.41, 4.04). Common pediatric illnesses (e.g. asthma,
respiratory infections, allergies) were positively associated with CRPS in bivariate analyses.

Conclusion

Adolescent CRPS is rare in the U.S., and adolescent vaccination was not significantly associated with CRPS hazard. Crude HRs for CRPS following vaccination were confounded by physical trauma, a known CRPS risk factor. Future research should consider health-related risk factors for CRPS in adolescents, particularly injuries, and inflammatory and mental illnesses.
SYSTEMATIC CAUSALITY ASSESSMENT OF ADVERSE EVENTS FOLLOWING HPV VACCINATION IN ITALY

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Background / Objectives

WHO recommends that serious adverse events following immunization (AEFIs) should be monitored and evaluated using a standardized algorithm for causality assessment. “Per protocol”, after a comprehensive analysis of the event and the concomitant factors, the relationship between the vaccine and the AEFI could be classified as “consistent”, “not consistent”, “undetermined” or “unclassifiable” (1). Despite WHO recommendations, the AEFI causality assessment manual is, in the clinical practice, rarely adopted. In Italy, AEFIs, spontaneously reported from physicians, healthcare workers or patients to the National Drug Authority (AIFA) routine surveillance system, are classified only by temporal criteria. Therefore, the AEFIs report, published yearly by the AIFA, lacks information about the strength of correlation between events and vaccines (2). In this work, we aimed at evaluating the systematic use of Causality Assessment algorithm of AEFIs following HPV vaccination in Italy.

Methods

In the Apulia region of Italy (about 4,000,000 inhabitants), from 2008 to 2014, 438,294 HPV vaccine doses were administered to females aged 12, 18 and 25 years. We selected severe AEFIs following HPV vaccination reported between 2008 and 2014 to the AIFA routine surveillance system. We applied the WHO causality assessment criteria; for AEFIs requiring hospitalization, we repeated the assessment obtaining additional information from individual medical records.

Results

Of 14 severe AEFIs following HPV vaccination (reporting rate: 3.2 x100,000 doses), 8 (57.1%) led to hospital admission. After causality assessment, 7 AEFIs were classified as consistent, 3 undetermined, 2 not consistent, 2 unclassifiable. Among hospitalized cases, 4 AEFIs were classified as consistent, 2 as not consistent, 1 as undetermined and 1 as unclassifiable; adding information from medical records, we obtained similar outcomes with the exception of the “undetermined” AEFI that changed in “not consistent”.

Conclusion
Severe AEFIs following HPV vaccination are rare. The systematic use of the causality assessment algorithm showed that only half of them could be related to vaccination. Information from the AIFA routine surveillance system, in the absence of a causative analysis, provides a distorted picture of the HPV vaccine safety and should be interpreted with caution.

References

1. WHO. Causality Assessment of an Adverse Event Following Immunization (AEFI), User manual for the revised WHO classification. WHO/HIS/EMP/QSS. March 2013

DRAW UP A PROTOCOL FOR THE USE OF VAGINAL SELF COLLECTIONS IN ‘NON-RESPONDER’ WOMEN IN TUSCANY HPV PRIMARY SCREENING PROGRAM

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Background / Objectives

A key feature of a self-collected HPV testing strategy is the move of the primary screening activities from the clinic to the community with the efforts to increase the affordability and availability of HPV DNA tests. Within the self sampling project, ongoing in ISPO, Florence, women aged 35–64 years, residents in Florence, who had been invited, by the screening programme, in the past screening round and had failed to respond were eligible for the study. A random sample of 5200 eligible women was randomly assigned to one of the following arms: control arms with a standard invitation letter to perform HPV test at the clinic on a pre-fixed date; two intervention arms: a group was directly sent the “home based” dry self-sampler device (nylon FLOQSwab® Copan, Italy) another group was directly sent the “home based” self-sampler device (nylon FLOQSwab® Copan, Italy) and 1 ml of preservation and transport solutions (MSwab® Copan, Italy). As a prerequisite for carrying out the study, we have investigated sensitivity and reproducibility of HPV test carried out on Cobas®4800 (ROCHE®) in HPV16 plasmid samples obtained by swirling the FLOQSwab® in ThinPrep® (TP, HOLOGIC®) and in MSwab®+TP®.

Methods

Starting from HC2® (Hybrid Capture®2, QIAGEN®) high risk calibrator (plasmid 100 HPV16 copies/μl) we prepared a series of dilutions in TP® and MSwab®+TP® (rate 1:4). For each test, calibrator was absorbed on FLOQSwab® and then swirled in assay preservation solutions. For each sample clinical sensitivity (5000 HPV copies/reaction) and LOD (Limit of Detection) were evaluated in 20 replicates comparing the results with Roche LOD (600 copies HPV16/ml). FLOQSwab® adsorption was taken into account in order to guarantee the actual copies number.

Results

All 12500 HPV16 copies/ml (5000 HPV 16 copies/reaction) replicates in MSwab®+TP® and TP® are positive. Only 65% of 600 copies of HPV 16/ml replicates in TP® and 60% in MSwab®+TP® result positive. It was found that LOD is 1200 copies of HPV16/ml, since all replicates in MSwab®+TP® and 95% of those in TP® are positive. FLOQSwab® adsorption was about 230 μl.

Conclusion
FLOQSwab® LOD for HPV16 is 1200 HPV16 copies/ml. Compared to LOD provided by Roche, results show that FLOQSwab®, retained part of the viral load, shows a higher LOD. However near to the clinical sensitivity limit, FLOQSwab®’s performance was as expected. Against this different FLOQSwab® performance, the MSwab® analysis buffer has been shown to have good performance, both for much lower and at the limit of clinical sensitivity viral loads. From the results reported, it appears that MSwab® has a slightly higher analytical sensitivity than TP®, which could however result in a lower clinical sensitivity.
TIME AND TEMPERATURE STABILITY OF SELF-TAKEN SAMPLES FOR HPV SELF-SAMPLING

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Background / Objectives

The Capital Region of Denmark is currently implementing HPV self-sampling to screen non-attenders as a new offer in the organized screening program. From the pilot implementation study, offering 24,000 non-attenders an Evalyn self-sampling brush (Rovers, Oss, Netherlands), we have previously described that only 0.3% of the returned brushes returned an invalid result upon HPV testing using the BD Onclarity HPV assay. However, temperatures differences across a calendar year, prolonged storage after sampling or during transport in the mail from the woman to the lab could potentially affect the analytical stability of the dry, self-taken samples. To strengthen the evidence for use of self-sampling, we investigated the analytical stability of the Evalyn dry brush for HPV testing as a function of time and ambient temperatures.

Methods

To simulate self-taken samples, we used residual cervical swabs (Copan Universal Transport Media, SSI, Copenhagen) from Danish women undergoing routine diagnostic HPV testing at Copenhagen University Hospital. Brushes were dipped in residual media, left to dry and then stored at three different temperatures (room temperature, 4°C and 30°C) prior to being analyzed using the clinically validated BD Onclarity HPV assay at four different storage time points, T=0 (baseline), 2, 4, and 8 weeks. Analytical quality of the samples was assessed using the Ct value on the BD Onclarity HPV test internal control Human Beta Globin Control (HBB). Up to four brushes were derived from each swab sample, allowing for longitudinally comparison of different study points. After storage and prior to Onclarity testing, the brush heads were removed and rinsed in 3 ml BD CBD medium. 1.0 ml was used for HPV testing in concordance with manufactures specifications.

Results

The mean Ct value of the HBB outcome per sample was compared. No statistical difference was observed in HBB Ct values between baseline and T=2 weeks, 4, and 8 weeks regardless of storage temperature (4°C; p=0.951, Room temperature; p=0.763, 30°C; p=0.203). All samples were reproducible with respect to HPV result and the Ct values of the individual HPV genotype groups were stable throughout the time points.
Conclusion

This data conclusively shows that dry brushes used for HPV self-sampling are analytically stable with respect to human and HPV DNA up to 8 weeks after the actual sampling, as well as over temperature conditions ranging from 4C to 30C. This provides important data for implementation of HPV self-sampling worldwide under different temperature and environment conditions.
INCREASING SCREENING ATTENDANCE AMONG LONG-TERM SCREENING NON-ATTENDERS: RANDOMIZED HEALTHCARE POLICY


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Background / Objectives

The organized cervical screening program of Stockholm, Sweden reaches a 10-year population coverage of 96%, with the remaining 4% of the population constituting a high risk group for cervical cancer. The organized coordination and quality assurance allows for pilot implementation of novel screening strategies designed to increase coverage among long-term non-attenders. We performed a randomized health services study within the real-life organized screening program.

Methods

A comparison of the population registry with the regional screening registry identified that 16,437 out of the 413,487 resident women between 23 and 60 years of age had not taken a cervical screening test in at least 10 years, despite annual renewed invitations. Among these long-term non-attenders, 8000 women were randomized to either a) ordering a self-sampling kit using an open source e-Health application b) mailing a HPV self-sampling kit directly to the woman c) an invitation to call the coordinating midwife of the screening program with questions and concerns regarding screening; and d) standard annual renewed invitation letter (routine practice). HPV positive women were referred directly to colposcopy. Participation rates by study arm and outcome of screening tests were identified by registry linkages.

Results

Overall participation, defined as returning a self-sampling kit (or other screening participation) by arm was as follows: a) 10.7%; b) 18.7%; c) 1.9%; and d) 1.7%. The relative risk of participating in study arm a was 6.3 (4.4-8.9), 11.0 (7.8-15.5) in arm b, and 1.1 (0.7-1.7) in arm c, compared to routine practice (repeat renewed invitation with new appointment) in study arm d. HPV prevalence among women who returned
kits in study arms a and b was 12.2%. In total, 63 women were referred to colposcopy, out of which 44 women attended.

**Conclusion**

Offering self-sampling increased attendance, even among women who had not responded to more than 10 invitations with appointments in an organized program. Attendance was higher when kits were sent directly but offering women to order a kit did increase attendance at lower costs.
THE CHOICE TRIAL: A RANDOMIZED, CONTROLLED EFFECTIVENESS TRIAL OF HPV SELF-SAMPLING FOR NON-PARTICIPANTS IN AN ORGANIZED CERVICAL CANCER SCREENING PROGRAM

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Background / Objectives

Offering cervico-vaginal self-sampling for high-risk human papillomavirus testing (hrHPV self-sampling) to non-participants in a cervical cancer screening program may increase uptake depending on the delivery mode of the self-sampling offer. We compared the effectiveness of different approaches for delivering a self-sampling offer to non-participants in an organized program in terms of screening up-take, and analyzed the proportion of self-samplers that received appropriate follow-up.

Methods

The study included 9,791 women aged 30-64 from the Central Denmark Region who have not participated in cervical cancer screening despite invitation and one reminder. They were randomized 1:1:1 to either: 1) direct mailing of a HPV self-sampling kit (directly mailed group); 2) mailing of an offer to order a self-sampling kit by either e-mail, text message, phone, or webpage (opt-in group); or 3) mailing a second reminder to contact a general practitioner (GP) for usual care, viz. cytology (control group). Women offered self-sampling were informed that they could also receive usual care if wanted. The self-sampling kit comprised a brush device (Evelyn Brush) for hrHPV testing using Roche Cobas® 4800. Performing an intention-to-treat analysis, we estimated the up-take 180 days post intervention, including self-samples taken at home and cytologies taken at a GP. Self-samplers’ compliance with GP follow-up was measured 90 days after a hrHPV-positive test result.

Results

The up-take was significantly higher in the directly mailed group (37.0%) than in the opt-in group (29.9%) (absolute participation difference (PD): 7.1%, 95% CI: 3.1-11.1%) and the control group (24.1%) (PD: 13.0%, 95% CI: 8.8-17.0%). Of 118 hrHPV-positive self-samplers, 91.0% (107) attended follow-up. Self-samplers were
significantly less likely than controls to have been screened in the previous screening round (30.8% vs. 12.8%, RR: 0.42, 95% CI: 0.34-0.51). We estimated an overall participation rate of 71% in the directly mailed group, 68% in the opt-in group, and 65% in the control group. The direct mailing strategy increased the absolute overall participation among invited women by 6.0%, (95% CI: 5.8-6.2%).

Conclusion

Direct mailing of self-sampling kits to non-participants proved to be the most effective strategy for increasing screening participation. Using timely opt-in procedures yielded an only limited participation gain compared with a second reminder to attend regular screening. Most hrHPV-positive women had appropriate follow-up. Implementing self-sampling in the Danish screening program may increase overall up-take and help recruit under-screened women, thereby increasing the program's effectiveness.
HOME-BASED HPV SELF-SAMPLING TO INCREASE CERVICAL CANCER SCREENING PARTICIPATION: A PRAGMATIC RANDOMIZED TRIAL IN A U.S. HEALTHCARE DELIVERY SYSTEM

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Background / Objectives

Women who delay Pap screening are at increased risk for cervical cancer. In countries with organized screening programs, population-based randomized controlled trials (RCTs) have shown that mailing HPV self-sampling kits to underscreened women increases participation. Our objective was to evaluate the effectiveness of this approach in a U.S. healthcare system.

Methods

We conducted a pragmatic RCT within Kaiser Permanente Washington (an integrated healthcare system) to compare 2 programmatic approaches for increasing screening among women aged 30-64 years who were overdue (≥3.4 years since last Pap). The control arm included usual care (annual patient reminders and ad hoc outreach by clinics). The intervention arm included usual care plus a mailed self-sampling kit for HPV testing by Roche Cobas assay. Women and their healthcare providers were notified of the home HPV results and providers were responsible for encouraging women to receive appropriate follow-up: diagnostic colposcopy if HPV16/18+ and additional in-clinic screening (Pap or co-test) if unsatisfactory or positive for non-HPV16/18 types. Unlike similar trials in other countries, HPV-negative women were still recommended to receive in-clinic screening because home HPV testing is not an approved screening strategy in the U.S. Screening uptake was defined as any of the following within 6 months of randomization: 1) in-clinic screening; 2) returning a kit that was HPV-negative or HPV16/18+; or 3) returning a kit that was unsatisfactory or positive for non-HPV16/18 types, followed by in-clinic screening.

Results

From 2014-2016, we randomized 16,242 women (8116 control; 8126 intervention) with a median age of 51 years. Screening uptake was higher in the intervention than
control arm (28.3% vs. 19.5%; relative risk=1.45, 95%CI:1.37-1.54). Within the intervention arm, 13.1% of women returned a kit and 15.4% attended in-clinic screening without returning a kit. 11.5% of self-sampling kits were HPV+ (3.1% HPV16/18+ and 8.4% positive for non-HPV16/18 types only).

Conclusion

Mailing HPV self-sampling kits to underscreened women increased screening uptake compared to usual care alone. Screening uptake in our intervention arm was comparable to pooled uptake from a meta-analysis of non-U.S. trials (28.3% vs. 23.6%).[1] Our results suggest that approximately half of women who choose to get screened after receiving a mailed home HPV kit will choose in-clinic screening over self-sampling in a hybrid screening approach. Additional findings from this study will describe clinical outcomes, demographic and health predictors of screening uptake, and self-sampling experiences and attitudes.

References


ClinicalTrials.gov, NCT02005510
COMPARATIVE EVALUATION OF TWO CERVICOVAGINAL SELF-COLLECTION METHODS TO DETECT THE PRESENCE OF CLINICALLY SIGNIFICANT HUMAN PAPILLOMAVIRUS INFECTION.


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Background / Objectives

PCR based high-risk human papillomavirus (hrHPV) testing on self- and physician-collected specimens have shown similar sensitivity to detect a high-grade cervical lesion (CIN 2+). However, limited evidence is available on the performance and acceptability of different sampling devices. We compared two self-collection methods to detect clinically significant hrHPV infection in Norway.

Methods

310 women referred to the treatment for histologically verified CIN 2+ completed self-collection using a dry brush (Evalyn Brush, Rovers, Netherlands) and a dry swab (FLOQSwabs, Coban, Italy), and filled a questionnaire. At the hospital, a physician took an additional cervical specimen (PreservCyt, Hologic, USA). Cytology specimens were blindly analysed by one cytotechnician and one pathologist. Self-specimens and a physician-specimen (reference) were analysed for the presence of 14 hrHPV DNA using three commercially available HPV assays; Anyplex (Seegene, South Korea), Cobas (Roche, USA) and Xpert HPV (Cepheid, USA). Agreement between self- and physician-specimen was assessed with kappa (κ) statistics with bootstrapped 95% confidence intervals (CIs). Absolute and relative sensitivities with 95% confidence intervals were computed using a matched-pair design.

Results

Analyses included 251 women with matched triplets. Overall hrHPV positivity rate was 89% in the reference specimen using Anyplex, and 86% using Cobas and Xpert HPV.

Overall agreement for hrHPV positivity between self- and physician-specimens was highest at 94% using Anyplex on Evalyn (κ = 0.68, 95% CI: 0.53-0.83), and lowest at 82% using Xpert HPV on FLOQSwabs (κ = 0.47, 95% CI: 0.35-0.59). Anyplex detected 95% of the CIN2+ lesions. Corresponding sensitivities for Cobas and Xpert HPV were 93% and 94%. The ability of any hrHPV test to detect CIN2+ from a brush-
specimen was similar to the reference, whereas significantly lower sensitivities were demonstrated using a swab. Both devices were well accepted, but women considered a brush easier, less painful and less uncomfortable than a swab. Generally, we did not observe any differences on perceptions on self-collection by sociodemographic status.

Conclusion

We observed significant device effects in detection of the hrHPV DNA and CIN2+ using three validated HPV assays. There were also differences on the acceptability of the sampling devices. If self-collection is considered as an alternative to provider-collection in national screening programmes, both hrHPV assays and sampling devices have to be validated.
FC 08-07
HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA IN HUMAN PAPILLOMAVIRUS SELF-SAMPLING OF SCREENING NON-ATTENDERS VERSUS ROUTINELY SCREENED WOMAN

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Background / Objectives

Self-sampling for Human Papillomavirus (HPV) offered to women who do not participate in organized cervical cancer screening is an increasingly popular method to increase screening coverage. The rationale behind it is that under-screened women harbour a high proportion of undetected precancerous lesions since ~50% of disease is routinely detected in underscreened women. In 2014 the “Copenhagen Self-sampling initiative” (CSI) offered HPV self-sampling to screening non-attenders in the Capital Region of Denmark. We compared the ≥CIN2-detection rate between screening non-attenders, who participated in self-sampling, and women attending routine screening (The HORIZON cohort).

Methods

23,632 women who were qualified as screening non-attenders in the Capital Region were offered HPV-based self-sampling using an Evalyn brush (Rovers, Öss the Netherlands). 4824 (20.6%) women returned a self-sample brush, and HR-HPV-positive women were referred for cytology and HPV-testing as follow-up. The entire cohort and a reference cohort (3347 routinely screened women from the HORIZON cohort), were followed for histopathology-confirmed ≥CIN2. Odds ratio and positive predictive value of ≥CIN2-detections between the two populations were estimated

Results
Women participating in self-sampling had the same ≥CIN2-detection rate as routinely screened women (OR= 1.03; 95% CI: 0.75-1.40). The positive predictive value of ≥CIN2 detections was, however, higher in screening non-attenders than routinely screened women (36.5% vs. 25.6%, respectively). Among all detected ≥CIN2, women were slightly more likely to have ≥CIN3 detected if they were CSi-attenders (78.6% of all ≥CIN2 diagnoses were ≥CIN3), than if they were routinely screened (HORIZON population) (72.1%). However, in total, 18 women were diagnosed with cervical cancer in the screening non-attenders population, versus only one in the reference population.

**Conclusion**

Self-sampling to non-attenders had similar detection rates for ≥CIN2 as routine cytology-based screening, reinforcing the importance of self-sampling to screening non-attenders in organized cervical cancer screening. The proportion of high-grade CIN lesions among all biopsies was high, demonstrating the efficiency of the approach. The major finding was a large increase in cancer detection in the self-sampled group, which underlines the importance of reaching underserved women to reduce morbidity and mortality from cervical cancer.
HPV test using self-sampling device is useful and effective in non-attendees of cervical cancer screening in Japan: In municipal population based screening in Izumo city

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Background / Objectives

In Japan, incidence and mortality of cervical cancer is increasing especially in young women in their late 20s and early 30s. One of the considerable factors is the low coverage of screening which is only 32.7% according to the National Livelihood Survey 2013. We tried to use self-sampling HPV test to approach non-attendees in Izumo city, which has implemented HPV co-testing for cervical cancer screening since 2007. 2,120 out of 12,546 women who were between 25-45 years old and did not attend municipal cervical cancer screening from 2010 to 2014 conducted HPV self-sampling test in 2015. At our presentation in EUROGIN 2016, the acceptability of self-sampling HPV test was very good. We want to make sure if HPV test using a self-sampling device is really useful and effective for non-attendees. So we investigate how many women who conducted self-sampling HPV test went to the medical facility and received the cytology, especially as a municipal cervical cancer screening after they got the result of HPV test.

Methods

The candidates of this study were 2,120 women living in Izumo city, who conducted HPV self-sampling test in 2015. We followed up on their results of cytology and HPV test using physician collected samples. We sent a letter and a questionnaire to those who were HPV positive and did not receive municipal screening to find out if they receive screening in any other setting or not.

Results

In 2,120 attendees of self-sampling HPV test, 152(7.2%) of women were screen-positive and 1,968(92.8%) women were screen-negative. 123(89.2%) of screen-positive women received cervical cancer screening. 111 out of 123 receive municipal screening (cytology and physician-collect HPV test) in Izumo city, and 40 of them had a detailed examination. The detection rate of CIN2+ in those who conducted self-sampling HPV test is 10.8%, and 18 times as many as the rate of CIN2+ in general screening run by Japan Cancer Society, which covers one third of population based cervical cancer screening in Japan. 239(12.1%) of screen-negative women receive municipal screening. 362 women (17.1% of the total 2,120) who conducted self-sampling HPT test went to the medical facility to undergo cytology as municipal screening. It is also 2.9% of total non-attendees in 5 years.
Conclusion

We believe that using self-sampling HPV test is a very useful and effective way to approach non-attendees of municipal cervical cancer screening. In Japan, there are many areas including small islands with few or no available facilities. Not only for non-attendees but also for women in low resource area in Japan, self-sampling HPV test has a possibility to improve the health and wellness of Japanese women.
PRIMARY HPV SCREENING USING THE COBAS® HPV TEST ON SELF-COLLECTED DRY CERVICOVAGINAL SAMPLES FROM UNDERSERVED GREEK WOMEN. PRELIMINARY RESULTS OF THE GRECOSELF STUDY

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Background / Objectives

To assess the performance of HPV-based cervical cancer screening in underserved women in Greece using the cobas® HPV Test on self-collected cervicovaginal samples, in comparison to historical real-life results of cytology-based screening. Secondary objective is to assess the acceptability of the Roche® self-collection device for cervicovaginal specimens.

Methods

Women 25-60 years old who do or do not attend cervical cancer screening and reside in rural areas of Greece are being recruited for the study. Approximately 12,700 women will be enrolled over 30 months starting May 2016. Women are contacted by midwives, forming a nationwide midwifery network set for the study purposes, via public announcement at their place of residence, and are provided, after giving their written informed consent, with a self-sampling kit (Roche®) (cotton swab and dry vial) along with the necessary instructions. Each woman collects the specimen and fills in a questionnaire specifically designed to assess cervical cancer screening participation and outcome history during the last 10 years, and the acceptance of the self-sampling procedure. Samples are tested using the cobas® HPV Test, Roche®, which detects HPVs 16 and 18 separately, and HPVs 31,33,35,39,45,51,52,56,58,59,66 and 68 [other high-risk (OHR)] as a pooled result. Women found positive for HPV are being referred for colposcopy. In case of abnormal colposcopic impression a biopsy is taken. If the histology report is within normal limits the woman is referred to routine screening, if there is Cervical
Intraepithelial Neoplasia (CIN) grade 1 or 2 or worse (CIN2+) she is referred to follow up or appropriate treatment respectively.

Results

Between May 2016 and March 2017 6,818 samples were collected, 6,156 were tested, of which 433 (7.0%) were HPV positive, 228 colposcopies were performed and CIN grade 1, 2 and 3 was detected in 17, 12, and 10 cases respectively. HPV16 positive cases were found in 14.2% and 63.6% among the low-grade (CIN1) and high-grade (CIN2+) lesions respectively. Moreover there had been a case of vaginal intraepithelial neoplasia (VaIN – OHR positive) and a case of cervical adenocarcinoma (HPV16 positive). The prevalence of high-grade disease or cancer among HPV positive women examined was 10.5% and among tested women overall 0.4%, about half than expected, since only about half of the HPV positive women have been examined colposcopically so far.

Conclusion

The preliminary report of the GRECOSELF study shows that HPV testing with individual HPV 16/18 genotyping on self-collected cervicovaginal samples is a feasible and effective cervical cancer prevention method for Greek women residing in rural distant areas.
THREE-YEAR EFFICACY OF THE QUADRIVALENT HPV VACCINE IN A COHORT OF HIV-POSITIVE WOMEN

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Background / Objectives

To assess the efficacy of the qHPV vaccine at 3 years in our cohort of HIV-positive women.

Methods

HIV-positive females participating in a multi-centre study of the qHPV vaccine were administered three doses at 0, 2 and 6 months. Demographic and clinical data were collected as well as samples for cervical cytology, classified by Bethesda criteria, and HPV DNA tested by Linear array assay. Persistent qHPV, genital warts and CIN2+ were assessed. Persistent cases of qHPV were defined as new HPV 6/11/16/18 that remained present in samples from ≥2 consecutive visits or as qHPV present in the last sample.

Two-year data was compared to findings in HIV-negative women to provide context to our results. To improve comparability of the cohorts, HIV-positive women with history of genital warts, cervical disease and cervical procedure were excluded as these were exclusion criteria among the HIV-negative women. A composite endpoint comprised of the three previous endpoints was utilized.

Results

278 females were eligible for the intention-to-treat (ITT) population (≥1 dose of vaccine, ≥1 follow-up visit). At first vaccination, median age was 39 years (IQR: 34-45), median CD4 count was 499/mm3 (IQR: 380-684), median CD4 nadir was 230/mm3 (IQR: 120-337) and 72% had a suppressed HIV viral load (<50 copies/mL). Median follow-up was 3.2 years. In the per-protocol efficacy (PPE) population (3
doses of vaccine within 1 year, ≥1 follow-up beyond month 7, naive to the relevant qHPV type), persistent qHPV = 0.9 per 100 person-years (95% CI: 0.4-1.8), genital warts =1.0 per 100 person-years (95% CI: 0.4-2.0), CIN2+ = 0 per 100 person-years.

Within 2-year follow-up of HIV-positive women, composite endpoint incidences in the PPE, NRT and ITT groups were 1.4, 2.3 and 3.7 per 100 person-years, respectively. Among HIV-negative vaccinated women, incidences were 0.1, 0.5 and 2.7 per 100 person-years. Among HIV-negative unvaccinated women, incidences were 1.5, 2.0, 3.9 per 100 person-years.

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† Muñoz et al., 2009.

Conclusion

This study demonstrates overall low rates of vaccine failure with low rates of infection and/or disease. However, the rates of qHPV-related disease were notably higher than rates in vaccinated HIV-negative women based on published studies and equivalent to rates in unvaccinated HIV-negative women.

References

THREE-YEAR EFFICACY OF THE QUADRIVALENT HPV VACCINE IN A COHORT OF HIV-POSITIVE WOMEN

E. Mcclymont, M. Lee, J. Raboud, S. Blitz, F. Coutlée, S. Walmsley, N. Lipsky, D. Money

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Two-year data was compared to findings in HIV-negative women to provide context to our results. To improve comparability of the cohorts, HIV-positive women with history of genital warts, cervical disease and cervical procedure were excluded as these were exclusion criteria among the HIV-negative women. A composite endpoint comprised of the three previous endpoints was utilized.

Results

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person-years. Among HIV-negative unvaccinated women¹, incidences were 1.5, 2.0, 3.9 per 100 person-years.

Conclusion

This study demonstrates overall low rates of vaccine failure with low rates of infection and/or disease. However, the rates of qHPV-related disease were notably higher than rates in vaccinated HIV-negative women based on published studies and similar to rates in unvaccinated HIV-negative women.

References

IMPACT OF BASELINE COVARIATES ON THE IMMUNOGENICITY OF 9-VALENT HPV VACCINE IN MEN AGE 16-26 YEARS

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Background / Objectives

A 9-valent HPV (6/11/16/31/33/45/52/58) (9vHPV) vaccine was developed to provide protection against the HPV types already covered by the quadrivalent HPV (6/11/16/18) (qHPV) vaccine and the five HPV types most commonly associated with cervical cancer worldwide after HPV 16/18. Antibody response to prophylactic HPV vaccines is the basis for their effectiveness in preventing infection and disease related to vaccine HPV types. Here, we present the combined results of an analysis of 9vHPV vaccine immunogenicity in men age 16-26 years in clinical trials.

Methods

Immunogenicity analyses of two clinical trials of the 9vHPV vaccine (protocols 003 [NCT01651949] and 020 [NCT02114385]) were conducted by competitive Luminex immunoassay in males age 16-26 years in a per-protocol immunogenicity population (PPI) consisting of subjects seronegative at Day 1 for the tested HPV type. Immunogenicity was summarized in populations defined by age at vaccination (≤21 or >21 years of age), sexual orientation (heterosexual men [HM], men having sex with men [MSM]), race, and region of residence. Immunogenicity in a historic clinical trial of the qHPV vaccine in men age 16-26 years was also considered.

Results

Of the randomized subjects, 1665 (99.8%) received at least one injection of 9vHPV vaccine. More than 99% of subjects who received 9vHPV vaccine and were in the PPI population were seropositive to the respective vaccine HPV type at 4 weeks post-Dose 3. For all subjects, geometric mean titers (GMT) for all nine HPV types were robust across age, sexual orientation, race, or region of residence. The magnitude of anti-HPV response to 9vHPV vaccine tended to decrease with increase in enrollment age. It was lower in MSM compared with HM with GMT ratios (MSM/HM) at 4 weeks post-Dose 3 ranging from 0.58 to 0.74, depending on the HPV type in the 9vHPV vaccine trials. GMTs were also lower in MSM versus HM in the historic qHPV vaccine trial, with GMT ratios ranging from 0.49 to 0.66. GMTs remained lower in MSM compared with HM after adjusting for age and region of residence in both vaccine clinical programs.

Conclusion

The 9vHPV vaccine was strongly immunogenic, as shown by high seroconversion rates (>99%) for all vaccine HPV types and robust HPV antibody responses.
regardless of race, geographic region, age, or sexual orientation. The lower immunogenicity in MSM compared with HM was not due to differences in baseline characteristics of age or region of residence and does not appear to have a meaningful clinical significance, as the qHPV vaccine was previously shown to be highly efficacious to prevent HPV infection and disease in MSM.
PREVENTING HPV RELATED DISEASES: AN HEALTH TECHNOLOGY ASSESSMENT OF THE NINE-VALENT VACCINE IN ITALY

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Background / Objectives

The human papillomavirus (HPV) is the most common viral infection of the reproductive system and the second viral agent responsible for cancer. About 5% of cancers can be attributed to HPV; the most common is cervical cancer, but also other cancers are due to HPV. Five-years survival rates are very low except for cervical cancer thanks to the screening. Genotypes 16 and 18 are responsible of around 70% of cervical cancers, but considering also 45, 31, 33, 52, 58 and 35, the proportion reaches 90%. Most of them are preventable through vaccination. The objective of this report is to assess the impact of the implementation of a universal HPV vaccination campaign with a nine-valent vaccine in Italy using the rigorous methodology of the Health Technology Assessment (HTA).

Methods

A HTA has been developed considering all the available evidence on epidemiological, clinical effectiveness, safety, cost-effectiveness, organizational, social and ethical aspects with a focus on Italian population.

Conclusion

A great amount of evidence is available regarding HPV epidemiology, vaccine efficacy and safety and the economic impact of a national vaccination program, but this is the first attempt to collect them together using HTA. The report highlights that
a nine-valent universal vaccination is cost-effective in reducing the risk of HPV-related cancers and diseases. The introduction of a nine-valent vaccine extends the protection to an increasing number of genotype and, consequently, can avoid a huge amount of neoplastic lesions in different sites and reduce the burden of disease. In fact, around 88% of anal, 70% of vaginal, 50% of penile and 43% of vulvar cancers are due to HPV. The nine-valent vaccine represents an investment for public health as demonstrated by the favorable cost-effectiveness profile under the perspectives of both the National Health Service and the society. This is strictly related to a reduction of healthcare costs to manage HPV-related diseases and to an increasing patients’ quality of life. From an organizational point of view, schools have been identified as one of the most effective setting for immunization campaigns. Additionally, amelioration in the invitation letters, communication strategies and/or activities aimed at strengthening the involvement of health care workers emerged as possible determinants of a higher vaccination coverage and a reduction of access inequality. A universal vaccination with nine-valent vaccine is an ethical choice for the society due to the higher clinical benefit-non-maleficence ratio than available alternatives for both the single vaccinee and the entire society considering also the incremental benefit due to the herd immunity.

References


SAFETY OF HUMAN PAPILLOMA VIRUS 9-VALENT VACCINE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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1 Federal University of Rio Grande do Norte (Brazil), 2 State University of Campinas (Brazil)

Background / Objectives

Vaccination against human papillomavirus (HPV) has been progressively implemented in most developed countries for approximately 10 years. In order to increase the protection of the vaccines, a 9-valent vaccine (HPV9) was developed, which provides protection against nine types of the virus, but is not yet used in prevention programs. Studies evaluating its safety are rare. Thus, in this study we performed a meta-analysis of three clinical trials assessing adverse effects in women randomly vaccinated with HPV9 or tetravalent vaccine (HPV4), with the objective of analyzing whether the HPV9 is as safe as HPV4.

Methods

A systematic review and metaanalysis of the HPV vaccines' safety in women was made. Randomized controlled trials (RCT) published between 2011 and 2016 were identified from searches of PubMed, Embase, Scopus, Web of Science and the SciELO databases.

Results

The studies selected 27,465 women who received one of the two vaccines. Results showed that pain (OR 1.72; 95% CI 1.62-1.82) and erythema (OR 1.29; 95% CI 1.21-1.36) occurred significantly more in HPV9 group. However, there was no significant difference between groups for the following adverse effects: headache (OR 1.07; 95% CI 0.99-1.15), dizziness (OR 1.09; 95% CI 0.93-1.27) and fatigue (OR 1.09; 95% CI 0.91-1.30) and the occurrence of serious events related to vaccination was similarly rare among those vaccinated.

Conclusion

Therefore, our findings demonstrate that HPV9 in female patients is as safe as the tetravalent vaccine.

References


FC 09-05
9-VALENT HPV VACCINE EFFICACY AGAINST RELATED DISEASES AND DEFINITIVE THERAPY: COMPARISON TO HISTORIC PLACEBO POPULATION

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Background / Objectives

The 9-valent human papillomavirus (HPV) vaccine protects against the same four HPV types as the quadrivalent HPV vaccine and five additional oncogenic types. The pivotal efficacy study of the 9-valent vaccine was controlled with the quadrivalent vaccine. Since the trial had no placebo group, a direct comparison with an unvaccinated population was not possible. Here, we present efficacy analyses comparing the 9-valent vaccine group with a historic placebo population.

Methods

Three international, randomized, double-blind studies were conducted using the same methodology. In the efficacy study of the 9-valent vaccine (Protocol V503-001; NCT00543543), 7106 and 7109 women received the 9-valent or quadrivalent vaccine, respectively. In the historic efficacy studies of the quadrivalent vaccine (FUTURE I [NCT00092521] and II [NCT00092534]), 8810 and 8812 women received the quadrivalent vaccine or placebo, respectively. Cervical cytologic testing was performed regularly. Tissue samples from biopsy or definitive therapy (loop electrosurgical excision procedure, conization) were assessed for HPV DNA.

Results

Among women negative to 14 HPV types prior to vaccination with the 9-valent vaccine, the incidence of high-grade cervical disease and cervical definitive therapy related to the nine HPV types was reduced by 97.4% (95% CI 91.0, 99.5) and 96.6% (95% CI 90.5, 99.1), respectively. The 9vHPV vaccine did not prevent disease related to HPV types detected at baseline but significantly reduced high-grade cervical disease related to other types.

Conclusion

Effective implementation of the 9-valent vaccine may substantially reduce the burden of cervical disease and related health care costs.
HIGH VACCINE EFFECTIVENESS AGAINST PERSISTENT HPV INFECTIONS UP TO SIX YEARS POST-VACCINATION WITH THE BIVALENT VACCINE IN A COHORT OF YOUNG DUTCH FEMALES

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Background / Objectives

Monitoring vaccine effectiveness (VE) in large-scale vaccination programs is of great importance for assessing the population impact of immunization. This study aimed to estimate the VE of the bivalent HPV vaccine against 12-month type-specific persistent infection up to six years post-vaccination among young Dutch women.

Methods

In 2009, girls 14-16 years of age, who were eligible for the HPV catch-up vaccination campaign in the Netherlands, were invited for participation in a cohort study. Both vaccinated and unvaccinated girls were included and baseline measurements were performed before vaccination. Yearly, questionnaire data and vaginal self-swabs were obtained. Vaginal self-swab material was analyzed with the SPF10-LIPA system. Persistence was defined as at least two consecutive measurements testing positive for the same HPV type, preceded by a high-risk (hr) -negative measurement at baseline or two type-specific negative measurements during follow-up. Type specific hazard ratios were obtained through survival analysis by using the Prentice Williams-Peterson total-time approach, adjusting for ethnicity, age, and sexual behavior. We calculated VE as (1-hazard ratio)*100%.

Results

In total 1593 women (46% vaccinated, 54% unvaccinated) had an available baseline sample, were unvaccinated or vaccinated completely in accordance to the Dutch schedule (at that time 3 doses at 0, 1 and 6 months) and negative for high-risk (hr) HPV at baseline. High VE was observed against vaccine types HPV16 and HPV18 of 95% (95%CI 66%-99%) and 100% (hazard rates per 100 person years: unvaccinated 2.07 (95% CI 1.12-3.86) and vaccinated 0.00 (95%CI 0.00-0.14)), respectively. We observed significant cross-protection against HPV31 (73%, 95%CI 3%-92%). We estimated a VE of 16% (95%CI -14-38%) against all hrHPV-types combined, and a VE of 51% (95%CI 24-69%) against hrHPV-types included in the nonavalent HPV vaccine (HPV16/18/31/33/45/52/58).
Conclusion

The bivalent vaccine shows high effectiveness against 12-month persistent infections by HPV16 and HPV18 among young Dutch women, vaccinated at age 14-16 years while hrHPV negative at baseline, up to six years post-vaccination. Additionally, we found significant cross-protection against 12-month persistent infections by HPV31.
THE HEALTH ECONOMIC IMPACT OF CROSS PROTECTION DUE TO HPV VACCINE

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Background / Objectives

To provide data on the health and financial impact of cross and direct protection from HPV vaccines with a focus on duration of protection.

Methods

A previously validated HPV health economics model calibrated for France was adapted to model cross protection while varying the duration of effectiveness of cross protection and direct protection for the bivalent and the 9-valent HPV vaccines, respectively. Published reports of both clinical trial data and real world evidence show short term protection due to cross protection, while effectiveness from immunity to HPV type-specific virus like particles (VLP) is expected to provide long term protection.

Results

The results from the model show significant reductions in cancer cases, deaths and costs due to long-term protection by type-specific VLP-based vaccines. We consider outcomes for cervical and anal cancers in both male and female population. The 9-valent HPV vaccine compared to the bivalent HPV vaccine with short term (5 years) cross protection results in 74 (per 100,000) fewer cancer cases; 24 (per 100,000) fewer cancer related deaths; and saves €101,850 (per 100,000) over a 100 year time horizon.

Conclusion

Short lived cross protection results in additional cancer cases, cancer-related deaths and lower economic benefit compared to immunity resulting from HPV type-specific VLP-based vaccines.
DECONSTRUCTING EFFICACY AGAINST HIGH-GRADE DISEASE IRRESPECTIVE OF TYPE OF AS04-HPV-16/18 VACCINE AND HPV-6/11/16/18 VACCINE: A POST-HOC ANALYSIS FROM PHASE III TRIALS

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Background / Objectives

A recent systematic review reports substantial heterogeneity in estimates of efficacy against high-grade cervical diseases irrespective of type for AS04-HPV-16/18 vaccine (AS04-HPV16/18) and HPV-6/11/16/18 vaccine (4vHPV).[1] In the current post-hoc analysis, we further explore the reported differences in efficacy.

Methods

Case counts of Cervical Intraepithelial Neoplasia of grade 2 and 3 (CIN2/3) cases were extracted from the FUTURE I/II study (NCT00092521/NCT00092534, Intention-to-Treat naive cohort) [2] and the PATRICIA study (NCT00122681, Total Vaccinated naive Cohort). Cases were assigned to the following categories based on HPV types found in the lesions:

- Lesions with at least one HPV vaccine type* and without any non-vaccine type**
- Co-infections: Lesions with at least one vaccine type* and at least one non-vaccine type**
- Lesions where no vaccine type* was detected (non-vaccine types** only or no high-risk HPV)

Efficacy against lesions with vaccine types, irrespective of type as well as cross-protective efficacies with both inclusion and exclusion of the co-infection cases are calculated using the case counts (n) and totals (N) as extracted.

<table>
<thead>
<tr>
<th>PATRICIA</th>
<th>CIN2</th>
<th>Efficacy (95% CIs) [n vaccine/n control]</th>
<th>Efficacy against lesions with at least one HPV vaccine type* including co-infections with a non-vaccine type**</th>
<th>Efficacy irrespective of type</th>
<th>Cross-protective efficacy including co-infections of a non-vaccine type** with an HPV vaccine type*</th>
<th>Cross-protective efficacy excluding co-infections of a non-vaccine type** with an HPV vaccine type*</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>98,9% (93.9 ; 100)</td>
<td>63,3% (50.4 ; 73.2)</td>
<td>47,5% (27.3 ; 62.3)</td>
<td>15,9% (-20.6 ; 41.6)</td>
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<td></td>
<td>CIN3</td>
<td>CIN2</td>
<td>CIN3</td>
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<td>[1/93]</td>
<td>[60/163]</td>
<td>[59/112]</td>
<td>[59/70]</td>
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<tr>
<td>AS04-HPV16/18:</td>
<td>100% (81.8 ; 100)</td>
<td>92.1% (75.2 ; 98.4)</td>
<td>88.5% (62.4 ; 97.8)</td>
<td>81.3% (34.7 ; 96.5)</td>
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<tr>
<td>N control:</td>
<td>[0/22]</td>
<td>[3/38]</td>
<td>[3/26]</td>
<td>[3/16]</td>
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<tr>
<td>FUTURE I/II</td>
<td>100% (91.9 ; 100)</td>
<td>42.8% (20.1 ; 59.4)</td>
<td>26.8% (-4.1 ; 48.9)</td>
<td>-9.0% (-61.6 ; 26.3)</td>
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<tr>
<td>N 4vHPV:</td>
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<td>[57/101]</td>
<td>[57/79]</td>
<td>[57/53]</td>
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<td>[36/42]</td>
<td>[36/23]</td>
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</table>

**Conclusion**

No head-to-head efficacy trials that compare different HPV vaccines have been conducted and methodological differences between trials cannot be excluded. However, our post-hoc analysis suggests that efficacy against CIN2 and CIN3 irrespective of type is largely influenced by lesions in which no vaccine type was found, resulting in different estimates for AS04-HPV16/18 and 4vHPV.

*Vaccine types in AS04-HPV16/18: HPV-16/18, 4vHPV: HPV-6/11/16/18


**References**


**Funding:** GlaxoSmithKline SA

Conflicts of interest: MR, VB, NK and FS are employees of the GSK group of companies. MR, VB and FS also report shares from the GSK group of companies.
BIVALENT VACCINE EFFECTIVENESS AGAINST TYPE-SPECIFIC HPV DNA POSITIVITY: EVIDENCE FOR CROSS-PROTECTION AGAINST ONCOGENIC TYPES

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Background / Objectives

To calculate the bivalent vaccine effectiveness (VE) against high-risk HPV DNA positivity, using cross-sectional data from the Netherlands up to six years post vaccination.

Methods

We included all vaccine-eligible women from the PASSYON study, a biennial cross-sectional survey among 16- to 24-year-old STI-clinic visitors (2009-2015). Vaginal swabs were analyzed using a sensitive PCR-based reverse line blotting system (SPF10- LiPA25) which is able to detect the high-risk types 16/18/31/33/35/39/45/51/52/56/58/59. VE was estimated by a logistic mixed model corrected for demographics and (sexual) risk behavior, with a random intercept to account for residual clustering of HPV types within individuals. HPV DNA positivity was compared between women who reported to be vaccinated at least once and women who reported to be unvaccinated. VE was calculated as (1-adjusted Odds Ratio)*100%.

Results

We included 1087 vaccine-eligible women of the PASSYON study years 2011-2015. Of these women, who were 16- to 22-years-old, 53% tested positive for a high-risk type and 60% reported to be vaccinated. Among women with serum available (43%), the self-reported vaccination status agreed well with the HPV16/18 antibody concentration (AUC 92.3%), suggesting reliable reporting. The pooled VE against the vaccine types HPV16/18 was 89.9% (81.7-94.4); 92.3% (82.5-96.6) against HPV16 and 85.5% (66.0-93.8) against HPV18. Moreover, we calculated significant VE against the non-vaccine types HPV45 (91.0% [59.7-98.0]), HPV35 (57.1% [2.3-81.2]), HPV31 (50.0% [10.8-72.0]) and HPV52 (37.2% [9.2-56.6]). Vaccinated women were more often HPV59 positive (6.0%) than unvaccinated women (3.4%), resulting in a VE of -89.4% (-259.9-0.3). The pooled VE against all high-risk types was 32.9% (20.2-43.7).

Conclusion
We demonstrate a high VE against prevalent infection with the bivalent vaccine types. In addition, we found significant cross-protection against HPV types 45, 35, 31 and 52. The negative VE against HPV59 is notable and needs further investigation.
PILOT STUDY ON USE OF INNO-LIPA® HPV GENOTYPING EXTRA II WITH COLLI-PEE COLLECTED UCM PRESERVED URINE

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Background / Objectives

The INNO-LiPA HPV Genotyping Extra II assay can individually detect 32 HPV genotypes. Performance of this assay has been demonstrated on cervical scrapes, but no data are currently available regarding HPV DNA detection in first void urine. The aim of this pilot study is to determine whether the INNO-LiPA HPV Genotyping Extra II test is compatible with self-collected first void urine specimens.

Methods

18 Colli-Pee™ collected, preserved first void urine samples (16 HPV positive samples previously identified with the Riatol qPCR HPV genotyping assay (AML) and Multiplex HPV Genotyping assay (Diamex)) were analysed – samples originated from a cohort of women with self-reported prior HPV positive test results.

The participants collected the first void urine self-sample at home and sent the collection vial containing preservative uncooled by postal mail to the Antwerp University. Prior to the PCR tests 4 ml of urine/UCM mixture was concentrated on an ultrafiltration membrane and extracted with easyMAG® (bioMérieux).

Results

17/18 urine samples were successfully genotyped with the INNO-LiPA HPV Genotyping Extra II assay. An opened vial in the PCR instrument caused an invalid result for one sample. Two samples were HPV DNA negative as found by the Riatol qPCR HPV genotyping assay. For 13 out of the 15 remaining samples at least one high risk HPV type was detected by INNO-LiPA HPV Genotyping Extra II assay and confirmed by one or two of the other assays.

Conclusion

These preliminary results confirm that the INNO-LiPA HPV Genotyping Extra II assay is compatible with self-collected first-void urine. Confirmation of performance by testing larger series in a clinical setting is warranted.

References

EVALUATION OF BD ONCLARITY IN DETECTION OF CANCER AND PRE-CANCER IN WOMEN WITH ASCUS/LSIL IN CHINA

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Background / Objectives

About 30~40% ASCUS women and 80% LSIL women are high risk HPV (HR-HPV) positive, while the prevalence of CIN2+ is about 3~9% and 15%, respectively. This indicated that there may be clinical benefit in stratifying HR-HPV positive women with ASCUS/LSIL by HPV genotypes. In this study, we evaluated the clinical performance of onclarity HPV test for partial HPV genotypes among women with ASCUS/LSIL cytology.

Methods

320 (221 ASCUS and 99 LSIL) women with cytological ASCUS/LSIL were recruited in the study. All those participants were referred colposcopy and directed biopsy, four-quadrant cervical biopsy was conducted when no visible lesions were found under colposcopy. All of the cervical samples were tested by cobas HPV test and onclarity HPV test with nine typing channels: HPV16, HPV18, HPV31, HPV45, HPV51, HPV52, HPV33/35, HPV35/39/68 and HPV56/59/66.

Results

The agreement rate between cobas HPV test and onclarity HPV test was satisfactory for testing 14 types HR-HPV, HPV16, HPV18 and HPV non-16/18, as for positivity agreement rate and kappa value, those two tests also showed preferably performance except HPV 18. BD onclarity HPV test can provide more HPV genotypes information that can be used for evaluation of ASCUS/LSIL triage. On the basis of HPV16/18, other HPV types by their risk grade were sequentially added into 6 groups, marked as subG1 (HPV16/18/31), subG2 (HPV16/18/31/33/35/58), subG3 (HPV16/18/31/33/58/35/39/68), subG4 (HPV16/18/31/33/58/39/68/35/52), subG5 (HPV16/18/31/33/58/39/68/35/52/45) and subG6 (HPV16/18/31/33/58/39/68/35/52/45/51). We found that the AUC increased when HPV31, HPV33/58, and HPV35/39/68 were added into the groups (AUC: subG1=0.687, subG2=0.746, and subG3=0.755), while it decreased when HPV52, HPV45, HPV51, and HPV59/66/68 were added into other groups (AUC:
subG4=0.748, subG5=0.746, subG6=0.743, and pooled 14 HR-HPV=0.709). AR (absolute risk) of CIN2+ was lower among women with HPV52/45/51/66/68 positive than that of pooled 14 HR-HPV types positive in cytological ASCUS/LSIL women [17.1% (95%CI: 9.93%-27.8%) vs. 35.0% (95%CI: 28.0%-42.8%)]. Compared with women with HPV52/45/51/66/68 positive, the risk of CIN2+ among women with HPV 16/18, HPV31, and HPV33/58 positive were 3.35 (95%CI:1.89-10.1), 2.43 (95%CI:1.04-5.66) and 2.22 (95%CI: 1.17-4.23), respectively.

Conclusion

The management of women with HPV 16/18/31/33/58 positive should be different from women with other 9 types HPV positive in China. Women with cytology ASCUS/LSIL and HPV 16/18/31/33/58 positive referred to colposcopy seemed to be of better clinical performance.
ANALYTICAL STABILITY OF SUREPATH COLLECTED CERVICAL SMEAR SAMPLES FOR HPV TESTING

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Background / Objectives

Changing from cytology to HPV testing as the primary screening analysis in organized programs for Cervical Cancer Screening will increase the requirements for bio-banking of cervical screening samples with respect to audit and quality assurance. The quality of stored, extracted DNA is well documented but what about storage of original sample material? The cervical cancer screening in Denmark is predominantly conducted using SurePath (BD, Sparks, Maryland, US) for sample collection. Here we present data on the analytical stability of SurePath collected cervical samples for HPV testing with ≥7 months storage of original SurePath samples between baseline testing and re-test.

Methods

We collected 1216 samples (897 NILM and 319 ≥ASCUS samples) from Danish women undergoing routine screening in the Capital Region of Denmark. The samples were split into two aliquots. Aliquots were tested at baseline and after ≥7 months (4°C) using the clinical validated Onclarity HPV assay (BD, Sparks, Maryland, US). Stability of the SurePath samples were assessed by 1) clinical reproducibility of results between 1st and 2nd test, 2) analytical quality assessment using the mean Ct-value of the internal control (HBB) of the Onclarity assay as a proxy-marker of overall DNA quality after storage, and 3) HPV genotype specific Ct-values of positive samples were used to address the stability of HPV DNA. The mean Ct-values of the internal HBB control and the HPV positive results were compared using the one-way ANOVA test (SPSS version 22).

Results

The overall reproducibility (positive-positive and negative-negative) was 98.0% (N=1192) with 1.8% (N=24) being discordant. No significant difference in the mean Ct-values of the internal HBB control (HBB; p=0.667) between the baseline test and the 2nd test were observed. The discordant samples were Ct 32.1±1.4 versus the manufacturer defined cut off of Ct 34.2. Furthermore, no significant difference were observed in measured Ct-values of the individual HPV genotypes detected between baseline and 2nd test; HPV16; p= 0.773, HPV18; p=0.530, HPV31; p=0.701, HPV33/58; p=0.996, HPV35/39/68; p=0.923; HPV45; p=0.992, HPV51; p=0.722, HPV 52; p=0.896, HPV56/59/66; p=0.626.
Conclusion

In conclusion, SurePath collected cervical screening samples can be stored at 4°C for at least 7 months without significant deterioration of the clinical or analytical quality of the material with respect to HPV testing when using the Onclarity HPV assay.
Valgent-4 Clinical Validation of Three HPV Genotyping Tests on SurePath Screening Samples from the Danish Cervical Screening Program

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Background / Objectives

As the demand for human papillomavirus (HPV)-related cervical screening increases, novel HPV tests must be evaluated using well-annotated samples. The Validation of Human Papilloma virus (HPV) Genotyping Tests (VALGENT) framework is an international collaboration designed to facilitate the clinical validation and comparison of HPV assays that offer genotyping capabilities. Here we present the data from three assays; BD Onclarity HPV assay (BD), Genomica CLART4s (CLART) and Agena MassArray HPV assay (MA).

Methods

In total, the Valgent4 consists of 1000 consecutive screening samples and an enriched subset of 300 samples equally divided between samples with ASCUS, LSIL and HSIL, all collected in SurePath LBC medium from Danish routine cervical screening. Briefly, the Onclarity assay is a real time PCR assay that detects 14 HR-HPV genotypes in nine groups (16,18,31,45,51,52,33/58, 56/59/66,35/39/68), the CLART4 assay is a PCR-based Microarray full genotyping assay that detects 36 genotypes (the 14 HR-HPV types and 22 non-HR HPV types), and the MA is a full genotyping MALDI-TOF-based assay that detects 19 genotypes (the 14 HR-types and 5 non-HR-types). Here, the analysis is limited to HR-HPV genotypes only.

Results

Onclarity, CLART4 and MA detected 371, 455, and 514 positives samples respectively. 947 samples had normal cytology, 106 ASCUS, 121 LSIL and 124 ≥HSIL. Histological follow-up at 12 months after initial sample collection resulted in 118 normal histology results including 29 ungraded CIN, 43 CIN1, 31 CIN2, 64 CIN3 and 7 cancers. The assays showed overall good pairwise HR-HPV detection concordance of 87-92%. At individual HR-HPV genotype level, the concordance varied from 40-84% (Onclarity vs MA), 32-62% (Onclarity vs CLART4) and 32-66% (CLART4 vs MA). HPV16, 18 and 31 had the highest pairwise assay concordance. For samples with ≥CIN2 outcome, the pairwise assay concordance was higher for
almost all genotypes detected by the three assays. The sensitivity for detection of ≥CIN2 was 95%, 98% and 96% for Onclarity, CLART4 and MA, respectively.

Conclusion

The three genotyping assay had overall good concordance at HR-HPV level, whereas on genotype level the discordance became noticeable. Genotypes HPV16, 18 and 31 had the highest pairwise concordance between the three assays. Comparison with a validated standard comparator test, planned later, will allow verification whether these tests fulfill the requirements for use in cervical cancer screening.
OPTIMIZATION OF THE RIATOL QPCR HPV GENOTYPING ASSAY BY CHOOSING A THRESHOLD ASSURING SATISFACTORY ACCURACY TO DETECT HIGH GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA

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Background / Objectives

To identify the optimal viral load threshold of the in-house AML Laboratory RIATOL qPCR HPV genotyping assay (qPCR) (Antwerp, Belgium) assuring satisfactory accuracy to detect high grade cervical intraepithelial neoplasia (CIN2+).

Methods

The clinical accuracy of the qPCR to detect CIN2+ was assessed using a set of cervical samples compiled for the VALGENT-3 project. The VALGENT framework is designed to assess the analytical and clinical performance of HPV tests that offer limited to extended genotyping capability. “VALGENT-3” panel comprised 1,600 samples from Slovenian women aged 20-64 years (1,300 sequential cases from routine screening and 300 “enriched” abnormal samples – 100 HSIL, 100 LSIL and 100 ASC-US).

The VALGENT-3 panel contained 126 specimen of women with CIN2+ (used to assess sensitivity) and 1,167 specimen from women with 2 consecutive negative Pap smears (used to assess specificity). Performance relative to the Hybrid Capture 2 (HC2) was also analyzed as per the non-inferiority criteria defined by Meijer et al. in 2009. The trade-off between sensitivity and specificity with different viral load cutoffs was assessed by ROC curve analysis. The cumulative hrHPV load was defined as the logarithm of the sum of the type-specific loads of the 14 HPV types.

Results

The qPCR had a sensitivity and specificity for CIN2+ of 97.6% (CI: 93.2-99.5%) and 85.1% (CI: 82.9-87.1%) respectively when the lowest analytical cutoff was used. At a cutoff of 1.58 log copies/cell, qPCR had a sensitivity of 96.0% (CI: 91.0-98.7%) and a specificity of 89.5% (87.6-91.2%). At this cutoff, accuracy of the qPCR was non-inferior to the HC2: relative sensitivity of 1.00 [CI: 0.97-1.03 (p<0.001)] and relative specificity of 1.00 [CI: 0.98-1.02 (p<0.001)].
Conclusion

HPV tests that provide viral load measurements (or other quantifiable signals) allow flexibility to optimize accuracy required for use in cervical cancer screening.
Evaluation of Xpert® HPV in cervical specimens collected in SurePath preservative fluid: An interim analysis

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Background / Objectives

Currently, a large scale study is being conducted to verify compatibility of the Xpert® HPV assay (Cepheid) with specimens collected in Surepath Preservative Fluid. Primary aim of this study is to investigate whether both fractions (primary cells and cell pellet) can be considered as suitable input material for the Xpert HPV assay. For this interim analysis, endpoints are limited to results with clinical cutoff only, and matched to corresponding outcomes of the Riatol qPCR genotyping assay as standard comparator test (limited to HR-HPV types only).

Methods

In total, 100 samples were prospectively collected and processed according to the standard Surepath method for cytology. Two different cell fractions were obtained per sample, i.e. left-over primary sample and pelleted cells after gradient purification. Both cell fractions are stored at 4°C, and further processed on both the Xpert HPV assay and the Riatol qPCR genotyping assay. Clinical thresholds were applied accordingly during this analysis. Paired T-test was used to compare Ct-values of both fractions.

Results

Median age of study participants was 46 years (IQR = 31 – 56). The first 100 samples gave valid results for 96 paired samples on Xpert HPV. HPV prevalence in this subset was 27/96 (28.1%). In all tested sample pairs, no clinical difference was observed between primary sample and pelleted cells. When comparing Ct-values for HMBS (cell adequacy control), significantly higher HMBS signals were found in the primary sample versus the processed fraction (p <0.001), indicating less cells in this fraction. At clinical level, full concordance between the standard comparator test (Riatol qPCR genotyping assay) and the Xpert HPV assay was observed. Errors (4 errors/200 reactions; 2%), were observed both in primary sample fraction (n=3) and in pelleted cells (n=1).

Conclusion
This interim analysis indicates a good compatibility of the Xpert HPV assay with samples collected in Surepath Preservative Fluid. Both cell fractions, generated after standard Surepath processing, can be analyzed without pre-treatment on the Xpert HPV assay. Results are shown to be clinically valid, as no differences could be observed with a validated comparator assay (Riatol qPCR genotyping assay). Higher concentration of cells was measured in the pelleted fraction, however these preliminary results do not indicate influence at clinical level, as no discordant HPV results between both Surepath cell fractions could be observed.
FC 10-07
REPRODUCIBILITY OF HUMAN PAPILLOMA VIRUS TYPING WITH XPERT REAL-TIME PCR ON ARCHIVAL CYTOLOGY SAMPLES

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Background / Objectives

Since most cervical cancers are HPV-associated, primary cervical screening is changing to HPV test in many European countries. HPV testing techniques are mainly based on direct DNA hybridization or nucleic acid amplification. HPV DNA testing may be performed directly from residual liquid-based cytology (LBC) specimens. In some cases, particularly in retrospective studies or for quality control purposes, previously archived samples may be used. In these cases, DNA degradation may become a potential issue. The aim of this study was to evaluate the reproducibility of HPV typing on archived LBC-specimens with the Real Time PCR (RT-PCR)-based Xpert® HPV assay (Cepheid, Sunnyvale, USA).

Methods

A total of 150 LBC samples (ThinPrep, Hologic, Inc. Marlborough, MA-USA) with a previous positive HPV test with Hybrid Capture II (HCII, Qiagene GmbH, Hilden-Germany), were included in the study and divided in 3 groups. Group 1 included 50 samples that were typed with the xpert assay immediately after the positive HCII test. Group 2 and Group 3 included 50 cases each with a previous positive HCII test which had been carried out 12 and 36 months before, respectively.

The observed agreement between Xpert® HPV assay and the previous HCII test was 96% for the first Group, 90% for the second Group and 94% for the third Group. The observed agreement didn’t differ significantly among the tree groups (p = 0.606).

Conclusion

These results show that HPV-typing by the RT-PCR based Xpert assay is reproducible even in long-term stored archival LBC-specimens. This may be relevant particularly for retrospective studies or for quality control purposes.
FC 10-08
DEVELOPMENT OF A NOVEL MULTIPLEX TYPE-SPECIFIC QUANTITATIVE REAL-TIME PCR FOR DETECTION AND DIFFERENTIATION OF INFECTIONS WITH HPV2, HPV27, AND HPV57

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Background / Objectives

HPV types HPV2, HPV27, and HPV57 are etiologically associated with more than 65% of common warts (verrucae vulgares), the most frequent HPV-associated benign lesions of the skin. When common warts appear in the anogenital region they can be misdiagnosed as anogenital warts (condylomata acuminata), which are etiologically associated with HPV6 and HPV11. In children the mentioned misdiagnosis could have potentially serious legal consequences, since the appearance of novel wart(s) in a child’s anal or genital region can be considered as an indicator of sexual abuse and can potentially trigger legal action against the parents or household members. Thus, although routine detection of HPV types present in tissue specimens or swabs of common and anogenital warts is not generally recommended, it could be very helpful in some clinical circumstances and/or for legal purposes, especially in children. To the best of our knowledge, no quantitative real-time PCR (RT-PCR) allowing simultaneous amplification and differentiation of HPV2, HPV27, and HPV57 has been developed so far. The present study describes the development and evaluation of the first multiplex type-specific quantitative RT-PCR, enabling simple, rapid, sensitive, and specific concurrent detection and differentiation of HPV types HPV2, HPV27, and HPV57 in a single PCR reaction.

Methods

The novel HPV2/27/57 multiplex RT-PCR was designed and optimized on plasmid standards and clinical samples of common warts.

Results

The HPV2/27/57 multiplex RT-PCR with a dynamic range of seven orders of magnitude (discriminating 10 to $10^8$ viral genome equivalents/reaction) has an analytical sensitivity of at least 10 viral copies of each targeted HPV type/reaction, and no cross-reactivities were observed among the included targets. All three primer/probe combinations were efficient in amplifying 500 copies of targeted DNA in a background of $10^8$, $10^7$, 500, 100, and 10 copies of non-targeted viral DNA/reaction, and the performance of the HPV2/27/57 multiplex RT-PCR was additionally not affected by the presence of background human genomic DNA. When testing DNA isolates obtained from fresh-frozen tissue specimens of various
children’s warts, the results of the HPV2/27/57 multiplex RT-PCR were completely in line with the results of the conventional low-risk Alpha-PV PCR.

Conclusion

The newly developed HPV2/27/57 multiplex RT-PCR is an appropriate test for use in routine clinical laboratory settings and for studies focusing on the molecular epidemiology, pathogenesis, and natural history of HPV2/27/57-related lesions.
The 5-year incidence and clearance of type-specific HPV in a screening cohort in China

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Background / Objectives

We aimed to evaluate the 5-year incidence and clearance of type-specific high-risk HPV (hr-HPV) among a Chinese rural women cohort.

Methods

A screening cohort with 1,997 women aged 35-45 years was followed up with an interval of approximate 5 years. HPV genotyping (INNO-LiPA Extra, Innogenetics, Belgium) was performed on cervical samples collected from HPV positive women (Hybrid Capture II) in this cohort since 2005. The 5-year incidence and clearance of type-specific HPV and the relevant demographic factors were calculated.

Results

The 5-year overall incidence of hr-HPV was 15.6% and the clearance was 69.0%. HPV 16 related types had two times probability of HPV incidence (10.6%) after 5 years than HPV 18 related types (4.4%) (P<0.001), while the clearance of HPV 16 related types (74.1%) was lower than that of HPV 18 related (85.9%) (P=0.048). The incidence and clearance of HPV 16 was 2.7% and 70.9%. HPV 52 and 16 ranked the top of hr-HPV incidence and HPV 52 and 18 ranked the top of hr-HPV clearance. Sexual debut age was the main factors correlated with HPV incidence, with the adjusted RR of 1.478 (95%CI: 1.110-1.967).

Conclusion

The high incidence, persistence and low clearance prompted the importance of screening in HPV 16 related positive mid-adult Chinese women. Women with HPV 16 related repeated positive within 5 years should be considered as a high risk population for cervical cancer.
HIGH-RISK HUMAN PAPILLOMAVIRUS SCREENING ROLL-OUT IN NORWAY

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Background / Objectives

A shift of primary cervical screening from cytology to hrHPV detection, although only for women aged 34 to 69 years, implies a major shift in the technical infrastructure for screening. To develop real-world evidence for preferred cervical cancer screening strategies, we compared liquid based cytology (LBC) screening every 3 years (current screening modality) with high-risk human papilloma virus (hrHPV) testing every 5 years in Norway (health service study trial number 006_2014_10_RHS).

Methods

Between February 2015 and April 2017, approximately 140,000 women aged 34 to 69 years who returned for their routine, triennial cervical cancer screening were assigned hrHPV-testing (cobas® HPV Test (Roche Diagnostics) or LBC, based on even/odd day of birth. Cervical intraepithelial neoplasia grade 2, 3 and cervical cancer (CIN2+) was detected among 32,434 women who completed their follow-up of a positive screening test by early 2017.

Results

Screening attendance by age was similar in HPV-screening and LBC-screening, being 68% after 1st and 28% after 2nd reminder. The proportion of screening test positives was 5.4% in LBC-screening and 6.5% in HPV-screening, and declined by increasing age. HPV16/18 was detected in 20% of hrHPV-positives. Compared to LBC-screening, we observed 40% more biopsy and/or treatment referrals, 78% more CIN2+ and 50% more CIN3+ in HPV-screening.

Conclusion

HPV-screening was well accepted and detected more pre-cancers, suggesting that HPV-screening should replace LBC-screening. Randomized implementation of HPV-screening allows to monitor the performance of novel technology in real-life, reassuring the overall high performance of the program and mitigating the transition.
EXTENDED SCREENING INTERVALS: EVIDENCE FROM THE ARTISTIC TRIAL COHORT

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Background / Objectives

The UK National Screening Committee (NSC) based its recommendation that HPV testing should replace cytology in primary screening largely on the 2009 follow-up results of the ARTISTIC trial. The NSC must now decide on screening intervals in time for national roll out of primary HPV screening (due 2019). Options include extending the screening interval up to 10 years for HPV negative women and delaying recall for HPV positive women with normal cytology, as their infections are usually transient.

Methods

In the ARTISTIC Trial 24,510 women attending for routine cervical cytology in Greater Manchester in 2001-2003 were recruited. During the trial women were recalled 3-yearly and histology results were obtained from local laboratories. After 2009 histological follow-up and sample collection ended and the women returned to routine cytological screening with recall 3-yearly below age 50 and 5-yearly at age 50-64. We have followed the trial cohort to 2015 through national cancer registration for CIN3 and cancer and through linkage to the cervical screening call-recall system to obtain lifetime cytology records.

Results

The analysis included 24,496 women at round 1 and 13,591 at round 2 (30-48 months later). Follow-up via local histology laboratories and national cancer registration identified 505 cases of CIN3+ (including 22 invasive cervical cancers). The cumulative CIN3+ risk 10 years after a negative HPV test (0.31%, 95%CI 0.18-0.49 in the revealed arm) was similar to that 3 years after negative cytology (0.30%, 95%CI 0.23-0.41 in the concealed arm) and fell sharply with age, from 1.1% below 25 (95%CI 0.7%-1.8%) to 0.08% (95%CI 0.03%-0.20%) above 50.

Conclusion

We found a similar level of protection 10 years after a negative HPV test and 3 years after negative cytology. These data support a much longer screening interval after a negative HPV test than after a negative cytology test.

About three quarters of women with HPV infection and normal cytology clear their infections within about 3 years. Their risk of CIN3+ within this time is low (1.5%),
suggesting that the current policy of annual repeat testing and referral after 2 years is too conservative. Approximately 40% of women who remained HPV positive had cleared their initial infection and acquired a new HPV type. Cumulative CIN3+ risks in women with type-specific persistent infections are about 6 times higher than in women with new infections. Triage strategies based on HPV persistence would therefore reduce unnecessary referral of women with new (and largely transient) infections.
FC 11-03
4 - YEAR EXIT RESULTS FOR WOMEN WITH NO CIN2 OR WORSE DETECTED IN EARLIER SCREENING ROUNDS IN THE HPV FOCAL TRIAL


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Background / Objectives

The HPV FOCAL RCT compares liquid based cytology (LBC) with hybrid capture 2® (HC2) triage of ASCUS at entry and 2-years (Control Arm) to HC2 testing with LBC triage at entry (Intervention Arm). Women exit with HC2/LBC co-testing performed at four years in both arms. We examine exit results in subjects without cervical intraepithelial neoplasia or worse (CIN2+) detected in earlier trial screening.

Methods

Subjects were included if they were eligible for routine screening at the time of the exit screen. For the Intervention arm this was women HC2 Negative at baseline (HPVBaseNeg), or HC2 positive and negative for intraepithelial lesion or malignancy (NILM) at baseline and HC2 negative at 6-12 months retesting (HPVRev), or recommended for colposcopy where no CIN2+ was detected (HPVColpoNeg). For the Control arm this was women NILM or atypical cells of undetermined significance (ASCUS) and HC2 negative at baseline and at two years (CYTNeg), recommended for colposcopy at baseline and no CIN2+ found (CYTColpoNeg0) or recommended for colposcopy at 2-years, but not at baseline, and co CIN2+ found (CYTColpoNeg2). Results presented are based upon exit (4-year) co-testing where women were referred to colposcopy if HC2 positive or ASCUS or worse.

Results

9,552 women were randomized to the Intervention arm and 8,338 were eligible and attended the exit screen, 9,457 women were randomized to the Control arm and 7,424 were eligible and attended the exit screen: the breakdown of subjects is given in the attached table. The overall relative risk of CIN2+ at exit for Intervention versus Control was RR=0.86 (95%CI=0.58-1.26). For those with a single negative HPV tests versus those with two consecutive negative LBC tests (i.e. HPVBaseNeg versus CYTNeg) the relative rate at exit contesting was RR=0.69 (0.45-1.07). Women having earlier negative colposcopy were at elevated risk in both arms compared to others eligible for routine exit screening: Intervention Arm RR=10.0 (5.5-18.3); Control Arm RR=5.0 (2.1-11.5).

Table: Rates of CIN2+ Identified at 48 Month Exit Cotesting by Study Arm Subgroup

<table>
<thead>
<tr>
<th>HPVBaseNeg</th>
<th>HPVRev</th>
<th>HPVColpoNeg</th>
<th>CYTNeg</th>
<th>CYTColpoNeg0</th>
<th>CYTColpoNeg2</th>
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<table>
<thead>
<tr>
<th>Number</th>
<th>7869</th>
<th>166</th>
<th>303</th>
<th>7308</th>
<th>108</th>
<th>80</th>
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</thead>
<tbody>
<tr>
<td>CIN2+</td>
<td>35</td>
<td>2</td>
<td>14</td>
<td>47</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Rate/100 (95%CI)</td>
<td>0.44 (0.32-0.61)</td>
<td>1.2 (0.33-4.3)</td>
<td>4.6 (2.8-7.6)</td>
<td>0.64 (0.48-0.75)</td>
<td>4.6 (2.0-10.4)</td>
<td>1.3 (0.2-6.8)</td>
</tr>
</tbody>
</table>

**Conclusion**

Women recommended for colposcopy with no significant lesion detected were at elevated subsequent risk and their careful surveillance is indicated.

**References**

Ogilvie, G et al, HPV for cervical cancer screening (HPV FOCAL): Complete Round 1 results of a randomized trial comparing HPV-based primary screening to liquid-based cytology for cervical cancer, IJC 140 (2017):440-448
CANCER CASES IDENTIFIED IN A RANDOMIZED IMPLEMENTATION OF PRIMARY HPV-TESTING IN THE NORWEGIAN CERVICAL CANCER SCREENING PROGRAMME

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Background / Objectives

High risk Human Papilloma Virus (HPV) testing is currently implemented in a randomized controlled fashion as the primary test in the Norwegian cervical screening programme. We present detailed evaluation of the cancer cases identified.

Methods

The implementation involves women in the age-group 34-69 years in four Norwegian counties, counting approximately 285,000 women. The follow-up algorithm after abnormal HPV-test is more aggressive than for abnormal cytology, and more women are referred to immediate biopsy, and thereby potentially earlier detection of cancers. To compare symptomatic and screening detected cancer cases among those allocated to HPV test or cytology, we included women with at least 15 months follow-up since screening. Description of screening results (cytology/HPV status/genotype), screening history, symptoms, FIGO-stadium and age of the cancer-diagnosed women are presented.

Results

By March 2016, approximately 140,000 women have been screened, half with HPV test and half with cytology. Around 32,000 women have had adequate follow-up time. A total of 25 cancer cases were identified; 14 cases among HPV-screened (12 squamous cell carcinoma, 2 adenocarcinoma) and 11 among cytology-screened (9 squamous cell carcinoma, 1 adenocarcinoma, 1 other cervical cancer type). 86% of the cancer cases was diagnosed in women below 50 years after primary HPV test compared to 46% in the cytology group. More than 50% of the women diagnosed with cancer were screened sub-optimally. Around 80% of the cancers were related to HPV16 and HPV18, and the majority of the cancers were FIGO stadium I. Updated results will be presented at the conference.

Conclusion

As we expected, observed number of cancer cases were comparable in HPV-screening and cytology screening, suggesting high performance of HPV-testing in routine screening.
DETECTION OF CIN2+ IN WOMEN WITH NORMAL CYTOLOGY USING A 3-TYPE HPV mRNA TEST

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Background / Objectives

Despite a well-established cervical cancer screening program in Norway, the incidence of CxCa in young women is increasing, peaking at 35 years. Twenty five percent of all women diagnosed with CxCa had normal cytology within 3 years of cancer diagnosis. We wanted to estimate the detection rate of CIN2+ in women with normal Pap smears by rescreening Pap smears from HPV mRNA 16, 18, and 45 positive samples. HPV 16, 18 and 45 cause 90% of cervical cancers in young women.

Methods

From April 2016, the Department of Pathology, University Hospital of North Norway, introduced a study by rescreening all normal Pap smears that had a positive HPV mRNA test (PreTect SEE). Women with revised cytology were followed up according to national guidelines.

Results

Of 26 948 women with Pap smear, 184 (0.7%) had normal cytology and a positive HPV mRNA test. After rescreening of the index cytology, 63 women had abnormal cytology. At present 42 women have had colposcopy, resulting in 7 women with normal biopsies, 21 CIN1, 9 CIN2 and 5 CIN3. The positive predictive value of CIN2+ among women with biopsy was 33.3 % (14/42).

Conclusion

By testing all women with normal cytology with a specific HPV mRNA test, an increase in screening program sensitivity can be achieved. When more women with CIN2+ are detected in the first screening round, fewer women will develop cervical cancer before next screening. The volume of rescreened smears (0.7%) is very low but adds significant improvement of screening sensitivity and increases quality in educating the screeners by rescreening presumably false negative Pap smears.
THE CLINICAL AND ECONOMIC IMPACT OF HPV EXTENDED GENOTYPING FOR THE INDIVIDUALIZED RISK MANAGEMENT OF PATIENTS: RESULTS OF AN ECONOMIC MODEL

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Background / Objectives

Denmark has a well-established cervical cancer screening program with nationwide screening guidelines issued by the National Board of Health. In women aged 30–59 years, guidelines recommend cytology-based screening with high-risk HPV testing for ASC-US triage. In women aged 60–64, primary HPV testing is recommended. Current algorithms utilize pooled HPV assays where either no HPV genotypes or only types 16/18 are differentiated. New technologies are available which can differentiate all 14 high-risk HPV types, potentially offering greater ability to stratify patients based on individual risk.

In this study, a health economic model was developed to estimate the impact of adopting extended HPV genotyping within the Danish cervical cancer screening program. Current screening strategies using no or partial HPV genotyping were compared with strategies using extended genotyping in terms of number of colposcopies and CIN2+ detection.

Methods

A budget impact model was constructed in Excel using data from the published literature on population size, HPV prevalence and disease outcomes. For ages 30–59 years, we compared 1) HPV triage of ASC-US without genotyping with 2) HPV triage of ASC-US using extended genotyping. For ages 60–64 years, we compared 1) HPV primary screening using HPV16/18 genotyping with 2) HPV primary screening using extended genotyping. In the extended genotyping algorithms, women with HPV16, 18, 31, 33, 45, 52, or 58 were sent to colposcopy, while women with lower risk genotypes (35, 39, 51, 56, 59, 66, or 68) were sent to a 1-year follow-up.

Results

Preliminary analyses indicated that using extended HPV genotyping for ASC-US triage in women aged 30–59 would reduce the number of colposcopies by 29.7%, at the cost of a slight (4.8%) increase in the number of CIN2/3 cases referred to 1-year follow-up instead of immediate colposcopy. Extended genotyping for HPV primary screening at
ages 60–64 would reduce colposcopies by 13.9%, while slightly (4.6%) increasing the number of CIN2/3 cases referred to 1-year return instead of immediate colposcopy.

Conclusion

Extended HPV genotyping may potentially reduce colposcopies in the Danish population with minimal sacrifice for disease detection. The CIN2/3 cases referred to 1-year return in the extended genotyping algorithms were attributed to lower risk HPV genotypes and likely had low probability of progressing to cancer within a year. Further analyses will be presented at the conference, including the budgetary impact of extended genotyping and the performance of extended genotyping for HPV primary screening of women aged ≥30.
HC 11-07
HPV Primary Screening Pilot Study: molecular testing of potential triage strategies for HPV-positive women


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Background / Objectives

The objective of this study is to evaluate and compare different strategies for the triage of women with a HPV-positive primary screening test. Clinical performance in terms of sensitivity, specificity, PPV, NPV will be calculated both cross-sectionally and longitudinally for each triage strategy. The overall aim of the study is to define optimal algorithms for triage of HPV DNA positive women from primary HPV screening.

Methods

In partnership with CervicalCheck, The National Cervical Screening programme, CERVIVA are undertaking a longitudinal observational HPV primary screening study which will evaluate different triage strategies for management of a HPV-positive primary screening test. Cervical cytology samples from approximately 13,000 women undergoing routine cervical screening will be tested for HPV DNA (cobas 4800 HPV test) and mRNA (Aptima HPV assay). All HPV-positive women will be further assessed with cytology and a panel of molecular tests including HPV16/18 genotyping, p16INK4a/Ki-67, and specific methylation markers. The performance of different triage strategies will be examined both cross-sectionally and longitudinally over two screening rounds for detection of CIN3+.

Results

To date 8500 woman have been recruited into the study. The median age of the population is 39 years. HPV DNA testing, performed on 7301 samples, shows a 14.7% positivity rate. HPV mRNA, performed on 7394 samples, gave a 12.7% positive rate. HPV mRNA had a significantly lower positivity rate in women under the age 40 years and women with a negative cytology (p=0.001 and p=0.0015). Second round testing identified 32% of HPV positive women were positive for HPV 16/18 and 30% had an abnormality on cytology. In a smaller subset 38% were positive for p16/Ki-67.

Conclusion
Overall prevalence of HPV mRNA is lower than HPV DNA in the study population. Here we present the preliminary cross-sectional data in relation to each of the putative triage tests.
GENOTYPING AND CYTOLOGIC TRIAGE OF HPV POSITIVE WOMEN FOR THE DETECTION OF CERVICAL HIGH-GRADE LESIONS

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Background / Objectives

The VASCAR (viral testing alone with Pap triage for screening cervical cancer in routine practice) study was a single-center demonstration project initiated in Montreal, Canada in 2011 among more than 23,000 women attending routine cervical cancer screening. In a secondary phase of VASCAR, we determined genotype-specific risks of disease progression to biopsy-confirmed cervical intraepithelial neoplasia of grade 2 or worse (CIN2+) associated with HPV types 16, 18, and/or the other 12 (pooled) high-risk types, compared to Pap cytology. We also assessed the diagnostic performance of HPV genotyping compared to cytological triage.

Methods

Women aged 30-65 were originally screened for HPV using the Hybrid Capture® 2 (HC2) Test. Women with positive results were triaged using conventional cytology, and those with positive Pap cytology results (≥ASC-US; atypical squamous cells of undetermined significance) were referred to colposcopy. We retrospectively genotyped 1396 cervical specimens that were HPV+ with HC2 using the Roche’s cobas® 4800 HPV system, and extracted the women’s medical history. We evaluated diagnostic performance of triage tests in the first year of follow-up among women positive for: (1) HPV16; (2) HPV18; (3) HPV16 and/or HPV18 and; (4) one or more of the other 12 HPV types. Using hierarchical and exclusive categories of HPV positivity (any HPV16; else HPV18; else 12 other HPVs), we correlated HPV status at enrollment with detection of histologically confirmed CIN2+ by estimating hazards ratios (HR) with 95% confidence intervals (CI) using Cox proportional hazards regression.

Results

Of the 1396 women, 1092 (78%) were classified as normal, 136 (10%) had CIN1, 80 (6%) had CIN2, 81 (6%) had CIN3 and 7 women had cancer, throughout the entire follow-up period. Sensitivity of HPVs 16, 18, 16 and/or 18, and any high-risk HPV for prevalent CIN2+ (n=76) were 35.5% (CI:24.9-47.3), 9.2% (CI:3.8-18.1), 43.4% (CI:32.1-55.3), and 64.5% (CI:52.7-75.1), respectively. Conversely, cytology triage
(ASC-US+) had a sensitivity of 92.0% (CI:83.4-97.0). Corresponding specificity values were 84.0% (CI:81.9-86.0), 95.0% (CI:93.7-96.1), 79.7% (CI:77.4-81.8), 33.4% (CI:30.9-36.0), and 73.6 (CI:71.1-76.0). Compared to cobas HPV- and HC2 HPV+ women, the HRs were 7.3 (CI:3.8-14.3), 3.9 (CI:1.5-10.2), and 2.7 (CI:1.4-5.2) for women with any HPV16, HPV18, and 12 other types, respectively. Compared to women with normal cytology, the HRs for AS-CUS, LSIL, and HSIL (SIL: squamous intraepithelial lesion) were 3.7 (CI:2.5-5.7), 5.0 (CI:3.1-8.0) and 16.5 (CI:11.0-24.7), respectively.

Conclusion

Cytology and genotyping seem to be comparable in triaging women with positive HPV results on screening.
5-TYPE HPV mRNA NEGATIVE WOMEN IN TRIAGE OF ASC-US/LSIL MAY RETURN TO SCREENING AT 3-YEAR INTERVAL – AN HISTORICAL PROSPECTIVE COHORT STUDY

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Background / Objectives

To compare the risk of CIN3+ among women who had a normal cytology (non-exposed cohort) at study start with women who had an HPV mRNA negative ASC-US/LSIL in triage (exposed cohort).

Methods

After exclusion of women who had a previous history of CIN1+ and HSIL, we identified 1063 women who had an HPV negative triage of ASC-US/LSIL over the years 2006 through 2011, and a control cohort of 25 948 women who had a normal cytology during 2006/2007. All women, aged 25-69 at study start, were residents of the counties Troms and Finnmark, Norway, and were followed through December 31, 2014. The HPV test targeted E6/E7 mRNA from the types HPV16, 18, 31, 33 and 45 (PreTect HPV-Proofer, PreTect AS). All analysis were done in SPSS version 24.0 with Chi-square test, T-test and survival analyses.

Results

The exposed cohort were significantly younger than the non-exposed cohort. The crude cumulative proportion of CIN3+ were 2 and 8 per 1000-w.-yrs. at 42 and 78 months of follow-up for the non-exposed cohort, and 14 and 26 (95% CI: 9-43) per 1000-w.-yrs. for ASC-US-/LSIL-women. The exposed cohort had significant more extensive follow-up than the control cohort. Over the entire study period 20 cervical cancers were diagnosed in the non-exposed cohort (incidence 15.3/100 000 w.-yrs.) compared to none in the exposed cohort.

Conclusion

Women who have a negative mRNA-test for HPV16, 18, 31, 33 and 45 at triage for ASC-US/LSIL have low risk for CIN3 within the first two screening intervals after triage, and may return to screening at 3-year interval.
VALIDATION AND IMPLEMENTATION OF A NEXT-GENERATION qPCR DIAGNOSTIC TOOL FOR HUMAN PAPILLOMAVIRUS TYPE 67 SCREENING

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Background / Objectives

Cervical cancer prevention in the post-cytology era mainly relies on primary HR-HPV screening. Current epidemiological findings have indicated an underestimated role of possible High-Risk HPV (pHR-HPV) types in cervical carcinogenicity and urge for increased availability of genotype-specific information. This could be suggestive for expansion of existing HPV genotyping assays with pHR-HPV types. It is determined pHR-HPV67 is rarely prevalent in cervical cancer, notwithstanding, its close relation to only HR-HPV types. Our objective was to optimize and validate a Next-Generation pHR-HPV67 qPCR assay. These qPCR assays were applied in a HPV67 epidemiological case study.

Methods

A triple-target HPV DNA qPCR assay was developed. The occurrence of cross-hybridisation with other genotypes within the same α9-species was evaluated by testing the assays with confirmed positive samples. Limit of quantification (LOQ) and limit of detection (LOD) were determined by a dilution series of synthetic gene fragments. Reproducibility and repeatability were performed by testing HPV67 positive samples in duplicate series and over different days. The epidemiological case study comprised 273 samples, gathered in April 2017, enriched with 100 samples of each aberrant cytological category (LSIL, HSIL).

Results

The cross-hybridization assay confirmed high specificity for HPV67 of the different sets. A LOQ concentration of 33.21 copies/μL and 12.67 copies/μL was obtained for the assays targeting HPV67E6, E7 and L1 respectively (analogue results for E6 and E7). A concentration of 33.21 copies/μL (E6), 6.33 copies/μL (E7) and 3.17 copies/μL (L1) was still detectable in 85% of the cases (LOD). Belgian women with HSIL had a HPV67E6, E7 and L1 prevalence of 8.16% (95% CI: 4.19%;15.28%), 8.16% (95% CI: 4.19%;15.28%) and 7.14% (95% CI: 3.50%;14.01%), respectively (no significant differences: p>0.05, Chi Square test). In LSILs, a HPV67E6, E7 and L1 prevalence of 12.04% (95% CI: 7.17%;19.51%), 12.96% (95% CI: 7.88%;20.59%) and 9.26% (95% CI: 4.19%;15.28%)...
CI: 5.11%;16.21%) respectively (p>0.05, Chi Square Test) was determined. Within the screening population, a HPV67E6, E7 and L1 prevalence of 2.22% (95% CI: 1.01%;4.71%), 1.83% (95% CI: 0.78%;4.21%) and 1.83% (95% CI: 0.78%;4.21%) was obtained (p>0.05, Chi Square Test).

Conclusion

Slightly divergent but not significant differences in HPV67L1 prevalence were observed when compared to the HPV67E6 and E7 prevalence. Possible explanations are a variation in PCR efficiency or alternative integration via the HPV L1 gene. The multiple-targeting aspect of the Next-Generation qPCR assay led to the more exact detection of HPV67 and can contribute to an increase in accuracy of HPV detection.
PRESENCE OF KOILOCYTOSIS IN LOW-GRADE CYTOLOGY OF hrHPV-POSITIVE WOMEN IS A NEGATIVE PREDICTOR FOR CIN3+

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Background / Objectives

At the beginning of 2017 The Netherlands converted to hrHPV-based cervical cancer screening with cytological triage of positive cases. A strong increase in colposcopy referrals is foreseen most of which seem unwarranted. Thus, reduction of unjustified referrals will have priority. Koilocytosis is considered as a cytopathic effect of a productive HPV infection but the relation with subsequent diagnosis of high-grade Cervical Intraepithelial Neoplasia (CIN) is unclear. The aim of this study was to investigate if the risk for CIN3 or more (CIN3+) differs between hrHPV-positive ASC-US/LSIL with or without koilocytosis and whether the presence of koilocytosis could justify a more conservatively follow-up regime.

Methods

Retrospective cohort study, using data from the nationwide network and registry of histo- and cytopathology in The Netherlands (PALGA). HrHPV-positive ASC-US/LSIL follow-up cytology of 1 201 women was used from the former cytology-based cervical screening programme. Reporting of koilocytosis was assessed as well as detection rates of CIN1 or less, CIN2 and CIN3+, stratified by the presence or absence of koilocytosis. Crude and adjusted odds ratios (ORs) were calculated.

Results

Koilocytosis was present in 40.1% of hrHPV-positive ASC-US and 45.9% of hrHPV-positive LSIL. CIN3+ is significantly less often found when koilocytosis was reported (7.8% for hrHPV-positive ASC-US with koilocytosis versus 15.8% without koilocytosis). For hrHPV-positive LSIL this was 11.7% versus 20.2%. The crude and adjusted ORs for CIN3+ were 0.45 for hrHPV-positive ASC-US and 0.52 for hrHPV-positive LSIL.

Conclusion

The presence of koilocytosis is a negative predictor of CIN3+. The risk of hrHPV-positive ASC-US combined with koilocytosis for CIN3+ is in the same range as
hrHPV-positive/cytology negative cases and these cases could be followed conservatively by repeat cytology after 6 months. However, the results of this study should be confirmed by the first data derived from the new HPV-based screening programme.
FC 11-12
MEASURING CYTOLOGY REPRODUCIBILITY IN THE NEW DUTCH CERVICAL SCREENING PROGRAM

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Background / Objectives

The shift from primary cytology screening to HPV-screening with cytology triage is expected to influence the performance of cytology reading. We initiated an educational program prior to the start of the renewed screening program to refresh classification criteria.

Methods

Two partially overlapping cytology sets of 100 liquid-based cytology slides (ThinPrep) were collected, derived from a blinded co test pilot done previously (the DuSC study), stratified for age and anticipated percentage of abnormalities (i.e. 30%). After examination by an expert panel of 3 cytotechnologists and a pathologist a consensus diagnosis was determined. The sets were ring-studied in the 5 screening laboratories. The first set was followed by an adjudication session with each lab by the national reference official, in which results were discussed and individual classifications were aligned to classification guidelines and consensus diagnosis. Then, after a washout period, a second set of 100 cases with 50 overlapping cases was offered to the same laboratories. Cytotechnologists were asked to individually examine all slides and score them according to KOPAC and Bethesda classification. Pathologists examined only the non-NILM cases.

Results

Discrepancies in cytology reading were examined between i) NILM (especially reactive cellular changes) and ASCUS and vice versa: cases with few atypical changes (< 5 cells per slide) were regarded as difficult for classification of NILM versus ASC-US. Notably, distinguishing between NILM and ASC-US is important because it is the threshold for referral to a gynaecologist in the new HPV-screening program; ii) cytotechnologists and pathologists: pathologists showed moderately higher scores of classifications in the non-NILM’s, iii) laboratories, iv). Missed LSIL/HSIL cases. Wide variation in classification was found in sporadic cases due to technical or obscuring factors (dark staining, thick cell groups, or low cell counts).
Guidance was given on the criteria of the Bethesda classification and according to the company guidelines.

**Conclusion**

The results show considerable variation in cytology classification between cytotecnologists and pathologists and between laboratories. These results are the starting point of an ongoing external quality control (EQA) and educational program on standardization and optimal cytology classification results within the Dutch cervical screening program. The participants evaluated the learning sets as an important educational tool.
Background / Objectives

**Background** - The FAM19A4/miR124-2 methylation test (QIAsure Methylation Test, QIAGEN) is a novel assay, designed by Self-screen, that can be used for the triage testing of HPV-positive women or women with ASC-US. The test is a quantitative methylation-specific PCR that detects hypermethylation of the genes FAM19A4 and hsa-mir124-2 in cervical scrapes and self-collected samples. Within the framework of the VALID-SCREEN (H2020) project, the performance of the FAM19A4/miR124-2 methylation test will be validated in different, well characterised clinical cohorts from different European countries.

**Objective** - Determine the inter-laboratory agreement of the FAM19A4/miR124-2 methylation test on HPV-positive cervical scrapes collected in different European screening cohorts.

**Methods**

In total 695 HPV-positive cervical scrapes from five different European screening settings were included, i.e. Slovenia (SL, n=97), Spain (SP, n=239), Scotland (SC, n=96), Germany (G, n=159), and Denmark (D, n=104). Cervical scrapes had been collected in PreservCyt (SP, SL, SC, and G) or Surepath (D) and DNA extraction was performed according to local procedures. The samples were tested locally and sent to the reference lab for retesting. Both test and reference lab performed separately bisulftite-conversion followed by FAM19A4/miR124-2 methylation test (QIAsure Methylation Test) according to manufacturers’ instructions. Testing at both sites was performed blinded to the results of the other lab, and compared afterwards.

**Results**
Overall inter-laboratory agreement was 90.5% (629/695; 95%CI:88-92) with even higher agreement values in women with CIN3+ (i.e. 96.7%; 29/30; 95%CI:80-100). Agreement values for the five screening settings ranged from 83.9% to 96.8%. Overall kappa value was 0.77 (range laboratories: 0.64-0.91) indicating good inter-laboratory agreement. Discordant test results related to samples of women without clinical relevant disease having methylation values around the clinical cut-off of the assay.

**Conclusion**

The inter-laboratory agreement of the FAM19A4/miR124-2 methylation test (QIAsure Methylation Test) was consistently high for the different European screening settings. Thus, the FAM19A4/miR124-2 methylation test is a good and reliable molecular triage assay suited for full molecular screening (i.e. HPV-DNA testing in combination with QIAsure Methylation Test).

**References**

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THE SCOTTISH HPV ARCHIVE - A RESOURCE FOR BASIC AND TRANSLATIONAL RESEARCH

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Background / Objectives

Continuous research is crucial to improve our understanding for better management of HPV associated diseases. Avenues of research include mechanistic studies of HPV infection, lifecycle and pathogenesis; innovation in HPV detection technologies; biomarker discovery; development of prophylactic agents and better treatment technologies. A population based sample archive, with well-annotated and quality controlled clinical materials, assists in such research.

Methods

The Scottish HPV Archive1, setup in 2009, is a biorepository of cervical samples from women in Scotland. The archive received government core-funding for the first 5 years and then has been sustained via research funding and a revenue model based on sample provision. At the outset, archival and data management procedures along with an integrated inventory system were established. Generic Research Tissue Bank approval was obtained for sample storage and for data linkage to national databases for cervical screening, immunisation, colposcopy and cancer.

As a dynamic archive, the samples constitute residual material from different collections and include samples from women attending routine screening in addition to research collections associated with specific inclusion criteria. Current collection contains over 40,000 samples, which include 34,321 liquid based cytology, 7,913 DNA and 913 self-taken vaginal swabs. Samples are annotated with HPV infection results and genotypes, cytology and histology results and vaccination status. Quality assessment is performed regularly to assess best storage conditions for viable cells, DNA, RNA and protein. Access to samples is obtained through application to the archive steering committee2.

Results

The archive has been associated with much activity and output; to date, 37 applications have been approved for use of samples and/or data with ~14,000 samples provided. The requests are associated with research into HPV epidemiology
(4, 10.8%), biomarker development (23, 62.2%), validation and assessment of HPV detection assays (9, 24.3%), and data linkage studies (1, 2.7%). The requests have been both from United Kingdom (31, 83.9%) and international partners (6, 16.2%); and 13 (35.1%) involved commercial collaborations. The archive has contributed to 19 peer reviewed publications, 57 international conference submissions and has been a part of 14 equitable grant awards since its setup.

**Conclusion**

In the eight years since its establishment, the Scottish HPV Archive has proved to be a valuable resource for researchers. Our aim is to continue to engage with scientific and clinical community to ensure the archive can adapt to reflect and accommodate key and contemporary research priorities.

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METHYLATION BIOMARKERS TO TRIAGE HPV POSITIVE SUREPATH COLLECTED SCREENING SAMPLES

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Implementation of primary human papillomavirus (HPV) screening will require triage of high-risk (hrHPV)-positive women to efficiently identify those with high risk of cervical high-grade intraepithelial neoplasia (CIN) and cancer, but equally importantly, to deselect hrHPV-positive women who are at low risk. Here, we evaluate the QiaSure methylation assay measuring the human biomarkers FAM19A4 and mir124-2 in combination.

Methods

Post-cytology residual SurePath samples from a group of 502 hrHPV positive women undergoing routine cytology screening at Hvidovre Hospital, Denmark, were collected (age 30-65, average: 49 years). HPV testing was done using Onclarity HPV test (BD Diagnostics, Sparks, MD) or CLART2 HPV array (Genomica, Madrid). Women with cytology abnormalities and/or hrHPV positive were referred to follow-up in concordance with Danish Guidelines. In total, 361 of the 502 women had histology registered in the Danish Pathology Databank within 12 months after the positive screening sample. Samples were reflex tested using the QiaSure methylation assay (Qiagen, Hilden, Germany). All molecular testing was performed in concordance with manufacturer’s specification. Clinical performance estimates of reflex methylation for the detection of ≥CIN3 were determined.

Results

Among the 361 women with histology, 8 had CxCa, 54 CIN1, 24 CIN2, 61 CIN3, 214 were normal and 1 had inadequate histology. For ≥CIN3, hrHPV/methylation analysis showed 77% sensitivity, PPV of 40% and NPV of 93%.

Conclusion

In a primary screening setting for women ≥30 years of age, where referral for colposcopy directed biopsies is defined by hrHPV status, the use of QiaSure methylation assay works with SurePath collected cervical samples. The resulting sensitivity, PPV and NPV support that the QiaSure methylation assay can be
considered as part of a unified molecular work flow for future molecular cervical screening, saving laboratories the work load of reflex cytology on hrHPV positive screening samples.

References

This work was supported by the SME Instrument in the Horizon 2020 Work Programme of the European Commission (Valid-screen 666800).
METHYLATION PATTERN SWITCH BETWEEN LOW AND HIGH GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA: IMPLICATIONS FOR PROGRESSION MODELS, ROBUST TRIAGE, AND CANCER RISK

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Background / Objectives

Human papillomavirus (HPV) infection accounts for an estimated 530,000 cervical cancer cases and 270,000 deaths annually, with the majority (86% of cases and 88% of deaths) occurring in developing countries. Infection with high-risk HPV types can lead to mild, moderate or severe cervical intraepithelial neoplasia (CIN1, 2 or 3) with a small percentage of persistent HPV infections and CIN3 subsequently developing into cancer. Study of viral persistence and molecular pathways in precancerous lesions is critical for an understanding of cervical carcinogenesis and optimal prevention strategies.

We assessed DNA methylation levels of host and HPV genes within the microenvironment of individual discrete lesions to look for significant associations between HPV genotype, methylation, and lesion severity in cervical surgical tissues and corresponding exfoliated cell specimens.

Methods

354 CIN were macrodissected from surgical specimens provided by 127 women who underwent loop electrosurgical excision procedures (LEEP). Samples were HPV genotyped and DNA methylation of EPB41L3 and viral regions of HPV16L1 and L2, HPV18L2, HPV31L1 and HPV33L2 were measured by quantitative pyrosequencing of bisulfite converted DNA1,2,3.

Results

Adjacent CIN of different grades usually contained the same hrHPV types. However, methylation patterns differed significantly and were much more characteristic of histopathological grade. Methylation levels in all CIN1 were the same regardless of whether a CIN1 was adjacent to CIN3 or was the highest diagnosis on the cervix. There was a significant trend of increased methylation with disease progression from normal and CIN1 to CIN3 (p<0.0001). A popular notion is that cervical carcinogenesis is a continuous progression from CIN1, CIN2, and finally CIN3 to cancer. However, experimental evidence indicates that CIN1 is not necessarily a direct precursor of
CIN3, instead different grade lesions can develop directly in a simultaneous or staggered timeframe. Our results indicate that elevated methylation characteristic of CIN3 seems to be related to a discrete molecular “high methylation” switch from the normal state rather than a gradual secular increase. HPV genotype and methylation results in LEEP biopsies and corresponding exfoliated cells were similar. 99% of CIN3 and 88% of CIN1 had the same or matching HPV types. There was a significant correlation between LEEP and cervical scrapes for both EPB41L3 (Spearman r=0.2033, p=0.0253, n=121, and HPV (Spearman r=0.2156, p=0.0237, n=110).

**Conclusion**

Our study supports the use of DNA methylation testing as a prognostic biomarker of CIN3 and cancer risk and it may be used as a robust triage for hrHPV positive women.

**References**


Diagnostic value of methylation markers in cervical cancer screening

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Background / Objectives

DNA methylation analysis has been assessed as a potential biomarker for early cervical cancer detection. In this review, we summarize the studies analyzing the diagnostic potential of methylation markers in cervical scrapings by Quantitative Methylation Specific PCR (QMSP).

Methods

All studies, until February 1 2017, were systematically searched from three electronic databases (Pubmed/Medline, Embase and Cochrane). Studies on cervical scrapings that used (Q)MSP for methylation analysis and histology as the golden standard were retrieved. Sensitivity and specificity of methylation markers were extracted to assess the diagnostic values of methylation markers. Data were stratified for studies using methylation markers analysis as primary test versus those as triage test after primary HPV screening.

Results

In total 699 studies were retrieved, of which 68 studies describing 89 genes fulfilled our criteria. Preliminary analysis revealed 21 methylation markers as primary test comparing normal/low-grade squamous intraepithelial lesions (LSIL) versus high-grade (H)SIL and cancer. Six genes (including EPB41L3 and JAM3) were identified with relatively high sensitivity (49%-100%) and specificity (67%-100%) to detect HSIL. Eight genes (including EPB41L3 and JAM3) showed high sensitivity (70%-100%) and specificity (78%-100%) for detecting cancer. Methylation analysis as triage test in HPV positive women resulted in 19 genes, of which 11 genes (including EPB41L3 and JAM3) showed combined high sensitivity (53%-100%) and specificity (71%-100%) for HSIL or worse, which was comparable or higher than other triage strategies.

Conclusion
The preliminary results of this review reveal that multiple methylation markers have been analyzed in either primary or triage test. Especially, triaging hrHPV positive women by methylation analysis is interesting for implementation in population-based screening where sensitivity can be even improved by combining markers without losing specificity. However, to confirm the relevance of selected methylation markers, further validation needs to be performed in large population-based settings.
FC 12-06
FAM19A4/MIR124-2 METHYLATION ANALYSIS FOR CERVICAL CANCER SCREENING IN WOMEN LIVING WITH HIV

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Background / Objectives

Women living with HIV (WLHIV) are at increased risk for developing cervical intraepithelial neoplasia (CIN) and cervical cancer. Countries with a high HIV incidence, such as South Africa, often lack effective cervical cancer screening programs due to insufficient resources, lack of infrastructure and limited access to health care. Reliable tests for early cervical cancer and precancer detection that are suitable for this setting are urgently needed.

This study evaluates the performance of FAM19A4/miR124-2 methylation markers (QIAsure Methylation Test) on physician-taken cervical scrapes to detect cervical cancer and CIN grade 3 (CIN3) in WLHIV in South Africa.

Methods

Samples from a prospective observational multi-centre cohort study were used for this analysis. In this study, two cohorts were included: a cohort of WLHIV who were invited for cervical cancer screening (n=321) and a referral cohort consisting of women referred for further evaluation of a cervical abnormality in a gynaecologic outpatient department (n=108). Cervical scrapes collected from all subjects were used for methylation analysis of FAM19A4 and miR124-2 genes by the QIAsure Methylation Test. High-risk HPV (hrHPV) status and histology endpoints were available for all subjects. Methylation levels in samples of HIV seropositive women were compared to samples of HIV seronegative women. Performance of reflex methylation analysis among hrHPV-positive women for detection of CIN3 or worse (CIN3+) was determined in the cohort of WLHIV.

Results

Methylation levels increased with severity of cervical disease in both study cohorts and were above the set threshold in all samples of women with cervical cancer. When compared to samples of HIV seronegative women, methylation levels in samples of WLHIV were significantly higher in all histology groups except cervical cancer. Stratifying hrHPV-positive women with reflex methylation analysis showed a CIN3+ sensitivity of 72.9% and a specificity of 76.1%.
Conclusion

In this South African cohort of WLHIV, reflex methylation analysis of hrHPV-positive cervical scrapes detects all cervical carcinomas and has an acceptable sensitivity and specificity for CIN3+ detection. The applicability of the test on self-collected samples and its objective nature makes it a promising screening tool for low-resource settings.
FC 12-07
HPV DNA METHYLATION AS A BIOMARKER FOR IMPROVING RISK STRATIFICATION AND CLINICAL MANAGEMENT OF HPV-POSITIVE WOMEN

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Background / Objectives

While HPV DNA testing has greater sensitivity compared to cytology, specificity is lacking, and triage tests are required to distinguish benign HPV infections from precancers. A promising triage option is HPV DNA methylation (DNAm). Increased HPV DNAm has been associated with precancer in four major carcinogenic types (HPV16, 18, 31, 45). We hypothesize that DNAm is an important step in carcinogenesis common to all HPV types. To test this hypothesis, we conducted a nested case-control study evaluating the association of HPV DNAm with cervical precancer for 12 carcinogenic HPV types.

Methods

For 12 HPV types, we selected 30 cases of cervical intraepithelial neoplasia grade 3 (CIN3) and 30 controls without abnormalities from a population of HPV-positive women. HPV DNAm in viral L1 and L2 genes (about 9 CpG sites per type) was measured using next-generation bisulfite sequencing. We calculated odds ratios (OR) using logistic regression for the association of DNAm with precancer of DNAm and assessed the possible discrimination between infection and precancer using areas under the curve (AUC). For each HPV type, we calculated specificity at a fixed sensitivity of 85% and weighted back to all HPV-positive women to estimate the risk in methylation positive and methylation negative subjects. These estimates were compared with established management thresholds (colposcopy referral).

Results

We observed significant associations of higher DNAm with precancer in all but 3 sites (OR range 4-28.0). For each HPV type, the highest AUCs were 0.91 (HPV59), 0.86 (HPV18), 0.85 (HPV39), 0.84 (HPV16), 0.82 (HPV45), 0.81 (HPV35), 0.77 (HPV52), 0.74 (HPV58), 0.75 (HPV31), 0.73 (HPV33) and 0.71 (HPV56 and HPV51). At fixed Se of 85%, the Sp for DNAm across HPV types was similar to that of cytology, and ranged from 26.7% (HPV51) to 90.0% (HPV59). Weighting back to all HPV-positive women, the risk of CIN3+ in methylation–positive women was clearly above the colposcopy referral threshold.

Conclusion
We observed a strong association of increased HPV DNAm with precancers across 12 HPV types, suggesting that DNAm is a general phenomenon in the transition from infection to precancer. For most types, clinical performance of DNAm was comparable to or exceeded that of cytology. Next, we will analyze a combined panel of DNAm sites from each HPV type in a large screening population. We plan to develop an assay that provides risk stratifying information based on HPV genotyping and DNAm for the clinical management of HPV-positive women, which can be measured in a variety of specimen types, including self-collected samples, which are not amenable for cytology.
CLINICAL VALIDATION OF POU4F3 METHYLATION AS A NEW BIOMARKER OF CERVICAL PRECANCER AND CANCER IN A TRIAGE OF HRHPV POSITIVE WOMEN

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Background / Objectives

The ongoing TRACE prospective, multicenter study provided a thorough clinical evaluation of the POU4F3 methylation by using the CONFIDENCE Marker™ RUO test in HPV triage by comparison to cytology triage. A new version of POU4F3 methylation test, called CONFIDENCE Marker™ (IVD-CE) was developed with the intended use of triaging hrHPV positive women aged 30 years or older and giving an indication about the women's current CIN2+ risk.

Methods

CONFIDENCE Marker™ (IVD CE) measures the relative methylation level of the promoter region of the gene called POU4F3 by quantitative methylation specific real-time PCR (qMSP) technology compared to the reference gene COL2A1 (providing a so-called Methylation index). Clinical performance of the CONFIDENCE Marker™ (IVD CE) was assessed on hrHPV positive (CONFIDENCE HPV™) LBC samples (CIN2- n=187; CIN2+ n=26) selected from the TRACE study collected from subjects aged 30 years or older. The results of the CONFIDENCE Marker™ (IVD CE) for CIN2+ and CIN3+ clinical endpoints and their agreement with the CONFIDENCE Marker™ RUO test results in the TRACE study was calculated.

Results

The CONFIDENCE Marker™ (IVD CE) achieved sensitivity of 88.5% (69.8-97.6%) with the relative sensitivity of 0.96 (0.77-1.19) and specificity of 69.6% (47.1-86.8%) with the relative specificity of 1.13 (0.79-1.59) for the histologically confirmed samples and 88.5% (69.8-97.6%) with a relative sensitivity of 0.96 (0.77-1.19) and 75.9% (69.2-81.9%) with a relative specificity of 0.99 (0.88-1.11) calculated on all samples, respectively for CIN2+ histological endpoint in the age group 30-65 of hrHPV positive women. The relative values were assessed by comparison to the CONFIDENCE Marker™ RUO test results in the TRACE study. The overall agreement of the two CONFIDENCE Marker™ test workflow results was 96.2% (80.4-99.9%) and 100% for CIN2+ and CIN3+ endpoints, respectively. The current analysis is focused on the baseline cross-sectional clinical results, the 3 years follow-up of the study is ongoing.

Conclusion
Based on the relative sensitivity and specificity values obtained, TRACE’s clinical evaluation of the CONFIDENCE Marker™ RUO can be considered valid for the CONFIDENCE Marker™ (IVD CE) as the results do not show a significant statistical difference.

On the basis of our findings, one of the first IVD-CE validated methylation assay, the new CONFIDENCE Marker™ (IVD CE) detecting the POU4F3 methylation as a triage test of hrHPV positives appears to be a promising method. We can reasonably assume that its quantitative nature offers the potential of an objective and discriminative risk assessment tool in the prevention and diagnostics of high-grade CIN lesions and cervical cancer.
ASSOCIATIONS OF EPB41L3 DNA METHYLATION WITH CERVICAL INTRAEPITHELIAL NEOPLASIA IN WOMEN LIVING WITH HIV-1 IN BURKINA FASO AND SOUTH AFRICA

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Background / Objectives

To evaluate the association of DNA methylation of the human gene EPB41L3 with high-grade cervical intraepithelial neoplasia (CIN2+) and HIV-related factors among women living with HIV-1 (WLHIV) in Burkina Faso (BF) and South Africa (SA).

Methods

Case-control study of WLHIV aged 25-50 with histology-determined CIN2+ (cases, N=152) or without lesions (≤CIN1, controls, N=210). Methylation levels of EPB41L3 were measured by pyrosequencing of exfoliated cervical specimens. Among 185 controls that were followed over a median 16 months (endline), methylation levels were measured among 26 incident CIN2/3 and 159 controls that remained ≤CIN1 at endline. Methylation levels were dichotomized using the 66.7 percentile among controls in each country for high/low cut-off.

Results

The median methylation levels for EBP41L3 were significantly higher among women with prevalent CIN2/3 compared to those with ≤CIN1 in both countries (Cuzick p for trend by CIN grade <0.001). Women with CD4+ count ≤200 cells/mm3 were more likely to have higher levels of EPB41L3 methylation compared to women with CD4+ >350 cells/mm3 at baseline (BF: adjusted Odds Ratio [aOR]=7.45, 95%CI 1.53-36.22; SA: aOR=2.74, 95%CI 1.16-6.47; adjusted for HR-HPV and CIN status). Among 36 women with prevalent CIN2+ at baseline who had not gone for treatment by endline visit in SA, women with persistent CIN3, or CIN2 which progressed to CIN3 had higher baseline EPB41L3 median methylation levels.
compared to women who spontaneously regressed to ≤CIN1 (Mann-Whitney p=0.016).

Conclusion

Methylation of human gene EPB41L3 DNA is elevated in prevalent CIN2/3, and incident and persistent CIN3 cases, and independently associated with lower CD4 count. DNA methylation based assays could be useful in settings with limited resources for management, by identifying women most likely to have CIN3 that will persist or progress.
Cervical cancer detection by DNA methylation analysis in urine


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Background / Objectives

Cervical screening programs using cervical cytology, are or will be replaced by primary hrHPV testing in several countries. Women who test hrHPV-positive require a secondary (triage) test to prevent over-referral and over-treatment. Analysis of DNA methylation of host cell tumor suppressor genes in cervical scrapes provides promising triage strategy for hrHPV-positive women. Urine collection is expected to increase the uptake of cervical screening programs, and hrHPV testing in urine appears promising. We aimed to test whether DNA methylation analysis in urine provides a novel more patient-friendly strategy to detect cervical cancer.

Methods

Cervical scrapes and urine samples from cervical cancer patients and healthy female controls (n=40 each) were tested for hrHPV DNA presence and DNA methylation of 6 genes with known performance of cervical (pre)cancer detection in cervical scrapes.

Results

A high concordance was found between hrHPV DNA testing on cervical scrapes and urine samples. Also DNA methylation levels in paired cervical scrapes and urine samples showed a good correlation. DNA methylation levels of all 6 genes were significantly increased in urine samples of cervical cancer patients compared to controls. Receiver operating characteristics (ROC) analysis of the 6 methylation markers in urine showed AUCs ranging from 0.88 to 0.95.

Conclusion

DNA methylation testing in urine is feasible and has a high accuracy to detect cervical cancer. These data warrant further exploration of methylation markers in urine-based cervical screening programs.
GYNTECT®, A DNA METHYLATION MARKER PANEL-BASED DIAGNOSTIC TEST SHOWS VERY HIGH SPECIFICITY IN THE TRIAGE OF CERVICAL CANCER SCREENING SAMPLES

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Background / Objectives

A change of the current screening algorithms to a HPV-based screening setting is discussed in several countries due to higher sensitivity of HPV testing compared to cytology. Reliable triage methods are, however, essential in such a setting to avoid overtreatment and higher screening costs. Specific DNA methylation patterns may provide a suitable tool especially with regard to keeping false positive rates low.

Methods

Cervical scrapes collected in PreservCyt® from women with cervical cancer (5 cases), CIN 1-3 (74 cases) and normal cytology (Pap I; 200 cases) were assessed for methylation of the marker regions ASTN1, DLX1, ITGA4, RXFP3, SOX17, and ZNF671 (GynTect® assay). All samples had previously been tested for HPV by the cobas® HPV assay. Moreover, for all patients with CIN or cancer and for 59 of 200 patients with Pap I data for p16/Ki67 dual staining (CINtec Plus® test) were available.

Results

All samples from women with cervical cancer, 61.2% of CIN3, 44.4% of CIN2 and 20.0% of CIN1 cases were scored positive for the GynTect methylation assay. The specificity within the Pap I group was 98.5%, thus showing an exceptionally low false-positive rate. Overall, the number of methylated marker regions increased proportionally to lesion severity, which is in contrast to CINtec Plus® and cobas® HPV, of which both detect all CINs irrespective of severity grade. Specificity of CINtec Plus in the Pap I group was similar, even though the tested cohort was smaller. We plan to have CINtec Plus results for all 200 Pap I samples before the Eurogin conference. Specificity of the cobas HPV in the Pap I group was 92%.

Conclusion

DNA methylation analysis of the above marker panel (GynTect® test) in cervical scrapes consistently detects cervical cancer and the majority of CIN3 as well as a subset of CIN1/2 lesions, whereas the positivity rate among cytology-normal samples is extraordinarily low. Altogether, the GynTect® assay based on detection of six
methylation markers may provide an excellent tool for triage within cervical cancer screening.
FC 12-12
BETA-GLOBIN CYCLE THRESHOLD VALUE AS A PREDICTOR OF SUFFICIENT DNA YIELD FOR HPV METHYLATION ANALYSIS

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Background / Objectives

Since several countries adopted HPV testing as a primary screening method for cervical cancer, different triage strategies for high-risk HPV positive women are being evaluated in order to avoid misinterpretation and mismanagement of patients. Reflex cytology, partial HPV genotyping and host and viral methylation are the most commonly evaluated strategies to date. The aim of our study was to assess whether there is a correlation between the beta-globin cycle threshold (Ct) value and the concentration of extracted DNA in order to predict sufficient yield of DNA for methylation.

Methods

For the purpose of this study, we have evaluated 195 samples that were tested with clinically validated HPV test Abbott RealTime High Risk HPV test (RealTime; Abbott, Wiesbaden, Germany) at the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia between January 2016 and March 2016. The internal control of the RealTime is based on amplification of the 136-bp region of the beta-globin gene. Furthermore, DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and DNA concentration was measured with Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, USA). Samples with DNA concentration above 2.5 ng/ul were considered eligible for further HPV methylation analysis.

Results

Ct values for beta-globin ranged between 20.26 to 31.25, with an average Ct value of 24.57. Of the 195 samples tested, 92 had DNA concentration above 2.5 ng/μL, 98 samples below 2.5 ng/μL and 5 below measurable concentration (range: 0.144-40,0 ng/ul). Our analysis showed that 93.8% of the samples with Ct values below 22.00 (15/16), 87.7% of the samples with Ct values between 22.00 and 24.00 (57/65) and 29.0% of the samples with Ct values between 24.00 and 26.00 (18/62) had sufficient DNA yield for methylation, respectively. Additionally, only two out of 52 samples with the Ct value above 26.00 had a concentration above the cut-off value (one sample with Ct value 26.15 had DNA concentration 3.42 ng/ul and one sample with Ct value 26.28 had DNA concentration 4.28 ng/ul).

Conclusion
In HPV primary screening settings a well-balanced triage strategy will be needed to identify women with higher risk of underlying cervical intraepithelial neoplasia grade 2 or worse. Our results suggest that Ct values of beta-globin of clinically validated HPV test RealTime can be used as a preliminary indicator for sufficient DNA concentration needed for methylation analysis.
LONGITUDINAL PERFORMANCE OF HPV 16 METHYLATION PREDICTING CERVICAL PRECANCER AND CANCER: A 10-YEAR COHORT STUDY IN CHINA


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Background / Objectives

To evaluate the predictive ability of methylation of the most common human papillomavirus(HPV16) L1 region and Long Control Region(LCR) for cervical precancer and cancer. And to assess the correlation of methylation between different CpG sites, as well as between methylation and HPV viral load.

Methods

A hospital-based case-control study consisting 27 HPV16 single positive women was designed to identify the significant CpG sites. Then 10-year population-based screening cohort conducted in cervical cancer high-incidence area (Shanxi province) was used to valid the identified CpG sites. The cohort included 1742 women followed up in 2005, 2010 and 2014 using HPV testing and cytology. Women with any positive screening result received colposcopic examination and biopsy if necessary. Based on the combination of biopsy result at 3 follow-up visits, women were classified into CIN2+ persistence/progression group and regression group to assess the longitudinal performance of previously identified CpG sites. DNA extracted from cervical cytology specimens was quantified for methylation levels at 35 CpG sites throughout HPV16 L1 and LCR. The Mann-Whitney U test was used to compare the methylation pattern between different biopsy results. And spearman-test was used to assess the relation between different CpG sites and between HPV viral load and methylation.

Results

HPV 16 methylation increased with the grade of cervical precancer(p<0.001). The median methylation level for CIN1/normal, CIN2, CIN3+ was 11.16%, 11.54% and 23.19%, respectively. Specially, methylation of 14 CpG sites (L1:5602、5608、5611、5617、5709、5726、6367、6389、6457、6650、7034、LCR:7455、7535、7553) was significant higher among women with CIN3+ than <CIN3. Considering literature review, another 10 CpG sites were also evaluated during follow-up. 77 women diagnosed with CIN2+ were HPV16 positive in 2005. After 5 years, the methylation of 6650 was significantly higher among CIN2+ persistence/progression group, another CpG(nucleotide position 31) also showed the predictive ability for CIN2+ after 10 years. Correlation was found between many different CpG sites, especially, the correlation coefficient between 6367, 6389 and 52 were both more
than 0.7. No strong relationship was identified between HPV viral load and methylation level stratified by biopsy result. The max correlation coefficient was no more than 0.4.

Conclusion

HPV 16 methylation of L1 and LCR can be used as biomarker for cervical precancer and cancer. The methylation of 6650 in L1 showed promising predictive ability for the progression of cervical precancer. The correlation was identified between different CpG sites, but HPV viral load was not related to methylation level.
DEVELOPMENT OF A NEW HIGHLY ACCURATE DNA METHYLATION CLASSIFIER FOR PREVALENT AND INCIDENT CERVICAL PRECANCER


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Background / Objectives

Cervical cancer, caused by persistent infection with high risk (hr) HPV affects ~500,000 women globally and causes ~260,000 deaths annually. Although HPV immunisation has been successfully implemented it will take decades to see an effect of the new nonavalent vaccine. Highly sensitive hrHPV testing is likely to become the dominant primary screen. However, hrHPV infection is common and only a fraction of women are at risk of developing cancer. 40% of hrHPV+ women are cytology negative and triage by proposed adjunctive tests such as p16 (in conjunction with ki67) are insufficient. A molecular test is needed to identify clinically significant HPV infection.

We aim to identify and validate novel DNAm biomarkers which, in combination with our existing DNAm classifier, will aid development of a new improved classifier for identification of high grade CIN and ultimately improve the current cervical cancer screening gold standard. To achieve this, we propose to measure DNAm of predefined sites in HPV16, 18, 31 and 33 in a set of 350 hrHPV+ women with normal cytology. In addition we will measure genome scale human DNAm with RRBS (Reduced representation bisulfide sequencing) followed by machine learning using MS-SPCA to identify ~100 sites which consistently appear in the best ranking models. Then, the best sites will be further sifted by additional multivariate data modelling to provide us with a minimum number of required classifier sites. Finally, these selected sites will be validated in a second set of 200 well characterised cervical samples.

Methods

DNA was purified from frozen LBC samples for methylation testing using Qiagen DNA extraction kits following standardized methods. The RRBS method was designed to obtain 30 million reads per sample using 50bp SE reads corresponding to 20X depth coverage of each CpG. All specimens used in the project from the ARTISTIC and Scottish HPV Archive will be tested for HPV DNAm by pyrosequencing. The HPV sites tested are selected according to established knowledge about their role and importance.

Results
The preliminary results indicate that this approach significantly improved risk classification tool to triage hrHPV+ women to colposcopy. The new classifier is expected to come close to the high sensitivity of current hrHPV tests (90-95%) but deliver a substantially higher specificity and PPV (both ~70%) than current molecular reflex tests (30-40% and 40-50% respectively).

Conclusion

This new algorithm would allow more efficient utilization of colposcopy services while hrHPV+ women negative for the triage classifier could be followed up at suitably frequent intervals to safely catch most, if not all, triage false negatives.

References

Background / Objectives

Background: Alterations in the host cellular immune response allow persistent infections with High-Risk Human Papillomavirus (HR-HPV) and development of premalignant cervical lesions and cervical cancer (CC). Variations of immunosuppressive cytokine levels in cervix are associated with the natural history of CC. Objectives: To assess the potential role of genetic host immunity and cytokines serum levels in the risk of developing CC, we conducted a case–control study paired by age.

Methods

Methods: Peripheral blood samples from patients with CC (n = 200) and hospital controls (n = 200), were used to evaluate nine biallelic SNPs of six cytokine genes of the adaptive immune system by allelic discrimination and cytokines serum levels by ELISA.

Results

Results: After analyzing the SNP association by multivariate logistic regression adjusted by age, CC history and smoking history, three Th2 cytokines (IL-4, IL-6 and IL-10) and one Th3 (TGFB1) cytokine were significantly associated with CC. Individuals with at least one copy of the following risk alleles: T of SNP (−590C > T IL-4), C of SNP (−573G > C IL-6), A of SNP (−592C > A IL-10), T of SNP (−819C > T IL-10) and T of SNP (−509C > T TGFB1), had an adjusted odds ratio (OR) of 2.08 (95% CI 1.475–2.934, p = 0.0001), an OR of 1.70 (95% CI 1.208–2.404, p = 0.002), an OR of 1.87 (95% CI 1.332–2.630, p = 0.0001), an OR of 1.67 (95% CI 1.192–2.353,
p = 0.003) and an OR of 1.91 (95 % CI 1.354–2.701, p = 0.0001), respectively, for CC. The burden of carrying two or more of these risk alleles was found to have an additive effect on the risk of CC (p trend = 0.0001). Finally, the serum levels of Th2 and Th3 cytokines were higher in CC cases than the controls; whereas IFNG levels, a Th1 cytokine, were higher in controls than CC cases.

Conclusion

Conclusion: The significant associations of five SNPs with CC indicate that these polymorphisms are potential candidates for predicting the risk of development of CC, representing a risk allelic load for CC and can be used as a biomarker of susceptibility to this disease1.

References

FC 13-01
COMPRESSED COMPARTMENTAL MULTI-TYPE HPV MODELS – CAN THEY BE USED TO INFORM CERVICAL CANCER SCREENING?

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Background / Objectives

Numerous options exist for cervical cancer prevention with new screening and vaccination modalities. Microsimulation models are used to investigate these options and predict the cost-effectiveness of integrated strategies but are computationally intensive. Besides, because the outcomes of micro-simulation models are not available in closed form, the execution of probabilistic sensitivity analyses, or parameter estimation, by Bayesian procedures remains challenging. Compartmental models may also be considered. They are fast and provide numerical closed form solutions, but the number of compartments rapidly increases with the number of HPV types and complexity of the screening algorithm.

Methods

We developed a compartmental mathematical progression model that integrates natural history of cervical carcinogenesis along multiple HPV types and screening interventions. The full model was compressed to achieve executable and efficient model, even when dealing with complex screening. The model outcomes are available in closed form so that the parameter uncertainty can be assessed by Bayesian procedures. The models were compared to a microsimulation approach in terms of overall and type-specific HPV prevalence of intermediate stages, screening outcomes and cancer incidence.

Results

The outcomes of the compressed compartmental model were stable over different levels of compression and stayed between the simulation error bounds of the microsimulation model for all overall HPV infection states. A small difference was observed in a fraction susceptible versus immune.

Conclusion

Good approximation properties of compressed compartmental models enable us to assess uncertainties surrounding the natural history of cervical carcinogenesis and screening decisions in a computationally undemanding way.
The cost-effectiveness of national HPV immunization programmes in six European tender-based settings

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Background / Objectives

Vaccination of preadolescent girls against HPV 16/18 within national immunization programmes has been established in most European countries, but coverage varies substantially between countries. In addition, HPV vaccines in many countries are purchased via national public procurements that reduce the cost per vaccinee considerably. This study sought to evaluate the cost-effectiveness profile of gender-neutral national HPV immunization programmes in settings with organized tender procedures for the acquisition of the HPV vaccine.

Methods

A previously published Bayesian synthesis framework was expanded to account for the full spectrum of the HPV-related cancers in both males and females and for all herd immunity effects from vaccinating girls as well as boys. Our analysis assessed the cost-effectiveness of a sex-neutral vaccination programme within 6 European countries (Austria, Belgium, Croatia, Latvia, the Netherlands and Sweden) for which we collected publicly available information on national tender procedures. Country and site-specific incidence data from the last edition of the Cancer Incidence in Five Continents (CI5) were used to inform the model and national mortality data were obtained from the World Health Organization mortality database.

Results

The incremental cost-effectiveness ratios (ICERs) of girls-only vaccination compared to no vaccination ranged from €500 (95% Crl: 0 - 1,000) per life-year gained in Latvia to €5,000 (95% Crl: 4,000 - 6,000) per life-year gained in Austria, while the incremental cost-effectiveness ratios of the sex-neutral vaccination programmes compared to girls-only vaccination ranged from €4,000 (95% Crl: 3,000 - 6,000) per life-year gained in Croatia to €26,000 (95% Crl: 20,000 - 33,000) per life-year gained in Sweden. The ICERs remained below the country-specific GDP thresholds for cost-effective intervention, recommended by the WHO. Ninety-five percent of the variation in ICERs for sex-neutral vaccination among countries could be explained by coverage among girls, vaccination cost, cervical cancer incidence and survival, and oropharyngeal cancer incidence in males.

Conclusion
Gender-neutral vaccination against HPV is likely to be cost-effective in settings where tender procedures can be organized for the acquisition of the HPV vaccine in national immunization programmes. This finding seems generalizable over a wide range of epidemiologic and economic constraints.
Cost-effectiveness of expanding the HPV vaccination program to include preadolescent boys in Sweden

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Background / Objectives

Since 2012, vaccinating against human papillomavirus (HPV) in preadolescent girls in schools has been a part of the national vaccination programme for children in Sweden. HPV vaccination coverage among girls has since been around 80%. The main goal with the introduction of the vaccine was to protect girls from infection with high-risk types of HPV that may cause cervical cancer. The vaccine also prevent other cancer types of which some are prevalent among men, such as oropharyngeal, anal, and penile cancer. The aim of this study was to assess the cost-effectiveness of including HPV-vaccination for preadolescent boys in the Swedish national immunization program by comparing health effects and costs of all HPV-related disease in a situation with a gender neutral vaccination programme compared to only vaccinating girls.

Methods

We used a dynamic compartmental model to simulate the transmission of HPV 16/18 in the population, accounting for indirect effects of vaccination through herd immunity. The model accounted for sexual behaviour, such as age preferences of sexual contacts and men who have sex with men. The main outcome was number of individuals with all HPV-related cancers as well as CIN. The data in the model were based on epidemiological studies, demographic statistics, cancer registers and other Swedish population-based healthcare and sociodemographic registers that capture all healthcare interactions. Estimates were calibrated to fit Swedish empirical data.

Costs included in the analysis were those incurred when treating HPV-related cancer and CIN, production losses during sick-leave, and acquisition and administration of the vaccine. Health effects were measured as quality-adjusted life years (QALY). The time horizon was set at hundred years, and both effects and costs were discounted with 3% annually. All health effects and costs were accumulated over the time horizon and used to create the incremental cost-effectiveness ratio (ICER). Several variables, such as price of the vaccine, vaccination coverage, vaccine effectiveness, and herd immunity were varied in sensitivity analyses to illustrate their impact on the results from the cost-effectiveness analysis.

Conclusion
Preliminary results indicate that a gender neutral vaccination programme will reduce HPV-related cancer and CIN during the model's time horizon, both due to direct effects of the vaccine as well as indirect effects decreasing HPV prevalence in the population. The potential cost-effectiveness of a gender neutral programme is dependent on the price of the vaccine, the lower the price the more favourable it is to vaccinate boys from a societal perspective.
OPTIMAL IMPROVEMENTS TO CERVICAL CANCER PREVENTION: EXAMPLE FROM AUSTRALIA


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Background / Objectives

There are now a variety of tools for cervical cancer prevention. HPV vaccination and screening programs are in place in several settings, however coverage is often sub-optimal. Improving participation is important, but improving vaccine uptake will involve different approaches to those required to increase screening participation or adherence to recommended follow-up. To assist in prioritizing and resourcing efforts, here we explore the impact of different potential improvements to participation in cervical cancer prevention programs, and which improvements would have the greatest impact.

Methods

Using a well-established model of HPV transmission, vaccination, natural history and screening, we assessed the relative impact of several improvements to HPV vaccination and screening participation, using Australia as an example. These improvements included: i) increasing HPV vaccine coverage in females (from current 78%); ii) increasing HPV vaccine coverage in females and males (current male coverage 72%); iii) reducing the proportion of women never screened; iv) increasing screening participation at the recommended interval; v) improving attendance for follow-up by women under surveillance following a previous abnormal screening test. The impact of improvements in screening participation were assessed separately for cohorts offered vaccination and for unvaccinated cohorts.

Conclusion

The findings of this analysis will provide important information about how to prioritise efforts in increasing participation in cervical cancer prevention programs.
BACKGROUND / OBJECTIVES

To assess the public health and economic impact of a gender neutral vaccination program for 9-14 year olds with a nine-valent human papillomavirus (HPV) in Sweden.

METHODS

A previously validated transmission dynamic model was adapted and calibrated for Sweden. The natural history of cervical cancer, CIN 1-3, vaginal cancer, vulvar cancer, anal cancer, and genital warts, was simulated in the model. The current screening program for cervical cancer in Sweden was included in the model. In the model a gender neutral vaccination program (boys and girls) for 9-14 year olds with a nine-valent HPV vaccine was compared to a girls only (9-14 year old) quadrivalent HPV vaccination program. The vaccination coverage and other inputs to the model were collected from relevant local sources where available. Life-long duration of protection was assumed in the model for vaccine HPV types for both vaccines and the model time horizon was set to 100 years. Costs and QALYs were discounted by 3%. Sensitivity analyses were performed on comparisons with adherence rates and discounting.

RESULTS

The gender neutral vaccination program resulted in an added reduction of 22% in cervical cancer incidence and mortality for females, and 13% additional decrease in anal cancer incidence and 12% mortality in females and 32% decrease in incidence and mortality in males. An additional 110,148 cases genital warts in girls and 466,960 cases in boys would be prevented if a gender neutral vaccination with a nine-valent vaccine would be introduced compared to the current scenario in Sweden. Gender neutral vaccination with a nine-valent HPV vaccine as compared to the quadrivalent vaccine resulted in an additional overall 14.5% decrease in disease specific costs.

CONCLUSION

The burden and costs related to various 6/11/16/18/31/33/45/52/58 HPV-related conditions, especially cervical and anal cancers, could be substantially reduced by the introduction of a nine-valent HPV vaccination program for females and males in Sweden.
NEW EVIDENCE WITH REGARD TO TEST CHARACTERISTICS FROM A MODELLING STUDY

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Background / Objectives

The natural history of cervical cancer is only partly known, as most precancerous lesions are either treated or regress naturally prior to becoming clinical. Also, data on test characteristics is lacking because randomized controlled trials are never performed. However, microsimulation models allow us to mimic the natural history of cervical cancer. Calibrating unknown parameters in these models, such as durations of disease states, test characteristics and demographic assumptions, to observed data provides us with more insight into unobservable processes. Recently, large cohort studies have been added to this observed data providing new possibilities for analyses. In this study, we explored what could be learned from a microsimulation model about test characteristics when screening for cervical cancer.

Methods

The established MISCAN-Cervix microsimulation model was calibrated to the Dutch setting based on the latest data from the Netherlands Cancer Registry (NCR), the nationwide network and registry of histological and cytopathology in the Netherlands (PALGA) and recently published cohort studies on HPV detection rates. To identify any differences in HPV-test sensitivity by disease grade, sensitivity was calibrated for each disease grade separately (i.e. cervical intraepithelial neoplasia (CIN)1, CIN2, CIN3 and cervical cancer). To test if false-negative tests were more likely to be attributable to the same (hard to reach) lesions, we allowed for the chance of systematically missing individual lesions with cytology testing in the model.

Results

We found that the model fitted the observed data best when the sensitivity of HPV tests increases by disease grade. Especially the sensitivity for CIN1 was considerably lower than for higher CIN grades. For cytology testing, the fit of the model was best if we allowed for systematically missing about 11% of individual lesions.

Conclusion

The model was much better able to fit the observed data when adjusting HPV test sensitivity by disease grade and systematically missing a percentage of lesions at cytology testing. A possible explanation for an increasing sensitivity of HPV-tests by disease grade is that the infection persists for a longer period of time, was able to spread more, and therefore more easily detected. Systematically missing lesions at
Cytology testing is probably caused by the fact that some lesions are harder to reach with the cervical smear brush or spatula, such as adenocarcinoma. These findings could have clinical implications for screening practices as the screening guidelines depend heavily on test characteristics.
HEALTH AND ECONOMIC IMPACT OF HPV TESTING COMPARED TO CYTOLOGY: WHAT IS THE OPTIMAL PRIMARY CERVICAL CANCER SCREENING STRATEGY FOR CANADA?

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Background / Objectives

To examine the incremental effectiveness and cost-effectiveness of switching from cytology-based routine screening to primary HPV testing in Quebec (Canada), assuming 9-valent HPV vaccination.

Methods

We used HPV-ADVISE, an individual-based transmission-dynamic model of 18 HPV types and related diseases calibrated to Canadian-specific data. We compared cytology-based screening vs. switching to primary HPV-testing (Cobas 4800 test & triage of HPV-positive women by cytology) in 2018. For vaccination impact predictions, we modelled Quebec’s vaccination program: vaccination coverage=80%, start of vaccination=2008, gender-neutral 9-valent vaccination. For our base-case Cytology screening scenario, we used Quebec’s proportion of women who ever had a cytology test, and current age distribution of first screening and adherence to screening intervals. For HPV testing scenarios, we varied age at start of screening and screening intervals. We used a health care provider perspective, 3% discount rate, 2018-2050 time horizon, and $40,000/QALY-gained cost-effectiveness threshold. Predictions are annual averages for a population of 10 million.

Results

In Quebec, switching to HPV screening was predicted to result in substantial cost savings vs. Cytology screening (savings of $27-38 million/year on average), under scenarios where age at start of screening was 25-35 years old, and intervals between tests were between 5-10 years. However, the only HPV screening strategy investigated with equal or lower rates of cervical cancer was when assuming age at start is 25 years and interval between tests is 5 years. Older age at start or wider intervals led to a predicted increase in cervical cancer cases. In terms of cost-
effectiveness, HPV screening every 10 years initiated at age 30 years was predicted to be the optimal scenario. Although this scenario could lead to a small increase in cervical cancers, it would also result in a substantial decrease in false positives that are referred to colposcopy. Consequently, the model predicts that the gains in Quality-Adjusted Life-Years (QALY) related to screening outcomes outweigh the QALY loss related to the increase in cancer cases.

Conclusion

In Quebec, switching from Cytology to HPV screening is predicted to produce substantial cost savings and important reductions in false positive rates, if there is good adherence to the recommended screening intervals. However, the only scenario predicted to decrease cervical cancer rates is 5-yearly HPV screening initiated at age 25 years. Finally, HPV-screening every 10 years initiated at age 30 is likely the most cost-effective scenario, although it could lead to a slight increase in the number cervical cancers.
HEALTH-RELATED QUALITY OF LIFE IN THE PREVENTION, SCREENING AND MANAGEMENT OF CERVICAL DISEASE: A SYSTEMATIC REVIEW

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Background / Objectives

Cost-effectiveness analyses (CEAs) of interventions to prevent cervical cancer require estimates of the health-related quality of life (HRQoL) effects of screen tests and subsequent treatment. The results of such simulation analyses can be highly sensitive to the HRQoL weights employed. Accordingly, accurate assessment of HRQoL is essential for the generation of reliable CEA estimates. We reviewed the literature regarding HRQoL in cervical cancer prevention and management in order to appraise the current evidence regarding this important input to CEA.

Methods

We searched the MEDLINE, Scopus and EconLit databases for studies that estimated HRQoL in cervical cancer prevention and management published January 1995-December 2015. The primary inclusion criterion was for studies that assess HRQoL using the EQ-5D instrument. Data were abstracted from eligible studies on setting, elicitation group, sample size, elicitation instruments, health state valuations, study design and follow-up. We assessed the quality and comparability of the studies with a particular focus on the HRQoL reported across states and groups.

Results

Fifteen papers met the inclusion criteria. Most used patient elicitation groups (n=11), 2 used the general public and 2 used a mix of both. Eight studies were cross-sectional and seven were longitudinal in design. Six studies used both the EQ-5D-3L and the EQ-VAS together with other measures of overall HRQoL or condition-specific instruments. Studies employing both the EQ-5D and specific measures of anxiety found that the EQ-5D tended to be insensitive for differences in anxiety scores detected by alternative instruments. Extensive heterogeneity was observed across study characteristics.

Conclusion

Our results reveal the challenges of sourcing reliable estimates of HRQoL for use in CEAs of cervical cancer prevention. The EQ-5D appears insufficiently sensitive for some health states. Research will be required to determine if the adoption of the more recent five-level EQ-5D-5L enhances sensitivity over the three-level EQ-5D-3L employed in the literature reviewed here. Another, more general problem is the
paucity of HRQoL estimates for many health states and their change over time. There is scope for more detailed longitudinal analysis of the HRQoL burden of cervical cancer related healthcare interventions.
FC 13-09
CLINICAL & COST-EFFECTIVENESS OF HPV PRIMARY SCREENING & DUAL-STAIN CYTOLOGY IN THAILAND

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Background / Objectives

In Thailand, cervical cancer is a leading cause of death among women. The Ministry of Public Health is focused on reducing cervical cancer with national screening efforts, one of which has increased access to pap tests. The burden of cervical cancer remains high, however. This study seeks to evaluate the clinical and cost-effectiveness of HPV DNA primary screening as compared to pap primary algorithms, from a payor perspective, with several triage strategies, including p16/Ki-67 dual-stain cytology and colposcopy.

Methods

A Markov model was used to compare 4 strategies for women ages 30-65 (per country recommendations), across a 100-yr horizon, assuming a 5-yr primary screening interval for the strategies evaluated: 1) HPV DNA primary testing every 5 yrs with pooled high-risk positive results sent to colposcopy; 2) HPV primary testing with pooled high-risk positive results triaged with p16/Ki-67 dual-stain cytology; 3) pap primary with ≥ASCUS results to colposcopy; 4) pap primary with ≥ASCUS results triaged with p16/Ki-67 dual-stain cytology. Values for HPV DNA testing and pap screening sensitivity and specificity were obtained from the Addressing THE Need for Advanced HPV Diagnostics (ATHENA) trial. Dual-stain cytology data are from the Primary ASCUS LSIL Marker Study (PALMS) and ATHENA trials. Costs were derived from a national tertiary care hospital in Bangkok. Costs and quality adjusted life-years (QALYs) were discounted at 3.5% annually. Sensitivity analyses were conducted to assess impact of cost and clinical inputs on incremental cost-effectiveness ratios and outcomes.

Conclusion

HPV DNA primary screening with colposcopy triage offers comparable cost-effectiveness to HPV DNA primary screening with dual-stain cytology triage. For pap primary testing, colposcopy triage was dominant to dual-stain triage due to the low cost of colposcopy and pap in Thailand. All HPV strategies were cost-effective ($20,000 US threshold), but in Thailand, the convention is to use a GDP threshold closer to $6000, thus testing more than every 5 yrs, though cost-effective, may not be acceptable. Because HPV primary testing offers greater sensitivity than pap, such
strategies may provide greater value when longer screening intervals cannot be avoided. Notably, HPV DNA primary testing with dual-stain cytology triage represents the optimal strategy to reduce cervical cancer, though this would require investment in tests and triage. All HPV primary strategies modeled allow for early detection of cervical precancer and cancer, reduction in mortality, and lower treatment costs. These factors will grow even more important as Thailand works to implement a sustainable national screening program.
Cost analysis of Human Papillomavirus related cervical diseases and genital warts in Swaziland

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Background / Objectives

Human papillomavirus (HPV) has proven to be the cause of several severe clinical conditions on the cervix, vulva, vagina, anus, oropharynx and penis. Several studies have assessed the costs of cervical lesions, cervical cancer (CC), and genital warts. However, few have been done in Africa and none in Swaziland. Cost analysis is critical in providing useful information for economic evaluations to guide policymakers concerned with the allocation of resources in order to reduce the disease burden.

Methods

A prevalence-based cost of illness (COI) methodology was used to investigate the economic burden of HPV-related diseases. We used a top-down approach for the cost associated with hospital care and a bottom-up approach to estimate the cost associated with outpatient and primary care. The current study was conducted from a provider perspective since the state bears the majority of the costs of screening and treatment in Swaziland. All identifiable direct medical costs were considered for cervical lesions, cervical cancer and genital warts, which were primary diagnoses during 2015. A mix of bottom up micro-costing ingredients approach and top-down approaches was used to collect data on costs. All costs were computed at the price level of 2015 and converted to dollars ($).

Results

The total annual estimated direct medical cost associated with screening, managing and treating cervical lesions, CC and genital warts in Swaziland was $16 million. The largest cost in the analysis was estimated for treatment of high-grade cervical lesions and cervical cancer representing 80% of the total cost ($12.6 million). Costs for screening only represented 5% of the total cost ($0.9 million). Treatment of genital warts represented 6% of the total cost ($1 million).

Conclusion

According to the cost estimations in this study, the economic burden of HPV-related cervical diseases and genital warts represents a major public health issue in Swaziland. Prevention of HPV infection with a national HPV immunization programme for pre-adolescent girls would prevent the majority of CC related deaths and associated costs.
References


FC 14-01
POSITIVE SOCIAL MEDIA CAMPAIGN EFFECT ON YOUNG WOMEN’S ATTENDANCE RATE TO CERVICAL CANCER SCREENING IN NORWAY

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Background / Objectives

Norway has an organized national screening program against cervical cancer, where all women 25-69 years are recommended to take a screening test every third year. The overall coverage, and particularly among the youngest women, aged 25 to 29, is unsatisfactory. When the program started in 1995, the attendance in this age group was at its peak, at 73,0 percent. The coverage rate has decreased continuously since, to an all-time low in 2012, at 52,7 percent. By connecting different actors concerned with the low coverage, the idea was to create a yearly campaign using different media channels, but with particular emphasize on social media networks. Involved in the campaign were: The Norwegian Cancer society, Det Nye, a glossy magazine for young women and The Norwegian Cancer Registry.

Methods

A national campaign #sjekkdeg (“get checked”) was launched in September 2015 after a young blogger diagnosed with cervical cancer started blogging about her disease in March 2015. The campaign focused on young women and included: short films featuring young, female Norwegian celebrities, editorial pieces in the magazine Det Nye, press coverage in other mainstream media, blog and social media activity with the hashtag #sjekkdeg. The campaign was followed up with a new campaign September 2016. The number of registered screening tests, and 3.5 year screening coverage by age, were calculated from the national screening databases at the Cancer Registry of Norway.

Results

By the end of 2016 the number of registered screening tests among women in the age group 25 to 29 increased by 10 886. The 3.5 year screening coverage, increased from 55,9 percent in 2014 to of 62,1 percent at the end of 2016. There was also an increase in 34 109 women attending screening in the whole screening population between 25-69 years from 66,5 to 68,8 percent.

Conclusion

The raised awareness on cervical cancer in Norway has contributed to increased attendance to the screening program. It is reasonable to think that a large proportion of this increase can be attributed to the #sjekkdeg-campaign. This campaign
indicates that unconventional thinking can be useful, and that employing new media channels that reaches the target audience directly can affect the screening coverage.

The results from the two years of the campaign has ensured the parties in the collaboration that the work should continue, with new campaign periods.
A NATIONAL SURVEY OF CANADIANS ON HPV: COMPARING KNOWLEDGE, BARRIERS AND PREVENTIVE PRACTICES OF PHYSICIANS TO THOSE OF CONSUMERS

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Background / Objectives

This Canadian survey of physicians and consumers aimed to explore similarities and differences in knowledge, barriers and preventive practices about HPV between the two groups.

Methods

General Practitioners (GP) (n=337) and Obstetrician Gynaecologists (OB/GYN) (n=81), vaccinated women (VW) (n=337) and unvaccinated women (UW) (n=802) aged 18-45, and men (M) (n=200) aged 18-26 were surveyed in May and June 2016 using an online questionnaire. A probability sample of the same size would yield a margin of error of +/- 4.8% for physicians and +/-2.7% for consumers, 19 times out of 20. Two posters with more detailed individual information about both groups were presented at the IPVS meeting in Cape Town, South Africa in March 2017.

Results

83% GPs recommend or administer HPV vaccine to adults. 93-98% of consumers said doctors are trustworthy sources of information. 99-100% of physicians compared to VW (93%), UW (85%) and M (59%) somewhat or strongly agree that vaccination is an important aspect of disease prevention. A higher proportion of patients were concerned about vaccine safety (VW (26%), UW (40%) and M (36%)) than were physicians (5-11%). 58-61% of consumers were generally cautious about taking any vaccine. Cost was seen as the highest barrier to getting vaccinated by 90-95% of physicians; however only 18-20% of consumers considered cost a barrier. Consumers accurately answered a majority of questions about HPV, however physicians rated consumers’ understanding of HPV to be low (11% very good and 48-56% somewhat good knowledge). Among those already vaccinated, VW (30-34%) and VM (13-31%) said physician recommendations/discussions did motivate them to be vaccinated. In the unvaccinated group, UW (38-55%) and UM (49-57%) said physician recommendations and discussions would motivate them to
be vaccinated. 60-66% of physicians say they routinely discuss HPV vaccination with patients.

Conclusion

Divergent views about HPV knowledge, barriers and preventive practices exist between physicians and consumers. These divergent views should be considered and addressed during physician education and consumer counselling.
FC 14-03
Vaccinating against human papillomavirus is not associated with risky sexual behaviours among men who have sex with men in Australia

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Background / Objectives
A recent meta-analysis has concluded that vaccinating against human papillomavirus (HPV) does not lead to risky behaviours among females but there has been no studies examining this association among men who have sex with men (MSM). We aimed to examine the association between sexual behaviours and HPV vaccination status among men who have sex with men.

Methods
We analysed MSM aged 16-40 years attending the Melbourne Sexual Health Centre (MSHC), Australia, for their first visit in 2016. Chi-squared test was used to examine the differences in sexual behaviours (e.g. number of male partners and condom use in last 3 and 12 months) between vaccinated and unvaccinated MSM.

Results
A total of 1332 MSM were included in the analysis with a median age of 27 (IQR 23-31). Six percent (n=81) of MSM had been vaccinated against HPV. The median number of male partners in the last 3 and 12 months was 2 (IQR 1-5) and 5 (2-10), respectively. The proportion of men used condoms always in the last 3 and 12 months was 39.2% (n=797) and 36.5% (n=774), respectively. There were no significant differences in number of partners and always condom use in both last 3 and 12 months between vaccinated and unvaccinated MSM (p>0.05).

Conclusion
Vaccinating against HPV is not associated with increased number of sexual partners and condomless anal sex practice among MSM, particularly among sexually-active men attending a sexual health service.
SAFETY MESSAGES INCREASE MOTHERS' WILLINGNESS TO VACCINATE AGAINST HPV: A RANDOMIZED TRIAL

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Background / Objectives

U.S. HPV vaccination rates are well below national targets and there is a need to identify messaging approaches that can increase acceptance of HPV vaccine. The objective of this study was to determine if messages about the relative safety of HPV vaccination and the strength of the recommendation increased mothers' willingness to vaccinate against HPV.

Methods

1,097 mothers of 9-13-year-olds living in the U.S. completed a national Web-based survey in August 2014. The analyses presented here focused on the 63.9% (n=701) who reported no HPV vaccine administration. The study used a 3x2 randomized between-subjects design (strength of recommendation x safety information). Illustrated vignettes depicted one of 3 levels of provider recommendation strength (brief mention; strong recommendation; strong recommendation + personal disclosure of vaccination of own children), and either the presence or absence of information regarding the relative safety of vaccination compared to common daily activities (e.g., playing soccer). The outcome was willingness to have the child receive HPV vaccine, measured on a continuous sliding scale ranging from 0 (definitely would not) to 100 (definitely would). Perceived benefits of vaccination were assessed with 5 items administered prior to viewing the intervention and included as a covariate in the analysis of covariance (ANCOVA).

Results

Overall mean willingness to receive HPV vaccine was 59.7 (SD = 35.4). ANCOVA indicated that provision of relative safety information increased willingness to vaccinate (M=63.1 vs. M=56.2; F=7.0, p<.01). Perceived benefits were also significantly related to willingness to vaccinate against HPV (F=214.9, p<.001). Strength of recommendation and child sex were not associated with willingness to vaccinate and there were no significant interactions.

Conclusion

Our results suggest that provider communication about the relative safety of HPV vaccine and the benefits of vaccination in general may increase rates of HPV vaccine acceptance. While strength of recommendation did not have an effect, this may have been due to the difficulty associated with replicating a personal physician’s recommendation via an online survey. As a next step it will be important to test the
relative safety messaging in settings where HPV vaccine can be administered and the effects on vaccine uptake can therefore be evaluated.
Background / Objectives

Reminder-recall letters (RR) are an effective means of increasing vaccination in the United States. However, opposition to HPV vaccination, which is viewed by many as a sexual health intervention, can lead to efforts to thwart RR systems through changes in public health policy. When opposition emerges, however, there is a ‘window of opportunity’, when coalitions can form to provide important evidence-based communication to change the course of policy development.

Methods

Using a case study approach, we examine one example of a coalition’s emergence and its efforts to provide rapid evidence-based advocacy in the U.S. state of Indiana. Data inform a point-in-time analysis of state RR communications for HPV vaccination, HPV vaccination completion, opposition emergence, evidence-based advocacy and policy activity. The study time period is from Jan 2012-May 2017.

Results

The Indiana State Department of Health (DOH) initiated RR letters to increase HPV vaccination uptake for girls in 2012, with subsequent RR cycles for both girls and boys annually. Vaccination completion rates steadily increased, reflecting the RR intervention. Opposition emerged in 2015, following a failed state legislative bill to establish an 80% HPV vaccination completion goal, with the focus of stopping the RR program. Executive policy from the state government ensued in an effort to undermine the RR effort. Within 24 hours of the attempt to shut down the RR program, a coalition emerged with a rapid, coordinated advocacy response using social media and news outlets. This coalition, including groups from the academic and public health communities, continues today and works with the new government (new governor and legislative committee chair). The RR intervention also continues, and a new legislative policy is under consideration to allow the DOH to establish a strategic plan to reduce HPV related cancer.

Conclusion

Broad-based, coordinated and rapid communications by community partners with public health evidence to policy makers can have a positive policy impact. The ability
to identify partners and leaders, and to coordinate communications is crucial to an effort’s success. Sustained partnerships are helpful, but even in the case of Indiana, a nascent group can also be effective if coordinated, on message and timely. These efforts must be based in the community and not in government or industry to be effective.
SCHOOL NURSES’ ATTITUDES TOWARDS AND EXPERIENCES OF AN HPV VACCINATION PROGRAMME

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Background / Objectives

Healthcare providers have an important role for the HPV vaccination programme to be successful. In Sweden one in five girls is not vaccinated against HPV. A population based study among Swedish school nurses who vaccinated against HPV at the early start of the national vaccination programme in 2013 [1], showed that most nurses were in favour of the vaccination programme. However, the majority had experienced difficulties. Most nurses had been contacted directly by parents who were concerned regarding the vaccine safety and effectiveness. The aim of this study was to investigate school nurses’ attitudes towards and experiences of HPV vaccination four years after its implementation. It was hypothesised that school nurses had more favourable attitudes towards the vaccination programme, perceived less barriers with the vaccinations and had higher level of perceived knowledge about HPV vaccine compared to our previous study in 2013.

Methods

School nurses (n=736) from all counties in Sweden completed a questionnaire in spring in 2016.

Results

Overall, the school nurses had more favourable attitudes towards the HPV vaccination programme (p=0.015) and reported less barriers (p<0.001) compared to the study in 2013. More than half of the nurses (n=415, 56%) strongly agreed that boys should also be offered the vaccine (p<0.001). There were no differences in school nurses’ perceived knowledge about HPV in order to inform and to answer questions about the vaccine to the girls or to the parents. More than half of the nurses (n=409, 56%) reported that they needed more education about HPV. Almost all nurses (n=659, 90%) had been contacted by parents with questions about the vaccine, and most questions were related to vaccine safety.

Conclusion

School nurses have a more favourable attitude towards the vaccination programme against HPV compared to three years earlier, although almost all nurses had been contacted by parents with diverse questions and concerns. Thus, it is essential to
provide ongoing education and training for school nurses who are key healthcare professionals for providing information about HPV to parents and pupils.

References

The New Zealand HPV vaccination programme - the road to comprehensive access.

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Background / Objectives

This presentation will describe the development of the New Zealand (NZ) HPV vaccination programme from commencement in 2008 to 2017 with comprehensive free access as well as future plans for the NZ school programme.

Challenges and coverage rates will also be discussed.

Methods

Information in this presentation is gleaned from literature review, government websites, the author's own knowledge and experience as a regional immunisation coordinator and unpublished research undertaken for her PhD.

Results

The NZ HPV vaccination programme commenced in late 2008 with a catch-up programme for girls born in 1990-1991. The school programme started in 2009 for Year 8 girls (age 12). Females could also access Gardasil in primary care until their 20th birthday. Gardasil, with a three-dose schedule at 0, 2 and 6 months was utilised for all eligible groups until January 2017.

From January 2017, enhanced access:

- Gardasil 9 replaces Gardasil (4)

- Gender neutral vaccine - offered to boys and girls in Year 8 at school

- Dose schedule change: <15s two doses at 0 and 6-12 months; 15+ three doses at 0, 2 and 6 months

- Expanded age eligibility: vaccine now funded in primary care for males and females aged 9 to 26 years

Future developments: In 2018 the Ministry of Health plans to introduce the vaccine programme to Year 7 students (age 11). This may lead to cost savings as the vaccine will be delivered concurrently with the Boostrix vaccine.

Overall coverage rates for girls have never met targets, set at 75% for three doses. Reasons for decline include safety concerns and age the vaccine is offered in school.
is too young. Lower HPV knowledge is associated with poorer acceptance of the vaccine.

Conclusion

School based HPV vaccination started in 2009 for girls only. From 2017 NZ has a comprehensive gender neutral programme. In 2018 the vaccine will be offered in Year 7. This may lead to savings in service delivery, but may also lower acceptance, as (young) age has been a factor in parent's decision making.

Coverage rates in NZ have never been high. Reasons for decline need to be addressed in order to increase acceptance.

References


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KNOWLEDGE, ATTITUDE, PRACTICE AND BEHAVIOR OF WOMEN ATTENDING GYNECOLOGICAL CLINIC TOWARDS CERVICAL CANCER AND PAP SMEAR SCREENING IN EASTERN INDIA

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Background / Objectives

In an effort to decrease the toll of cervical cancer, by its knowledge, prevention and treatment services in the community, we assessed the knowledge, attitude, and practice regarding cervical cancer screening among Eastern Indian women.

Methods

A total of 481 women were randomly selected from those who visited the outpatient clinic at MAGS Medical & Research Center, Kolkata, India irrespective of reason(s) for the visit. A pre-tested structured questionnaire covering socio-demographic characteristics, knowledge, attitude, and practice related to cervical cancer screening was administered.

Results

We found a significant lack of awareness regarding cervical cancer and its screening methods in Indian women. Only 24.5% of them had ever heard of cancer cervix and this was quite low when compared to other developed and many of the developing nations. During analysis the mean age of study group was found to be 44.29±10.036 years, 95.2% were married with mean age at marriage being 22.71±2.99 years and mean parity being 1.82. Although, depth of knowledge regarding Pap smear and cervical cancer was also found to be quite shallow in this study but it was significantly higher in those with higher educational level and higher income group. Attitude towards Pap smear test was found to be positive as more than two third of the participants aware of the test assumed it to be beneficial. The role of education and economic stability was also established in regard to perceived benefits of pap smear and this distribution was significant statistically (P value 0.049 and 0.015). It was seen in this study that the positive attitude towards the test was translated into right practice and behavior.

Conclusion

This study revealed the limited knowledge of Indian women about the susceptibility of cervical cancer, and the necessity of cervical cancer screening among the women. Inadequate public health education, lack of patient-friendly health services, socio-cultural health beliefs, and personal difficulties were the most salient barriers to screening.
FC 16-01
DETERMINANTS OF HPV E6-E7 MRNA OVEREXPRESSION IN WOMEN HPV DNA POSITIVE - PRELIMINARY RESULTS FROM NTCC2 STUDY


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Background / Objectives

Cervical cancer screening by Human Papillomavirus (HPV) DNA testing with cytology triage is more effective than cytology based screening. Compared to cytology, the HPV DNA test shows higher sensitivity, which allows better protection and longer screening intervals, although a lower specificity which makes it necessary to triage the women resulted positive. We have been conducting a large randomized clinical trial (New Technologies for Cervical Cancer 2 [NTCC2], NCT01837693)(1) within organized population-based screening programs in 5 Italian regions using HPV DNA as the primary test, with the aim of evaluating the HPV E6-E7 mRNA test (Aptima HPV assay, Hologic) and the p16/ki67 double staining (CINtec plus test, Roche) as the triage test in comparison to cytology.

Methods

Women were tested with HPV DNA assay (Cobas 4800 HPV assay, Roche, or Hybrid Capture 2 (HC2), Qiagen). Those positive were triaged with cytology and tested for mRNA and p16/ki67 double staining. Women with positive or inadequate cytology were referred to colposcopy, while those with negative cytology were randomised to immediate colposcopy or to 1-year HPV re-testing. Women will be followed up for at least 5 years, until the next screening round. Here the baseline results of mRNA positivity according to age, cytology, HPV type, and HPV viral load, are presented.

Results
More than 42,000 25-64yo women have been recruited from April 2014 to April 2017. Here are included data from 35,877 samples collected up to June 2016; 2,651 (7.4%) were HPV DNA positive. Up to now, 2,453 samples have been tested also by Aptima, and 1,649 (67.2%, 95% CI 65.4–69.2) gave a positive result. Positivity was similar in all age groups, and it was higher in women with positive cytology (82.7%, 95%CI 79.0–86.0; 93.5%, 95%CI 88.3–96.8, for low and high grade, respectively) than in those with negative cytology (60.8%, 95%CI 58.5–63.1) and in women HPV16 infected (81.8%, 95%CI 76.4–86.4) compared to those infected by other high risk types (72.6%, 95%CI 69.8–75.3). Finally, positivity increased with HPVDNA viral load from 10.6% (95%CI 6.9–15.3) for women with HC2 relative light unit ratio (RLU) between 1 and 1.99, to 85.0% (95%CI 82.2–87.6) for women with HC2 RLU >10.

Conclusion

If used as a triage test, the mRNA test, due to the observed positivity rate, would determine a 5% immediate colposcopy referral, compared to 2% with cytology triage. Thus, the number of women referred to colposcopy would increase if the 1-year referral for HPV DNA-positive/mRNA-negative cases is maintained. Only longer interval for these women might make triage by this test more efficient than by cytology, as long as the mRNA test shows very high sensitivity.

References

A THREE-TIERED SCORE FORMAT FOR KI-67 AND P16INK4A IMPROVES CONSISTENCY AND VALIDITY OF GRADING CIN LESIONS

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Background / Objectives

Accurate histological grading of cervical intraepithelial neoplasia (CIN) is essential for clinical management. However, CIN grading has a moderate inter- and intra-observer agreement. We investigated the reproducibility of the performance of a score system based solely on the cumulative score value of the biomarkers Ki-67 and p16ink4a (immune-score), and compared the results to consensus pathologist CIN grading based on slides stained for H&E, Ki-67 and p16ink4a.

Methods

Three expert gynaeco-pathologists received H&E slides of 115 randomly selected cervical tissue specimens, selected the most abnormal area and rendered a diagnosis (diagnosis 1). At a later time point, the individual pathologists independently scored corresponding Ki-67 and p16ink4a immunostainings by a three-tiered immune-score system. Next, a diagnosis was made based on both the H&E and immunostainings (diagnosis 2). The consensus diagnosis 2 was used as the Gold Standard. Consistency of diagnosis 1, 2 and immune-score was determined by Spearman Correlation coefficients, Kappa values and absolute agreement between pathologists. Validity of the diagnosis 1, 2 and immune-score was determined by sensitivity and specificity for CIN2+ and CIN3+ graded by the Gold Standard diagnosis.

Results

Gold Standard diagnoses revealed 35 specimens without dysplasia, 20 CIN1, 17 CIN2, 22 CIN3 and 21 specimens with SCC. The highest consistency between pathologists was found for the immune-score, with a Spearman correlation coefficient of 0.907, and a maximum Kappa of 0.829 and absolute agreement of 92%. Immune-score showed a higher sensitivity for Gold Standard CIN2+ and CIN3+ than diagnosis 1 and 2, with a sensitivity for marker scores 3 to 6 being higher than 97.0% and an increase in specificity from 70.0% to 85.9%, respectively.

Conclusion
CIN grading based on a simple three-tiered Ki-67 and p16\textsuperscript{ink4a} immune-score has a higher reproducibility and accuracy in terms of consistency and validity than classical histological and immunohistochemical CIN grading. Moreover, this immune-score defines more accurately where on the trajectory of development of cervical cancer via CIN1 to CIN3 the cervical lesion is situated. This is important for the clinician to decide on cervical treatment or a wait and see policy.
FC 16-03
P16/KI67-BASED TRIAGE FOR HISTOLOGIC HSIL-RISK WOMEN IN 12-18 FOLLOW-UP: P16/KI67 TWICE-POSITIVITY AND COLPOSCOPY FIRST-NEGATIVITY.

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Background / Objectives

All world-wide recommended algorithms in cervical cancer secondary prevention have limitations in precancers detection. Simultaneous co-expression of anti-proliferative and proliferative proteins in p16/Ki67 test is clinically used to select high-grade cervical intraepithelial lesions. We evaluated whether twice-positivity p16/Ki67 test – in first test and in one year follow-up – can improve detection of these.

Methods

8824 automated proceeded LBC (a study period 08/2015 – 04/2017), including 2063 as cotesting with DNA high-risk HPV, have been performed in secondary cervical cancer prevention. Immunocytochemical p16/Ki67 double staining was done in 372 cases using automated preparation system. 180 women with ASC-H or higher or ASC-US/LSIL cytology and with HPV-positive status were referred to colposcopy with biopsy. 35 patients with histological LSIL or less (biopsy first-negativity), reached follow-up cotesting with p16/Ki67 test and biopsy in 12-18 months.

Results

Diagnostic value of twice-positivity p16/Ki67 test for histologic HSIL (hHSIL) in the second follow-up biopsy was evaluated. Follow-up p16/Ki67 test was positive in 11 women – 8 hHSIL and 3 histologic LSIL cases were diagnosed in biopsy. 1 hHSIL was p16-Ki67 twice-negative. Sensitivity/specificity/PPV/NPV of p16/Ki67 for hHSIL in the second biopsy was 89/88/73/96 (CI 95%) respectively.

Conclusion

A twice-positive p16/Ki67 test can be a precise biomarker in triage patients for hHSIL-risk in cervical cancer screening. In women with abnormal screening test results after first biopsy, p16/Ki67 could be sufficient as alone diagnostic test in referring to follow-up biopsy in 12-18 months.
A NOVEL WHOLE GENOME SEQUENCING METHOD TO ACHIEVE A COMPREHENSIVE MAP OF ALL HPV16 INTEGRATION SITES ACROSS THE HUMAN GENOME

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Background / Objectives

Our study will have 3 main goals. 1) Through our novel approach utilizing the latest in NGS techniques, we will extensively map all HPV integration events across the human genome for each cancer and pre-cancer patients in our initial cohorts. 2) Using in-house developed analytic software, we will combine each patient’s integration maps and identify integration patterns that could be used for future targeted approaches, and 3) the whole genome human data will also be used to identify all chromosomal rearrangements within the tumors and the proximity of these events to the cataloged integration sites.

Methods

For this study we have chosen to use 10X Genomics Technology in combination with Illumina whole genome sequencing to provide us with the most accurate, phased human genomes. Our pilot study consists of 16 HPV16+ women (8 cancers, 4 pre-cancers (CIN3) and 4 control HPV16+ non-cancer samples (CIN2)).

Results

We have performed whole genome sequencing on all 16 women from our pilot study. All 16 of the pilot samples have been processed through our cloud based analytic pipeline that aligned each sample to an HG19-HPV16 hybrid reference, for each sample their HPV16 integration sites have been recorded as well as all other chromosomal abnormalities have been identified and recorded.

Conclusion

We have developed a method that combines the latest in NGS technology to create a comprehensive list of HPV integration and chromosomal abnormalities for each of the 16 women in our first pilot study. This data will be used for potential development of targeted assays.
WHOLE EXOME SEQUENCING TO FIND NEW BIOMARKERS FOR DETECTION OF CIN3

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Background / Objectives

Our team has developed a methylation classifier to detect CIN3 that could be used routinely as a molecular diagnostic tool. Although this classifier already has good sensitivity and specificity our aim was to improve it further. We used whole exome sequencing to find new biomarkers to add to the classifier.

Methods

The study was designed to compare the baseline and dyskaryotic samples of HPV positive women who developed CIN3 (cases) to women who did not (normal controls) and to HPV negative women (negative controls). The samples at baseline did not show any sign of dyskaryosis. From the archived material of the ARTISTIC trial, we selected 27 cases (2 samples per case, one at baseline, one at time of diagnosis) that were matched to 27 normal controls by age and HPV type to the cases. Twelve negative controls were also randomly selected. In total, we sequenced the whole exome of 93 samples on an IonProton using the IonAmpliSeq Exome RDY kit. We developed an in-house bioinformatics pipeline.

Results

First, we validated our bioinformatics pipeline on the top 29 candidate single nucleotide polymorphisms (SNPs) using Sanger sequencing. All SNPs were successfully validated which showed that the sequencing and analytical methods were appropriate. Then, using a principal component analysis with both germline and somatic mutations, we showed a clear separation between both types of controls and the cases. We found that the cases harboured mutations at baseline and time of CIN3 diagnosis that were not present in any of the controls. We are now in the process of validating the top 200 variants using Fluidigm and Illumina technologies. More details of our ongoing investigations will be reported.

Conclusion

We have validated the use of whole exome sequencing on the IonProton platform to identify new biomarkers for detection of CIN3 and produced a list of 200 candidate SNPs to improve our classifier.
MICRORNA DETECTION IN CERVICAL SCRAPES ALLOWS FOR THE TRIAGE OF HPV-POSITIVE WOMEN IN CERVICAL SCREENING

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Background / Objectives

Screening for cervical cancer by primary high-risk HPV (hrHPV) testing has just been introduced in the Netherlands. Because hrHPV testing also results in the identification of women with clinically irrelevant transient hrHPV infections, additional triage markers are required to identify women at risk of high-grade cervical intraepithelial neoplasia (CIN) or cancer. We expect microRNAs (miRNAs) to provide promising triage markers, since they are 1) often deregulated in cancer and 2) easily detectable in small amounts of clinical material. We previously identified 8 miRNAs with altered expression in cervical (pre)cancer due to either methylation-mediated silencing or chromosomal alterations. In this study, we evaluated the clinical value of the 8 miRNAs to triage hrHPV-positive women in cervical screening.

Methods

Quantitative TaqMan RT-PCR was used to determine expression levels of the 8 candidate miRNAs in RNA isolated from cervical scrapes of hrHPV-positive women without disease (n=66), with high-grade CIN (n=121), cervical squamous cell carcinoma (SCC, n=29) and cervical adenocarcinoma (AC, n=9).

Results

Six out of 8 miRNAs showed significantly (p < 0.05) differential expression between scrapes of hrHPV-positive women with and without high-grade CIN and cancer. Using logistic regression analysis a miRNA classifier was built, which detected a major subset of high-grade CIN (~65%), all but two SCC (93%) and all AC (100%).

Conclusion

Altered miRNA expression is detectable in hrHPV-positive cervical scrapes of women with underlying high-grade CIN and cancer. Our miRNA classifier provides promising results for the triage of hrHPV-positive women.
Co-expression of HPV E6, E7 mRNA and PD-L1 in Cervical Cytology Samples: Prognostic Implications

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**Background / Objectives**

The immune system has been shown to control HPV infection in some women and not in others leading to pre-cancerous lesions and subsequently cancer. The relatively high regression rate of cervical intraepithelial lesions (CIN) has similarly been attributed to engagement of the immune response directed against neoplastic cells. Recent advances in immune-oncology have shown the dramatic effects of PD-1/PD-L1 inhibitors in epithelial tumors including squamous cell carcinoma and adenocarcinoma, the major cancer subtypes in the female genital tract. Here, we present a novel assay that combines RNA in situ hybridization for HPV E6, E7 mRNA and PD-L1 cell surface staining on squamous cells in liquid-base cervical cytology specimens. This assay could provide actionable data supporting therapeutic options.

**Methods**

ThinPrep liquid based cervical cytology samples were obtained from both normal patients and patients with HSIL cervical cytology. Cells were isolated and hybridized in suspension for HPV E6, E7 mRNA using the HPV OncoTect 3Dx (CE-IVD) kit. Following post-hybridization washes, cells were stained with PD-L1 using the OncoTect iO (CE-IVD) kit then counterstained with cell cycle reagent. Cells were analyzed on a CytoFlex flow cytometer (Beckman Coulter).

**Results**

PD-L1 expression was low in the normal HPV negative samples and variable in the HSIL samples using a standard 1% cut-off for positivity in squamous cells. Of interest, PD-L1 expression was higher in cells expressing HPV E6, E7 mRNA compared to cells lacking transcriptional activity.

**Conclusion**

PD-L1 expression is variable in samples with abnormal cervical cytology. The prognostic implications of PD-L1 expression in cervical pre-cancer remains to be determined, however, we present in this study a means for fine quantification of PD-L1 expression in both HPV transcriptionally active and normal cells. This combination disease markers should facilitate further studies of the role in progression or regression of cervical pre-cancer.
Association between PD-L1 mRNA expression and HPV infection in cervical adenocarcinoma and squamous cell carcinoma

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Background / Objectives

PD-L1 expression in different subtypes of cervical cancer is largely lacking. We investigated the expression of PD-L1 mRNA in human papillomavirus (HPV)-negative and HPV-positive adenocarcinoma (CADC) and squamous cell carcinoma (SCC) to determine its prevalence and the association between PD-L1 expression level and HPV infection.

Methods

RNA ISH was performed with an RNAscope 2.5 (Advanced Cell Diagnostics) according to the manufacturer’s instructions. 85 cases of formalin-fixed paraffin embedded tissue sections were evaluated for mRNA expression of PD-L1 and HPV. All cases were tested HPV DNA using WTS-PCR and multi-infected cases were tested HPV DNA using LCM-PCR. HPV and PD-L1 expression were visualized with standard bright-field microscopy and reviewed by two pathologists. Positivity was defined as 1 to 3 spots in one target cell (20-40X), scored 1. If the signal was 4 to 10 spots in one cell, more than 10 spots in one cell and lower than 10% cells are with clustered spots or more than 10 spots in one cell and more than 10% cells are with clustered spots will be scored 2, 3 or 4, respectively. To obtain significance in the difference between groups was performed by Chi-square test using SPSS 24.0 software.

Results

Our results showed that the PD-L1 positivity was found in 20 out of 71 (28.2%) CADC cases, while it was found in 4 out of 14 (28.6%) SCC cases. PD-L1 mRNA was expressed in 11 out of 32 (34.4%) HPV mRNA positive CADC and SCC cases and that was 13 out of 53 (24.5%) HPV mRNA negative cases. In HPV DNA positive group, PD-L1 positivity was found in 15 out of 50 (30.0%) cases. In HPV DNA-negative group, PD-L1 positivity was found in 9 out of 35 (25.7%) cases. Above all, PD-L1 positivity was slightly higher in SCC and HPV-positive group than that of CADC and HPV-negative group. But no significant differences were observed in these groups.

<table>
<thead>
<tr>
<th>Table 1 PD-L1 mRNA score for RNAscope ISH</th>
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<td>HPV/PD-L1</td>
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<table>
<thead>
<tr>
<th></th>
<th>WTS-PCR HPV+</th>
<th>WTS-PCR HPV-, LCM-PCR HPV-</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HPV mRNA+</td>
<td>HPV mRNA-</td>
<td></td>
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<tr>
<td>CADC</td>
<td>13(65.0%)</td>
<td>12(75.0%)</td>
<td>25(69.4%)</td>
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<tr>
<td></td>
<td>7(35.0%)</td>
<td>4(25.0%)</td>
<td>11(30.6%)</td>
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<td>0(0.0%)</td>
<td>0(0.0%)</td>
<td>0</td>
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<td></td>
<td>9(81.8%)</td>
<td>2(18.2%)</td>
<td>11</td>
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<tr>
<td></td>
<td>9(81.8%)</td>
<td>2(18.2%)</td>
<td>11</td>
</tr>
<tr>
<td>SCC</td>
<td>8(66.7%)</td>
<td>2(100.0%)</td>
<td>10</td>
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<td></td>
<td>4(33.3%)</td>
<td>0(0.0%)</td>
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<td></td>
<td>10(71.4%)</td>
<td>4(28.6%)</td>
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**Conclusion**

We didn’t observe differences of PD-L1 mRNA expression between SCC and CADC or HPV+ and HPV- cervical cancer. This may due to limited SCC cases. Further research can be done to investigate the association between HPV and PD-L1 expression patterns in squamous cell carcinoma and adenocarcinoma of the cervix.

**References**

KERATIN 17 (K17) IS A PROGNOSTIC BIOMARKER OF CERVICAL CANCER: ENDOCERVICAL GLANDULAR NEOPLASIA.

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Background / Objectives

Although p16INK4a and other diagnostic biomarkers have been established as sensitive and accurate diagnostic biomarkers for cervical squamous cell carcinoma (SCC) and endocervical adenocarcinoma, they have limited power to predict the survival of patients following the diagnosis of cervical cancer. By contrast, we discovered by laser capture microdissection and mass spectrometry, that keratin 17 (K17) could predict the overall survival of patients with squamous cell carcinoma (SCC) more accurately than grade, stage, or any other clinicopathologic features¹,². K17 status, however, has not been previously evaluated in glandular neoplasms of the endocervical mucosa. Based on the concept that both squamous and glandular lesions of the cervix are driven by similar pathogenetic mechanisms, we hypothesized that K17 overexpression could also be a prognostic biomarker for endocervical glandular neoplasia.

Methods

Cases of endocervical adenocarcinoma, adenocarcinoma in situ (AIS), benign glandular lesions, and normal endocervical mucosa were selected from the archival collections of formalin-fixed, paraffin-embedded tissue blocks from the Departments of Pathology at Stony Brook Medicine and the University of Massachusetts. Immunohistochemical staining for K17 was performed by an indirect immunoperoxidase method and K17 expression was scored based on the PathSQ score, the proportion of cells that showed strong (2+) staining.

Results

K17 was highly expressed in 21/32 (65.6%) cases of adenocarcinoma in situ (AIS) and in 75/90 (83%) of adenocarcinoma cases. K17 staining was detected in a mean of 33.94% of malignant cells and was strongest at the periphery of pseudoglandular groups and at the invasive front of tumors. K17 was not detected in benign glandular lesions but was found in subcolumnar reserve cells, most notably in microglandular hyperplasia. High levels of K17 expression were significantly associated with decreased patient survival. While the 40-89% K17+ category did not significantly differ from the <40% category (HR = 2.03, p=0.13), those with a 90%-100% K17+ scores had 3.47 times higher risk of death as compared to the risk of death for those with a <40% K17 score (p=0.01).

Conclusion
In summary, K17 expression in normal endocervix was limited to subcolumnar reserve cells while expression in the columnar endocervical epithelium was highly specific for glandular neoplasia of the cervix. Most importantly, we further determined that high K17 expression is a powerful, negative prognostic biomarker that can be used to identify patients that have the shortest survival probability following the diagnosis of endocervical adenocarcinoma.

References


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Background / Objectives

The aim of this study was to investigate the genetic susceptibility of functional polymorphisms involved in the regulation of reactive oxygen species (ROS) - NADPH oxidase (NOX) subunit (p22phox) and myeloperoxidase (MPO) in cervical carcinogenesis.

Methods

A total of 862 women, 312 cases (46 LGSL+ 32 HGSIL+ 234 ICC) (mean age of 46.20±13.2 years old) and 550 controls (mean age of 42.44±13.38 years old). In this population were determined the polymorphisms of p22phox (CYBA C242T, rs4673) and MPO (G463A, rs2333227) by PCR-RFLP. Statistical analyses were qui-square and binary logistic regression. The results were significant for P<0.05.

Results

In our population, we confirmed that age and smoking habits were risk factors for development of cervical cancer (OR=1.04, 95%CI [1.02-1.05], P<0.0001; OR=2.47, 95% [1.36-4.47], P=0.003, respectively). The A carriers of MPO polymorphism were about 5-fold of increased risk for cervical cancer (OR=5.41, 95% [2.15-13.64], P<0.0001), being dependent of age (OR=3.38, 95% [0.85-13.48], P=0.085) and independent of smoking habits (OR=3.85, 95% [1.33-11.11], P=0.013). In p22phox polymorphism, the TT genotype was a tendency for increased risk in cervical cancer (OR=3.57, 95% [0.85-13.48], P=0.057), being age and smoking habits dependents.

Conclusion

Epithelial lesions, target for HPV, are exposed to ROS, therefore, functional polymorphisms involved in the modulation of this species may influence the susceptibility for cervical cancer. The A carriers of MPO and CC genotype of ph22phox polymorphisms, associated with lower activity of macrophages, may contribute to higher susceptibility to cervical carcinogenesis.
INTER- AND INTRA LABORATORY QUALITY MONITORING OF HPV TEST PERFORMANCE IN THE DUTCH CERVICAL CANCER SCREENING PROGRAM

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Background / Objectives

From January 2017 primary screening for cervical cancer in the Netherlands has switched from cytology to HPV based screening. The programme expects to examine well over 500,000 samples per year distributed over five CCKL or ISO15189 accredited laboratories. To achieve uniform performance between the five sites all laboratories are equipped with identical Roche Cobas4800 systems, three per laboratory. Furthermore, the Cobas 4800 HPV test kit production lot, laboratory reagents and assay procedures are all standardized between the laboratories.

Methods

To develop a quality control programme to monitor and continuously ensure the quality of HPV based screening results within and between the Dutch screening laboratories and to compare their performance with international peer laboratories outside the Dutch screening network. This integrated quality monitoring system is based on:

A verification and release programme for acceptance testing of equipment upon installation and upon repair or major service. In addition, this programme is used to test and release (new lots of) critical reagents.

2. A run control programme with a manufacturer-independent control sample in each HPV run.

3. External Quality Assessment to monitor inter-laboratory performance by participating in both international inter-laboratory comparisons with proficiency panels and in national comparisons based on inter-laboratory exchange of clinical materials from the screening program.

Results

Dedicated HPV control panels were designed and used for acceptance of systems and kits prior to the start of the programme. These panels have been instrumental to achieve the required level of performance standardization within and between
laboratories. Furthermore, the results to be presented demonstrated that the observed inter-run, inter-system as well as inter-laboratory variations in the Ct values of the results were small and well within acceptable ranges.

From the start of the screening programme an independent run control sample was included in each assay run. This control is completely independent from the assay supplier both in design, development, production and result interpretation. The independent run control, called User Defined External Control (UDEC), is examined in every run in each laboratory. UDEC results are used to monitor the (variation in) assay performance within and between the screening laboratories; it is not aimed for acceptance of individual runs.

Conclusion

The quality monitoring programs have been implemented to monitor assay performance in the Dutch hrHPV screening network of five laboratories participating in the recently renewed national screening program for cervical cancer.
P16/Ki67 DOUBLE STAINING FOR TRIAGE POSITIVE RESULTS IN PRIMARY CERVICAL CANCER SCREENING BASED ON DNA HPV TESTING.

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Background / Objectives

Human papillomavirus (HPV) DNA testing is globally recommended in primary cervical cancer screening. Effective triaging of HPV-positive women plays a crucial role for detection cancer precursors. p16/Ki67 has been studied for distinguishing of high-grade cervical intraepithelial lesion (HSIL) risk by co-expression of anti-proliferative/proliferative markers. We investigated a diagnostic value of p16/Ki67 as the second-step in HPV-based screening.

Methods

From 8824 cervical cancer screening tests (including 1718 cotesting, 345 LBC with reflex HPV and 372 p16/Ki67 tests), a group of 189 cases was selected based on 4 end-points: positive high-risk HPV status, LBC and double immunocytochemical p16/Ki67 test (DS) in automated preparation systems and performed colposcopy with biopsy as follow-up.

Results

Total number of histologic HSIL/DS positivity was 33/30 - for positive 16 or 18 types HPV (16/18HPV+) 21/20 and for positive non-16 or non-18 types (n16/n18HPV+) 12/10, for histologic LSIL was 70/23 - for 16/18HPV+ 28/12 and for n16/n18HPV+ 42/11, and for negative was 84/16 – for 16/18HPV+ 37/10 and for n16/n18HPV+ 47/6. Sensitivity/specificity/PPV/NPV of DS for hHSIL were 91/74/42/97 respectively. In retrospective analysis, total number of biopsies needed in p16/Ki67-based triage was 71 comparing to 180 in LBC-based triage.

Conclusion

p16/Ki67 can be a clinically important diagnostic test for detecting hHSIL in HPV-positive women that may reduce number of performed invasive procedures and increase patients comfort. In consequence, incorporating of p16/Ki67 test clinical algorithms could reduce total costs of secondary cervical cancer prevention.
HPV-positive women with normal cytology remain at increased risk of CIN3 after a negative repeat HPV test


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Background / Objectives

In human papillomavirus (HPV) screening, a repeat HPV test is often recommended after positive HPV and normal cytology (HPV-pos/cyt-neg) but its absolute risk of cervical precancer (CIN3+) over two screening rounds needs to be assessed.

Methods

We assessed the five-year risk of HPV infection and CIN3+ in HPV-pos/cyt-neg women with a negative repeat HPV test and in double negatives (negative HPV and cytology) in the POBASCAM cohort. We obtained histology data from the Dutch pathology registry (PALGA).

Results

HPV infection risk was 20.4% (19/93) in HPV-pos/cyt-neg, repeat HPV-negative women and 3.2% (294/9,185;p<0.001) in double negatives. Corresponding CIN3+ risks were 2.0% (4/199) and 0.2% (41/18,549,p<0.001). Infection risks were also increased in type-specific analyses of HPV16,31,33,39,52,56 and 58.

Conclusion

HPV-pos/cyt-neg women continue to have an increased CIN3+ risk, also when the repeat HPV test is negative. Therefore, intervals in primary HPV screening should be determined separately for HPV-positive and -negative women.
Non-inferiority of Onclarity HPV genotyping compared with HC2 in a German HPV-screening pilot project (WOLPHSCREEN)

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Background / Objectives

Only new HPV tests that show similar sensitivity and specificity for the detection of CIN3+ as HC2 or GP5+/GP6+ should be used in cervical cancer screening programs. We evaluated the performance of Onclarity for the detection of HR-HPV compared with HC2 in a German HPV screening program and the utility of Onclarity genotyping for the triage of HR-HPV positive women compared with p16/Ki-67.

Methods

Women attending for their 1st, 2nd or 3rd HPV&Pap screening round in WOLPHSCREEN were included. Participants were 30-70 years old, hysterectomy was an exclusion criterion. All underwent Pap smear and HC2 co-testing with 5 years intervals for women with normal findings. In 2015/16 ThinPrep samples of 4,699 participants were tested with Onclarity in a non-interventional followed by an interventional trial with 2,781 women in 2016/17. In this second phase women were called for colposcopy when Onclarity tested positive for HPV 16, 18, 31, 33, 45 or 58.

Results

The overall HR-HPV prevalence was 7.0% with Onclarity and 8.47% with HC2. Only 40/4,301 HC2 negative samples were tested positive with Onclarity (0.93%). Sensitivity of Onclarity for CIN2+ was 93% (40/43), specificity 94.2%, NPV 99.9% and PPV 10.7%. Genotyping for HPV 16, 18, 31, 33, 45 or 58 and p16/ki-67 triage showed an identical sensitivity of 79.1% for CIN2+.

Conclusion

The overall performance of Onclarity HR-HPV testing was non-inferior to HC2 in WOLPHSCREEN. Onclarity showed a better specificity than HC2 while sensitivity for CIN2+ was slightly lower. Primary screening with Onclarity and the use of genotyping to triage HR-HPV positive cases seem feasible.
Significant reduction of cervical cancer incidence within a primary HPV screening pilot project in Wolfsburg, Germany (WOLPHSCREEN)

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Background / Objectives

A number of randomized controlled trials (RCTs) showed that screening with HPV testing, either as co-testing with cytology or as stand-alone test, led to a significant reduction in cervical cancer incidence compared with cytology based screening. However, evidence for a similar efficacy outside of RCTs is still lacking. Here we report on the 11 years follow-up of the Wolfsburg primary HPV screening pilot project (WOLPHSCREEN).

Methods

26,624 women were recruited between Feb 2006 and Dec 2016. Participants had to be at least 30 years old, hysterectomy was an exclusion criterion. All underwent co-testing with Pap smear and HR-HPV testing (HC2). Women with normal findings had their next screening round after 5 years, Pap+/HC2+ case were immediately transferred to colposcopy, while cases with discordant findings had repeat testing after 12 months with referral to colposcopy in case of persistent positive findings.

Results

Overall 305 CIN3+ cases were diagnosed, including 31 invasive cervical cancers. The 5 years incidence of CIN3+ was 0.96% (95% CI: 0.85%-1.09%) in the first round and dropped significantly to 0.16% (95% CI: 0.10% - 0.26%) in subsequent screening rounds. The observed decline in cervical cancer incidence was even steeper from 0.1089% (95% CI: 0.07%-0.16%) in the first to 0.0167% (95% CI: 0.00%-0.06%) in subsequent rounds. The vast majority of CIN3+ cases were diagnosed at first colposcopy (277/305). In the second screening round the remaining cancers were explained by failure of screening tests or colposcopy, while the majority of CIN3 were classified as new lesions.

Conclusion

The observed decline of CIN3+ and invasive cervical cancers in WOLPHSCREEN gives proof that significantly better cervical cancer prevention can be achieved with HPV-testing even outside of RCTs.
**EFFECTIVENESS OF SCREENING IN HPV VACCINATED WOMEN**


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**Background / Objectives**

With the first birth-cohorts of vaccinated women reaching the age of screening its characteristics (most notable positive predictive value) will change abruptly. There is a great need to redesign an appropriate screening protocol as right tools for the future cervical cancer screening programs. We report here the baseline characteristics and safety interim results of a randomized trial assessing the impact of infrequent vs. frequent cervical screening in human papillomavirus (HPV) type 16/18 vaccinated women.

**Methods**

Total female 1992-94 birth-cohorts (30129) were invited to and respectively vaccinated (9482 + 2036) as early adolescents (age 12-15 years) or as young adults (age 18 years) against HPV16/18 in a community-randomized trial (EUDraCT 2007-001731-55). Women in this trial will be screened at the age of 22, 25 and 30 years for cytological screening. In one arm, representing frequent screening, the participants receive information on all the cytological findings on each visit, in the other arm, representing infrequent screening, the participants receive this information only at the of 30, information on high-grade squamous intraepithelial lesion (HSIL)s is, however, given due to ethical reasons. In 2010-13, 4660 (49.1%) and 2036 (100%) of the HPV vaccinated women attended a baseline cervical screening visit, and in 2014-2017 4018 (42.4%) and 1326 (65.1%) of these women attended the 1st round of cervical screening at the age of 22 years.

**Results**

Overall prevalence of HSIL and any abnormal cytological findings at the 1st screening round were: 0.3% (n=11) and 5.7% (n=220) in those vaccinated as early adolescents, and 0.2% (n=3), and 4.6% (n=58) in those vaccinated as young adults, respectively. The incidence rate from the 1st screening round of HPV16/18 and other HPV DNA are being currently analysed and will be combined with the above cytological results. Our previous results from the adolescent group at the age 18
showed HPV16/18 and HPV31/33/35/45 prevalence range from 9.9% to 10.7% and 9.2% to 10.2%, respectively.

**Conclusion**

Our birth cohorts attending screening, as described above, are in close to ten years ahead of comparable observational studies or monitoring of vaccination program participating birth cohorts in other affluent countries. Our study will show the first results of safety, accuracy and effectiveness of infrequent vs. frequent screening for cervical cancer safety which can pave the way to the future synergistic vaccination/screening programs.
FIRST RESULTS OF THE EU-TOPIA PROJECT: TOWARDS IMPROVED CERVICAL CANCER PREVENTION IN EUROPE

Background / Objectives

Screening programmes vary substantially between countries and in most long-term effectiveness of screening has not yet been assessed. In 2015 the EU-TOPIA project has started. The objective of EU-TOPIA is to systematically evaluate and quantify the harms and benefits of the screening programmes for cervical, breast, and colorectal cancer in all European countries, and identify ways to improve health outcomes and equity for citizens. We will present an overview of the first result of this important EU funded project, focusing on the results for cervical cancer prevention.

Methods

First, we have harmonized key quality indicators across cancer sites and prioritized key indicators for the harms and benefits of screening. Second, we systematically evaluated literature on the effects (in terms of mortality or incidence reduction) of cancer screening across the European countries. This information will be used to validate decision models which will be used to find the optimal screening strategy. Third, we started constructing state-of-the-art decision models of the natural history of the cancers (based on the well-known microsimulation model MISCAN), using country-specific data. Fourth, a CATWOE ('Customers, Actors, Transformation, Weltanschauung, Owner, Environment') analysis tool was developed to identify barriers hindering implementation of optimal screening programs. This tool was filled in for six EU countries representing four European region (North, East, South and West). Fifth, the first workshop on monitoring screening programmes in a series of four workshops is planned. The aim of the workshops is to build capacity to conduct cancer screening evaluation independently.

Conclusion
We prioritized a set of 17 key quality indicators (7 benefits and 10 harms), harmonized across cancer sites. The literature search provided evidence that cervical cancer screening is proven to reduce mortality across Northern and Western Europe. However, no studies have been performed evaluating the direct effect of screening on mortality in other European regions. The CATWOE analysis identified the most important barriers and different levels of the screening programme in six exemplary countries. It was found that many barriers occur in each country/region, but also many specific to each country/region. Finally, the first workshop will be held in September 2017. Currently, already 60 persons (researchers and policymakers) registered from 25 different countries. In conclusion, important progress have been made within the EU-TOPIA project.
Background / Objectives

During 2012-2014 in Örebro County, Sweden, the organized screening program included women between 23 and 60. Women with normal cytology then used to exit the program at 55-60 years of age. The aim of this study was to see if HPV test (professionally collected and/or self-collected tests) could predict cervical histological changes and be used as screening option in this age group.

Methods

All women (between 55-60) with normal cytology in their exit sample in the screening program during the years 2012-2014, a total of 2030, were invited to participate in the study. All samples were genotyped for HPV DNA with CLART HPV2 (Genomica). A total of 249 of the 2030 (12.3%) women were positive for carrying any of the 35 HPV genotypes. Of these, 141/249 carried an intermediate or high risk HPV according to the IARC classification, group 1 and 2A and B. These 141 women were invited for a new visit with professional sampling, and also to use a self-sampling device, (Rovers Evalyn brush) and to have a cervical cone performed. Out of these 141 women, 99 have completed all the above these testing and also came for a follow up test 6 months after the histology sample.

Results

Concordance between self-sampling and professionally collected samples was seen in 82.4% and both test methods correlated in the same way to the histological results. Abnormal histology was seen in 19/99(19%) of the performed cones. Follow up tests after 6 months showed that 17/99(17%) of the women still had a HPV positive test.

Conclusion
Further results and conclusion will be presented at the conference, with focus on different HPV genotypes, the concordance with histological findings and HPV persistence.
IF PERSISTENT HPV INFECTION CAUSES DISEASE, WHY ARE WE NOT MEASURING IT?


BD Diagnostics, Sparks, Maryland (United States of America)

Background / Objectives

There is universal agreement that HPV is required for the development of cervical disease and that HPV-induced disease is associated with a persistent HPV infection. The introduction of organized cervical cytology screening programs has resulted in a marked reduction in cervical cancer morbidity and mortality. Here, the relative insensitivity of cytology to detect disease was offset by the serial nature of the screening paradigm, where disease [resulting from persistent infection(s)] was often detected over several screening rounds. Many countries are now shifting to primary HPV screening using extended screening intervals. As we embark on this transition, the irony is that we seem to be ignoring some basic first principles of cervical cancer screening, namely the ability to monitor persistent HPV infection directly using extended genotyping (beyond HPV 16/18).

Methods

Here we argue that extended genotyping information provides a unique and necessary insight into a woman’s risk for developing disease:

1. It enables the physician to detect persistent infections which have a higher disease risk
2. It can guide management decisions, especially in short-term follow up scenarios
3. It enables comprehensive test of cure to be performed on treated women (not just for HPV 16/18)
4. It can identify non-HPV16/18 types with an increased CIN3 risk (e.g. HPV 31, 33 and 45) versus just recording “other-high-risk” infections, enabling them to be tracked over time
5. It provides critical information for downstream “risk-based colposcopy” follow up
6. It provides important information on vaccinated women with reduced HPV16/18 disease burden

We illustrate these points using data from the BD Onclarity™ PMA and CE Mark trials and examples from recently published literature. We also demonstrate that extended genotyping overcomes one of the primary deficiencies of the 12-other HPV pool screening approach, namely the inability to accurately assess a patient’s CIN3 risk due to masking (when 2 or more HPV types with different absolute risks result in a pooled average risk that is an underestimate of the highest individual genotype risk).
Conclusion

We conclude that extended genotyping permits an expansion of HPV testing to include all elements of risk management: (i) the detection of prevalent CIN3+ disease; (ii) estimating the 3-year risk for future CIN3+; and (iii) permitting unambiguous detection of persistent infections as another indicator of clinical risk.
LONG TERM SCREENING PERFORMANCE OF CYTOLOGY, HPV 16/18 GENOTYPING, AND E6 ONCOPROTEIN IN TRIAGING WOMEN WITH POSITIVE HIGH-RISK HPV TEST IN CHINA

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Background / Objectives

HPV genotyping and cytology test have been recommended for the triage of HPV positive women to colposcopy. E6 oncoprotein is a necessary agent of HPV driven oncogenic transformation as a potential biomarker in triaging HPV positive women. However the clinical performances of HPV genotyping, cytology and E6 oncoprotein test are almost based on cross-sectional studies and no data from prospective cohort are reported in Chinese women. This study was aimed to evaluate the long-term role of cytology, type-specific HPV and E6 oncoprotein triage based on the population-based cervical cancer screening cohort in mainland China.

Methods

We analyzed the cohort database of Shanxi Provincial of Cervical Cancer Screening Study from 2005-2014, 1734 women aged 45–55 were screened by the Hybrid Capture 2 (HC2), liquid based cytology (LBC) tests with experienced cytologist and visual inspection with acetic acid (VIA), and referred to colposcopy and biopsy if any test was positive. HPV16/18 E6 oncoprotein (E6) testing was performed on cervical samples with positive HC2 results. They were followed up in 2010 and 2014 with HPV testing, LBC and VIA (except in 2014). Based on screening results in 2005, cross-sectional and prospective clinical performance by visit, with 5-year and 10-year screening performance of CIN2+ were calculated.

Results

Among 290 HR-HPV positive women in 2005, incident sensitivity detecting CIN2+ for all triage methods decreased while incident specificity increased over time. During
the 10-year follow-up, cytology with ASC-US cut-off had the highest incident sensitivity (95.2%, 92.2% and 91.5% in 2005, 2010 and 2014, respectively), HPV16/18 E6 protein testing had the highest incident specificity (92%, 94.5% and 94.7% in 2005, 2010 and 2014, respectively), and HPV16/18 genotyping were at intermediate efficiency (sensitivity were 71.4%, 68.6% and 63.3%, and specificity were 70.6%, 74.6% and 74.9% in 2005, 2010 and 2014, respectively); positive predictive value of CIN2+ in cytology with ASC-US cut-off, HPV16/18 genotyping, and HPV16/18 E6 protein testing were 96.5%, 88.5%, and 83.3% in 2014, and negative predictive value were 38.8%, 40.0%, and 58.6%, respectively.

Conclusion

Genotyping for HPV16/18 could be recommended in triaging HPV-positive women while in situations without high quality cytologic screening. Positive HPV16/18 E6 protein testing might be a good potential biomarker for triage with its high predictive value of the long-term risk of CIN2+. 
FC 17-11
COMPARATIVE PERFORMANCE EVALUATION OF SCREENING TOOLS FOR POINT OF CARE CERVICAL CANCER SCREENING AND PRE CANCER TREATMENT AMONG WOMEN LIVING WITH HIV: CASE FOR INTEGRATING CERVICAL CANCER SCREENING WITH HIV TESTING AND COUNSELING CENTERS IN RESOURCE LIMITED SETTINGS.

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Background / Objectives

Human Immunodeficiency Virus (HIV) related immunosuppression predisposes co-infection with Human Papillomavirus (HPV) and increases the risk of cervical intraepithelial neoplasia (CIN) and cervical cancer. Centers for Disease Control (CDC) has included invasive cervical carcinoma among the AIDS-defining conditions. India shares 25% of global burden of cervical cancer and a third large burden of HIV-infected women [1,2] who currently continue to live longer due to improved access to antiretroviral therapy. A cross-sectional study was performed to determine the prevalence of HPV infection, risk of cervical pre-cancer and clinical performance validation of Visual Inspection with Acetic Acid (VIA), Pap cytology and HPV tests among women living with HIV/AIDS in Mumbai, India.

Methods

309 HIV Positive women attending cervical cancer screening services in a tertiary care centre between 2009-12 were enrolled for cervical cancer screening using Visual inspection with acetic acid (VIA), Pap cytology and HPV testing. Total 291 HIV Positive women were included in the analysis. 18 were excluded from analysis for incomplete investigations. Screen positive women by either of the screening tests were subjected to histopathology confirmation with colposcopy guided cervical punch biopsy.

Results

Screen positivity rate for cervical cancer screening by VIA, HPV DNA and Conventional Cytology test was 36.1% (105), 32.3% (94) and 16.4% (48) respectively. Using a Histopathology CIN 2+ threshold, the sensitivity of VIA, HPV DNA and Conventional cytology tests were 0.96 (95% CI: 0.78 - 1.00), 0.91 (95% CI: 0.71 - 0.99), 0.64 (95% CI: 0.41 - 0.83) and specificities were 0.70 (95% CI: 0.64 - 0.76), 0.71 (95% CI: 0.65 - 0.76), 0.98 (95% CI: 0.95 - 0.99) respectively.

Conclusion
The prevalence of HPV infection and CIN are significantly higher in the HIV-positive women in India. VIA performed equivalently to currently approved but expensive HPV DNA tests and was highly sensitive compared to conventional cytology. HPV DNA and cytology are currently not feasible in low resource settings due to its logistics, financial and technical requirements. It is feasible to integrate low cost VIA as a point of care cervix cancer screening modality that facilitate “See and Treat” approaches, which would improve compliance to pre cancer treatment with the current HIV testing and counseling centers in the country.

References


A new technique of DNA isothermal amplification techniques in cervical cancer screening

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Background / Objectives

Background: Nucleic acids isothermal amplification techniques are gaining a wide popularity as diagnostic tools due to their simple operation, rapid reaction and without using thermocycler machine, e.g. Aptima HPV E6&7 mRNA test (HOLOGIC). However, mRNA based test asks for strict specimens preservation which limits its use in low resource setting. Isomega HPV test kit (National Bio-Founder, China) is a new HPV DNA based on isothermal amplification technology that can detect 15 high risk HPVs with simultaneous genotyping of HPV16 and HPV18 in a single tube by real-time fluorescence. The test procedure is simple and less than 90 minutes for operation on low cost and simple equipment. Objectives: To evaluate the agreement of HPV detection between Isomega HPV test and cobas 4800 HPV test (Roche) and their performance for cervical cancer screening.

Methods

Methods: Isomega HPV test and cobas 4800 HPV test were performed on cervical specimens collected from 2,774 women aged from 30 to 64 in high risk areas of cervical cancer in China. The sensitivity and specificity in detection of CIN2+, and the agreement between Isomega HPV test and cobas 4800 HPV test were assessed.

Results

Results: The positivity rate of HPV 16 and 18 and other HR-HPV were 3.64%, 1.19%, 13.95% for Isomega HPV test and 3.53%, 1.29%, 13.63% for cobas 4800, respectively. The agreement between two methods was 94.77% (Kappa=0.818) for HR-HPV types, 99.60% (Kappa=0.943) for HPV16, 99.78% (Kappa=0.868) for HPV18 and 93.62% (Kappa=0.744) for other high-risk types. The sensitivity and specificity were 87.76%, 82.86% for Isomega, and 89.80%, 85.06% for cobas 4800.

Conclusion

Conclusions: Isomega HPV test showed high accordance with cobas 4800 HPV test with good sensitivity and specificity, would be a cost-efficient, fast and reliable HPV assay for cervical cancer screening in low resource setting.
Effectiveness of HPV testing in ASC-US to predict HSIL

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Background / Objectives

Cervical cancer screening management is changing drastically since the introduction of human papillomavirus (HPV) tests. HPV testing in ASC-US has been shown to improve the management of ASC-US by a better risk stratification of HSIL+ risk. We aimed to evaluate the accumulative risk at 5 years of HSIL+ at first screening visit for cervical cancer with a particular focus on ASC-US and HPV detection in Catalonia, Spain.

Methods

The study included 169358 women aged 25-65 living in Catalonia (Spain) participating for the first time in the opportunistic screening during 2010-11 at the NHS facilities and follow up until December 2015 for any further cervical cytology record. Kaplan Meier and Cox methods were used to estimate the time to HSIL+ and the hazard ratio of HSIL+.

Results

Women with ASC-US having a negative HPV had a similar HR than those having a negative cytology. While women with ASC-US and a positive HPV test had a similar HR of HSIL+ as that seen for LSIL cases (HR=29.4 and HR=25.5 respectively). Women ASC-US with unknown HPV data had an intermediate HSIL+ risk (HR=15.09).

For the first time in Spain we show the robustness of HPV testing in the prediction of HSIL+ lesions in women with a diagnosis of ASC-US at the primary health level.

A literature review on the topic will be presented.

Conclusion

In agreement with other settings we show the robustness of HPV testing in the prediction of HSIL+ lesions in women with a diagnosis of ASC-US at the primary health level in Spain.
Colposcopic and histopathologic evaluation in women aged 56-64 with HPV-persistence 1 and 3 years, respectively, from the organized primary HPV screening in Sweden.

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Background / Objectives

To evaluate the colposcopic and histopathologic findings in HPV++ women 56-64 years in an organized primary HPV screening program.

Methods

The organized screening program in Stockholm county randomised all resident women 56-60 years to either primary HPV screening with cytology triage or to primary cytology and HPV triaging for LSIL. In HPV screening, HPV+/Cyt- women had a repeat HPV test 1 year later. The repeat HPV test was changed to three years later in May 2013. Attendance rates were similar with the 2 policies. (HPV screen: 7325 women and cytology screen: 7438 women). All HPV++/Cyt- women were invited to colposcopy after 1 year, and HPV++ cyt-/+after 3 years, performed by the same expert gynecologist.

Results

After 1 year 52% (87/167) were HPV negative or declined participation. 80 women had a colposcopy. 74% were persistent for the same HPV type (59/80), the most common type HPV 16(27%). 51% (41/80) had atrophic epithelium. 40% (32/80) a transformation zone (TZ) type 3. 45% (36/80) had a colposcopic lesion, 15% (12/80) abnormal cytology from the endocervix and 24% (19/80) CIN2+ in the cervical biopsy or conisation following the colposcopy. Of the 16 women with HPV 16 persistence at the second visit 6 developed HSIL.

After 3 years, 58% (77/132) were HPV neg or declined participation. So far 36/55 HPV++ women have had a colposcopy and results are available for 18 patients 9 who were HPV++ Cyt-. 8/9 had atrophic epithelium, 6/9 a transformation zone (TZ) type 3, 3/9 had a colposcopic lesion, 4/9 abnormal cytology from the endocervix and 0/9 HSIL in the random cervical biopsy, (no cone biopsies included yet).
Out of 9 patients who were HPV++ and Cyt+ (7/9) had atrophic epithelium, (3/9) a transformation zone (TZ) type 3, (7/9) had a colposcopic lesion, (6/9) abnormal cytology from the endocervix and (2/9) HSIL in the cervical biopsy. More results will be presented.

**Conclusion**

Women were not aware of their randomization however similar attendance rates was noted in both arms. Colposcopic evaluation of cytologically negative, HPV ++ positive women resulted in a PPV for HSIL in histopathology of 24% (19/80) after 1 year. PPV for HSIL in case of HPV 16 persistence was 38% after 1 year. Results after 3 years will be presented.

Atrophy and TZ 3 are challenges while blind biopsies or diagnostic cone biopsies, HPV genotyping and cytology triage including endocervical sampling are options for this group of women.
EVIDENCE FOR CLINICAL APPLICATION OF EXTENDED HPV GENOTYPING IN PERSISTENCE TRACKING AND TEST-OF-CURE: A SYSTEMATIC REVIEW

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Background / Objectives

Management guideline originators have not yet included an analysis of the body of science published during the last decade about the clinical value of extended HPV genotyping (xGT) in follow-up of women with abnormal results and in test-of-cure. This targeted systematic review addresses key questions (KQ) that pertain to the effectiveness of including xGT results with follow-up testing for reducing cervical cancer mortality and incidence. KQ1 evaluates xGT for cumulative risk estimates in the scenario of persistence. KQ2 evaluates xGT for risk discrimination in the follow-up of women with prior abnormal results. KQ3 evaluates xGT in test-of-cure of women with prior treatment of cervical intraepithelial neoplasia (CIN)2 or CIN3.

Methods

We searched the Database of Abstracts of Reviews of Effects, the Cochrane Database of Systematic Reviews, PubMed, and the Health Technology Assessment database from 2000 through 2017 for relevant controlled trials and observational studies. We supplemented by hand-searching of retrieved article reference lists. Eligible studies included prospective studies of women and retrospective studies of residual specimens from women that were screened or tested using human papillomavirus DNA tests. The reference standard was cervical intraepithelial neoplasia 2 (CIN2) or CIN3 or CIN2+ or CIN3+ or invasive cervical cancer (squamous and/or adenocarcinoma). The timeframe was 1-year, or 3-year, or 5-year, or greater than 5-year for persistence tracking. The time frame was 6-month, 1-year, 18-month, or 2-year for follow-up and test-of-cure. Relevance screening, data extraction, risk of bias analyses, and quality assessments were performed. Critical appraisal methodology used design-specific quality criteria from the QUADAS evidence-based quality assessment tool of diagnostic accuracy studies, supplemented by the STARD checklist and GRADE methodology.

Conclusion
The available evidence supports the conclusion that reporting xGT results supports clinical decision making for follow-up of women by discrimination of risk. Based on large studies, xGT appears very promising as follow-up of persistence versus clearance, to discriminate risk and support risk-based clinical action steps by the principle of equal management for equal risk. Models for different management paradigms are described. The information in this report is intended to help guideline panels, policymakers, clinicians, and women make informed decisions about the selection of health care services, is intended as a reference, and not as a substitute for clinical judgment.
RISK OF CERVICAL CANCER AFTER ATYPICAL GLANDULAR CELLS FOUND AT SCREENING IN THE NETHERLANDS

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Background / Objectives

Atypical glandular cells (AGC) abnormalities are less common squamous cell abnormalities in cytology, but can be indicative a number of conditions that vary in severity. Understanding the risk of invasive cervical cancer after AGC are found at screening is important for management of these patients. In this study, we examined the risk of cervical cancer in the next primary screening round after AGC compared to the risk after normal cytology, low-grade squamous intraepithelial lesion/atypical squamous cells of undetermined significance (LSIL/AS-CUS) or high-grade squamous intraepithelial lesion (HSIL) abnormalities.

Methods

Using data from the Dutch Pathology database (PALGA) from 1 January 1996 to 31 March 2014, we conducted preliminary analysis on all primary screening rounds that had cytology results with AGC only, LSIL/AS-CUS only, HSIL only or normal cytology and investigated the result of the next primary screening round. Screening rounds with both squamous abnormalities and AGC cells, or with cancers diagnosed within the same screening round as the abnormality were excluded from analysis. All cervical cancer types were included. Crude rates of cancer diagnosis in the next screening round were calculated for each result category. Follow-up time was calculated from each date of primary smear until either a) the next primary screening round, b) a primary histology diagnosis, c) 8.5 years of follow-up or c) 31st March 2014, whichever came first. Hazard ratios (HR) were calculated using SAS 9.4.

Results

From 9,659,626 cytology smears included in our analysis, 3,156 cervical cancers were identified in the next screening round: 3,037 after normal cytology, 29 after AGC only, 29 after LSIL/AS-CUS only and 61 after HSIL only cytology. Crude rates per 100,000 women-years at risk were highest for the AGC only group (38.8 per 100,000) compared to other groups (normal: 8.7 per 100,000; LSIL/AS-CUS: 12.5 per 100,00; HSIL: 24.6 per 100,000). Compared to normal cytology, the HR was significantly higher for AGC only smears (4.3, 95% CI: 3.0, 6.2), with lower HRs for both HSIL only smears (2.9, 95% CI: 2.2, 3.7) and LSIL/AS-CUS smears (non-significant - 1.4, 95% CI: 1.0, 2.0).

Conclusion
Preliminary results indicate that women with AGC only abnormalities found on cytology screening have a higher risk of a cancer diagnosis at the next screening round compared to women with a squamous cell abnormality. This could be caused by false-negative follow up tests or suboptimal management of these women. Given that with primary HPV screening relatively more women with an AGC smear will be found, gynaecologists need to be aware of this increased cancer risk.
RISK FACTOR ANALYSIS OF RESIDUAL HSIL AFTER LEEP: A CLINICAL STUDY OF 1511 LEEP CASES AT OB&GY HOSPITAL OF FUDAN UNIVERSITY

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Background / Objectives

The purpose of this study was to analyze risk factors of residual cervical high grade squamous intraepithelial lesion (HSIL) after loop electrosurgical excision procedure (LEEP).

Methods

This retrospective study was carried out on 1511 patients with HSIL who underwent LEEP from January 2011 to December 2013 at Obstetrics and Gynecology Hospital of Fudan University. All patients were followed 3-6 months after the LEEP with ThinPrep cytologic test (TCT), HR-HPV test and colposcopy guided biopsy.

Results

Among the 1511 HSIL cases, 57 (3.8%) cases suffered residual HSIL or more serious lesion, including 48 cases with HSIL, 6 cases with invasive squamous cell cancer, 3 cases with adenocarcinoma. Residual rate are different among different age groups. The residual rate was 2.8% (4/143) in the age below 30 group, 3.0% (36/1202) in the age 30-49 group, 10.2% (17/166) in the age beyond 50 group. The residual rate of LEEP positive margin was obviously higher than the negative margin group (7.8% vs 2.9%). Cytologic abnormality showed higher residual than non-residual with significant difference (6.0% vs 1.7%). Patients with positive postoperative hrHPV had a higher residual rate than the negative group (1.4% (10/730) vs 3.4% (7/207)), while there was no statistical significance. There was statistical significance on perimeter and thickness between residual group and non-residual group (2.6±0.8 cm VS 2.9±0.7 cm, 0.6±0.3 cm VS 0.7±0.2 cm (P<0.05)), though depth showed no obvious difference (1.4±0.5 cm vs 1.5±0.3 cm, P>0.05). Different positive margin has diverse residual rate, with endocervical positive margin was 17.5% (10/57), positive margin undetermined was 8.2% (8/97), which is higher than margin negative group (2.9%, 36/1242). Ectocervical margin and deep margin positive showed no difference with margin negative ones. Multivariate logistic analysis showed that age (OR=2.9, P<0.05) and postoperative cytology follow-up positive (OR=3.0, P<0.05) were independent risk factors of residual lesion.

Conclusion
Aging, Endocervical positive margin and abnormal cytology follow-up were high risk factors that lead to postoperative residual lesion. Endocervical positive margin, positive margin undetermined were easier to suffer residual lesion than margin negative and ectocervical positive margin.
FC 18-06
HPV CELL-FREE DNA IN PLASMA AS AN USEFUL MARKER FOR MONITORING RELAPSE OF CERVICAL CANCER

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Background / Objectives

Patients with CC have a relapse rate ranging from 8% to 49%. Within two years of follow up, 62% to 89% of relapses are detected. Nowadays, the tests used to detect recurrence are imaging and cytopathology from the vaginal vault, but there are no available specific tests yet. Cell-free circulating DNA (cf-DNA) is a non-invasive biomarker easily obtained from plasma and serum. Several studies have shown the possibility to detect and quantify nucleic acids in the plasma of cancer patients and that the changes in cf-DNA potentially reflect the changes that occur during tumorigenesis. This non-invasive diagnostic tool may be useful in screening, prognosis and monitoring response to treatment. Therefore, the development and standardization of non-invasive laboratory tests that are able to identify tumour markers and to make early diagnosis of the disease recurrence increase the chance of cure by appropriate treatments. Objective: This study aimed to detect HPV DNA in the plasma of patients with CC, to assess its potential utility as an early marker of recurrence.

Methods

A tumour biopsy and a blood sample of patients with CC, attended in ICESP and HC Barretos, were collected before and after CC treatment. HPV genotyping was performed on the DNA obtained from the tumour. cf-DNA was extracted from plasma samples obtained pre and post-treatment and submitted to type-specific real-time PCR spanning the E6 region from HPV 16 and 18. Patients were followed for at least 2 years after treatment and plasma samples obtained at least once during this period.

Results

137 patients entered the study, 120 bearing HPV-16 positive cancers (87.6%), 12 HPV-18 (8.8%) and five harboured both HPV -16 and 18 DNA (3.6%). The presence of HPV DNA in pre-treatment plasma was observed in 58.8% (77/131) with viral load ranging from 204 copies / ml to 2,500,000 copies / mL. HPV DNA frequency in pre-treatment plasmas increased with advancing clinical tumour stage: I - 45.2%, II - 52.5%, III - 80.0% IV - 76.9%, (p = 0.0189). The presence of HPV DNA in the post-treatment plasma was detected in 27.3% (30/110). The average time of relapse was
3.1 years (2.7 to 3.5 years). HPV DNA was evidenced up to 460 days before clinical diagnosis of recurrence. Patients who had the presence of HPV DNA in plasma after treatment had a worse prognosis compared to those who were negative, strongly correlating to poor survival rates and shorter disease-free time intervals.

**Conclusion**

In patients with CC, HPV-DNA in the plasma can be a useful early marker for monitoring relapse and progression of the disease.
PREVALENCE AND RISK FACTORS FOR MULTIZONAL NEOPLASIA IN A COHORT OF HIGH-RISK WOMEN


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Background / Objectives

Multizonal Anogenital Neoplasia is defined as the presence of high-grade squamous intraepithelial lesion (HSIL) or carcinoma concurrently in two or more of the following sites or zones: Perianus, Anal canal, Vulva, Vagina or Cervix. In each zone, disease may be uni- or multifocal. Homerton Anal Neoplasia Service (HANS) is a tertiary referral centre, receiving referrals from across the UK. It screens women at risk of HPV related dysplasia or carcinoma with a multizonal assessment and provides treatment of HSIL. Multizonal assessment is a thorough examination of the genital tract, performed with the woman in lithotomy position with 5% acetic acid applied to the cervix, vagina, vulva, perianus and anal canal, which is then visualised using colposcope as a microscopic aid. Biopsies are taken of suspicious areas.

Aims: Identify disease prevalence in the anogenital region in a cohort of women at high-risk of multizonal neoplasia

Methods

Retrospective case note review of one hundred women referred to the HANS since January 2011 for multizonal assessment.

Results

Women were referred to the HANS Unit for multiple reasons, most commonly for known persistent vulval HSIL (n=19) and widespread anogenital dysplasia (n=17). Mean age was 49 years (range 26-85 years) with median follow up of 24 months. Half the women (50%) were immunosuppressed. Twenty six women had previous vulvectomy for HSIL, 13 women had a hysterectomy for cervical HSIL (CIN) and 25 women had a previous anogenital cancer. All women underwent a multizonal assessment and 42 women were found to have two or more zones of high grade dysplasia. Sixteen women with no perianal disease had anal canal HSIL, one of which had cancer. Over one quarter of women had a new zone of HSIL diagnosed (n=27). Six occult cancers were found at first appointment and two women progressed to cancer over follow up, one had a severe immune defect and the other woman was likely to have had an occult cancer at initial presentation.

The risk factors for multizonal neoplasia were analysed. Twenty seven women (64.3%) were immune suppressed in the multizonal neoplasia group (42) compared to twenty three (39.7%) of those without multizonal neoplasia (58). A history of
previous anogenital cancer was more common in the multizonal neoplasia group (38.1%) than in the women without multizonal disease (15.5%).

**Conclusion**

The absence of perianal disease does not preclude the presence of anal HSIL or occult cancer. Concentrating only on the referral zone would miss HSIL disease in other related anogenital tract sites. Multizonal assessment is essential to diagnose occult areas of HSIL or carcinoma in high risk women.
Is p16/ki67 dual-stained cytology essential at a colposcopy department?

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Background / Objectives

The main decrease of cervical cancer mortality and incidence was provided by cytology-based screening programs. The interpretation of cytology is pathologist-dependent, and due to that some countries shift screening to human papillomavirus (HPV) DNA detection. HPV test has higher sensitivity and higher negative predictive value for detection of cervical intraepithelial neoplasia (CIN). Nevertheless, HPV DNA testing has a lower positive predictive value, because most high-risk infection clear spontaneously. Recently, a widely-studied biomarker, p16/Ki-67, demonstrates to be useful on limiting the unnecessary referrals to colposcopy units. This property is based on the fact that simultaneous presence of p16/Ki-67 indicates deregulation of cell-cycle, mediated by transforming high-risk HPV infections.

The main goal of the study was to evaluate the utility of p16/Ki-67 dual-stained cytology, for identification of CIN in high-risk HPV-positive women, previously referred to a colposcopy unit.

Methods

It was performed a prospective cohort study of 46 high-risk HPV-positive women followed at a colposcopy unit. They were evaluated by p16/Ki-67 dual-stained cytology from February 2016 to March 2017. Statistical analysis was performed by STATA 13.1 program.

Results

The women included at the study, had a median follow-up time at the unit of three years (p5:6 months; p95:5 years) and a median age of 44 years (p5:29; p95:62). Of high-risk HPV-positive women, thirty-three (72%) had negative (NILM) cytology, ten (22%) had low-grade squamous intraepithelial lesion (LSIL) and three (6%) abnormal squamous cells of undetermined significance (ASC-US). Positive p16/Ki-67 was identified in twelve women with NILM cytology, in six with LSIL and in one with ASC-US cytology. The positive predictive value to CIN was 81.8% (95%CI:48.2-97.7) with a sensitivity of 90.0% (95%CI:55.5-99.7).

At the same sample, liquid-based cytology (ThinPrepÔ) had a sensitivity of 60.0% and specificity of 89.5%, HPV test (CobasÔ) had a sensitivity of 91.7%, specificity of 20.7% and a positive predictive value of 32.3%. Colposcopy had a sensitivity of 87.5% and a positive predictive value of 80.8%.
From positive p16/ki67 dual-stained cytology, 58% women were submitted to expectant treatment and 42% to invasive one. All women maintain medical surveillance at the unit.

Conclusion

The p16/Ki-67 dual-stained cytology demonstrates high sensitivity and positive predictive value to intraepithelial neoplasia however, it will be needed a larger sample and a higher time of follow-up to generate solid conclusions.
FC 18-09

CLINICAL-PATHOLOGICAL VARIABLES ASSOCIATED WITH CERVICAL CONIZATIONS SPECIMENS WITHOUT HIGH-GRADE INTRAEPITHELIAL LESION: A STUDY OF 221 CASES.

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Background / Objectives

Comparison of clinical-pathological variables in a series of cervical conizations associated with the presence or absence of high-grade intraepithelial lesion or carcinoma.

Estimation of the utility of the immunohistochemical staining for p16 to identify conization specimens without intraepithelial lesion or carcinoma.

Methods

We reviewed our conizations from 2010 to 2016. In every cases without ≥ CIN 2 ("negative cone"), 3 hematoxylin and one p16 slides were added. Former colposcopy biopsies were reassessed.

The clinical variables were: age, smoking, spreading of the colposcopic lesions, number of colposcopic biopsies, vaccination status against HPV prior to conization.

The collected pathological features were: former cytology result; HPV status and colposcopic biopsy diagnosis prior to conization; lesion length and intensity of the inflammatory infiltration in the colposcopic biopsy; initial and final diagnosis in negative cones after morphological reassessment and hematoxylin and p16 additional slides.

Pap smears were performed in liquid medium. HPV determination was performed with COBAS® 4800 HPV Test

Results

There were 221 conizations, with a mean age of 38 years and a 50.8% of smokers. In a 71.9% of the cases, the previous cytology was ≥ASCUS. In a 99.1% of women, HPV status was positive, being a 54.5% of them positive to HPV16 and 48.4% to neither types 16 or 18.
There were a 59,5% of the lesions affecting an unique cuadrant, with a 60,3% of single biopsies. A 55,9% of the women were vaccinared between the colposcopic biopsy and conization. A 97,1% of the colposcopic biopsies were ≥ CIN 2, with an average lenght of 3,37 mm. A 35,3% of such biopsies showed moderate to intense inflammatory infiltration.

Initially there were 44 (19.90%) "negative cones", which was shorten to 27 (12.21%) after morphological reappraisal and hematoxylin and p16 addition, being a 38,63% reduction. 4 " negative cones" were retrieved due to the morphological review, another 12 by using p-16 and, finally, one case was reclassified as low grade intraepithelial lesion.

Comparing clinical and pathological items we only found a statistical trend to younger age and lower lesion spreading in the "negative cones" and a statistical significance in favour to a larger size of colpscopic lesions in pathological cones.

**Conclusion**

In our series we can hardly see statistically significant differences between clinical-pathological variables of the pathological cones and the negative cone.

Before establishing a diagnosis of negative cone we should completely reassess the case and to perform immunohistochemical study for p16.

**References**

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COMPARISON OF PAIN CONTROL BY LIDOCAINE SPRAY AND PARACERVICAL BLOCK DURING LOOP ELECTROSURGICAL PROCEDURE: A RANDOMIZED CONTROL TRIAL

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Background / Objectives

Cervical cancer is second most common new case and third most common cause of death of cancer patient in developing country1. The loop electrosurgical excision procedure (LEEP) is worldwide used for diagnosis and treatment in CIN2-3, precancerous lesion since 19902. The anesthetic technique common use during LEEP are paracervical block and submucosal block3. The paracervical has some risk for local anesthetic systemic toxicity4. Açmaz et al report adverse effect from paracervical block 2 patients have bradycardia5. The new anesthetic technique lidocaine spray has report that effective use during endoscopy6, intubation7, fractional and curettage8, suctional and curettage5 and LEEP9.

This study is compare effective of pain control between paracervical block and lidocaine spray and adverse effect.

Methods

A randomized control trial was conducted in 132 women who underwent LEEP of cervix. The participants were randomly allocated to two groups. PB group were anesthetized by standard paracervical block using 10 mL of 2% lidocaine with 1:100,000 of epinephrine at 4 and 8 o’clock locations. LS group were locally anesthetized by four puffs (40 mg) of 10% lidocaine spray applied thoroughly to the cervix. The pain score at during anesthesia, during excision and 30 minutes post excision were compared.

Results

A total of 132 LEEPs were performed with 66 participants in LS group and 66 participants in PB group. The age, parity, menopausal status, indications and tissue specimen volume were not significant difference in both groups. The mean pain scores during excision were 5.2±0.3 in LS group and 4.1±0.4 in PB group (mean difference 1.1, 95%CI 0.8-2.1, p value=0.03). The pain score during anesthetized was significant lower in LS group (2.0±0.3 vs 3.1±0.3, mean difference 1.1, 95%CI 0.3-1.9, p value=0.008). There had no adverse effect in LS group compare with 8 cases in PB group (tinnitus, numbness and tachycardia with hypertension).

Conclusion
The pain score in local 10% Lidocaine spray group was significant higher from standard paracervical block during the LEEP procedure of cervix but had less adverse effect.

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References


CROSSWALKING EUROPEAN GUIDELINES ON THE MANAGEMENT OF VAGINAL DISCHARGE AND THE MANAGEMENT OF STI

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Background / Objectives

Screening guideline originators (WHO, IUSTI, CDC, country-specific) have not included STI testing within the contextual vaginal discharge or vaginitis guidelines and have not included vaginitis testing within the contextual STI screening and management guidelines. Women may present with a request for STI screening and have vaginitis, with or without an STI. Women may present with a complaint of abnormal vaginal discharge and meet criteria for STI testing, but this is not explicitly stated in vaginitis guidelines.

Methods

We searched PubMed, Guideline International Network, WHO, IUSTI, CDC, and the Worldwide Web from 2010 through 2017 for relevant guidelines. We supplemented by hand-searching of retrieved guideline reference lists. Eligible guidelines publications included screening for STIs in women and/or included diagnostic guidance for women presenting with abnormal vaginal discharge or symptoms of vaginitis or symptoms of vaginosis. Relevance screening, data extraction, and quality assessments were performed. Critical appraisal methodology used design-specific quality criteria from the AGREE reporting checklist, RIGHT reporting checklist, and the Standards for Developing Trustworthy Clinical Practice Guidelines.

Conclusion

The review supports the conclusion that clinical practice guidelines about STI screening and management do not provide guidance for concurrent diagnosis and management of vaginitis or vaginosis. Similarly, clinical practice guidelines about diagnosis and management of abnormal vaginal discharge or symptoms of vaginitis do not provide guidance for concurrent screening and diagnosis of STIs. Guideline panels could choose to add content to their specific guideline, or could choose to add linkages to relevant guidelines. Crosswalking of European guidelines are tabulated. The clinical criteria for STI screening that should be checked during a problem visit for vaginitis are provided. The clinical criteria for vaginal discharge assessment for vaginitis that should be checked during a visit for STI screening are provided. The information in this report is intended to help guideline panels, policymakers,
clinicians, and women make informed decisions about the selection of screening and diagnostic services, is intended as a reference, and not as a substitute for clinical judgment.
FC 18-12
RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY (REIMS) IKNIFE AND ITS CLINICAL APPLICATION IN THE TREATMENT OF CERVICAL ABNORMALITIES

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Background / Objectives

Cervical cancer and its precancerous form cervical intraepithelial neoplasia (CIN) commonly affect women of reproductive age. Fertility-preserving trachelectomy procedures are available, but if the excisional margins are not cancer-free, as is the case in 33% of procedures, these women must undergo a hysterectomy, therefore losing their child-bearing potential. Rapid Evaporative Ionization Mass Spectrometry(REIMS) analyzes electrosurgery-generated aerosols, using time-of-flight mass spectrometry to provide real time tissue identification without the need for sample preparation, raising the potential for use as an intraoperative diagnostic technique and improving the surgical and fertility outcome for one third of the women who undergo trachelectomy. We conducted a pilot study showing that REIMS can differentiate between cancerous and healthy cervical tissue thus presenting an innovative technique that could drastically improve fertility-sparing operations.

Methods

Cervical biopsies of 66 women were cut using a Covidien diathermy hand-piece. The surgical aerosol produced was transferred into a Waters Xevo G2-S mass-spectrometer. The tissue samples were then stained for histopathological validation. These diagnoses were used in multivariate statistical analysis of mass spectroscopic spectral data, including principal components and linear discriminant analysis performed using Offline Model Builder software. Correct classification rate was checked using leave one patient out cross-validation.

Results

The study showed correct classification with REIMS of 91%, with correct identification of cancer tissue of 88.5% and of healthy tissue of 92.5%. Ongoing sample processing is currently being undertaken to investigate the correct classification rate with REIMS between the different grades of CIN.

Conclusion

Frozen section is the current method for intraoperative assessment of margin status at the time of trachelectomy, and the concordance between intraoperative frozen
section and final histology has been quoted as 84%, significantly lower than the preliminary results of REIMS. In addition to providing real-time information, thus reducing anaesthetic time, REIMS has the potential to improve the accuracy of intraoperative margin detection. This could potentially increase success rates of trachelectomy, leading to a truly advanced fertility sparing technique in modern surgery. This principle is also under investigation for its use in CIN to be ruled out into the colposcopy clinic.
AGE DISTRIBUTION AND PROBABILITY OF HYSTERECTOMY IN GERMANY

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Background / Objectives

Hysterectomy is the most common gynecological surgery in many industrialized countries. In Germany, the hysterectomy rate is high in comparison to other European countries. The aim of this analysis was to determine the distribution of age at hysterectomy as well as the age-specific probability of undergoing a hysterectomy between the ages of 0-64 in the German female population.

Methods

Analyses were based on data from the MARZY study, a prospective, randomized, population-based cohort study investigating early detection of cervical cancer in western Germany. At baseline, 6 429 women were invited to attend cervical cancer screening. The distribution of age at hysterectomy as well as indications for hysterectomy were reported. Based on survival analysis, which accounts for censoring at the age of interview, and the inverse probability weighting (IPW) method, the age-specific probability of undergoing a hysterectomy was estimated. The IPW method corrected for missing date of hysterectomy. Simulated calendar-period specific survival curves (1939-1979, 1980-1989, 1990-1999, 2000-2006) were computed to show how age and calendar year determine the probability of undergoing a hysterectomy.

Results

Data on hysterectomy were available for 4 719 women. Of these, 961 women (20.4%) had undergone a hysterectomy. The main indication for hysterectomy was uterine fibroids (48%). A total of 850 women (88.4%) reported a date when their hysterectomy had been performed. The highest proportion of women were hysterectomized between the ages of 40-44 (24.6%). The IPW corrected probability of having a hysterectomy between the ages of 0-64 was 0.354. The age-specific probability of hysterectomy was highest in the 45-49 year age group (0.078). The age-specific probability of hysterectomy decreased between the years 1939 to 2006.

Conclusion
Data from the MARZY study allowed valuable conclusions to be drawn about the distribution of age at hysterectomy as well as the age-specific probability of undergoing a hysterectomy in Germany.
Type-specific Human Papillomavirus DNA Load in Association with Prospective Risk of Cervical Intraepithelial Neoplasia: A Useful Triage Tool

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Background / Objectives

The ASCCP guidelines recommend referring non-16/18 high-risk human papillomavirus (HR-HPV) positive women with cytology ≥ASCUS (ASCUS+) to colposcopy. In low-resource areas without cytology, other screening methods are needed to triage HPV positive women. Using direct prospective evidence, our study compared the risk of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) in non-16/18 HR-HPV and HPV 16/18 positive women stratified by viral load versus cytology, and evaluated the risk of CIN2+ by type-specific HPV viral load.

Methods

Using China's SPOCCS1 cervical cancer screening cohort, where 1,742 women were screened by liquid-based cervical cytology and Hybrid Capture 2 (HC2) at five-year intervals from 2005 to 2014, all HC2 positive samples were genotyped. Semi-quantitative viral load was measured by HC2 relative light units, with categories of low (1-9.99), moderate (10-99.99), and high (≥100) viral loads. Kaplan-Meier methods were used to estimate the ten-year cumulative incidence rate (CIR) of CIN2+ among pooled and type-specific HR-HPV women with baseline low, moderate and high viral loads, or ASCUS+.

Results

Among the 209 HR-HPV positive women at baseline, the highest CIR of CIN2+ were for women infected with HPV 16/18 (N=38/57), HPV 16 (N=36/57), non-16/18 HR-HPV (N=19/57), HPV 31 (N=7/57), and HPV 58 (N=7/57). Any HR-HPV, HPV 16/18, non-16/18 HR-HPV or HPV 16 positive women had significantly higher CIRs of CIN2+ at moderate or high viral loads than at low viral loads. Similar trends were observed for HPV 18, 31, 33, 52, and 58 positive women, but limited sample sizes prevented reaching statistical significance. For HPV16/18 women, the CIR of CIN2+ with ASCUS+ was 55.8% (95%CI: 42.2-67.4%) and CIRs with low, moderate, and high viral loads were, respectively, 25.7% (95%CI: 9.3-46%), 43.2% (95%CI: 26-59.3), and 62.6% (95%CI: 40.6-75.5%). For non-16/18 HR-HPV women, the CIRs of CIN2+ at low and moderate viral loads were, respectively, 5.1% (95%CI: 0.9-15.2%)
and 18.2% (95%CI: 21.6-54.9). The CIR of CIN2+ for non-16/18 HR-HPV positive women with ASCUS+ was 34.6% (95%CI: 21.9-47.6%) and comparable to that of non-16/18 HR-HPV high viral load (37.8%, 95%CI: 21.3-54.3%) (P>0.05).

Conclusion

HPV16/18 and non-16/18 HR-HPV viral loads could predict the ten-year CIR of CIN2+. HPV16/18 positive women should be directly referred to colposcopy regardless of viral load, as those with even a low viral load had a notably high risk of CIN2+. For non-16/18 HR-HPV women, using a viral load cutoff of ≥100 RLU had a comparable CIR of CIN2+ compared to ASCUS+ and presents a valuable triage tool for non-16/18 HR-HPV women in areas where cytology is not readily available.
WHOLE-GENOME SEQUENCING ANALYSIS OF HPV18 DIVERSITY IN THE NETHERLANDS

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Background / Objectives

Whole-genome Sanger sequencing was used to identify HPV18 variant diversity in the population. In addition conservation of HPV18 infections over time and the occurrence of HPV18 type-specific (TS) reinfection events were assessed. SNPs occurring in clearing infections and persistent infections were compared to identify possible differences between groups.

Methods

Vaginal self-samples were collected annually in up to four rounds from women (16-29y) participating in the Chlamydia trachomatis Screening Implementation program in the Netherlands. HPV-DNA detection and genotyping was performed using the SPF10-DEIA-LiPA25 system. Persisting and clearing HPV16 infections were selected and subjected to Sanger WGS. Persisting infections were defined as HPV18 positive by genotyping at two subsequent sampling moments. Clearing infections were defined as HPV18 positive initially, with at least one HPV18 negative follow-up sample.

Results

Complete genome sequences were obtained from 51 study participants having 24 persistent and 27 clearing infections, resulting in a total of 52 unique HPV18 variants (including one HPV18 reinfection event). Persistent infections were completely conserved through time, with up to three years between the initial sample and the last follow-up sample. One reinfection event was identified, initially considered a persisting HPV18 infection, with unique HPV18 variants at both sampling moments. The identified variants predominantly clustered with sublineages A3 (31 variants) and A1 (11 variants). Other sublineages identified in this cohort are A4 (2 variants), A5 (1 variant), B1 (5 variants) and B2 (1 variant). Although the dataset size is limited, SNP comparison did not identify strongly acting nucleotide differences resulting in infections clearing or persisting.

Conclusion

A remarkably high HPV18 variant diversity was found in a Dutch cohort from sequencing 51 HPV18 infections. Lineages A1 and A3 are predominantly present in the Netherlands. Persistent infections are completely conserved through time with up to three years follow-up. One HPV18 variant reinfection event was identified, initially considered a persistent HPV18 infection by conventional genotyping.
FC 19-04
NATIONWIDE AND COMPREHENSIVE HUMAN PAPILLOMAVIRUS GENOTYPING OF INVASIVE CERVICAL CANCERS

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Background / Objectives

The Swedish National Cervical Screening Registry collects and evaluates nationwide, comprehensive data as a basis for optimization of organized cervical cancer prevention. Most data are imported via exports from administrative databases at the healthcare providers, but the comprehensive biobanking system of all cases of cancer in the nation is also a carrier of population-based data that could be read and imported to the registry. As a proof-of-principle, we identified all cervical cancers diagnosed in Sweden during a 10 year period (2002-2011; 4254 confirmed cases), requested the archival blocks and subjected them to HPV genotyping.

Methods

The Swedish Cancer Registry was used to identify the cases and the diagnosing pathology lab. The diagnostic blocks were requested and sectioned at an accredited sectioning company. In between each case block, a blank block containing only paraffin was sectioned, as a control for contamination. The blank-block had to be negative in all tests and the case-block positive for beta-globin. Following DNA extraction, HPV genotype data were retrieved using beta-globin real-time PCR and HPV genotyping using modified general primer (MGP)-PCR (primer target L1) and Luminex. Confirmed invasive cancers that were “HPV-negative” by Luminex were also analysed by real-time PCR for HPV16 and HPV18 (primer target E7 and E6), and will be sequenced using Illumina technology.

Results

Although all pathology biobanks were - in principle - open access, not all agreed to participate. 2954/4254 (69%) cases were actually included and valid genotyping data could be obtained for 2852 cases (97% of included). The most common type was HPV16 (60% of HPV-positive, 50% of all valid cases), followed by HPV18 (19%/15%), HPV45 (8%/6%), HPV31 (3%/2%), HPV33 (2%), HPV52 (2%), HPV39 (1%), HPV70 (1%), HPV56 (1%), HPV35 (1%), HPV58 (1%) and HPV59 (1%). 96% of all HPV-positive tumours contained only one HPV type. Real-time PCR for E6/E7
performed only for “HPV negative” cases found that 12% of these were HPV16 positive and 7% were HPV18 positive.

**Conclusion**

Nationwide, comprehensive HPV genotyping of consecutive series of invasive cervical cancers is readily doable as part of the data importing tasks of a cervical screening registry, improving the possibilities to monitor the effectiveness of cervical cancer prevention and continued monitoring of the HPV-type specific disease burden.
HPV PREVALENCE AND RISK FACTORS ASSOCIATED WITH HIGH RISK TYPES IN A LOW INCOME POPULATION

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Background / Objectives

HPV is the most common sexually transmitted infection and is associated with cervical cancer in women – the fourth cause of mortality of female cancer in Brazil. Surveys on HPV prevalence and lifestyle factors relevant to HPV transmission are essential to monitoring the infection and plan prevention programs in low income countries to fight against HPV related cancers. Therefore, we aim to estimate the prevalence of genital HPV in women and men and associated lifestyle behavior.

Methods

A total of 860 women and men aged 16-25 years old of Northeast Brazil, enrolled in the POP-Brazil study, an ongoing nationwide HPV prevalence study, enrolling 7,505 participants between October 2016 and June 2017. DNA was extracted from specimens collected in Primary Care Units and HPV genotyping was performed by polymerase chain reaction amplification followed by hybridization (Linear Array Roche®). Demographic and sexual behavior were gathered by interview in the Primary care unit.

Results

The demographic characteristics of the sample reflect the general population of the region: the mean age was 20.5 (± 2.8) and the majority self-declared as brown skin color (64.4%), with household income less than US$ 500.00 per month. The overall prevalence of HPV was 56.2% (95% CI 50.0-62.4) and the prevalence of high-risk types was 38.2% (95% CI 32.1-44.2). Education level (PR=1.2; p= 0.42) and household income (p= 0.46) were not associated with high risk HPV. HPV presence was associated with smoking (PR=1.8; 0.007) and drug use (PR=1.4; 0.05) but not with alcohol consumption (PR= 1.3; p=0.15). Young people with HPV have higher number of sexual partner (PR=1.2; p< 0.01) but the age of first sexual intercourse was not associated (PR=1.04; p=0.2). Same-sex relationships did not increase the prevalence (PR=1.0; p>0.05) as well as the use of condom (PR= 1.1; p=0.54). The most prevalent types were 16 (11.2%), 58 (6.0%) and 59 (5.2%).

Conclusion
Brazilian infections by all HPV types and high-risk types in young people from Northeast region is very high. Certain lifestyle factors as smoking, drug use and number of sexual partners are associated with an increased prevalence of infection. Our data on HPV prevalence and behavior are crucial for evaluation of the effectiveness of the existing HPV vaccination recommendation in Brazil and planning of preventive strategies to reduce the burden of cervical cancer and other HPV-related tumors.
Detection of HPV- ZIKV Co-infections in Ecuadorian Women Using Two Real-Time PCR-based Methods in Cervical Cytology Samples

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Background / Objectives

Human papillomavirus (HPV) is the cause of cervical cancer. Zika virus (ZIKV) infection during pregnancy has been linked to birth defects. There is limited information about the detection of co-infections with these two pathogens.

We had the objective to detect the presence of HPV and ZIKV in cervical cytology specimens from 109 healthy women attending the Hospital Luis Vernaza (Guayaquil, Ecuador).

Methods

All samples were tested for high risk HPV genotypes (HPV-HR) using the Cobas HPV 480 and for ZIKV using a validated, laboratory-developed real-time RT-PCR (the ZCD assay). Cobas HPV has specific call-outs for HPV 16 and HPV 18 and combines detection of the remaining 12 genotypes.

Results

Patient age range was 22 to 68 years, with a median of 39.34[JJW1]. HPV DNA was detected in 19 women (17.43%). Eighteen (16.51%) were positive for a single marker (HPV-HR, HPV-16 or HPV-18) while one (0.9%) was infected by both HPV-16 and HPV 18. ZIKV was detected in 18/109 (16.51%) patients. Of the ZIKV positive women, the majority were under the age of 45 (13/18[JJW2] vs 5/18). We found 4 cases of HPV- ZIKV co-infection: two patients were positive for HPV-HR (ages 41 and 53) and one patient each was positive for HPV 16 (age 49) and HPV 18 (age 26). All four women were asymptomatic from the ZIKV infection.

Conclusion

These data on ZIKV detection provide additional supporting evidence for female-male transmission and demonstrated to the potential utility of screening for ZIKV in
cervical cytology specimens. To the best of our knowledge, this is the first report of HPV-HR-ZIKV co-infections.

References


FC 19-07
HPV16/18 E6 ONCOPROTEIN EXPRESSION IN INFECTIONS WITH SINGLE AND MULTIPLE HPV GENOTYPES AND ASSOCIATED THE RISK OF CERVICAL DISEASE

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Background / Objectives

To characterize the likelihood of HPV16/18 to express E6 protein in single and multiple type HPV infections, and to analysis their risk of cervical disease.

Methods

Women with normal histology (n=773), CIN1 (n=62), CIN2/3 (n=130), and cervical cancer (n=466) were analyzed for presence of 14 types of high-risk HPV DNA and HPV16/18 E6 oncoprotein using BD onclarity™ and OncoE6™ assay.

Results

Of 1431 subjects, 546 (38.16%) tested positive for HPV16/18. The frequency of E6 oncoprotein expression was significantly higher in single infections than in multiple infections for both HPV16-E6 and HPV18-E6 (HPV16: 80.2% vs. 61.6%, p<0.001; HPV18: 75.7% vs. 47.6%, p=0.011). In HPV16/18 coinfection, the positivity rate was 44.4% for HPV16-E6 protein and 40.7% for HPV18-E6 protein. Only two cases showed expression of HPV16-E6 and of HPV18-E6 at the same time. The overall positivity rate of HPV16 and HPV18 oncoprotein expression in HPV16/18 coinfection subjects was 77.8%, almost the same as in single infection (HPV16: 80.2%; HPV18: 75.7%). Multiple HPV infection clusters most likely to express E6 were HPV16/52 (71.4%), followed by HPV16/51 (60.0%), and the less were HPV16/45 (14.3%). In CIN2+, E6 positivity was 86.5% for HPV16 and 89.7% for HPV18 single infection, significantly higher than 70.5% for HPV16 (P<0.001) and 56.6% for HPV18 in multiple infections (P=0.004).

Conclusion

HPV16/18 E6 is more likely to express in women with single HPV infection than in women with multiple HPV. Multiple HPV infection clusters show distinctive propensity to express E6 oncoproteins. This could relate to possible intergenotypic competition as consequence multiple infections.
HUMAN Papillomavirus Prevalence in Portugal and Its Association to Other Microbial Pathogens

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Background / Objectives

Although Human Papillomavirus (HPV) is a necessary cause of cervical cancer, it is not a sufficient cause, so, others cofactors like co-infection with microbial pathogens could increase that risk. There is an association between the prevalence of high risk HPV genital infection and cervical cancer in adult women.

Methods

We studied 1067 patients mean age 37.2±9.9 (range: 17-76 years old) from multicenter hospitals for HPV detection and a sub-sample of these patients (n=172) for HPV and microorganism detection of the Gynecology/Oncology ambulatory of Santiago Hospital. The cervical samples were obtained for cytology, HPV, Ureaplasma parvum, Ureaplasma Urealyticum, Mycoplasma Genitalium and Mycoplasma Hominis detection. The method used for HPV detection and genotyping determination was Polymerase Chain Reaction followed by hybridization. The statistical methods used were Chi-square, ANOVA and binary logistic regression (SPSS v.22). Significance was attributed if P<0.05.

Results

From 1067 patients, 314 (29.4%) had HPV DNA positive among women with normal and abnormal cytology. The incidence of HPV DNA positive was highest in women aged 20-40 years old (n=196, 62.4%). We identified 33 HPV types, which HPV 16 was the most predominated (n=60 (14.6%) followed by HPV types 31 (n=39, 9.5%), 51 (n=38, 9.2%), 52 (n=36, 8.8%), 53 (n=35, 8.5%), and 66 (n=27, 6.6%). The HPV genotypes were classified in low-risk (LR) (n=39, 12.4%), high-risk (HR) (n=286, 91.1%), 2 or more HPV types (n=97, 30.9%). For molecular diagnostic tests, we found that the majority of women with HPV DNA positive presented normal cytology (n=176, 56.1%), followed by atypical squamous cells of undetermined significance
(n=100, 31.8%), 35 (11.1%) with low-grade squamous intraepithelial lesions and 3
(1.0%) with high-grade squamous intraepithelial lesions. In a sub-sample (n=172), we
found that the presence of genital microorganisms was increased 3.0 times with HPV
infection (OR=3.0, 95%CI [1.2-7.9], P=0.019). Furthermore, 2 or more HPV types
increased this risk for 3.2 times (OR=3.2, 95%CI [1.3-8.1], P=0.015). Ureaplasma
parvum was the microorganism more prevalent (71.4%); being 23.3% HPV positive
and 24.1% with 2 or HR of detected HPV.

Conclusion

The presence of genital microorganisms was increased with HPV infection, being the
presence of Ureaplasma parvum associated to HPV positive. We propose that the
screen for the presence of different microorganisms could be important in prevention
of severe dysplasias.
Population-based study on distribution of HPV infection and its risk factors among women in Inner Mongolia, China

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Background / Objectives

To explore the epidemiologic characteristics of human papillomavirus (HPV) infection among women from Jungar banner, Inner Mongolia, China, and analyze the related risk factors.

Methods

The cervical cancer screening program for women aged 35-64 has been conducted in Jungar banner, Inner Mongolia, China in 2016. CareHPV was applied as primary screening method followed by visual inspection with acetic acid/Lugol’s iodine (VIA/VILI) as triage method. The HPV and VIA/VILI positive women were referred to colposcopy and biopsy if necessary. Chi-square and stepwise logistic regression analysis were performed by using the SPSS21.0 statistical software.

Results

A total of 7659 women received careHPV test were included in the final analysis. Average age of the study population was (45.55±7.37) years. The overall HPV infection rate was 14.60%, and there was no difference between Han and the Mongol nationality with the infection rates of 14.54% and 15.26%, respectively (P > 0.05).

The HPV infection rates in ≤CIN1 (normal or cervical intraepithelial neoplasia grade 1) and CIN2+ (cervical intraepithelial neoplasia grade 2 or higher) were 14.14% and 82.98%, P=0.00; The HPV infection rates were different among the different age groups: 13.71% (≤44 years old), 14.26% (45-54 years old), 18.74% (≥55 years old), (χ²=15.93, P=0.00). Single factor logistic regression analysis shows that the age of sexual debut (≤20 years old, with OR=1.45, 95% CI:1.23-1.72), the first childbirth age (<25 years old, with OR=1.21, 95% CI:1.04-1.39), unmarried (OR=1.76, 95% CI:1.05-2.95), education levels at high school and below (OR=1.43, 95% CI:1.18-1.74), the history of reproductive diseases (OR=1.29, 95% CI:1.11-1.49) and multiple parity (>2 times with OR=1.23, 95% CI:1.02-1.47) would increase the Han's risk of HPV infection. However, only the first childbirth age (<25 years old) was significantly related with HPV infection in Mongol women with OR=1.60, 95%
Multi factor unconditional logistic regression revealed that the age of sexual debut, marital status, the history of reproductive diseases were significantly connected with HPV infection.

**Conclusion**

The overall HPV infection rate of women in Inner Mongolia was higher than that in rural areas of China. HPV positivity among women with CINII+ was significantly higher than that among women with normal cervix or CINI. Risk factors of HPV infection were different between Han and the Mongol nationality: Cervical cancer prevention propaganda should be strengthened to reduce the disease burden of cervical cancer.
Effects of vaccination on the epidemiology of HPV67 in a Belgian routine setting

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Background / Objectives

HPV vaccination programs with the HPV bivalent (HPV16/18) vaccine (Cervarix®, GlaxoSmithKline Biologicals, United Kingdom) or the recombinant HPV quadrivalent (HPV16/18/6/11) vaccine (Gardasil®, Merck & Co, Inc., USA) have been implemented in the majority of industrialized countries. According to the International Agency of Research on Cancer (IARC, World Health Organization, France) HPV67 is subdivided as a possible high-risk HPV. This study was performed to broaden the epidemiological knowledge of HPV67 in a Belgian Routine setting, and to evaluate the potential influence of vaccination status on its prevalence.

Methods

In total, 478,822 samples were evaluated with the Riatol qPCR assay. Self-reported vaccination status was known for 376,905 samples. A subset of 22,878 women reported to be vaccinated.

Results

HPV67 is found in 1.23% (95% CI: 1.20%-1.26%) of the screening population, with a significant (p<0.001, Pearson's Chi Square Test) higher prevalence in the diagnostic population 4.16% (95% CI: 4.04%-4.28%). In the screening population, multiple infections with other HPV genotype(s) occur in 51.36% (95% CI: 49.96%-52.76%). The most prevalent coinfections involve HPV39/51/16/59. Furthermore, HPV67 is associated with HPV18/39/31 in coinfections within the diagnostic population. HPV67 is significantly (p=0.021, Pearson's Chi Square Test) more prevalent in vaccinated women (3.36%; 95% CI: 3.06%-3.68%), compared to the non-vaccinated population (2.94%; 95% CI: 2.75%-3.14%). Similar results are obtained for other HPV types. In the proportion of women vaccinated with Gardasil® (0.13%; 95% CI: 0.11%-0.14%) vs Cervarix® (0.11%; 95% CI: 0.08%-0.13%) no significant differences (p=0.228, Pearson’s Chi Square Test) are found.
Conclusion

The overall prevalence of HPV67 in Belgian women is 1.86%. The changing coinfection patterns between screening and diagnostic populations should be topic of further research. Specific non-HPV16/18 genotypes are observed to be significantly more prevalent in vaccinated women, irrespective of vaccine type. These data hypothesize a continuous HPV genotype shift. In addition, HPV31/33 (together with HPV16 in α9-species) and HPV45/68 (with HPV18 in α7-species) display a significant reduction in prevalence in the vaccinated population. Phylogenetic related HPV genotypes share similar capsid epitopes, potentially eliciting cross-reactive immune responses after vaccination. Our findings indicate that selected possible high-risk HPV types as HPV67 are more frequently found in vaccinated women. It is therefore warranted to perform close surveillance on the transforming potential of these types, including HPV67.
ACCEPTABILITY OF SELF-SAMPLING IN NEW ZEALAND: A PILOT STUDY

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Background / Objectives

In New Zealand (NZ) there are major ethnic inequalities in cervical-cancer (cxca) screening, incidence and mortality with >50% of women diagnosed with cxca not screened and a further 11% unscreened for >3 years (the recommended screening interval). Thus, novel strategies for increasing screening participation are needed.

Internationally, offering self-sampling of cervicovaginal material has been shown to increase screening uptake but no NZ studies have yet been published. We undertook the first NZ study to examine the acceptability of self-sampling in women who self-identified as Māori (the indigenous people), Pacific Islander or Asian.

Methods

Women aged 30-69 years who had never been screened or who had been screened >6 years ago were identified through our collaborating clinic. Women were contacted by a nurse and asked to attend to: 1) examine three different self-sampling devices; 2) complete a questionnaire about the acceptability of the devices; 3) take a self-sample with ≥1 device; 4) have a cytology sample taken by a nurse (as per standard care). Samples were later tested “off-label” using the cobas HPV test (Roche).

Results

We aimed to recruit 120 women but only 56 took part. The majority (31; 56%) were of Pacific Island ethnicity, 12 (21%) were Māori, 9 (16%) were Asian, and 4 (7%) were of ‘other’ ethnicities. The median age was 39.5.

The Her Swab device was used by 51 women, 8 used the Delphi Screener, and 7 a swab-based self-sampling device (7 used >1 device).

Before trying any devices, 39 women (70%) said that they would prefer to self-sample next time they were due for screening, 11 (20%) that they would prefer a smear test, and 6 (10%) did not answer the question. After trying a device, fewer women (29; 52%) preferred to self-sample next time, slightly fewer women (7; 13%)
preferred a smear test, 8 (14%) expressed no preference, and 12 (21%) did not answer the question.

Before using a device 29 (52%) women thought that they would experience no or very little discomfort using the device, 24 (43%) that they would experience some or a lot, and 3 (5%) declined to answer. After using a device slightly more women (31; 55%) experienced no or very little discomfort, and fewer women (16; 29%) experienced some or a lot of discomfort, but more women (9; 16%) did not answer the question.

HPV testing was unremarkable with only one sample positive for HPV “other” oncogenic types out of 32 valid tests.

**Conclusion**

This is the first study of self-sampling in NZ and the first study to include Māori women. Although the sample size is small, the pilot study suggests that un- and under-screened NZ women find self-sampling acceptable and all sample types are feasible for HPV testing.
EVALUATION OF HIGH RISK HPV DNA DETECTION IN SELF-COLLECTED VAGINAL SAMPLES AND URINE IN A TEST-OF-CURE SETTING

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Background / Objectives

Self-collection is considered an alternative for improving coverage rates in cervical cancer screening, while its use in the test-of-cure setting (ToC) is not well established. Aim: Comparing the high risk HPV DNA (HPV) status of self-sampled vaginal fluid (VF) and first-void urine (FVU) to physician-sampled cervical scrapes (LBC) collected 6 months post first life-time treatment of high grade cervical lesions during the same visit, using a PCR-based clinically validated test.

Methods

VF (Qvintip, Aprovix), FVU and cervical smears (PreservCyt, Hologic) from women with pretreatment histology of CIN2 (N=100), CIN2/3 (N=24), CIN3 (N=250), AIS/CIN3 (N=11) and AIS (N=9) were tested with RealTime High Risk HPV (Abbott), followed by establishing correlation of results with cytology and colposcopy data. Dried FVs stored at room temperature and VFU samples, frozen within <1 h from collection, were placed into Cervi-Collect tubes (Abbott) prior to testing.

Results

Valid HPV results were obtained from all LBC samples, 99.7% VFs and 95.7% FVUs. 376 triplets with valid PCR results were available for analysis. In women with abnormal cytology (N=60), concordance between HPV results from VF/LBC (90.0%) was higher than that from FVU/LBC (78.3%); comparable HPV detection rates were found with VF/LBC pairs (55% [33] ea.), while fewer FVU samples were HPV-positive (43.3% [26]). In women with normal cytology (N=316), similarly high concordance between HPV results from VF/FVU and LBC samples was found (89.9% ea.), while a significantly higher HPV detection rate was observed with VF (21.8% [69]) compared to FVU (12.3% [39]) and LBC (13.0% [41]). In women with high grade lesions identified during colposcopy (N=29), high concordance between HPV results from matched self- and physician-collected pairs (VF/LBC 96.6%; FVU/LBC 89.7%) and comparable HPV detection rates with all three sample types (LBC 37.9%[11]; VF and FVU 41.4% [12] ea.) were found. In women with normal/low grade (NLG; N=196) and TZ3 (N=151) colposcopy, similar patterns in concordance between HPV results from VF/LBC and FVU/LBC pairs (NLG: VF/LBC 87.8%; FVU/LBC 84.7%; TZ3: VF/LBC 91.4%; FVU/LBC 92.1%) and significantly higher HPV detection rates from VF vs LBC compared to VF vs LBC (NLG: VF 30.6% [60], FVU 18.4% [36]), LBC
19.4% [38]); TZ3: VF 19.9% [30]), FVU 11.3% [17]), LBC 16.6% [25]) were observed.

Conclusion

All VF and the vast majority of FVU samples from HPV-positive women with high grade lesions at 6 months control by colposcopy were identified with RealTime High Risk HPV, suggesting that self-collected VF and FVU may be suitable for HPV-testing in the ToC-setting.
UNDERSTANDING WOMEN’S PERSPECTIVES AND INFORMATION NEEDS FOLLOWING A POSITIVE HPV SELF-SCREENING TEST RESULT

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Background / Objectives
At-home HPV self-screening with triage of high-risk HPV+ women to in-clinic follow-up may improve cervical cancer screening adherence. Understanding patient experience after a positive kit result is essential to optimize delivery and minimize negative perceptions of self-screening. We explored patient perspectives following a HPV+ self-screening result to identify information needs and emotional responses to this potential home-based screening modality.

Methods
We conducted a pragmatic randomized controlled trial in Kaiser Permanente Washington (an integrated healthcare system) to compare two programmatic approaches for increasing screening among women aged 30-64 years who were overdue (≥3.4 years since last Pap; see abstract #414 for details). Control arm included usual care (annual patient reminders and adhoc clinic outreach). Intervention arm included usual care plus an unsolicited mailed HPV self-sampling kit. We recruited 46 women who returned a kit and tested HPV+ (62% of invited; median age 55.5 years) to complete a semi-structured interview and a brief survey. Most women completed timely diagnostic evaluation (85% had a Pap and/or colposcopy, mean=15 [IQR=10-35] days between HPV+ result and first in-clinic procedure). Four coders analyzed transcripts using iterative content analysis.

Results
Seven themes emerged: 1) convenience of home test; 2) surprised by kit results because low perceived risk of HPV infection; 3) anxiety and urgency to follow up and discuss results with provider; 4) poor understanding of kit results and subsequent information-seeking through Internet, patient portal, and family/friends; 5) provider communications about results eased patient worry; 6) confusion about purpose and meaning of HPV versus Pap results; and 7) concern that HPV self-screening was inaccurate when follow-up Pap was normal. Most women strongly agreed their experience using the kit was positive; but, only 65% agreed they trusted the HPV result and 59% believed it was correct.
Conclusion

Although women liked the test’s convenience, communication about discordant home HPV and in-clinic Pap results led some to question the accuracy of self-screening. Patient-provider communication around self-screening is more complex than for reflex or co-testing, because clinician-collected Pap results are unknown at the time of the positive self-screen. Women need information about the differences between HPV and Pap tests and how findings from both are used in combination for screening and follow-up. To reassure women and keep them interested in self-screening, education is needed at three key points: when mailing the kit, releasing HPV+ results, and discussing in-clinic diagnostic findings.
Detection of cervical (pre)cancer on the basis of cervicovaginal fluid: possibilities for development of a selftest.

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Background / Objectives

Despite tremendous efforts over the last decades, current screening methods for cervical cancer still have limitations in sensitivity and/or specificity. Moreover, vaccines are not effective against all HPV types and efficiency is uncertain in case of previous infection. In the search for more specific and sensitive biomarkers, and additional challenge represents the application of these biomarkers in low- and middle income countries where the incidence of cervical cancer is highest.

The Cervico Vaginal Fluid (CVF) is composed of secretions originating from organs that are part of the female genital tract, including vagina, cervix, endometrium and ovaries; hence the proteome of this fluid contains a wealth of information concerning the physiological status of all of these organs. Since many studies have proven self-sampling as a good and acceptable sample collection method for subsequent DNA genotyping, cytology or immunohistochemistry, CVF may very well be suited for the development of a selftest for triage of suspected cases or screening in low- and middle income countries.

Methods

A differential proteomics study on CVF was performed using six CVF samples from healthy and six samples from precancerous women. Extracted proteins were run over a 2D-LC-MS/MS platform and quantified by spectral counting. Lists of identified CVF proteins were analyzed by Ingenuity pathway Analysis (IPA) to find out whether cervix cancer pathways were reflected in the CVF. A series of candidate biomarker proteins was further validated by ELISA or mass spectrometry (MRM).

Results

We identified alpha-actinin-4 (ACTN4) as a protein biomarker that could discriminate between the healthy and (pre)cancerous states with a sensitivity and specificity of resp. 84 and 86%. Based on the list of proteins that were differentially abundant in both types of CVF, a set of cervical cancer protein biomarkers interconnected within several cancer-related pathways was identified by Ingenuity Pathway Analysis (IPA). We quantified these biomarkers by ELISA or mass spectrometry (MRM) in CVF samples from healthy or precancerous woman in order to further increase the discriminative power in combination with ACTN4.

Conclusion
The cervical vaginal fluid may contain several biomarkers which, when used in an appropriate combination, could be used for development of an accurate cervical cancer screening test. Since collection of CVF is non-invasive, these biomarkers allow for the development of a self-diagnosis test to be used for screening, prediction or follow-up of cervix cancer.

References

PERFORMANCE EVALUATION OF A NEW SELF-SAMPLING DEVICE FOR HPV DETECTION AND GENOTYPING IN ROUTINE CERVICAL CANCER SCREENING

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Background / Objectives

The objective of this study is to evaluate the suitability of dry self-collected vaginal samples, using the Iunetest® self-sampling device, for the determination of human papillomavirus (HPV) by a comparison to HPV test results obtained from standard physician-collected samples.

Methods

We included 100 patients who came for routine cervical cancer screening and 100 patients with a previous diagnosis of intraepithelial neoplasia (CIN) grade 1 or greater (CIN1+). For each patient, two samples were collected for molecular HPV testing, one obtained by the physician (cytology specimen in Thin Prep) and the other by the patient, using the Iunetest® self-sampling device. Upon collection, samples were sent to the Pathology Department at the University Hospital Son Espases for analysis with PCR COBAS 4800. The presence or absence of CIN1+ was verified by colposcopy and biopsy for all women that tested positive in at least one of the samples. As a quality control measure, in cases where only one sample tested positive, both samples were retested using LINEAR ARRAY® HPV Genotyping Test (Hospital Universitario Santa Cristina). Finally, all samples were analysed for 14 high-risk HPV (hrHPV) and 2 low-risk HPV (lrHPV) types using multiplex fluorescent PCR (F-HPV typing™) at Labco Laboratories.

Results

Both sampling techniques proved to be comparable for cytological lesion detection through the detection of HPV-positive cases. The agreement between the two techniques was very good, with a Kappa index of 0.88 (CI 95%, 0.74 to 0.91) (p<0.001). One hundred and twenty-six women (63%) preferred self-sampling, mainly for its comfort (55%), which included privacy (29%) and time flexibility (27%).

Most frequent HPV types detected in both sampling methods were hrHPV-16 (27.5%), 52 (13%), 39 (9.5%), 66 (8%) and 58 (8%). Cases with a single HPV type infection were present in 47 physician-collected samples (59%) and 49 self-collected samples (62%). Cases with multiple HPV type infection were present in 32 physician-collected samples (40.5%) and 30 self-collected samples (37.9%).
Overall result concordance between the two sampling procedures was 90%, 99% for HPV-16, 99% for HPV-18 and 99.1% for the remaining hrHPV types.

**Conclusion**

Dry self-sampling is a valuable alternative to obtain samples for primary cervical cancer screening due to its reliability, comfort and low cost. As we know, most cervical cancer cases occur in women that do not attend screening. Self-sampling can not only cut costs of screening but also motivate more women to participate in cervical cancer prevention programs.

**References**


URINE HUMAN PAPILLOMAVIRUS HOME SELF-SAMPLING, A PROMISING STRATEGY FOR ENHANCEMENT OF UTERINE CERVICAL CANCER SCREENING IN A LARGE RURAL FRENCH COHORT WITH A 5-YEAR CLINICAL FOLLOW-UP (THE PAPU29 STUDY)


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Background / Objectives

To increase patient’s compliance with cervical cancer (CC) screenings, urine self-sampling HPV-DNA detection could be an alternative to clinician sampling (1). Home self-sampling can reduce screening difficulties, CC incidence and mortality. The aim of this French prospective PapU29 study was to evaluate self-collected urine HPV-DNA screening in 25-65 yo rural women non-attenders to organized cytological-based (OC)-CC screenings.

Methods

From 2008 to 2010, 15471 women were invited to cytology screening. All the responding women were assigned to “cytological first-line”(CFL) group. A urine HPV-DNA kit was sent to those non-responders at home (“HPV first-line”(HFL) group). Urine sample was send by mail to the virology labo from Brest university hospital for HPV DNA quantification by real-time PCR (2,3) and genotyping (InnoLiPA). Women with positive test were assigned for “cytological second-line” within 3 months, those with negative test were recommended for a cytology within 3 years. Participation rate was compared between CFL and combined CFL-HFL. The HFL group was followed-up for 5 years (2011-2016). This study was supported by the French "Ligue contre le Cancer". All women gave their signed consent.

Results

Participation rate (15260 eligible women, mean age: 46.6 yo (11.5)) was significantly increased between CFL and combined CFL-HFL (3.73% vs. 31.9%, p < 0.001) (> 50 yo women: 4.67% vs. 33.3%, p<0.0001). After urine HPV screening, abnormal
Cytology detection rate was increased (4.11% vs. 6.31%, p=0.078). Screening rate of precancerous lesions CIN2+ was increased (0.41% vs. 1.42%, p=0.11) (35-50 yo women: 2.55%, p=0.038). First case of HPV18 adenocarcinoma was identified in HFL. The most high-risk genotypes (HPV16, 18) were detected in positive HPVs with CIN2+ (p=0.019). The 5-year follow-up showed no CIN2+ in negative HPVs and only one in positive HPVs with normal cytology at the baseline.

Conclusion

The feasibility and reliability of first-void urine HPV home self-sampling was showed in rural women non-attenders in OC-CC screenings. The high rate of CIN2+ detection demonstrated the clinical benefit of using urine HPV in women at high risk of CC. Given today’s rise in HPV vaccination, we propose urine self-collect HPV as a primary CC screening strategy and a 5-year urine HPV screening interval. We recently found comparable results with vaginal self-sampling and a 3-fold higher acceptance for urine sampling (on-going analysis). Further large-scale randomized studies on difficult to reach women are warranted to validate our results with urine HPV screening.

References


URINARY HPV DNA TESTING AS A TOOL FOR CERVICAL CANCER SCREENING IN FRANCE

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Background / Objectives

Previous studies have shown that self-sampling for human papillomavirus (HPV) testing increases rates of compliance. With this purpose in mind and in collaboration with Cap Santé 49, we performed the CapU 1-2 studies to evaluate the acceptance of an urinary HPV test. Letters proposing an at-home urine self-sampling were sent to 5,000 women who not had a Pap smear over the past 3 years. The participating patients had to send their urine samples to the Angers Hospital Virology Laboratory. High-risk HPV (HR-HPV) were detected in 29 women using real time PCR. In follow-up, 28 women with positive urinary HPV results had a Pap smear or colposcopy done. The cytological results showed 9 abnormal Pap smears, among which histology studies confirmed 3 cases of cervical intraepithelial neoplasia grade III lesions (Ducancelle et al, 2015).

Since September 2016, a 3rd study, CapU3, aims to invite 13,000 women aged 35 to 65 who had not had a Pap smear over the past 7 years. 500-700 letters proposing an at-home urinary HPV testing are sent monthly. With the letter, the women receive an information note, a letter of consent, a sterile container, a procedure protocol, a bubble envelope and a prepaid return envelope. Women accepting to participate send their first-stream urine samples by mail to the Angers University Hospital Virology Laboratory using the bubble envelope and the prepaid envelope in accordance with a three-rule secure packaging protocol as recommended in France. The end of the study is scheduled for November 2018.

Methods

HR-HPV detection is performed using a real-time PCR (Anyplex II HPV28 Detection) that detects 28 genotypes. Patients with HPV positive results are encouraged to perform a cervical smear as soon as possible to detect the presence of cervical lesions. For HPV-negative women, a Pap smear within 1 year is recommended for those women who do not have regular gynecological follow-up.

Results

The preliminary response rate is 15.9% (635/4,000). After exclusion (for hysterectomy or refusal), the participation rate is 13.3% with 530 samples received. Among them, 492 specimens were already tested in which 58 were positive for at least 1 HR-HPV (11.8%). Among the cervical smears performed in positive patients, 1 high-grade cytological lesion has already been detected.
Conclusion

Because home HPV urinary testing are non-invasive and do not require medical attention, this method may be an alternative for women who are reluctant to use Pap smear. In the other hand, we show that Anyplex II HPV28 Detection is a valuable assay for urinary HPV testing as described.

References

IS HPV E6 ONCOPROTEIN DETECTABLE IN URINE AMONG WOMEN WITH INVASIVE CERVICAL CANCER?

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Background / Objectives

Cervical cancer (CxCa) is a major public health problem, especially in low- and middle-income countries (LMICs), where women have little access to CxCa screening; consequently 80% of CxCa related mortality occurs in LMICs. The development of screening methods that need less infrastructure thus represents an urgent medical need.

Objectives: To evaluate the feasibility of detecting HPV16 and/or HPV18 E6 oncoprotein in urine samples from women with invasive cervical cancer (ICC).

Methods

Between January 2017 and April 2017, sixteen women with previously untreated macroscopic ICC (FIGO stage IB or greater) with ages ranging from 25 to 64 years were recruited at the Barretos Cancer Hospital – Pio XII Foundation. At study enrollment, self-collected vaginal samples, physician-collected cervical samples and urine samples were obtained prior to colposcopy. Urine-based protocols to measure HPV-DNA via Cobas® HPV Platform (Roche, CA, USA) and E6 oncoprotein levels using the OncoE6™ Cervical Test (“E6 test”; Arbor Vita Corp., CA, USA) were developed and applied.

Results

The mean age of participants was 43.2 years. All vaginal self-collected samples tested positive for high-risk (HR) HPV-DNA: 11 for HPV16, 2 for HPV18, 5 for other HR-HPV types; 2 had multiple infections. Fifteen (93.8%) physician-collected cervical specimens and urine samples tested positive for HR HPV-DNA, with the same genotyping results observed in correspondent self-collected vaginal samples. Among HPV16 or HPV18 positives, corresponding E6 oncoprotein was detected in 10 of 13
vaginal self-collection samples, in 12 of 13 physician collected samples, and in 7 of 13 urine samples. HPV E6 oncoprotein types showed 100% correlation to HPV genotyping outcome.

Conclusion

Our results suggest that vaginal self-collection may be compatible with the E6 Test. Surprisingly, E6 oncoprotein was also detected in urine of ~54% of women with ICC, suggesting that a urine based E6 Test may be possible upon further protocol development. Vaginal and urine based detection of E6 oncoprotein could enhance CxCa screening coverage in LMICs.
HE TAPU TE WHARE TANGATA (THE SACRED HOUSE OF MANKIND): RESEARCH TO INFORM CERVICAL SCREENING STRATEGIES FOR INDIGENOUS MĀORI WOMEN IN NEW ZEALAND

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Background / Objectives

Indigenous Māori women experience substantially higher rates of cervical cancer than New Zealand European women do. The National Cervical Screening Programme (NCSP) consists of a three yearly Pap smear for women aged 20-70. However, this is an invasive and sometimes time consuming and/or costly procedure, and screening rates are significantly lower for Māori. HPV self-sampling presents an opportunity to overcome these barriers. This project explores the acceptability of HPV self-sampling for under-screened Māori women (aged >25 years, with no screen in >4 years). This research is funded by the New Zealand Ministry of Health and will inform the new NCSP in 2018.

Methods

This Kaupapa Māori (by Māori, with Māori, for Māori) mixed methods project explores the attitudes and beliefs towards HPV self-sampling of an under-screened Indigenous population using a distributed research model (DRM). This involved community based researchers (CBRs) and community members collecting the data. CBRs ran 19 focus groups/interviews with 94 under-screened Māori women in four regions. Preliminary focus group/interview data was analysed thematically and organised into potential barriers or facilitators to accepting this new technology. The analysis guided the development of a short survey that focus group participants disseminated to peers. Percentages were calculated from the survey data.

Results

Potential barriers to self-sampling were: concerns about accuracy/safety, concerns about the sample getting lost/contaminated, stigma of HPV being an STI, and concerns about the longer re-testing time (5 years if negative). Facilitators were: privacy/ease, competent practitioners, appropriate information, whole family approach. Survey data (to-date) shows: 90% of participants are enrolled with a Primary Health Organisation, 75% are likely/very likely to self-sample, and 90% are likely/very likely to seek further diagnosis if their HPV test is positive. The DRM has
been successful in engaging 309 eligible (considered hard to reach) survey participants.

**Conclusion**

The majority of under-screened Māori women who took part in this study are engaged in the health system, but they do not screen. This is a system failure. Both the focus group and survey data emphasise the acceptability of HPV self-sampling for under-screened Māori women, if accompanied by appropriate support. Almost all participants indicated they would seek further diagnosis or treatment if required. This suggests that with well introduced self-sampling, many currently under-screened Māori women would be screened (and treated if necessary). HPV self-sampling has the potential to save lives.
SELF CERVICAL COLLECTION FOR HPV, HHV-2 AND HIV-1 DETECTION IN WOMEN FROM LOWER AMAZON


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Background / Objectives

Self-cervical collection and epidemiological studies on anogenital infections by human papillomavirus (HPV), human herpes virus 2 (HHV-2) and human immunodeficiency virus 1 (HIV-1) in women from outlying regions to urban centers, such as the interior of the State of Pará, are limited. Objective: To estimate the prevalence of anogenital HPV and HHV-2 infections in association with, or not, to HIV infection and to verify the uptake of self cervical collection.

Methods

Recruitment of a cross-section of women who voluntarily sought assistance in eight different health services provided by the municipalities in Tapajos/Santarem on the lower Amazon in Pará, Brazil. The study utilized swabs from the cervix and anus along with peripheral blood obtained by trained technicians that were compared to samples from self-administered cervical collections. All specimens were preserved in Thin Prep and collected between August 2015 and August 2016. PCR products amplified by MY09/11 and GP5/6+ primers were sequenced to genotype HPV DNA. Real time PCR was used to detect HHV-2 nucleic acid.

Results

A total of 476 specimens from 112 women were analyzed. A high acceptance to the self-administered cervical sampling protocol was shown by the study population (84%, 94/112), which identified HPV DNA in 37 samples (39.4%). The most prevalent HPV type was HPV16. The prevalence of HHV-2 was 8.9%. The agreement rate between the clinical samples and the self cervical sampling was 65% (26/40) for HPV and 50% (4/8) for HHV-2. No HIV-1 infected women were detected.

Conclusion
A high prevalence of oncogenic HPV type with no dysplastic lesion was found in cervical and anal samples. The self-cervical collection had a high acceptability, especially among vulnerable women, that strongly suggests that this approach could be an important tool to increase access to diagnosis. We encourage public healthcare officials to include self-administered samples in future screening programs for cervical cancer and the identification of circulating STDs in women that live in low-resource and rural settings, such as the Amazon region of Brazil.

References

UTILITY AND FACTOR EVALUATION OF HPV DETECTION BASED ON URINE SAMPLES IN GENERAL CHINESE WOMEN

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Background / Objectives

Cervical cancer screening is mainly based on cervical exfoliated cell samples at present. To reach out women without access to routine screening, self-collection could be one promising alternative sampling choice. Urine sample collection seems to be convenient and lower resource demand. The utility and impact factors of HPV testing from urine samples were evaluated in subset population in a pilot HPV primary screening study in China.

Methods

34-64y women from Yangcheng and Xiangyuan in Shanxi Province consented to participate. Women self-collected at least 40ml urine sample and mixed with 10ml EDTA preservative before stored at 4°C. Another clinician-collected cervical sample was placed in ThinPrep™ Solution (Hologic, Inc., Marlborough, MA) or DCM solution (Qiagen, Gaithersburg, MD). Urine samples were tested with the Trovagene high-risk HPV assay (Trovagene, Inc., San Diego, CA) and cervical samples were tested with the cobas® HPV Test (Roche Molecular Systems, Pleasanton, CA) or the careHPV™ test (Qiagen, Gaithersburg, MD). At the time of urine collected, information were also obtained through face-to-face interview, including urinary frequency, time interval from the latest urination, intraday water intake and time interval from the latest drinking at home, water intake on site and urine collection times. Women were triaged, referred to colposcopy per the pilot study protocol and biopsies were obtained if clinically indicated.

Results

In total, 2038 women were enrolled. 1953 women were qualified as evaluable subjects and 85 women were excluded due to invalid urine testing results. The median age was 48 years old. And the HPV positive rate in cervical samples was higher than that in urine samples (18.5% vs. 16.7%, P=0.049). And the agreement rate was 84.7%. It was also indicated that intraday water intake (OR: 1.668, 95%CI: 1.196-2.325) could increase HPV positive rate. Whereas urinary frequency (OR: 0.699, 95%CI: 0.522-0.935) and drink water on site (OR: 0.616, 95%CI: 0.386-0.981). No significant differences were observed among other factors.

Conclusion
HPV positive rate in urine samples appears to be comparable to that in cervical samples. Intraday water intake, urinary frequency and water intake on site could affect HPV results in urine samples. Thus, it can be inferred that HPV detection based on urine still need to be optimized before applied in cervical cancer screening.
LONGITUDINAL STUDY OF HPV DETECTION IN PLASMA OF WOMEN WITH A RECENT HISTORY OF CERVICAL DYSPLASIA

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Background / Objectives

The presence of viral DNA offers a specific tumor marker for viral associated cancers. Some studies have reported the presence of HPV DNA in sera and plasma of women with cervical cancer, indicating the possible entry of cancer cells in circulating blood. On the contrary, only few studies have investigated the presence of HPV DNA in the bloodstream of patients with low grade or precancerous cervical lesions. The aim of this pilot study was to investigate the presence of high-risk HPV (hrHPV) in cervical and plasma samples of women with a recent history of low-grade cervical dysplasia (ASCUS or L-SIL) and to evaluate its persistence in bloodstream after 6 months.

Methods

Blood and cervical samples were obtained from 28 women referred to San Gerardo Hospital, Monza, Italy with a recent history of ASCUS or L-SIL. Routine cervical cytology (Pap test) and hrHPV detection was performed on cervical and plasma samples. HPV detection in plasma and cervical samples was also evaluated at a follow-up visit after 6 months. Nucleic acid extraction was performed using automated NucliSENS easyMAG system (bioMérieux). A full high-risk HPV genotyping assay (Papilloplex, GeneFirst) was performed on cervical samples. HPV 16, 18, 31, 33, 45, 51 and 52 detection was carried out by means of previously described genotype-specific "in house" real-time PCR assays; all assays were validated through the participation to the WHO LabNet HPV Proficiency Study.

Results

At baseline, positivity for one or more of the tested hrHPV types was demonstrated in 75% (21/28) of cervical samples with the most prevalent genotypes identified being HPV 16 (37.5%), HPV 51 (25%) and HPV 31 (10.7%). Overall, 8 women (28.6%) were found to be hrHPV positive in plasma at a least one time point. The same hrHPV type was detected in plasma and cervical samples at the same time point in 31.2% (5/16) of tested samples. Five women resulted positive for the same hrHPV type at both baseline and follow up; of these 3 showed HPV DNA persistence in plasma in spite of viral clearance from the cervix.

Conclusion
These preliminary results confirm that HPV DNA can be detected in peripheral blood samples of women with a recent history of cervical dysplasia. This pilot study has also demonstrated that HPV DNA can persist in bloodstream for up to 6 months and that viral DNA can be detected in plasma even after clearance of cervical infection. Further studies are required to evaluate the significance of hrHPV DNA detection in the circulatory system of women with transient or early stages of cervical dysplasia.
Suggesting ideal strategy of cervical cancer screening in Japan based on Fukui cervical cancer screening study

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Background / Objectives

The induction of HPV testing in cervical cancer screening has spread throughout the world for the detection of cervical cancer precursors.1-3 But an ideal strategy unified in the world is not determined yet. Therefore, this Fukui Cervical Cancer Screening (FCCS) study has two important objectives. One is to confirm the performance of cytology testing, human papillomavirus (HPV) testing, and co-testing with cytology and HPV testing in Japan. The other is to determine whether the different approach by HPV16 type, HPV18 type, and 12 other high-risk HPV (hrHPV) types is a beneficial method for the Japanese cancer screening population.

Methods

The study enrolled 7,584 women aged ≥25 years who were undergoing routine screening. All women underwent cytology and HPV testing. Women with abnormal cytology regardless of the HPV status, those with positive hrHPV results regardless of cytology results, and those randomly selected from among women with normal cytology and negative hrHPV results were referred for colposcopy. This study had four features: a) all the samples were liquid-based cytology samples b) the cobas 4800 HPV test, which can detect HPV16, HPV18, and 12 other hrHPV types separately, was used c) bias in the cytological diagnosis was very small because the cytology was evaluated in a single institution d) a central pathology review panel determined the histological diagnosis.

Results

The prevalence of hrHPV types, HPV16 type and HPV18 type was 6.8%, 1.2%, and 0.5%, respectively. Estimated sensitivities for cervical intraepithelial neoplasia grade 2 or worse for abnormal cytology, positive hrHPV, abnormal cytology or positive hrHPV either, and abnormal cytology or positive HPV16 either were 71%, 92%, 100%, 86%, respectively. Estimated specificities for abnormal cytology, positive hrHPV, abnormal cytology or positive hrHPV either, and abnormal cytology or positive HPV16 either were 33%, 21%, 21% and 33%, respectively. Abnormal cytology or positive HPV16 type either is higher sensitivity and higher specificity than only abnormal cytology.

Conclusion
The FCCS study is first clinical trial to determine the performance of Japanese cervical cancer screening. This study demonstrated that a cervical cancer screening strategy to perform colposcopy and biopsy for women either with abnormal cytology or with HPV16 genotype might have a good balance between benefit and potential harm in Japan.

References


EMERGING TECHNOLOGIES IN CERVICAL CANCER SCREENING, THE ETICCS INITIATIVE

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Background / Objectives

Cervical cancer is 18 times more common in the poorest as compared to the most advanced countries affecting women in the prime of their lives and weakening the development of their communities. While the knowledge and the technologies to effectively prevent cervical cancer are available as shown in affluent societies, the dramatic disparity largely results from the lack of effective screening programs for fragile and unreliable health systems often found in low and middle income countries.

Methods

ETiCCS (Emerging Technologies in Cervical Cancer Screening) is a non-profit initiative within the Department of Applied Tumor Biology, Institute of Pathology, Heidelberg University, which aims to advance and promote organized cervical cancer screening in underserved communities with a high cancer burden. Cervical cancer screening programs will only be successful if they are broadly accessible, acceptable and using effective screening tools. Leveraging emerging technologies and novel insight into the cervical cancer pathogenesis it is possible to develop organized screening programs also for remote settings. Various new tools are available to create innovative approaches including molecular screening methods, self-sampling devices, digital imaging devices and versatile electronic systems for program monitoring and evaluation.

Results

Our current scope of activities -in partnership with the WACA HPV initiative of the Global Health Institute, University of Antwerp- include (i) capacity building in 8 sub-Saharan countries, (ii) a long-standing collaboration with SAP™ to develop new ancillary tools to combine mobile and cloud-based information technology and (iii) studying innovative, scalable screening approaches based on primary HPV testing using home-based self-sampling and (iv) studying triage options including biomarker and cervicography.

Conclusion
The ETiCCS initiative is dedicated to curb the high cervical cancer burden in underserved and disadvantaged communities through organized, scalable cervical cancer screening by forging alliances with national and international stakeholders and key industries and by developing knowledge, capacity, and advocacy. Website: www.eticcs.org
AN ELECTRONIC DATA SYSTEM FOR AN ORGANIZED CERVICAL CANCER SCREENING PROGRAM IN A RURAL SETTING IN ETHIOPIA

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Background / Objectives

Primary HPV testing using home-based self-collection of the HPV sample is a promising screening approach for settings with a high burden of cervical cancer. The efficacy of such an approach depends on a high participation rate and a seamless follow-up of at risk women. As part of the ETiCCS (Emerging Technologies in cervical Cancer Treatment) initiative (www.eticcs.org) we developed and implemented a digital data management system that allows close monitoring of the screening process from sample collection at home to laboratory testing and clinical management for a rural community in the Gondar region of Ethiopia.

Methods

A digital prototype was developed with unique user interfaces for the community health worker, clinic nurse, lab technician, gynecologist and pathologist.

Sampling information captured by the community health worker during a home visit are stored on tablets and regularly synchronized with a clinic-based tablet at the time of sample delivery. The clinic-based tablet serves as a multidirectional hub for clinic, lab and referral information through synchronization to a central server to which the laboratory and gynecologist are also connected via browser. Server data can be accessed and corrected with audit trail by authorized person. Multilevel security includes password protection for users and encryption of sensitive private data.

Results

1000 women are being recruited in 2 rural communities in the Dabat district, Gondar, Ethiopia. Eligible women are visited in their home and invited to provide a self-sample for HPV testing, HPV-DNA positive women are invited to the local clinic for p16INK4a /Ki-67 testing. Samples are assayed in a central lab and women with abnormal findings are referred to the gynecologist for clinical management.

Conclusion
The built prototype greatly improves access and linkage to cervical cancer screening programs using home-based HPV self-sampling. The prototype is ideal for scale-up of an organized screening approach in rural settings.
The impact of migration on cervical screening behaviour

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Background / Objectives

The incidence of cervical cancer in Eastern Europe (EE) is significantly higher than Western Europe (WE) despite the introduction of screening and vaccination programs in many EE counties. The migration of women from EE has been hypothesised to contribute the rising incidence of cervical cancer in WE. The aim of this study was to explore the effect of migration to the UK on the cervical screening behaviours of EE-born women.

Methods

A mixed methods study using quantitative surveys and in-depth semi-structured qualitative interviews was conducted in the UK and Latvia. Women were recruited from three groups, migrant EE-born women (nEE), native English-born Caucasian women (nEN) and native Latvian-born women (nLV). Data were analysed using SPSS software and thematic analysis.

Results

489 surveys were completed and 66 interviews were conducted. Knowledge of the purpose of cervical cancer screening was lower in the nEE and nLV groups compared to the nEN. The nEE and nLV women believed that a cervical smear test was performed as part of a routine gynaecological examination. The natural history of cervical cancer and its association with HPV infection was poorly understood resulting in some women from nEE and nLV groups requesting more frequent smears. There was general distrust of the healthcare system in the country of migration and consequently there was a delay in engaging with screening services. nEE women either continued to have screening in their country of birth, have screening in England and additional smears in their country of birth and others did not participate in any form of screening. The screening behaviours and knowledge of the nEE and nLV group were similar, suggesting that there is little change following migration. However the length of stay in the country of migration may contribute to how much the nEE women adapt their screening behaviours.

Conclusion

The role of cervical cytology as part of a structured screening programme is poorly understood. The screening behaviours of many nEE women appears to be governed by their pre-existing knowledge of cervical cancer and screening prior to migration.
Targeted education both prior to and after migration may help to increase screening coverage.
EVIDENCE FOR CLINICAL APPLICATION OF EXTENDED HPV GENOTYPING IN CERVICAL CANCER SCREENING PARADIGMS: A SYSTEMATIC REVIEW

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Background / Objectives

Screening guideline originators (WHO, ASCO, country-specific) have not yet included an analysis of the body of science published during the last decade about the clinical value of extended HPV genotyping (xGT) in cervical cancer screening and triage, needed to support guideline panels’ recommendations. This targeted systematic review addresses key questions (KQ) that pertain to the effectiveness of including xGT results with screening for reducing cervical cancer mortality and incidence. KQ1 compares xGT versus partial genotype reporting/masking of a pool of other genotypes. KQ2 evaluates xGT as triage of primary HPV positive results, of ASCUS cytology, and as triage of cotesting.

Methods

We searched the Database of Abstracts of Reviews of Effects, Cochrane Database of Systematic Reviews, PubMed, and the Health Technology Assessment database from 2000 through 2017 for relevant controlled trials and observational studies. We supplemented by hand-searching of retrieved article reference lists. Eligible studies included prospective studies of women and retrospective studies of residual specimens from women that were screened or tested using human papillomavirus DNA tests. The reference standard was cervical intraepithelial neoplasia 2 (CIN2) or CIN3 or CIN2+ or CIN3+ or invasive cervical cancer (squamous and/or adenocarcinoma). The timeframe was baseline, or 1-year, or 3-year, or 5-year, or greater than 5-year. Relevance screening, data extraction, risk of bias analyses, and quality assessments were performed. Critical appraisal methodology used design-specific quality criteria from the QUADAS evidence-based quality assessment tool of diagnostic accuracy studies, supplemented by the STARD checklist and GRADE methodology.

Conclusion

The available evidence supports the conclusion that reporting xGT results supports discrimination of both current and future CIN2+ risks. Guideline panels must decide whether to separate reporting by individual genotypes or to group genotypes with similar risks into risk tiers. Based on large studies, xGT appears very promising as
triage in all screening paradigms to discriminate risk and support risk-based clinical action steps by the principle of equal management for equal risk. Models for different screening paradigms, including routine screening interval, enhanced screening interval, referral to colposcopy, and treatment are described. The information in this report is intended to help guideline panels, policymakers, clinicians, and women make informed decisions about the selection of health care screening and diagnostic services, is intended as a reference, and not as a substitute for clinical judgment.
FC 21-06
HIGHER RATE OF HISTOLOGICALLY CONFIRMED CIN 2+ WITH INCREASING USE OF HPV, LIQUID BASED CYTOLOGY AND p16/Ki-67 IN ROUTINE

A. Xhaja

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Background / Objectives

Annual conventional cytology (cc) is still the standard in the German cervical cancer prevention program. For the external quality control by the association of health insurance physicians (KV) a yearly report of the distribution of the histological results relating to the PAP groups is required. The sensitivity of a single cc for the detection of cervical intraepithelial neoplasia of grade 2 or higher (CIN 2+) is low. However, within the last decade HPV testing, liquid based cytology and new biomarkers (e.g. p16/Ki-67) have made it into routine use mainly in the triage of borderline and low grade cytological findings.

Methods

Cytomol is a commercial lab specialized in cervical cancer prevention. Here since the year 2000 HPV testing has been used at a progressive rate in triage and to a much lesser extent as a self-paid service in addition to cc. Since 2007 p16 testing, from 2010 on coupled with Ki-67 staining (all in liquid based cytology), is used in the triage of borderline and abnormal cytology as well as in cases with HPV positivity and normal cytology. Here we report the rate of histologically confirmed CIN 2+ cases correlated with the preceding cytological findings. Cytological diagnoses originally reported in the Munich Nomenclature II (MN; with the use of the unofficial Pap IIW category) until 30.6.2014, from then in the MN III (which is still the reporting standard in Germany) were translated to TBS (The Bethesda System). All the yearly Cytomol reports from 2007 till 2015 to the KV are analyzed.

Results

The finding rate for CIN 2+ lesions per year [Pap > ASC-US] in the screened population increased from 2007 until 2015 from 0.059% to 0.44%. The percentage of histological reports related to the different cytological groups remained almost the same over the years. The most significant increase was observed from 2007 until 2011. In parallel, a strong rise in cases of p16/ Ki-67 [2007: 292 p16, 2015: 6130 p16/Ki-67] diagnostics performed in the lab occurred. In parallel a systematic mode of review in cases with a history of abnormal cytological, histological, HPV and chlamydia findings and of actual borderline or abnormal cytology specimens had been established.

Conclusion
The introduction of new diagnostic techniques and/or improved cytological approaches in a routine lab for cytology seems to lead to a significantly increased sensitivity and specificity for CIN 2+. 
The Study of Folate Receptor-Mediated Staining Solution (FRD™) Used for Cervical Cancer Screening

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Background / Objectives

To evaluate the significance of the Folate Receptor-Mediated Staining Solution (FRD™) when used for cervical cancer detection, by comparing it with the results of the thin-prep cytology test and high risk HPV test and using pathological results as the gold standard.

Methods

The FRD™ is a special staining method for rapid visualization of CIN2+. Results are determined by the color changes of the stain. The FRD™ was applied on the cervix and the cervical canal separately, and the color change was observed. Brown or green indicates a negative result, and blue, bluish-black, or black indicates a positive result. The women included in this study were returning for evaluation by colposcopy based on an abnormal cervical cytology result, a positive HPV result, or showed symptoms of increased leucorrhea discharge or postcoital bleeding. The FRD™ test was performed before colposcopy. A biopsy, and histopathological examination were conducted as needed.

Patients with a positive FRD™ result of the cervical canal, colposcopy assessment type is II-III, or the result of cytology was AGC, were required to complete an ECC as well.

Results

1,504 women with histological findings were included in the study. CIN2+ was found in 561 patients (37.3%) including 50 patients with cervical invasive cancer (3.3%). CIN1 and negative cases accounted for 29.3% and 33.4%, respectively. Pap results included NILM in 394 women (26.2%), ASC-US in 476 women (31.6%), LSIL in 334 women (22.2%), ASC-H in 96 women (6.4%), AGC in 10 women (0.7%), and HSIL in 194 women (12.9%). The HPV positive rate was 89.0% (1338/1504). A positive FRD™ test was determined in 54.1% of the women (813/1504). The sensitivity to detect CIN2+ lesions for TCT, HPV, and FRD™ was 80.4%, 95.5%, and 77.7%, respectively. The specificity was 30.1%, 15.0%, and 60.0% respectively.

Conclusion

From this study we found that the results of the FRD™ and the cytology examination are similar when detecting CIN 2+. The FRD™ can indicate the tumor tendency of epithelial tissue in direct proportional to the organization of the morphological changes. Furthermore, the more serious the nature of lesion and the higher the level,
then the more accurate the results of the FRD™ were. Also, this study shows that the FRD™ results can accurately reveal the level of the epithelial tissue lesions.

Finally, in this study, we compared the sensitivity and specificity of the FRD™ to the cytology examination, and found that the FRD™ can meet the demands of clinical requirements needed for the detection of abnormal cervical lesions (CIN 2+). Furthermore, the FRD™ is an easy and very inexpensive method that can be used in less-developed countries.
CERVICAL CANCER SCREENING PARTICIPATION IN BELGIUM 2006-2012

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Background / Objectives

In Belgium, in 2012, cervical cancer (CC) screening was still opportunistic. We estimated the proportion of women aged 25 to 64 who participated in CC screening in 2006-2012 using individual health insurance data.

Methods

Data were provided by the Intermutualistic Agency (IMA), that compiled a database including all reimbursement records for screening Pap smears performed in Belgium between 2006 and 2012. Coverage was defined as the proportion of women from the target population who had a Pap smear taken within the last 3 years. Overuse was defined as the proportion of Pap smears taken that does not contribute to the coverage: (number of smears taken in 3 years /number of women screened in that period – 1)*100.

Until July 2009, reimbursement was not conditioned neither by age nor by screening interval, as recommended in European or Belgian guidelines (1 Pap/3 years in age group 25-64). Since July 2009, reimbursement was restricted to one Pap smear per two years.

Results

From 2006 to 2012, the coverage dropped from 58.7% to 53.7%. Overuse decreased from 80.5% in 2006, to 18.3%, in 2012.

In the age group 25-44, the coverage varied between 59.1% and 63.6%. The coverage dropped progressively by age (to 35.9% at age 60-64) and was lower among socially vulnerable groups benefiting from increased reimbursement: 41.6% versus 55.7% among other women. 91% of Pap smears were taken by gynaecologists and 9% by general practitioners.

Conclusion

CC screening coverage in Belgium is moderate and tended to decrease over recent years. Restriction of reimbursement diminished over-use. Organised measures are needed to optimise coverage over all levels of the target population.
Cervical cancer screening in Flanders

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Background / Objectives

In June 2013, the Flemish cervical cancer screening program started. Before 2013, screening was essentially opportunistic. An efficient call recall system was set up, only inviting women who are not adequately screened. The screening program motivates eligible women 25-64 y to be screened every three years, by means of a PAP smear.

Methods

The Belgian Cancer registry collects the test results of all cervical samples in a central cyto-histopathological registry and retrieves from the IMA/AIM reimbursement data of clinical acts which are relevant for the detection, monitoring and treatment of cervical cancer. Hence, the Cancer registry compiles an exclusion list that consists of all women for which a screening examination is not required for the next invitation round. Hence, the Centre of Cancer screening will exclusively invite women who are eligible for screening.

Results

The program faces several challenges:

Coverage remains stable around 63%. 37% women are never or rarely screened. Research into the socio-demographic characteristics of non-responders demonstrated a significant social gradient. Furthermore, Eastern Europe migrant women are under screened. Targeting disadvantaged and migrant women will be necessary to reduce inequity. Currently, qualitative research among women from low socio-economic status, is conducted to explore barriers to screening. Post-menopausal women do not seem to be aware of their risk of cervical risk. After 55y coverage drops to 50%.

The relationship between disability status and screening is currently under investigation.

25% of women with screen-detected abnormalities do not receive adequate follow up. A fail safe system will be set up.

Since 2010, the Flemish government has offered free HPV vaccinations to all girls in the first year of secondary education. About 82% of girls aged 13 are being vaccinated. In 2022, cohorts of young vaccinated women will enter the target population for screening.

Conclusion

- Targetting never of rarely screened women remains a priority.
- Changes in screening strategy (screening test, screening interval) may be necessary once women who were offered HPV vaccination, will reach the age of 25y.
Cervical Cancer Screening in Iran: Developing a New Method

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Background / Objectives

Cervical cancer incidence and mortality are low in Iran but the prevalence of HPV infection is increasing. On the other hand, there is no national population based program for screening of cervical cancer in Iran and a large number of women did Pap smear test based on the gynecologist prescription even annually and with different interval and inadequate accuracy. Ministry of Health of Iran planned a National cervical cancer early detection and screening Program from 2017.

Methods

Women aged 30-69 called to health care service centers in the first phase in four cities and were evaluated using cytology and HPV DNA test assays in cervical samples. In the next phase and based on new evidence and expert panel recommendation the age range of program decreased to 30-49. All samples were sent to a national lab we prepared for this purpose in Tehran. HPV DNA test was accomplished recruiting automated devices for purification, master mix preparation and electrophoresis steps. The viral DNA was amplified by conventional polymerase chain reaction using the PGMY09/11 set of primers. Experimental validation was performed for HPV DNA test according to the WHO HPV laboratory guidelines. Standard HPV plasmid of different genotypes and human cervical cancer cell lines (Hela and Ca-ski) was used to determine the analytical sensitivity of the assay. Real time PCR was used to assess the type-specific prevalence of high risk (HR)-HPV genotypes; HPV-16 and HPV-18 as predominant associated agents of cervical cancer.

Results

Among 20,000 cases tested from April 2017 in four cities, around 1400 cases were detected positive for HPV infection of any genotype using PCR-based HPV test, which is account for 7% of HPV infection prevalence. At the time of preparation of this manuscript the result of genotyping is not ready but We scheduled to do assay for 1000 cases daily so the presented results will update for the presentation we can present the result of more samples from more cities and genotyping in October.

Conclusion
We have just launched a national cervical cancer screening program with HPV testing and Cytology in Iran as the vast member in Middle East. We plan to up scale the program whole country step by step and with more automated method that help us prevent cervical cancer occurrence and decrease its mortality.
ASSOCIATION BETWEEN INTEGRATION OF HIGH-RISK HPV GENOMES DETECTED BY MOLECULAR COMBING AND THE SEVERITY AND/OR CLINICAL OUTCOME OF CERVICAL LESIONS

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Background / Objectives

High-risk human papillomavirus (HR-HPV) are causally associated with cervical cancer. Integration of HR-HPV DNA in cellular genomes is considered as a major event in cervical cancer development. Several techniques have been used to evaluate integration of HPV but most of them give an imperfect reflect of HPV physical status. Molecular Combing is a powerful innovative technology which allows direct and high-resolution visualization of HR-HPV genome integration pattern.

The aim of the EXPL-HPV-002 study is to evaluate the integration of 14 HR-HPV (16/18/31/33/35/39/45/51/52/56/58/59/66/68) by Molecular Combing as a biomarker of the severity and/or of the progression of cervical lesions.

Methods

The EXPL-HPV-002 prospective multicentric study will enroll about 600 women aged 25-65 in 2 clinical sites in the Czech Republic, referred to colposcopy after an abnormal Pap smear.

The study will be divided into two phases: (1) a transversal phase which will study the association between HPV integration status and colposcopy results and histological grades; (2) a longitudinal phase which is expected to last 36 months. This 2nd phase will study the association between HPV integration status and the progression of the lesion / infection.

HPV genotyping and Molecular Combing will be performed in central labs. All histological data will be analyzed by a central reading.

So far, one clinical site is active, and the first patient has been enrolled in June 2016. To date, 300 patients were enrolled, and about 65% of them are HR-HPV positive.

An interim analysis planned after 6 months evaluated 126 patients.
The EXPL-HPV-002 study will evaluate the diagnostic and prognostic values of HR-HPV integration status detected by Molecular Combing. Integration can prove to be a reliable biomarker that can specifically differentiate between women with a high risk from women with a low risk of developing cervical precancerous lesions or cancer. While the first will require immediate treatment, the other will require appropriate monitoring. Molecular Combing technology, as well as the first results of the interim analysis will be presented.
FC 22-02
HPV-16 variant´s and IGF1R overexpression induces resistance to radiotherapy in uterine cervical cancer

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Background / Objectives

Several causes of the variable radiotherapy (RT) efficacy have been studied without convincing results. If the HPV-16 infection has been hypothesized to be a predictor of poor response to RT (Ferdousi et al., 2010, Moreno-Acosta et al., 2017), the real clinical impact of HPV-16 and its prognosis significance is still to be demonstrated. In the recent studies (Zacapala-Gómez et al., 2016), was reported that factor receptor insulin-like growth1 (IGF1R) is over-expressed by effect of E6 AA-a, and E-G350 HPV-16 variants. Previous studies have shown a role for IGF1R in cellular radioresistance in cervical carcinoma; Moreno-Acosta et al., 2012, found that the overexpression of IGF1R is a predictive marker for patients (HPV16 (+)) undergoing Radiotherapy because overexpression of this receptor confers 28.6 times greater risk of treatment failure. The aim of the present study was to prospectively report the detection of HPV-16 variants, gene expression IGF1R and assess the relationship with treatment response.

Methods

Detection of HPV 16 variants of 19 patients by PCR-SSCP and direct sequencing and analysis of IGF1R gene expression by real-time PCR. Of these patients, 15 underwent exclusive radiotherapy and four underwent radiochemotherapy.

Results

Three months after treatment completion, out of the 15 patients receiving exclusive RT, 8 experienced complete responses: 3 with the European T350 variant (E-T350 and IGF1R low expression), 2 with the European G350 variant (E-G350) and IGF1R negative expression), 2 with an undetermined European variant (E-Nd) and IGF1R negative expression), and 1 with an Asian-American variant (AAa) and IGF1R negative expression. The other 7 experienced no complete response: Three patients were diagnosed a partial response (2 E-T350, 1 E- G350, and IGF1R overexpression), 3 had a stable tumor (2 E-G350, 1 E-Nd and IGF1R overexpression) and 1 experienced tumor progression (AAa and IGF1R overexpression).

Conclusion
The presence of E-G350 and non-european (eg. AA) variants and overexpression of IGF1R in the no complete response group could be related with radio-resistance. Larger prospective trials are needed to validate the presence of HPV-16 variants and IGF1R expression as a biomarkers of radioresistance.

References


Effects of HPV16 E6 and E7 oncogenes on genomic stability in HCT116 cells

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Background / Objectives

Genomic instability develops at early stages of HPV-infected neoplasias and is associated with deregulated expression of the oncogenes E6 and E7, which were both shown to induce centrosome abnormalities, multipolar mitosis and aneuploidy. The effects of HPV16 E6 and E7 on genomic integrity have been described in primary keratinocytes and in cervical cancer cell lines, which are either critical for long-term culturing or already chromosomally instable. To analyze the effects of the HPV oncogenes on genomic stability in a time dependent manner we intended to use chromosomally stable HCT116 colon carcinoma cells for the generation of clones that allow doxycycline inducible expression of HPV16 E6 and E7.

Methods

Western Blotting and RT-qPCR were performed to characterize HPV16 E6 and E7 expression in selected doxycycline inducible HCT116 clones. Effects on centrosome numbers and spindle poles formation during mitosis were analyzed using gamma-tubulin immunostainings. DNA damage in HCT116 clones induced for E6 and E7 expression was evaluated by staining of the phosphorylated histone component γH2AX, a marker for DNA double strand breaks. The number of aneuploid cells in response to HPV 16 E6 and E7 expression was determined by propidium iodide staining of the DNA and subsequent FACS analysis.

Results

Induction of both oncogenes elevated the number of interphase cells showing abnormal centrosome numbers. Additionally, the percentage of cells with abnormal spindle poles during mitosis was significantly increased. Both effects could already be observed after 48 hours of oncogene induction and were found to be elevated after longer induction phases. As a result of the deregulated distribution of chromosomes during mitosis, E6- and E7-expressing cells showed increased rates of DNA damage and aneuploidy.

Conclusion

In conclusion, HPV16 E6 and E7 induce genomic instability in HCT116 cells as indicated by abnormal spindle pole formation and increased DNA damage rates.
Subsequent analyses on gene copy number variations and differential methylation patterns will also help to understand how and how fast genomic stability is affected by the HPV oncogenes, deepening the insight into mechanisms and causes promoting malignant progression to cervical cancer.
FC 22-04
Staging of Cervical Pre-Cancer using single cell mRNA E6/E7 and cell cycle (OncoTect 3DX)

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Background / Objectives

New therapeutics directed at treating pre-cervical cancer changes prior to the development of cervical cancer require staging the molecular changes associated with transformation and carcinogenesis in order to treat at the earliest possible stage. To that end, we report the preliminary results of a study that uses a single, high throughput assay (Oncotect 3Dx) that defines the stages of squamous cell abnormalities that lead to cervical cancer.

Methods

We analyzed 227 samples that included 79 normals (NILM HRHPV DNA-), 72 low grade (NILM/ASCUS/LSIL HRHPV DNA+), and 76 high grade (HSIL HRHPV DNA+) collected in ThinPrep® liquid-based cytology media. Each sample was assayed using the 96-well OncoTect 3DX assay that quantifies E6, E7 mRNA and cell cycle on a cell by cell basis. In particular, the post-G0/G1% was calculated for each sample as a measure of cell proliferation. In addition, mean corpuscular volume (MCV) was determined for every cell in all samples.

Results

There was an inverse correlation between cervical abnormality stage normal-low grade-high grade and MCV with normal samples being 161 uM3, low-grade 131 uM3, and high grade 113 uM3 (Mann-Whitney P=<0.001). The post-G0/G1% also differed depending on the stage of abnormality with normal samples and high grade samples having the highest proliferation rate and low grade abnormalities having the lowest proliferation rate (Mann-Whitney P=0.03).

Conclusion

Using multiple parameters quantified using the OncoTect 3Dx assay, we were able to define normal cervical samples as E6, E7 mRNA-, MCV hi, post-G0/G1% hi; low grade cervical samples as E6, E7 mRNA +/-, MCV intermediate, post-G0/G1% low; and high grade cervical samples as E6, E7 mRNA +, MCV lo, post-G0/G1% hi. The ability to stratify cervical cancer abnormalities in an automated, high-throughput manner is advantageous for companion diagnostic applications.
FC 22-05
CELL ADHESION AND CELL-CELL SIGNALLING ARE AFFECTED BY HPV INTEGRATION, WHILE Deregulation of Specific Pathways Occur During the CIN3 to Cervical Cancer Transition

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Background / Objectives

Both HPV-positive and negative cervical cancers are associated with cell cycle deregulation, but the actual molecular events, remain elusive. To this end, we employed in vitro and in vivo models of cervical cancer by i) investigating the genomic and transcriptomic effects of the presence or absence of HPV in four informative cervical cell lines, and ii) validating these transcriptomic patterns in tissues from patients with normal, CIN3 and cervical cancer stages.

Methods

Whole exome sequencing and RNA sequencing were performed in a normal cervical cell line (HCK1T), in one HPV (−) C33A, and in two HPV (+) cell lines, HeLa [HPV18+] and SiHa [HPV16+], and in 12 samples of normal, CIN3 and cancer stages.

Results

RNA-sequencing revealed the main integration sites of HPV18 and HPV16 in chromosomes 8 and 13, respectively. Furthermore, a total of 212 genes (85 upregulated and 127 downregulated) were differentially expressed in HeLa and SiHa only. The majority of the downregulated genes are involved in processes of cell adhesion, cell-cell signaling and differentiation. The upregulated genes are involved in embryonic morphogenesis, apoptosis, cell cycle and in positive regulation of transcription. Whole exome sequencing revealed that 1,257 genes were mutated in HeLa and SiHa cells only, consistent with their expression profiles affecting processes of cell adhesion and development. Sixteen of these mutated genes were also differentially expressed between HPV [+] and HPV [-] cells. We validated these data in clinical samples from normal, CIN3 and cervical cancer tissues. The CIN3 and cervical cancer transcriptomes exhibited minimal similarities. With more than 2,000 transcripts differentially expressed among CIN3, cervical cancer and normal tissues, <400 genes showed similar expression patterns. Chemotaxis and immune-related processes were downregulated in CIN3 patients, while cell cycle and mitosis
were upregulated only in cervical cancer patients. Consistent with this, most of the transcriptional regulators controlling the immune and defense response, several cytokines and interferon regulatory factors, were downregulated, while in cervical cancer, regulators controlling cell cycle progression, were upregulated. Interestingly, many pluripotency-related genes displayed elevated gene expression only in CIN3, suggesting the establishment of a transient pluripotency-like phenotype.

**Conclusion**

These combined data imply that the presence of HPV in cervical cells initially leads to aberrant expression of genes controlling cell-cell signaling and cell adhesion, while at the precancerous stages, distinct genes and pathways are deregulated during the transition from CIN3 to cervical cancer.
DISCOVERY OF BIOMARKERS FOR IN VIVO IMAGING OF CERVICAL PRECANCERS IN THE STUDY TO UNDERSTAND CERVICAL CANCER EARLY ENDPOINTS AND DETERMINANTS

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Background / Objectives

Cervical cancer is the second-leading cause of cancer death in women globally, with a disproportionate burden on developing countries. Human papilloma virus (HPV) vaccination will not eliminate cervical cancer in the short term, so screening programs will remain essential for decades. HPV DNA testing is a highly sensitive screening method now being implemented in more settings, but its low specificity mandates triage (secondary) testing for positively screened women to avoid overtreatment harms which may include adverse pregnancy and fertility outcomes. Currently, triage options in low income settings are limited. Specific biomarkers that could be readily detected during the patient encounter through in vivo imaging present a novel promising triage strategy. The discovery of membrane biomarkers of cervical cancer and precancerous lesions may therefore enable the development of specific, sensitive, low cost in vivo detection tests for prevalent precancers.

Methods

The Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED), which includes approximately 3000 women referred for colposcopy from 2003 to 2011 with abnormal screening results, was designed to investigate cervical carcinogenesis and improve the ability to predict which women with HPV infections and low-grade lesions will progress to cancer. Gene expression levels were determined from mRNA microarrays of SUCCEED tissue from 128 patients at all stages of progression to cervical cancer (1), and differential expression of genes across the spectrum of normal, cervical intraepithelial neoplasia (CIN), and cancerous tissues (alpha=0.05 for cancer vs normal expression and for CIN vs normal expression) was detected.
Results

Based on the above criteria, 48 genes encoding for proteins with membrane-bound Gene Ontology annotations that could be amenable to in vivo staining and visualization were identified. Nineteen genes were prioritized for further investigation according to plausibility of plasma membrane localization, altered expression during early carcinogenesis, cervical expression, antibody availability, and enzymatic activity, of which 15 had increased and four had decreased expression in CIN tissues.

Conclusion

Validation of candidate proteins through immunohistochemical staining of SUCCEED tissues is currently under way, which will be followed by the investigation of the in vivo imaging potential of validated candidates using both antibody-based and enzyme-activated optical imaging methods. Finally, promising candidates will be moved forward to clinical studies evaluating their clinical utility for triage to immediate treatment after positive cervical cancer screening results.

References

FC 22-07
DETECTING CERVICAL CANCER VIA ELEVATED HPV ONCOPROTEINS E6/E7 – ACCURACY OF THE ONCOE6™ CERVICAL TEST

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Background / Objectives

Cervical cancer remains a major cause of cancer related mortality among women living in low and middle income countries (LMICs). A decrease in mortality has been achieved in regions where population wide screening was implemented, yet existing screening technologies have failed to reduce mortality in many LMICs. Reasons for the lack of effective implementation of cervical cancer screening in many regions in need are complex; the infrastructure needs (pathologist, complex instrumentation, cold chains requirements) of existing screening modalities and high false positive rate with regard to detection (and subsequent treatment) of true malignancy are contributing factors.

Methods

Cervical cancer is one of the very few cancers where the molecular cause can be pinpointed in nearly 100% of cases: elevated expression of the viral encoded E6/E7 oncoproteins as the consequence of “molecular accidents” allowing such deregulation. This knowledge suggested a very plausible and direct way to detect HPV-induced malignancy via detection of elevated E6/E7 oncoprotein levels. The OncoE6™ Cervical Test (“E6 Test”) has been developed to (i) achieve highest specificity by direct detection of a cancer causing agent, the HPV encoded oncoprotein E6, and to (ii) be robust, and easy of use; this, and the simple training requirements allow implementation in virtually any setting. The E6 Test has been used in studies in many LMIC settings worldwide, and it has consistently revealed high clinical specificity (~ 99%) and positive predictive value for CIN3+. In several instances, the E6 test detected high-grade cervical disease where traditional methods (cytology, colposcopy) have failed to do so.
Conclusion

We will present a synopsis of the clinical accuracy of the E6 Test in a variety of real world settings, including studies on HPV driven oropharyngeal cancer, and we will critically discuss typical use scenarios for the E6 Test.
 Genome-wide microRNA profiling of hrHPV-positive self-samples: Promising triage markers for early detection of cervical cancer

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Background / Objectives

The effectiveness of cervical screening programs is hampered by suboptimal participation rates. Offering cervicovaginal self-sampling for high-risk HPV (hrHPV) testing has been shown to increase the participation. Since only a minority of hrHPV-positive women is at risk of cervical cancer, further stratification (triage) is needed to avoid overtreatment. MicroRNAs (miRNAs) represent a potential class of triage markers and their deregulation has been implicated in cervical cancer. At present, little is known about genome-wide miRNA expression patterns in cervical precancerous lesions (CIN3) and, most importantly, it is unknown whether deregulated miRNA expression is detectable in self-samples. In this study we set out to determine genome-wide miRNA profiles in hrHPV-positive self-samples in order to identify miRNAs detectable in self-samples that can predict the presence of CIN3 and cervical cancer.

Methods

Small RNA sequencing (sRNA-Seq) was conducted to determine genome-wide miRNA expression profiles in 77 hrHPV-positive self-samples (36 of women without cervical disease during follow-up (≤CIN1), 37 of women with CIN3 lesions, and 4 of women with squamous cervical carcinomas (SCC)). Logistic regression analysis was performed to identify the best miRNA panel with the highest combined sensitivity and specificity for CIN3 detection. Candidate miRNAs were validated by qPCR in an independent cohort of 164 hrHPV-positive self-samples (101 ≤CIN1, 49 CIN3, 14 cervical cancers).

Results

Classification of sRNA-Seq data resulted in the identification of 8 differentially expressed miRNAs with an area under the curve (AUC) of 0.89 for CIN3 detection. Six out of eight miRNAs could be validated in an independent self-sample series by qPCR, showing that CIN3 and cervical cancer associated miRNAs can be detected in hrHPV-positive self-samples.

Conclusion
This study is the first to determine genome-wide miRNA profiles in self-samples and reveals that miRNA expression analysis offers a promising novel molecular strategy for CIN3 and cervical cancer detection in hrHPV-positive self-samples. Moreover, our small RNA-Seq data will lead to a better understanding of the contribution of miRNA diversification in cervical carcinogenesis.
Prognosis of donor patients according to the characteristics of Patients derived xenograft (PDX) tumor in gynecological cancer.

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Background / Objectives

Patients derived xenograft (PDX) reflects molecular and cellular characteristics of the donor tumor and is an important model in the study of cancer biology. PDX models also have been developed to apply more personalized strategy against cancer, such as evaluation of drug efficacy and biomarker validation. The purpose of this study is to evaluate the prognosis of donor patients according to the characteristics of PDX tumor in gynecological cancer.

Methods

Cancer tissues from gynecological cancer patients (ovary, cervix and uterine cancer; total 107 cases) were fragmentized to 3 mm pieces and transplanted into athymic nude mice. The volume of each PDX tumor were measured using a digital caliper for up to 1 year and 4 months after transplantation \([\text{Short} \times \text{Short} \times \text{Long}) / 2 = \text{vol. (mm3)}\). The largest PDX tumor in each patient's cases were selected, thereafter, engraftment and growth rate were calculated in PDX tumor (Success of implantation is a confirmed growth-up in PDX tumors; Growth rate is slope value of growth equation per PDX). Donor patient's prognosis were evaluated by survival rate calculated using survival significance methods, applied with the previously reported cut-off value (Finder et al, PLoS One. 2012;7(12):e51862.).

Results

Engraftment results showed that implantation succeeded PDX tumor cases had a tendency of poor prognosis than failed cases in 5 year disease free survival, \((\text{Log rank p} = 0.2446, \text{HR} 3.246, 95\% \text{ CI} = 0.6876 \text{ to } 15.33)\). As for the growth of PDX tumors, fast growing cases showed a tendency of poor prognosis in 5year disease free survival \((\text{Log rank p} = 0.1562, \text{HR} = 2.982, 95\% \text{ CI} = 0.4712 \text{ to } 18.87)\) and showed a significant poor prognosis in 5year overall survival \((\text{Log rank p} = 0.0374)\) than slow growing PDX tumors.

Conclusion

This study reveals concordance of aggressive cancer biology, with fast growing in PDX tumors and poor prognosis of the donor patient. These findings may be an important resource for studying cancer biology and supporting PDX model for developing personalized strategy against cancer.
References

Key words: Patients derived xenograft (PDX); Gynecological cancer; Prognosis;
FC 23-01
META-ANALYSIS ON THE PROGNOSTIC SIGNIFICANCE OF P16INK4A AND HPV DNA IN ANAL SQUAMOUS CELL CARCINOMAS

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Background / Objectives

Anal squamous cell carcinomas (ASCC) represent the most common histologic entity among anal cancers. Oncogenic human papillomavirus (HPV) types play an etiological role in a large proportion of ASCC as indicated by the detection of HPV DNA in up to 90% of cases frequently accompanied by overexpression of the cell cycle regulator protein p16INK4A on immunohistochemistry (IHC). By analogy to head and neck squamous cell carcinomas, it has been suggested that HPV DNA and p16INK4A status might be of prognostic relevance in ASCC patients. However, the reported survival rates for ASCC patients, stratified by these two markers, differ among the published studies.

We aimed to determine the prognostic relevance of oncogenic HPV DNA and p16INK4A status among all published literature in a systematic review and meta-analysis.

Methods

A broad search string was designed to identify all published studies analyzing p16INK4A expression by IHC and providing survival data in patients diagnosed with ASCC. Overall survival (OS) was analyzed performing Cox Regression including p16INK4A IHC, HPV DNA status and clinical data as covariates. Authors were contacted to obtain lacking information or data.

Results
16 studies were found to be eligible for inclusion in the final analysis. From 8 of them we obtained the individual patient records comprising 666 ASSC cases. 84.3% of 555 ASCC tested positive for oncogenic HPV DNA. 81.8% of 658 ASCC demonstrated overexpression of p16INK4A on IHC. Patients with ASCC demonstrating p16INK4A overexpression had a significantly longer median OS than patients without p16INK4A overexpression (36 vs. 28 months (m), respectively), hazard ratio (HR)=0.42 (95% confidence interval (CI), 0.30-0.61) in a pooled analysis. Patients with HPV DNA-positive ASCC also demonstrated a significantly better median OS compared to HPV DNA-negative ASCC patients (39 vs. 26 m, respectively), HR=0.39 (95% CI, 0.27-0.57). Hazard ratios for gender (female vs. male), T-stage (T3/4 vs. T1/2), N-stage (N+ vs. N0), M-stage (M+ vs. M0), HIV status (positive vs. negative) and age were 0.42 (95% CI, 0.30-0.59), HR=2.88 (95% CI, 2.06-4.04), HR=1.92 (95% CI, 1.37-2.70), HR=3.29 (95% CI, 1.60-6.02), HR=1.30 (95% CI, 0.66-2.33), HR=1.02 (95% CI, 1.01-1.03), respectively.

Conclusion

p16INK4A overexpression and oncogenic HPV DNA are detected in a large proportion of ASCC patients and predict better survival compared to p16INK4A- or oncogenic HPV DNA-negative ASCC patients. The obtained data will be further analyzed in multivariate analyses. In the future, we will use digitized Kaplan-Meiers to meta-analyze the 16 studies and use available individual patient data from 8 studies to validate statistical methods.
VAGINAL AND ANAL HRHPV INFECTION AMONG FEMALE SEX WORKERS IN AMSTERDAM, THE NETHERLANDS: PREVALENCE AND CONCORDANCE

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Background / Objectives

Condom use is high among female sex workers (FSW) in Amsterdam, but because of limited condom induced protection against human papillomavirus (HPV) infection, FSW may be still at high risk of HPV infection and HPV-related diseases. We aimed to study risk factors, prevalence and concordance of genital and anal high-risk (hr) HPV infection among FSW in Amsterdam.

Methods

In 2016, FSW aged ≥18 years having a consultation regarding sexually transmitted infections (STI) with the Prostitution and Health Center (PG292) in Amsterdam were invited to participate. Participation entailed taking a vaginal and anal self-swab. Demographics and sexual behavior data were collected in the consultation and HPV DNA was analyzed using SPF10-PCR-DEIA-LiPA25-system, version 1. Uni- and multivariable logistic regression analyses were performed to assess determinants of type-specific vaginal and anal hrHPV infection. Determinants of vaginal and anal hrHPV infection were uni- and multivariably assessed using logistic regression with generalized estimating equations (GEE).

Results

We included 304 FSW with a median age of 29 years (IQR 25-37). The STI prevalence at moment of inclusion was 9% and the prevalence of vaginal and anal hrHPV among participants was 46% and 55%, respectively. The most prevalent vaginal hrHPV infections were types 31 (11.8%), 52 (10.2%), 51 (8.6%), and 16 (8.2%). The most prevalent anal hrHPV infections were types 51 (14.1%), 31 (12.8%), 16 (11.5%) and 18 (12.2%). The highest concordance between vaginal and anal infections was found in types 31 (5.6%), 52 (4.6%), 18 (4.3%) and 16 (3.9%). A risk factor for both vaginal and anal hrHPV was opposite anatomical site of infection (OR 1.32, 95%CI 1.24-1.40; OR 1.40, 95%CI 1.31-1.50, respectively). Additionally, a risk factor for vaginal hrHPV was region of birth (Eastern Europe OR 0.95, 95%CI 0.93-0.97; America’s OR 0.96, 95% CI 0.94-0.99; other regions OR 0.95, 95% CI 0.93-0.98; compared to the Netherlands).
Conclusion

Vaginal and anal hrHPV prevalence is high among FSW in Amsterdam, the Netherlands. Even in multivariable logistic regression using GEE, concordance between vaginal and anal type-specific hrHPV infections was high.
REduction in Sexual Activity Following a Diagnosis of Anal High-Grade Intraepithelial Lesion (HSIL) Among Gay and Bisexual Men (GBM)


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Background / Objectives

To assess the impact of a diagnosis of anal HSIL on subsequent sexual activity in GBM.

Methods

The Study of the Prevention of Anal Cancer (SPANC) enrolled GBM in Sydney who had never previously undergone high-resolution anoscopy (HRA). At baseline and 6-month visits, a behavioural interview, cytological ± histological assessments were performed. We examined the association between a baseline HSIL diagnosis and subsequent changes in sexual behaviour.

Results

Among 617 GBM enrolled (median age 49 years; 35.7% HIV-positive), 518 (84%) attended 6-month follow-up. The number of participants reporting any recent casual sex declined among 232 (37.6%) men diagnosed with HSIL (76.8% to 67.7%, p=0.050) but not in those negative for any squamous intraepithelial lesion (SIL) at baseline (76.0% to 70.1%, p=0.239). There was also a reduction in median partner numbers in the previous 6-months among men with HSIL (9 vs 5, p=0.048) but not among SIL-negative men (7 vs 5.5, p=0.128). Among both men with HSIL and men without SIL at baseline, the number of episodes of receptive penile-anal sex, rimming, fingering, fisting and toys remained unchanged. Neither presence nor duration of pain or bleeding following baseline HRA were associated with subsequent reduction in partner numbers.

Conclusion
GBM in SPANC reduced casual sexual contact and partner numbers following a diagnosis of HSIL. This could be a conscious decision based on a perception of reducing future risk of anal cancer or a fear of transmitting the causative high-risk HPV infection to partners. Further investigation of the reasons behind this change in sexual activity is warranted.
FC 23-04
PREDICTORS OF 12-MONTH PERSISTENT HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS (HSIL) IN A COHORT OF GAY AND BISEXUAL MEN

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Background / Objectives

Gay and bisexual men (GBM), particularly HIV positive GBM, are at greatly increased risk of anal cancer. The anal cancer precursor HSIL is so highly prevalent in GBM that it is clear most HSIL do not progress to cancer. We examined predictors for 12-month HSIL persistence and clearance in GBM with HSIL at study baseline, with the aim of identifying clinically-useful predictors of risk of progression to anal cancer.

Methods

Participants were 617 GBM from the ongoing Study of the Prevention of Anal Cancer conducted in Sydney, Australia. They completed detailed demographic and behavioural questionnaires and underwent cytological, histological and HPV assessments of anal canal samples at baseline, 6- and 12-months. Composite HSIL was defined as either cytological and/or histological detection. Among those with HSIL at baseline, clearance and persistence were defined by non-detection (double negative) and persistent (double positive) detection of HSIL at both 6- and 12-month visits, respectively.

Results

By March 2017, 485 participants had completed their 12-month visits and of these 435 (89.7%) attended all three of the baseline, 6- and 12-month visits. A total of 390 men (63.2%) had both cytological and high resolution anoscopy results available
from each visit. Of these, the median age was 49 years and 137 (35.1%) were HIV-positive. Among 159(40.8%) who had composite HSIL at baseline, 44(27.7%) had HSIL detected at baseline only and 89 (56.0%) had HSIL which persisted at all three visits. HSIL in older men was much less likely to clear (p=0.005) than in younger men. HIV status was not associated with HSIL clearance. HSIL-AIN3 lesions were half as likely to clear as HSIL-AIN2 lesions (HR 0.42, 95% CI 0.20-0.85, p=0.016). Larger lesions (more than one octant) were also less likely to clear (HR 0.33, 95% CI 0.009-1.25, p=0.005). HPV16 positivity at baseline was strongly associated with decreased rates of clearance of HSIL (RR=0.15, 95% CI 0.006-0.36). Clearance was lowest in those who had HPV16 (p<0.001) and type-specific non-HPV16 high risk HPV (p<0.001) at both 6- and 12-month visits.

Conclusion

Among men with HSIL at baseline, HSIL persisted for at least 12 months in over half the participants and one in five had no evidence of HSIL at two subsequent visits. Both baseline and persistent high risk HPV and in particular HPV16, strongly predicted lack of clearance of HSIL. Two HPV tests separated by at least 6 months may identify a subgroup of men with HSIL that is likely to be persistent, and thus at high risk of progression to cancer.
Baseline low- and high-risk HPV prevalence in rectal swabs from men prior to selective immunisation with the quadrivalent HPV vaccine in Scotland

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Background / Objectives

The quadrivalent vaccine prevents infection with HPV types 6, 11, 16 and 18 and has been shown to induce strong and sustained neutralising antibody responses that confer protection against infection and associated disease. Such data stem from population-based surveillance of women who have largely been part of catch-up cohorts from a school-based programme. We aimed to collect baseline data on rectal HPV prevalence from a cohort of men who attended sexual health services, prior to implementation of a selective immunisation programme for men-who-have-sex-with-men (MSM) up to age 45.

Methods

Approximately 1200 rectal swabs were obtained from males attending for sexual health services in Edinburgh, Scotland. Swabs had originally been collected for (routinely indicated) Chlamydia trachomatis testing. Residual material was subject to molecular HPV genotyping using automated extraction and a luminex-based assay which detects 24 HPV types including all established high-risk types and all those included in the quadrivalent vaccine. At time of abstract preparation, results are available for 1064 samples.

Results

HPV prevalence in this population was high; 782/1064 (73%) were HPV positive and 531/1064 (50%) were positive for at least 1 of the types within the quadrivalent vaccine. When vaccine types were counted individually (including as part of a mixed infection) HPV 6, 11, 16, and 18 accounted for 156, 74, 362, and 80 infections respectively. Of those positive for at least 1 of the 4 vaccine types, none were positive for all 4 types.

Conclusion

These preliminary data indicate that the quadrivalent vaccine has the potential to have a significant impact on the prevalence of HPV in this population given that 50% are infected with one of the types included in the quadrivalent vaccine. Comprehensive data which stratify HPV status by age and HIV status will also be presented.
References


INCREASED RISK OF HIGH-GRADE ANAL INTRAEPITHELIAL NEOPLASIA (HGAIN) IN PATIENTS WITH ANAL WARTS ASSOCIATED WITH HERPES SIMPLEX TYPE 2, GONORRHOEA AND OTHER STI’S

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Background / Objectives

Anal cancer rates are rising in men and women worldwide with anal intraepithelial neoplasia (IN) thought to be a precursor. Anal warts have been associated with high rates of IN especially in HIV infection, and anal cancer has been recognised as arising from anal warts. Cofactors other than HPV are likely to be associated with the development of anal cancer and its precursors. A surgical data base with epidemiological and histological data from patients surgically treated for anal warts was created at Royal Perth Hospital in 1996, and findings from this database have been previously reported.1,2

Methods

Patients who underwent surgical excision of anal and/or perianal condylomata acuminata or mapping biopsies from December 1995 to November 2016 were included in the analysis. Demographic data were collected including sex, sexual preference, lifetime sexual partners, history of gonorrhoea, or chlamydia and serological data for syphilis, herpes type 2 antibody (HSV2) and HIV 1 and 2 antibody at the time they were enrolled for surgery. Anal HPV testing by Digene Hybrid Capture II for high-risk strains (hrHPV) was included as a standard of care from June 2005 onwards.

Results

463 patients were included in the analysis, the majority of whom were MSM (367). Almost one third were HIV positive and had high-grade squamous intraepithelial neoplasia (HSIL). Overall 75% of the samples tested positive for hrHPV, with HIV positive men having 95% hrHPV. HSV-2, gonorrhoea, and syphilis were associated with the risk of HGAIN: OR 14.3 (95%CI 6.20-33.1), OR 10.4 (95%CI 4.32-25.0) OR 9.83 (95%CI 4.20-23.0) respectively.

Conclusion
The association of STI’s such as gonorrhoea, syphilis and genital herpes with the presence of HGAIN deserves further study. Given rates of gonorrhoea are increasing in the MSM community, patients should be counselled to avoid STI acquisition and use condoms to reduce their risk of anal cancer. Detection of HGAIN is problematic with high resolution anoscopy clinics being scarce. A history of gonorrhoea, syphilis or HSV-2 could be used to triage patients at risk of HGAIN to these clinics.

References


Predictive value of methylation markers in anal swab samples for persistent anal HSIL

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Background / Objectives

Gay and bisexual men (GBM) are at increased risk of HPV-associated anal cancer. Screening for the precursor high-grade squamous intraepithelial lesions (HSIL) analogous to cervical cytology screening has been proposed but there is currently no agreed algorithm. Anal HSIL is highly prevalent in GBM, however it is clear that most anal HSIL do not progress to cancer. Identification of biomarkers to establish patients at highest risk of cancer are therefore needed. Change in DNA methylation is recognised as an early essential step in carcinogenesis, and is predictive of cervical HSIL and cancer. It is thought that DNA methylation patterns may also predict anal HSIL. Here, DNA methylation was evaluated as a potential tool to identify persistent anal HSIL among GBM.

Methods

Samples were obtained as part of the Study of the Prevention of Anal Cancer, a 3-year natural history study of anal HPV and related disease in GBM aged 35 years and older. HSIL was defined as a composite of cytology and histology diagnoses (ie had one or both cytological possible HSIL (pHSIL) and/or histological HSIL). Persistent HSIL was defined as HSIL detected at all of baseline, 6 and 12 months clinic visits. DNA was extracted from anal cytology PreservCyt samples from the baseline clinic visit, and subjected to bisulfite conversion. Methylation-specific
quantitative PCR (msqPCR) on promoter regions of CADM1, MAL and miR-124-2 genes were performed, with the positive threshold for each marker set at 1.5%, 0.5% and 0.5% methylation, respectively. Sensitivity and specificity of each marker for detecting prevalent baseline and persistent HSIL were calculated.

Results

Of the 165 participants included, 52 (31.5%) were HIV-positive and 69 (41.8%) had composite HSIL at baseline. In total, 23 (13.9%) had persistent HSIL at 12 months post-baseline. Sensitivity and specificity, positive and negative predictive value (PPV, NPV) of each methylation marker are shown in the table.

<table>
<thead>
<tr>
<th>Marker</th>
<th>HSIL diagnosis</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
<th>% PPV (95% CI)</th>
<th>% NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAL</td>
<td>Baseline</td>
<td>62 (50-74)</td>
<td>41 (31-52)</td>
<td>43 (34-54)</td>
<td>60 (47-72)</td>
</tr>
<tr>
<td>MAL</td>
<td>Persistent</td>
<td>74 (52-90)</td>
<td>42 (33-51)</td>
<td>18 (11-28)</td>
<td>90 (80-96)</td>
</tr>
<tr>
<td>mi-R-124-2</td>
<td>Baseline</td>
<td>52 (40-64)</td>
<td>41 (31-52)</td>
<td>39 (29-50)</td>
<td>54 (42-66)</td>
</tr>
<tr>
<td>mi-R-124-2</td>
<td>Persistent</td>
<td>57 (35-77)</td>
<td>42 (33-51)</td>
<td>15 (8-24)</td>
<td>85 (74-92)</td>
</tr>
<tr>
<td>CADM1</td>
<td>Baseline</td>
<td>36 (25-49)</td>
<td>73 (63-81)</td>
<td>49 (35-63)</td>
<td>61 (51-70)</td>
</tr>
<tr>
<td>CADM1</td>
<td>Persistent</td>
<td>48 (27-69)</td>
<td>70 (62-78)</td>
<td>22 (12-36)</td>
<td>88 (81-94)</td>
</tr>
</tbody>
</table>

Conclusion

Methylation markers MAL, CADM1 and miR-124-2 in baseline anal swab DNA may have higher sensitivity and similar specificity for the detection of persistent HSIL than for the detection of baseline HSIL, however larger numbers and further studies are needed to evaluate markers for detection of persistent HSIL.
Is the persisting HPV genotype on anal swab the causative genotype in HGAIN lesions?


Background / Objectives

To study the correlation between persistent type-specific HPV infection on anal swabs and the causative HPV type in HGAIN lesions in HIV+ MSM.

Methods

A cohort of HIV+ MSM was followed for two years with 6-monthly anal swabs. Swabs were tested for HPV DNA and typed using the SPF10-PCR-DEIA-LiPA25v1 system. Men in whom at least 4/5 anal swabs were positive for the same HPV type were considered to have a persistent anal HPV infection. After this 24-month period men were assessed by High Resolution Anoscopy (HRA), and biopsies were taken. For the present analysis, we selected MSM with persisting anal HPV infections on swab and HGAIN on histology (N=30). After reviewing of HE and p16 slides for the worst diagnosis (AIN2 or AIN3), worst lesions collected using laser capture microdissection were tested for HPV with the same system which resulted in identification of the causative genotypes. We assessed the correlation between persistent type-specific HPV infection on swab and the causative HPV type of HGAIN lesions.

Results

After exclusion of 5 patients in whom the worst diagnosis upon re-examination was less than HGAIN, 51 biopsies from 25 men remained. Worst lesion found on patient level was AIN2 in 9 men and AIN3 in 16 men. On the anal swabs, 12 men had persisting HPV infection of a single HPV genotype (3 lrHPV, 9 hrHPV), and 13 with multiple types. Of the men with multiple persisting HPV genotypes, one man had lrHPV genotypes only, 4 had hrHPV genotypes only and 8 had both lrHPV and hrHPV genotypes. HPV6 was the most frequently found persisting genotype (36%). On biopsy, four men had (multiple) HGAIN lesions with different HPV genotypes and 21 men had one HGAIN lesion with a single causative HPV genotype. The most
frequently found genotype in HGAIN lesions was HPV 16 (28%, 7/25). The causative HPV type (1 lrHPV and 24hrHPV) was the same as the persisting type in 11/25 (44%) men with HGAIN: 2/9 (22%) in AIN2 and 9/16 (56%) in AIN3. In 8/25 men (32%) the causative type was not persistent, but was detected in at least one of the swabs. In 6/25 men the causative type was never found in anal swabs (24%).

**Conclusion**

A persisting genotype was marked as a causative genotype in only 44% of the men with a HGAIN lesion, with a remarkable difference in correlation with AIN2 (22%) and AIN3 (56%). In 32%, the causative genotype was detected in at least one of the swabs and in 24% the causative genotype was never detected on swab in the 2 years prior to HRA. These results demonstrate that the causative genotype is often not persistently or not detected on swabs. Therefore, serial anal swabs may have limited value in screening for HGAIN, but AIN3 is linked to persistence more often than AIN2.
HPV TESTING USING XPERT HPV ON SELF-COLLECTED VAGINAL SWABS VS. CLINICIAN-COLLECTED CERVICAL SAMPLES

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Background / Objectives

HPV testing of self-collected vaginal swabs may help expand access to cervical cancer screening in low-resource settings. We compared the utility for screening of Xpert HPV when run on self-collected vaginal swabs vs. clinician-collected cervical samples.

Methods

At a colposcopy and a primary care site in Cape Town, South Africa, 585 HIV-negative aged 30-65 years were recruited. Self-collected vaginal swabs and clinician-collected cervical samples were tested using Xpert HPV. This assay detects high risk HPV in 5 channels: HPV16, HPV18,45, HPV31,33,35,52,58, HPV51,59, HPV39,56,66,68. Outcome of cervical intraepithelial neoplasia grade 2/3 or cancer (CIN2+) was determined by colposcopy and histology for all women.

Results

Sensitivity of Xpert HPV to detect CIN2+ was similar in self- (85.7%) and clinician- (88.3%) collected samples, but specificity was lower in self- (77.0%) vs. clinician- (87.3%) collected samples. Sensitivity could be retained at high levels if screen-positive was defined as positivity for one or more of the three channels detecting HPV16,18,45,31,33,35,52,58 (84.4% vs 87.0% self vs. clinician, respectively). Restricting to these channels, specificity improved to (82.3 vs 90.5% self- vs. clinician, respectively). Defining screen-positive based on more stringent cycle thresholds (Ct) and allowing sensitivity to be 80%, resulted in specificities of 87.3% and 94.7% in self- vs. clinician-collected samples. If a second clinician-collected sample is obtained and tested from self test-positive women, at 80% sensitivity, specificity can be improved to 92.9%.

Conclusion

HPV testing on self-collected samples has excellent sensitivity. Specificity can be improved by HPV type selection and more stringent Ct cut-offs. Specificity can be improved further with secondary triage with HPV testing on clinician-collected samples.