

# **EUROGIN 2018**

## **Abstracts**

### **PART III - POSTERS**

00336

# THE DEVELOPMENT OF A PRIMARY CELL MODEL FOR THE INVESTIGATION OF DOUBLE STRAND DNA REPAIR IN HPV-ASSOCIATED GYNAECOLOGICAL CANCERS

## 01. Viral and molecular biology

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### **Background / Objectives**

The DNA damage Repair (DDR) pathway is crucial for maintaining chromosomal integrity following challenges by genotoxic insults and is potentially a key determinant of cellular sensitivity to multiple modalities with potential to treat squamous gynaecological cancers including chemotherapeutics, ionising radiation and PARP inhibition. HPV is acknowledged to manipulate the DDR pathway at multiple junctures to facilitate viral replication, and a greater understanding of the consequences of this on double strand DNA (dsDNA) repair in HPV positive tumours may enable an enhancement of therapeutic efficacy and the identification of biomarkers to indicate resistance to conventional treatment.

HPV positive tumours of the oropharynx and genital tract are generally considered more radiosensitive than HPV negative tumours. In vitro studies have demonstrated that in the presence of HPV E6 and E7, cells can exhibit delayed dsDNA repair kinetics, potentially due to the preferential recruitment of DDR proteins to sites of viral DNA (1,2). To our knowledge a comprehensive study of dsDNA repair kinetics in cells derived directly from HPV positive tumours has not been undertaken. We therefore set out to develop primary cells from cervical and vulvar cancers to use as a model for the study dsDNA repair following exposure to ionising radiation.

### **Results**

Fresh tumour (and adjacent normal) tissue was collected intra-operatively from 14 patients undergoing surgical procedures for the management of squamous cancers of the cervix or vulva at Liverpool Women's Hospital, UK. Following initial processing the tissue was incubated in selective media on Cellbind plates until explants formed. Explanted cells that were successfully passaged had DNA and RNA extracted for mycoplasma testing, HPV 16&18 E6 and E7 PCR and Short Tandem Repeat analysis to confirm origin from primary tumour sample. Cell pellets were subjected to

p16 staining and DNA/RNA ISH for high risk HPV. All cell lines demonstrated pan-cytokeratin staining.

Primary cells (passages 3 to 6) were then assessed for radiation sensitivity by clonogenic assay, and this data was correlated to the evaluation of DDR proteins including RAD51, ATM, H2AX and FANCD2 by immunofluorescence and Western blotting at serial timepoints post 2Gy of ionising radiation.

## References

HPV positive and negative primary cells derived from gynaecological squamous cancers and adjacent normal tissue can be successfully developed and used as a comparative model to evaluate dsDNA repair in these tumours. This resource should allow further characterisation of the important relationship between HPV and the DDR pathway, which is desirable to refine the current treatment protocols for HPV positive cancers and look towards the development of new therapeutic strategies.

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00527

## ANALYSIS OF HUMAN PAPILLOMAVIRUS AND DNA PLOIDY IN CERVICAL LESIONS

### 01. Viral and molecular biology

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#### Background / Objectives

Cervical cytology remains the main screening method for cervical cancer, although the detection of human papillomavirus (HPV) oncogenic types has been gaining ground in screening programs. In addition to these methods, aneuploidy identification is a marker of neoplastic transformation and it is associated with progression from precancerous lesions to invasive cancer. The objective of this study was to investigate the ploidy behavior and the presence of high-risk HPV types (hr-HPV) in precancerous cervical lesions.

#### Results

One hundred and two women were referred to colposcopic and biopsy examinations after an abnormal cytology result. Cytological samples were collected in ThinPrep® solution for molecular identification of HPV types by Microarray DNA after PCR amplification. Slides were stained by the Feulgen method for nuclear analysis. DNA ploidy analysis met the requirements established by the 4th European Society for Analytical Cellular Pathology (ESACP) Consensus in DNA image cytometry (DNA-ICM).

#### Conclusion

From 102 patients, 75.3% were positive for hr-HPV and 53.9% had an aneuploid profile. Among patients with aneuploid DNA, 92% were positive for hr-HPV types. According to histological result, 45% of cases without lesion were positive for hr-HPV and 15% were aneuploid. Among cases of cervical intraepithelial neoplasia (CIN) grade 1, 64% were positive for hr-HPV and 20% revealed aneuploidy. In CIN 2 cases, 79.9% were positive for both hr-HPV and aneuploid DNA. All CIN 3 patients were positive for hr-HPV, while 82.4% of CIN 3 patients were aneuploid. All cases of squamous cervical cancer (SCC) were DNA aneuploid, while 66.7% were positive for hr-HPV. No DNA was found in the sample diagnosed as adenocarcinoma in

situ (AIS), but this case was aneuploid. Both cases diagnosed as adenocarcinoma (AC) were positive for hr-HPV and proved to be aneuploid. High risk HPV types were present in 84.2% of CIN 2+ (CIN 2, CIN 3, AIS, AC or SCC) cases with a significant relationship. The relationship between aneuploidy and CIN 2+ cases was also significant, with aneuploidy being present in 82.5% of CIN 2+ cases. When both hr-HPV and aneuploidy were positive, they were present in 68.4% of CIN 2+ cases. HPV 16 was the most frequent HPV type observed, followed by types 31, 35, and 58.

## References

DNA aneuploidy was increasingly observed in relation to the precancerous lesions severity progression, whereas oncogenic HPV types were observed in a more balanced manner. Thus, the use of hr-HPV identification along with a DNA ploidy profile could be useful to identify high-grade lesions and indicate which lesions have the greatest potential for progression.

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00622

# HIGH THROUGHPUT MOLECULAR TESTING FOR HPV: PERFORMANCE OF THE ONCLARITY™ HPV ASSAY ON A NEW HIGH THROUGHPUT SYSTEM VERSUS THE VIPER™ LT SYSTEM

## 01. Viral and molecular biology

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### Background / Objectives

The BD COR™ System is a new high throughput, automated and modular molecular platform designed to address the pre-analytical challenges of today's clinical laboratories. The BD COR™ System focuses on providing efficiency, competitive performance and flexibility by simplifying sample sorting complexities, reducing hands on time and the potential for manual errors, and providing scalability to satisfy a broad range of molecular testing needs. The system is intended to offer a portfolio of assays that provide diagnostic solutions in the areas of microbiology and women's health. The study described here compares performance of one assay, the BD Onclarity™ HPV Assay, tested on the BD COR™ System versus the BD Viper™ LT System using simulated specimens stored in BD SurePath™ Preservative Fluid and Hologic PreservCyt® Solution.

### Results

Contrived panels were prepared by spiking BD SurePath™ and PreservCyt® media with quantified C-33A (background cervical epithelial cells), SiHa (HPV16), HeLa (HPV18) and MS751 (HPV45) cells to simulate cervical specimens comprising HPV infection at low target levels to challenge the system. Spike levels for each of the tested genotypes included Negative (C-33A only), High Negative (C5), Low Positive (C95) and Moderate Positive (3xC95) as determined using the BD Onclarity™ HPV Assay on the BD Viper™ LT System. For each media type, a total of 81 replicates were tested for each spike level of each genotype across 3 BD COR™ and 3 BD Viper™ LT systems. A two one-sided test method (TOST), using an  $\alpha$ -level equal to 0.05, was used to assess equivalence across the two test systems. A pre-determined allowable margin for the difference in mean Ct.score values between BD COR™ and BD Viper™ LT systems was assigned to be  $\pm 0.75$ . Performance at each of Negative,

C5, C95 and 3xC95 levels for each of HPV16, HPV18 and HPV45 genotypes was assessed across 3 BD COR™ systems relative to the reference of 3 BD Viper™ LT systems.

#### **References**

Performance of the BD Onclarity™ HPV Assay using the BD COR™ high throughput molecular system when tested with contrived panels is equivalent to the same assay performed on the BD Viper™ LT System.

\* The BD COR™ System is under development and is not available for sale or use.

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# INCIDENCE OF CERVICAL LESIONS ASSOCIATED WITH HUMAN PAPILLOMAVIRUS INFECTION IN WOMEN LIVING IN TRIBUTARY COMMUNITIES OF AMAZONAS RIVER - BRAZIL

## 02. Epidemiology and natural history

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### Background / Objectives

**Introduction:** Cervical cancer remains a word public health problem. The relationship between this neoplasia and human papillomavirus infection is well established. In Brazil, the National Cancer Institute (INCA) predicts that there will be 16,300 new cases of the disease in 2018. There are no reports of cases about this neoplasm in the riverine populations of the Amazon River and its tributaries, leaving this population deprived of medical assistance and programs of cancer prevention.

**Objective:** The present work aims, by using a liquid-based cervical cytology followed by a Human Papillomavirus (HPV) genotyping, to identify the incidence of HPV infection, a precursor lesion of cervical cancer, in cervical samples from women living in the riverside of Negro and Madeira River.

### Results

**Method:** 123 cervical samples were collected in a liquid medium (Cellpreserv) and automatically processed on KLP 2000 equipment (KolplastÔ). Two cytologists analyzed the cellular material subjected to conventional Papanicolaou staining and classified the results by Bethesda System (2011). The HPV genotyping was performed using the MicroArray (EuroimmunÔ) method in duplicate. Data were analyzed statistically by the Mann-Fisher exact test and the chi-square test. The study was duly approved by the Ethics Committee of the Santo Amaro University - SP (Brazil Platform - CAAE: 61414216.4.0000.0081).

### Conclusion

**Results:** Of the 123 cellular samples, 65 samples were from Negro River riverside population and among of them 12.32% showed squamous intraepithelial lesions with

neoplastic potential such as ASCUS (1.54%), LSIL (6.15%) and HSIL (4.62%). The highest incidence of HPV types were 16, 45, and 61 (21.43%). Others 58 cellular samples were from Madeira's riverside population and 6.7% showed cervical intraepithelial lesions with neoplastic potential, of them ASCUS (1.72%), LSIL (1.72%) and HSIL (3.45%) The incidence of HPV infection, in both regions, was 31 (25.20% samples analyzed by molecular test, being HPV 16 (4,87%) and HPV 45 and 53 (3,25%) the most prevalent types. The highest incidence of HPV infection was in women aged less than 25 years (43.75%) and over 50 years (27.27%), compared to women aged between 26 and 49 years ( $X^2 = 9.64$   $p = 0.0081$ ). Viral infection was more frequently found among single women than married women ( $p = 0.0412$ ).

## References

**Conclusion:** Our results showed a high risk of developing cervical neoplasia in young and single women due to the great incidence of high-grade squamous intraepithelial lesions (HSIL) associated with HPV types of high oncogenic risk found in women living in the riverside communities near the Madeira and Negro River, tributaries of the Amazon River.

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00027

# PHYLOGENETIC CHARACTERISATION OF HIGH RISK HUMAN PAPILLOMAVIRUS GENOTYPES ISOLATED FROM GAMBIAN WOMEN

## 02. Epidemiology and natural history

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### Background / Objectives

High risk human papillomavirus and its variants are known to cause approximately 90% of cervical cancer, a leading cause of death amongst women globally. Oncogenicity varies according to the HPV genotype, as well as the lineage of some genotypes. HPV is markedly heterogeneous and more than 150 genotypes have been identified, which are classified into types, lineages, and sub-lineages based on analysis of the structural late gene protein (L1 gene).

Aim of study: To sequence and identify HPV isolates from reproductive aged women attending a sexual health clinic in Banjul, Gambia, and compare these with previously published sequences of isolates from other geographical locations.

### Results

A 450 base pair region of L1 gene was amplified by PCR using PGMY 09/11 consensus primers. Amplified PCR products were purified and sequenced, and BLASTn searches of the generated sequences were carried out for initial HPV genotype identification. The Gambian sequences were then aligned with other representative sequences of HPV genotypes using MUSCLE and a Maximum Likelihood phylogeny was generated in MEGA7 under the conditions of the T92+G model with 1000 bootstrap replicates. The measure of divergence within and between genotype specific clades was also calculated using MEGA7

### Conclusion

Six different high-risk, and two probable high-risk, HPV types were detected which were HPV58, HPV52, HPV16, HPV51, HPV56, HPV66, and HPV73 and HPV53, respectively, with the newly generated sequences sharing 90-100% nucleotide similarity with previously published sequences of these different HPV genotypes in GenBank. The most common genotype detected was HPV52. Phylogenetic analysis confirmed the identity of the Gambian samples of which each grouped within its respective HPV genotype clade. Within each genotype Gambian samples clustered with reference-sequences from different geographical regions but no distinct geographical lineages were identified. Overall only low levels of inter and intra divergence between the representative viral sequences were detected

## **References**

Low levels of divergence between the Gambian L1 gene HPV genotype sequences and those from other geographical localities illustrate a probable rapid radiation event of these specific HPV genotypes. However, owing to the low levels of diversity between the sequences and the lack of geographically distinct lineages it was not possible to identify potential origins of HPV in Gambia. Current results could illustrate either a recent invasion event of these HPV genotypes into Gambia or, more likely, could be recent movement of HPV from Gambia mediated by the historical movement of people.

## **References**

00033

# IDENTIFYING BIAS CAUSED BY MULTI-TYPE HPV INFECTIONS IN TYPE-SPECIFIC PROGRESSION PARAMETER ESTIMATES

## 02. Epidemiology and natural history

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### Background / Objectives

Type-specific progression and clearance parameters are typically estimated for each HPV type separately. However, such estimates may be influenced by multi-type infections. The parameters can also be estimated simultaneously for all HPV types by applying a multi-type model. Our aim with this study was to investigate the differences between single-type and multi-type estimation procedures for type-specific parameters and how different conditions influence these estimates.

### Results

A simplified progression model was built for two (generic) HPV types with given type-specific progression and clearance parameters. The model was used to produce a simulated data set from which the type-specific parameters were estimated in two ways: 1) single-type estimates for both types separately, and 2) multi-type estimates for both types simultaneously. The single- and multi-type estimates were compared to the original parameters with which the data was produced. To investigate the conditions under which the estimates differ, the procedure was repeated with different progression and clearance parameters.

### Conclusion

The multi-type estimation procedure provided reliable and accurate estimates of the progression and clearance parameters. Under the considered conditions single-type estimates included up to 40% bias. The error in single-type estimates was largest when there was a 50% difference between the two type-specific progression rates and smallest when the types were both progressing at an intermediate rate.

### References



Single-type estimates for progression and clearance may be biased by multi-type HPV infections. Our results with two HPV types give motivation to extend the inference to a realistic multi-type model.

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# DECIPHERING THE KINETICS AND ECOLOGY OF HUMAN PAPILLOMAVIRUS (HPV) GENITAL INFECTIONS IN YOUNG WOMEN

## 02. Epidemiology and natural history

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### Background / Objectives

Human papillomaviruses (HPVs) are responsible for one third of infection-induced cancers [Total et al. 2011 Prev Med]. Most studies focus on chronic infections and cancers, and we know little about the early stages of the viral infection [Alizon et al. 2017 Viruses]. In particular, the roles in infection clearance or persistence of the immune system, the microbiota and the virus genetics remain poorly understood. We designed a longitudinal study to unravel the course of HPV genital infections in young women.

### Results

This study combines mathematical modelling, bioinformatics and clinical research. We follow longitudinally 150 young women (aged 18 to 25) visiting an STI detection centre in Montpellier (France). HPV negative women visit every 4 months and HPV positive women visit every two months. All women are required to perform 8 self-samples at home between visits. At each visit, a gynaecological exam is carried out and samples are collected to measure HPV virus load, systemic anti-HPV antibodies induction, local cytokine levels, and determine HPV genotype and local immune cell response. These data consist in time series, which will be used to fit mathematical models. In addition, we sample genital microbiota using eSwabs to analyse its interaction with HPV infection.

## **Conclusion**

Our preliminary data show that HPV prevalence is high in the study population but the longitudinal follow-ups are very consistent in terms of types. We are also able to analyse local immune cells using a ten color panel by flow cytometry and to detect specific cytokines.

## **References**

This study will provide us with one of the most detailed natural history studies of acute HPV infections in young women and their interactions with the host immunity and the vaginal microbiota. It will also allow us to investigate related issues regarding HPV genetic diversity, vaginal microbiota dynamics and sexually transmitted infections in general.

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00070

# SIGNIFICANT DISPARITY IN THE PREVALENCE OF LOW-RISK BUT NOT HIGH-RISK HPV GENOTYPES AMONG HIV POSITIVE MSMs AS COMPARED TO HEALTHY WOMEN

## 02. Epidemiology and natural history

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### Background / Objectives

Although the anus and the cervix share some anatomical/histological similarities, in particular with respect to the presence of a susceptible transitional zone, it is previously shown that anal HPV infection tends to be more diverse and persistent than HPV infection of the cervix. This diversity tends to widen when one compares cervical HPV infections among healthy women with anal HPV infections among HIV positive MSMs.

In a country where a-free-of-charge HPV vaccine is provided in a gender-neutral manner, it is of interest to understand the details of which genotypes predominate in which anatomical site and among which population group. The aim of this epidemiological study is to assess disparities in the distribution of HPV genotypes among HIV positive MSMs and healthy screening women in Austria.

### Results

Out of a total of 813 sexually active men (n= 271) and women (n=542), 355 HPV positive adults were selected for this analysis. Anal swab samples collected for STD screening among HIV positive MSMs and cervical swab samples collected as part of the routine cervical cancer screening program were additionally examined for 40 HPV genotypes using a reverse line blot hybridization method. Sociodemographic and behavioral data were collected using structured questionnaires.

## Conclusion

Considering all 813 study participants the prevalence (95% CI) of overall HPV infection was 90% (86.3-93.4) among HIV+ MSMs and 20.5% (17.0-23.8) among healthy women. HPV positivity was significantly associated with age, age at sexual debut and number of life time sexual partners (LSP) in both study population, but smoking showed significant association only with cervical infection. Although HIV+ MSMs have higher prevalence of all detected genotypes as compared to cervical infections in women, a closer look into those HPV-positive study participants (n=355) revealed that MSMs have a statistically significant higher proportion of LR-HPV types whereas the HR-HPV genotypes predominate among women. Multivariable adjusted OR (95% CI) for the distribution of LR-HPV among this high risk population was 7.3 (2.25-23.5) whereas that of HR-HPV types was 0.19 (0.05-0.79).

## References

Considering existing data (based on laser capture microdissection) which suggest that LR-HPV types might be occasionally associated with anal cancer, our finding of significant predominance of LR-HPV needs a closer scrutiny. The observed discrepancy in the distribution of HR- and LR-HPV among these two population groups suggests that factors other than the number of life time sexual partners (significantly higher in HIV+ MSMs) might play a role in the acquisition and pathogenesis of HPV.

00102

# PREVALENCE OF HIGH-RISK HPV IN A REGION OF PORTUGAL: ANALYSIS OF DATA FROM PATIENTS REFERRED FROM CERVICAL CANCER SCREENING

## 02. Epidemiology and natural history

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### Background / Objectives

In Portugal's North region, a population cervical cancer screening was implemented. Women were tested for high-risk HPV and referred to consultation if they tested positive for HPV16 or HPV18 or if they had other high-risk HPV (hr-HPV) associated with cytology abnormalities. The aim of this study was to characterise the population referred to the Inferior Genital Tract Unit of Centro Hospitalar Póvoa de Varzim/Vila do Conde, in particular the prevalence of different HPV serotypes.

### Results

We conducted a retrospective study analysing the clinical cases of 138 women referred to consultation in our Unit after cervical cancer screening during an eighteen-month period (from January 2017 to June 2018). Clinical data – such as demographic data, motive of referral, HPV serotype(s) and colposcopy and biopsy results – was extracted and analysed. Results were compared to those of the Cervical Lesions Observed by Papillomavirus Types-A Research in Europe (CLEOPATRE).

### Conclusion

The women were aged between 25 and 64 and none of them were immunised against the HPV virus. The main reason for referral was hr-HPV and atypical squamous cells of undetermined significance (ASC-US) in the cytology, followed by presence of HPV16 or 18, and in third place by hr-HPV and a cytology showing low-grade intraepithelial lesion (LSIL). The most prevalent serotype was HPV 16 (present in 20% of women), followed by HPV 31 (11%); HPV 18 was present in 8% of women;

hr-HPV other than these were present in 60% of the population studied. In particular, serotypes not covered by the nonavalent vaccine (HPV 68, 39, 66, 51, and 59) were found 71 times in the population studied (HPV 68 was in fact the third most common serotype). Infection by multiple serotypes affected 32% of women, being more frequent in the younger population.

## **References**

In our (non-vaccinated) population, the most common serotypes are 16 and 31, which is in line with the results of the CLEOPATRE study. In contrast, the rate of multiple serotype infection was significantly higher than the one described in that study (32% vs 7%). In addition, the serotypes 68, 39, 66, 51, and 59 were quite common in our population. This finding raises questions about the potential negative impact in the disease evolution in the future, after nonavalent vaccination. Further studies and epidemiological monitoring is, therefore, necessary in our particular population.

00127

## ANALYSIS OF HPV 18 DIVERSITY AMONG WOMEN WITH DIFFERENT MORPHOLOGY DIAGNOSIS IN RUSSIAN FEDERATION

### 02. Epidemiology and natural history

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#### Background / Objectives

Cervical cancer takes first place in morbidity of women of reproductive age in Russian Federation. HPV 18 is detected in about 14% of invasive cervical cancer. Despite numerous investigations in the world there is still lack information about HPV 18 diversity in Russian population.

#### Results

This part of work is preliminary to whole genome sequencing of about 1000 samples of HPV 18 type from RF with different morphology diagnosis. To evaluate distribution of HPV 18 variants, URR and E6/7 genes from 35 samples were sequenced by Sanger method. 22 samples have histology confirmed CIN2+diagnosis, 11 – negative for intraepithelial lesion and malignancy.

#### Conclusion

All samples belong to A variant, among them one sequence obtained from patients with carcinoma in situ diagnosis clustered together with A1 lineage reference sequence, and one from patient with LSIL – together with A4 variant reference sequence. Most of other sequences were close to A3 lineage. There was neither correlation with histology/cytology diagnosis nor with viral integrated/nonintegrated form.

Interestingly, two samples from patients with NILM cytology diagnosis formed separate branch with 0.7% dissimilarities from closest variant A4. Both samples have integrated viral form.



## References

Molecular epidemiology of HPV is a blank space in Russian Federation despite high rate of cervical cancer mortality and morbidity. We obtain preliminary data on distribution of HPV18 lineage on territory of Russian Federation. HPV 18 population is quite uniform and belong to A lineage, as it was shown for HPV 16. Yet we cannot elicit any influence of HPV 18 variant on morphology diagnosis due to small amount of samples.

00136

## A CLINICAL AUDIT ON HPV PREVALENCE AMONGST CERVICAL LESIONS IN MALTA

### 02. Epidemiology and natural history

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#### Background / Objectives

Human Papillomavirus (HPV) infection has been established as the main causative agent of cervical lesions worldwide. HPV may rest in a latent phase up to the trigger of a transformation event, where lesions are induced; these varying from LSILs to HSILs which may eventually progress to SCC. Different HPV sub-types may cause different lesion severities. The aims of this retrospective clinical audit are mainly to assess the prevalence of HPV-induced lesions together with the most prevalent hr-HPV subtypes in Malta and to correlate the HPV genotypes to histology and cytology results.

#### Results

The study by Falzon et al. (2013) analysed all CIN3 and invasive carcinomas over the period 2000-2013 in Malta, using a Multiplex HPV genotyping assay and RT-PCR techniques on FFPE tissue. HPV Prevalence Data was obtained through the study carried out by Zahra et al. (2018) which included the different hr-HPV sub-types in Malta over 3 years (2015-2018). 96 HSIL LLETZ Histology cases, excised from 2011-2017 were extracted from Mater Dei Hospital Laboratory records. COGNOS® Software was used to extract the data required. All LBC Specimens, with a suspected lesion from April 2018 up to June 2018 were assessed for the respective result of HPV genotyping.

## Conclusion

The study by Falzon et al., (2013) indicated that between the period 2000-2013, 91% of invasive cervical carcinomas were found to be HPV positive, with HPV 16 being the most prevalent genotype. HPV 16, followed by HPV 56 were also the most prevalent genotypes in the study carried out by Zahra et. Al (2018). Results obtained through the Mater Dei Hospital Histopathology laboratories demonstrated that the LLETZ specimens diagnosed as HSIL from the period 2011-2017 were most prevalent in the age group 31-35 (35.4%), followed by the 26-30 age group (22.9%). 16.7% of HSIL LLETZ cases were diagnosed in the 36-40 age group whilst 12.5% and 11.5% of cases fell in the >40 and the 20-25 age groups respectively. One case was reported to have had a HSIL LLETZ excision under 20 years of age.

## References

This study has shown that in concordance with worldwide studies, HPV16 is the most prevalent HPV subtype in Malta, causing the majority of invasive cervical carcinomas, however HPV56 is the second most prevalent HPV subtype. The fact that certain high-risk cytologically abnormal cases had a negative HPV result indicates the possible presence of additional hr-HPV subtypes. This study has provided information about the distribution of HPV genotypes in Malta, as well as the HPV genotypes mostly associated with high-grade cervical lesions. It is also part of an ongoing study involving the use of molecular biomarkers for prediction of HPV transformation events, possibly leading to optimised techniques for improved patient diagnoses.

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00206

A Cohort Study on the cervical precancerous lesion demographic character, prevalence and Practice on its association with Human Papillomavirus Virus subtypes between Iranian populations

## **02. Epidemiology and natural history**

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### **Background / Objectives**

Retrospective study has reported that cervical cancer is the most common gynecologic malignancy around the world. At the first sight, based on the long-time interval between the pre-cancerous phase and this malignant progression, and the simple available screening test, in the other hand the well-known correlation between cervical lesion (mainly squamous cell subtype) and human papillomavirus, its prevention seems to be simply achieved. Despite all of this concept near 270000 woman's death per year is due to this malignancy.

This study aimed to assess the demographic data on Human papillomavirus infected patient and the reliability of the present screening test, and to establish any correlation between the sub type of human papilloma virus (HPV) and cervical lesion progression among Iranian woman in Mashhad.

### **Results**

This cohort study was conducted on 562 patients with cervical intraepithelial neoplasia and cancer, who was referred to the Gynecology Clinic at Ghaem Hospital- Iran- Mashhad from Nov 2016 to Feb 2018. All patients demographic, familial, nutritional, clinical feature, previous screening (consist of Pap smear and HPV typing test) and diagnostic test (colposcopic biopsy samples) results were collected and analysis by SPSS.

### **Conclusion**

Among 562 patients who included in the study, cervical intraepithelial lesion grade 1, 2&3 and cancer cases were 430, 100 and 32 patient, respectively. The mean age was 36+/-9 years in pre-cancerous lesion group versus 47.2+/-8 years in malignant cases. No significant difference was identified between cervical lesion and weight, smoking, alcohol drinking and parity. ( $p > 0.05$ ). In analysis of the contraceptive method usage between patients with cervical lesion there were found many cases of cervical intraepithelial lesion who had used barrier (condom) even and unfortunately most of the patient had no method of contraception at all. Present screening Guideline in Iran is based on the adequacy of Pap test among women over 30 years old, meanwhile, there is no need for screening, HPV infection among women, so far not in younger age; but in this study, the mean age of first sexual intercourse was 19 years old and even under the age of thirty there were detected multiple cases of cervical pre-cancerous lesion.

## References

An organized cervical cancer screening is a necessity for Iran as more than 500-900 women in middle age diagnosed with an invasive cervical cancer every year cannot be ignored. This recommendation should be taken into account by the National Health System of Iran and Muslim countries with shared culture and behavior patterns. Cobas HPV test could be consideration in countries Muslim country with appropriate budget, resources and facility.

00295

Prevalence of Human Papillomavirus among adolescents after introduction of school-based HPV vaccination in Norway

## 02. Epidemiology and natural history

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### Background / Objectives

The objective of the study is to assess the effect of school-based HPV-vaccination by comparing type-specific HPV prevalence between vaccinated and non-vaccinated women of the first cohort offered vaccination in Norway.

### Results

Facebook advertisement was used to recruit women born in 1997, the first cohort to be offered school-based HPV vaccination. Self-samples were collected from the vagina, using Evalyn Brush®, Rovers. All samples were HPV-typed using modified general primers (MGP)-PCR followed by hybridization of type-specific oligonucleotide probes (Luminex technology), detecting and genotyping 37 HPV types. Sexual habits were ascertained through a short questionnaire. Self-reported vaccination status was validated via linkage to the Norwegian Immunization Registry (SYSVAK). Multivariate Poisson regression was used to calculate prevalence ratios (PR). All presented PRs are adjusted for lifetime number of sexual partners, age at sexual debut and time since last intercourse.

### Conclusion

Of 562 women who completed the registration form, 315 returned a self-sampling device and were included in the study. Among the 312 women with complete data, 239 (76.6%) had been vaccinated with at least one dose of quadrivalent HPV (qHPV) vaccine prior to sexual debut. Prevalence of any HPV type was similar for both vaccinated (38.5%) and unvaccinated (41.1%) women (PR: 0.97, 95% CI: 0.63-1.48). Prevalence of at least one high risk HPV type was 19.2% in both vaccinated and unvaccinated women (PR: 1.07, 95% CI: 0.58-1.99). Prevalence of qHPV types was

low in the cohort overall at 1.9%. Prevalence of qHPV vaccine types was 0.4% for the vaccinated women and 6.8% for the unvaccinated (PR: 0.04, 95% CI: 0.00-0.42).

## References

Among the first cohort of women receiving school-based HPV-vaccination in Norway, overall prevalence of any HPV was similar for vaccinated and unvaccinated women. We observed lower prevalence of qHPV vaccine types among those who received at least one dose of qHPV vaccine before sexual debut, after adjusting for sexual behavior. However, this result was based on small numbers and should therefore be interpreted cautiously.



00388

Human papillomavirus (HPV) cytology and genotype distribution in vaccinated women from East-Flanders, Belgium

## 02. Epidemiology and natural history

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### Background / Objectives

The human papillomavirus (HPV) vaccination program for young women has been introduced in Flanders (Belgium) since 2010. Monitoring of changes in the prevalence of HPV infection has been proposed as a useful tool to detect breakthrough infections and possible shifting in genotype distributions. However, data on HPV prevalence under vaccination are limited. The objective of this study was to evaluate the prevalence of HPV infection and genotype distributions among vaccinated women attending a public hospital.

### Results

Women presenting at the gynecological department of AZ Jan Palfijn hospital in Ghent were recruited during 110 consecutive days. Cervical smear samples were collected to perform cervicovaginal cytology. The Cobas<sup>®</sup> HPV Test was performed to identify HPV types 16 and 18 while concurrently detecting other high-risk (HR) types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) without individual identification. Vaccination information was collected using standardized questionnaires.

### Conclusion

Out of 2979 specimens, information about the vaccination status was available in only 114 (3.8%) cases. Ninety-five (83%) patients were non-vaccinated (23-63 year). In the group of 19 (17%) vaccinated women (21-46 year), one patient was vaccinated with Cervarix®, seven with Gardasil®, one with Gardasil 9® and for three patients vaccine type could not be specified. In the vaccinated group, three specimens were classified as NILM (one HR HPV+), three as LSIL (all HR HPV+), eleven as ASC-US (eight HR HPV+), one as ASC-H (HR HPV+) and one as HSIL (HR HPV+). All detected HPV types were identified as other-high risk types except one ASC-US specimen that showed positivity for HPV16 and other high-risk types.

## **References**

There is a need for a longitudinal monitoring program to determine postvaccinal changes in genotype distributions and breakthrough infections. Since the interpretation of our findings is hampered by the lack of sufficient vaccination data, future research should include an optimized strategy in retrospective data collection (e.g. consultation of the national vaccination register) and full genotyping.

00454

## BREAST CANCER ASSOCIATED TO HPV AND CONCOMITANT PARTNER WITH A HISTORY OF PENILE CANCER

### 02. Epidemiology and natural history

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#### **Background / Objectives**

In the last twenty years, many papers have described the relation between breast cancer and Human Papillomavirus (HPV). Recently, we have published an article relating the presence of HPV identified by multiplex nested Polymerase Chain Reaction (PCR) tests in the tumor tissue in 50 of 100 breast cancer diagnoses in the Federal University of Ceará (UFC) Hospital. After this publication we started a research which was approved by the Ethics Committee No 2.396.348-2017 of UFC, in order to identify how HPV approached the ductal system in the breasts of the patients examined at the Grupo de Educação e Estudos Oncológicos (GEEON). The objective of this case report is to present a case of a woman with breast cancer that had a partner with a history of penile cancer.

#### **Results**

After January 2016, all patients in GEEON breast service have been investigated for HPV diagnosis history in previous cervical cancer screenings followed by dermoscopy of the nipple and biopsy of suspected HPV-contaminated areas, oral and vaginal swab examinations, Immunohistochemistry tests for P16 in the breast tumor biopsies and PCR testing of the tumor sample and punch biopsy of the nipple in order to confirm HPV. A 37-year-old patient named MLS with a 3cm diameter malignant tumor in her upper right quadrant of the right breast, when asked about HPV, did not know if she had genital HPV, but knew that her husband had penile cancer associated to HPV diagnosed 3 years prior. We took a core biopsy of the tumor and a dermoscopy-guided punch biopsy of an area of the nipple tissue with abnormally high vascularization to identify traces of HPV in the tumor and the nipple. In addition, the patient underwent oral and vaginal swab tests to identify traces of HPV.

#### **Conclusion**

HPV 16 and 33 were identified oral cavity of the patient with breast cancer. In the paraffin-shaped biopsy of the breast tumor and of nipple, were identified HPV 6 and 11. The p16ink4a was positive in breast cancer sample. But cervical and vaginal samples were negative for HPV. The HPV 6 and 11 were also identified in the penile specimen of her partner and p16ink4a was expressed too.

## **References**

Presence of HPV at various genital and non-genital sites of an illustrative case of breast cancer patient and partner with penis cancer opens up prospects for further studies that may elucidate the actual natural history of the virus especially with regard to breast cancer. Nipple tissue and Breast Cancer positives to same genotypes of penile cancer of sexual partner could suggest that the virus could reach the ductal system of her breast through sexual intercourse.

00466

## CERVICAL ADENOCARCINOMAS, HUMAN PAPILLOMAVIRUS TYPES AND ASSOCIATION WITH AGE; AN ASSOCIATION STUDY

### 02. Epidemiology and natural history

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#### Background / Objectives

Background/Objectives: Cervical cancer is the second most common type of cancer in women. Infection by high-oncogenic risk human papillomaviruses (HPV) is the principal causal factor of the development of cervical cancer. HPV 16 and 18 predominate in squamous cell carcinomas and adenocarcinomas, followed by HPV 45. The objective of this study was to evaluate the specific HPV types present in women diagnosed with cervical cancer and identify associations with age and histological type.

#### Results

The hematoxylin-eosin stained slides were reviewed and the cases were then selected according to the histological diagnosis obtained. The paraffin blocks corresponding to those slides were separated to perform further testing using molecular techniques. A total of 106 paraffin-embedded tissue blocks from biopsies performed in cervical cancer patients, 84 cases of squamous cell carcinoma and 22 cases of adenocarcinoma, were selected. HPV detection and genotyping were performed using the INNO-LiPA HPV Genotyping assay (Innogenetics)

## Conclusion

The age of the patients at cervical cancer diagnosis ranged from 26 to 91 years, with a mean of 52.3 years (95% confidence interval (95%CI: 49.3 - 54.7). The mean age of the patients with a diagnosis of adenocarcinoma was 46 years (95%CI: 40.7 - 51.3) compared to 53.9 years (95%CI: 49.7 - 56.2) for the women with a diagnosis of squamous cell carcinoma. Women diagnosed with adenocarcinoma were significantly younger than those diagnosed with a squamous cell carcinoma ( $p=0.04$ ). In 56.6% of these cases, HPV 16 was found as a single infection and in 14.3% in combination with other HPV types as a multiple infection. HPV 18 was found in 12 cases: in 7.5% as a single infection and in combination with HPV 16 in 3.8%. Positivity for HPV 16 in single infection or in combination with other HPV genotypes was significantly associated with a diagnosis of squamous cell carcinoma while adenocarcinomas were associated with HPV 18 in single or multiple infections. A significant association was found between age <50 years at the time of cervical cancer diagnosis and a histological diagnosis of adenocarcinoma (OR = 4.31; 95%CI: 1.45 - 12.80;  $p = 0.007$ ). Women <50 years of age with cervical cancer were 5.41 times more likely to test positive for HPV 18 when compared to those infected with HPV 16.

## References

The variation in the prevalence of HPV types according to age may be the result of a progression of precursory lesions of cervical adenocarcinoma containing HPV 18 considering that this type is often integrated into the host genome.

00558

## HIGH RISK HUMAN PAPILLOMAVIRUS IN A GROUP OF PORTUGUESE WOMEN

### 02. Epidemiology and natural history

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#### **Background / Objectives**

Human Papillomavirus (HPV) is the etiological agent for cervical cancer and genital warts. Worldwide, cervical cancer is the fourth most common cancer in women and the high risk HPV (HR-HPV), namely HPV 16 and 18 are responsible for the most of the cases. The objective was to analyze the HR-HPV frequency in a group of women referred for HR-HPV testing.

#### **Results**

Clinical samples from 3117 women were performed by Cobas® HPV test (Roche Molecular Systems, CA, USA), this assay detected HPV 16 and HPV 18 and 'Other HR-HPV' (-31,-33,-35,-39,-45,-51,-52,-56,-58,-59,-66 and 68). Positive samples for 'Other HR-HPV' were sequenced for genotyping using MY09/11 primers.

#### **Conclusion**

HR-HPV frequency was 20.8% (649/3117). Among the positive samples, 'Other HR-HPV' was the most common (72.8%; 473/649). HPV 16 and 18 were detected only in 22.8% (148/649) and 7.4% (48/649) of the cases, respectively. 7.4% (48/649) of the positive women were infected with more than one HPV (34 with 'Other HR-HPV' + HPV 16; 8 with 'Other HR-HPV' + HPV 18; 5 with 'Other HR-HPV' + HPV 16 + HPV 18 and 1 with HPV 16 + HPV 18). Sequencing of 'Other HR-HPV' is ongoing and preliminary results shown the majority frequency for HPV 31 (11.7%) followed by HPV 56 (9.1%) and 8.9% for the HPV 66.

#### **References**

The HR-HPV frequency is high (20.8%), 30.4 % of these women were infected with HPV 16 or HPV 18 which is a high frequency. This study reveals the importance of the implementation of screening programs, and the use of HPV detection.

00607

Human Papillomavirus infection in gynecological cancers in Latvia: From the epidemiological data to clinical impact

## **02. Epidemiology and natural history**

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### **Background / Objectives**

According to the United Nations (UN) definition, the Baltic countries belong to the group of countries whose economy is in transition. This transition period has created changes in life-style, priorities, and living standards. On a global scale, the New Independent States (NIS) of the former Soviet Union have an intermediate incidence of cervical cancer, the main etiologic factor of which is Human Papillomavirus (HPV), a major sexually transmitted disease (STD). Recently, the prevalence of all STD has exploded in these countries. In parallel with the increase of STD and because of the lack of any organized cancer screening in the new independent states of the former Soviet Union, the incidence and mortality rates of cervical cancer are rapidly rising. The objective of this presentation is to outline the most recent data on HPV and related cancers in Latvia provided by the Catalan Institute of Oncology (ICO) Information Centre on HPV and Cancer.

### **Results**

We present the ICO Information Centre on HPV and Cancer Fact Sheet 2017 (2017-07-27) that provides the most recent data on HPV and related cancers in Latvia.

### **Conclusion**

Latvia has a population of 923,264 women ages 15 years and older who are at risk of developing cervical cancer. Current estimates indicate that every year 284 women are diagnosed with cervical cancer and 135 die from the disease. Cervical cancer



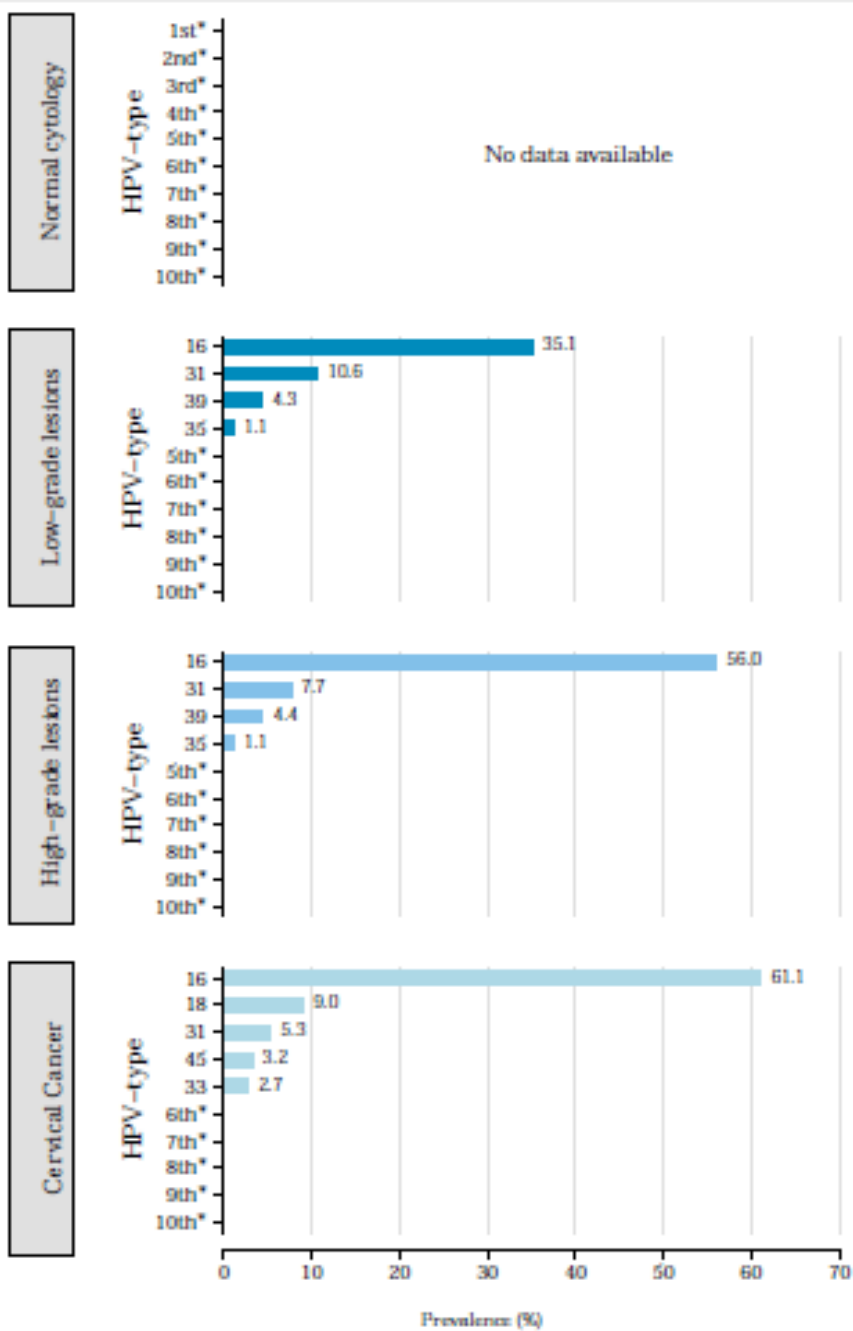
ranks as the 5th most frequent cancer among women in Latvia and the 2nd most frequent cancer among women between 15 and 44 years of age. Data is not yet available on the HPV burden in the general population of Latvia. However, in Northern Europe, the region Latvia belongs to, about 4.5% of women in the general population are estimated to harbor cervical HPV-16/18 infection at a given time, and 77.0% of invasive cervical cancers are attributed to HPV 16 or 18.

## References

The incidence rate of STD in the Baltic countries is increasing, the average age of patients suffering from STD is decreasing, the specificity of the diagnostic methods used for STD needs to be improved. Women and girls in these NIS countries are conservative in many key characteristics of “high-risk” sexual behavior, such as age at onset of sexual activity, number of partners, and casual sex partners. HPV-positive and HPV-negative groups are clearly distinguished by the same variables identified as the key risk factors of HPV infection and cervical intraepithelial neoplasia (CIN) in Western countries. Surveillance of STD should be intensified where needed. Additional or better-quality data should be collected including reasons for testing, denominator data to estimate positivity rates, diagnostic methods, concurrent STD, sexual orientation, and country of acquisition. More analytical rather than descriptive epidemiology is needed.

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00609

Epidemiology of oral HPV infection in tonsil tissue

## 02. Epidemiology and natural history

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### Background / Objectives

Human Papillomavirus (HPV), is the causative agent of cancers of the genital tract, including cervical cancer. While cervical cancer prevalence is declining due to effective cervical screening procedures and HPV vaccination programmes, the prevalence of HPV positive Head and Neck Squamous Cell Carcinoma (HNSCC) has been increasing, particularly in young men. HPV is strongly associated with oropharyngeal carcinoma (OPC), a subset of HNSCCs. The prevalence of HPV positive OPC is projected to surpass HPV positive cervical cancers by 2020.

Despite a thorough understanding of the viral life cycle (prevalence, pathogenesis, and persistence) of cervical infection, understanding of the viral infection in the oropharynx is limited; with no screening practices, the trend is for individuals, predominantly male, to present late to clinic with established OPC. Better understanding of the viral lifecycle of HPV within the oropharynx to determine how HPV causes OPC is thus timely. Few studies examine the prevalence of oral HPV in tissues that are known to develop HPV-related OPC; the tonsil.

### Results

We will investigate the frequency and pathogenesis of active oral HPV infections in subjects undergoing routine tonsillectomy. qPCR and western blot will be used to assess the viral strain, oncogenic gene and protein expression, and integration status, of HPV within the tonsil. An accompanying questionnaire addressing the individuals' lifestyle choices (sexual behaviour, smoking and alcohol use) will be analysed by generalized linear modelling to examine whether these behaviours influence the prevalence and pathogenesis of oral HPV infection.

### Conclusion

This study is only in its early stage, and as such only produced preliminary results. Thus far we have recruited one post-doctoral researcher to lead research work (in post since October), and obtained ethical approval to carry out our work. We have started to collect tonsil samples and questionnaires from subjects at the Royal Derby Hospital (N=30 to date), and are expanding our scope to include two additional nearby hospitals. We have optimised qPCR protocols for the initial HPV screen, targeting the HPV L1 gene, and internal control, targeting subject  $\beta$ -actin.

## References

Overall this project will characterise the natural history of oral HPV infection within the disease-relevant tissue of healthy individuals, the tonsils. It will describe the locality of viral infections within the tonsil, and show whether HPV is able to complete its full lifecycle or integrate into the host genome. It will also determine whether gender is associated with the prevalence and pathogenesis of oropharyngeal HPV. As such, this work will begin to assess both the prevalence of oral HPV DNA, and how HPV infection is prognostic for the subsequent development of HPV-OPC.

00063

## VERY LOW PREVALENCE OF VACCINE HUMAN PAPILLOMAVIRUS (HPV) AMONG VACCINATED SEXUALLY ACTIVE YOUNG WOMEN: A STUDY IN EASTERN FRANCE

### 05. HPV prophylactic vaccines

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#### Background / Objectives

In France, less than 20% of girls remain fully immunized, despite the availability of safe and effective HPV vaccines for over ten years[1]. We estimated the overall prevalence and distribution of HPV in vaccinated, sexually active young eastern French vaccinated women who were screened for cervical cancer by cytology and HPV testing.

#### Results

High-risk HPV (HR-HPV) prevalence, genotype-specific prevalence and extent of multiple infections were assessed in 125 cervical samples from females with available vaccine data using hc2 assay and INNO-LiPA assay in our regional university hospital referral center in France. HPV status was analyzed in accordance with cytological data.

#### Conclusion

In our series, mean age was 23 years ( $\pm 3.3$ ). Overall prevalence of HR-HPV was 52% and was correlated with the lesion grade. The diversity of HPV genotypes was broad. The overall prevalence of genotypes covered by the quadrivalent vaccine was

low (5.9%); with 4.2%, 0%, 0.8% and 0.8% for HPV 16, HPV 18, HPV 6 and HPV 11 respectively. Single HR-HPV infections were identified in 11%, 21% and 47% of women with NILM, ASC-US/-H and LSIL respectively. Multiple infections with HR-HPV were detected in 28% of the specimens; only 24.5% of women with NILM presented infections with 2 HR-HPV genotypes or more, vs 28% of women with ASC-US/-H and 35% of women with LSIL.

## References

Among HPV-vaccinated young women, HR-HPV are detected at a high rate, and an association with the grade of cytological abnormalities was observed. However, HPV 16 and 18, both targeted by the vaccines, are remarkably rare among young French women since program implementation.

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00106

## IMPACT AND EFFECTIVENESS OF THE QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE ON ORAL AND ANAL HPV INFECTIONS AND RECURRENT RESPIRATORY PAPILLOMATOSIS

### 05. HPV prophylactic vaccines

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#### Background / Objectives

The real-world impact and effectiveness of quadrivalent HPV vaccination (4vHPV) on cervical HPV infection and disease was previously assessed [1]. Our aim was to review the evidence related to impact and effectiveness of 4vHPV on endpoints not previously assessed: oral and anal HPV infections and recurrent respiratory papillomatosis (RRP).

#### Results

Medline and Embase were searched for observational studies (published before April 12, 2018) evaluating real-life benefits of 4vHPV. Impact was defined as population prevented fraction of abnormalities by comparing risk of specified endpoints before and after vaccination program introduction or trends over time. Effectiveness was assessed as proportion of prevented endpoints, comparing vaccinated and unvaccinated individuals.

#### Conclusion

Data was extracted from 8 studies (Table 1), with 88% reporting effectiveness measures. Studies included both women and men, from the age of 12, with the

exception of the RRP study which was in children between 0-14 years of age. Studies came from Australia (n=1, RRP), and the US (n=7, anal [n=4] and oral [n=3]). Vaccination reduced vaccine-type (VT) anal infection by >60%, and VT oral infection by 80-100%. Variability in results was likely due to heterogeneity of the studies (populations; cohorts; sexual preferences; vaccine coverage rates; time since start of vaccination program). The incidence of RRP was reduced by 87% 4 years after introduction of vaccination. [Legend to table: E/I – Effectiveness/Impact; M/F – male/female; MSM – men having sex with men; VT-HPV – vaccine-type HPV; HGAIN– high-grade anal intraepithelial neoplasia; RRP - recurrent respiratory papillomatosis].

## References

Reductions in HPV infections and lesions, occurring in both genders, are becoming increasingly evident. This indicates that the vaccine prevents infections and lesions in all organs affected by HPV and provides additional support for 4vHPV benefits.

## References

[1] Garland et al. Clin Infect Dis. 2016; 63(4):519-27.

Study	Endpoint	E/I	M/F (age)	Measure	% reduction
Schlecht, PLoS One 2012	Anal infection	E	F (12-19)	VT-HPV infections (3 doses vs non-vac)	62.0
Schlecht, JID 2016	Anal infection	E	F (12-19)	VT-HPV Incidence rate ratio (vac vs non-vac)	64.0
Swedish, CID 2012	Recurrent HGAIN	E	MSM (18+)	incidence rate (vac vs non-vac)	35.0
Swedish, PLoS one 2014	Anal condyloma	E	MSM (26+)	incidence rate (vac vs non-vac)	49.3
Schlecht, PLoS One 2012	Oral infection	E	F (12-19)	VT-HPV infections (3 doses vs non-vac)	79.8
Kahn, STD 2015	Oral infection	E	MF (12-24)	VT-HPV infections (3 doses vs <3 doses)	100.0
Chaturvedi, JCO 2018	Oral infection	E	MF (18-33)	all HPV infections (vaccinated vs non-vac)	27.4
Novakovic, JID 2018	RRP	I	MF (0-14)	Incidence post-vac (2016) vs pre-vac (2012)	87.5

00256

## PREVALENCE OF HPV INFECTION AND HYPOTHETICAL EFFECT OF THE NONAVALENT VACCINE IN WESTERN HUESCA (SPAIN)

### 05. HPV prophylactic vaccines

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#### Background / Objectives

Cervical cancer is one of the most common cancers all over the world. In developed countries is health care problem, but with a relative low mortality due to the vaccination and the screening. The available vaccines includes 2, 4 or 9 HPV types. Our aim is to estimate the percentage of infections and high grade lesions that could be avoided with the 9 types vaccine Gardasil 9®.

#### Results

The target population were women between 25 to 65 years in our regional health area. The period of study was from May 2012 to June 2018, in this time the screening program was based on HPV. Cytologies were collected with Thinprep®. HPV test was performed with COBAS 4800®. The majority of positive cases to not 16/18, and randomly some of the positive 16/18, were genotyped with Clart HPV4®. The percentage of avoidable infection with Gardasil9® was calculated. The agreement between both technologies was calculated.

Furthermore we selected all the colposcopy biopsies from February 2016 to June 2018 with HSIL o carcinoma and with genotyping on the cytology with Clart HPV4®. The percentages of avoidable HSILs and carcinomas with Gardasil9® and coinfections were calculated. The agreement between cytology and lesions was calculated.

#### Conclusion

In the time period has been performed 19669 HPV test, being positive 2881 (prevalence of 14,65%). From them 1628 (56.51%) were genotyped with Clart HPV4 ®. The agreement to 16, 18, and other high risk types were 96.3, 92.1 and 95.6%, respectively. Hypothetically 66,65% of the HPV infections could be avoided with Gardasil9® .

Due to the screening program from February 2016 to June 2018 521 colposcopy biopsies has been made, with 177 (33.97%) HSILs and 12 (2.30%) carcinomas. In 171 (90.47%) there was a genotyped in previous cytology and later the biopsy was genotyped with Clart HPV4 ®. With Gardasil9 ® could have been avoided 145 HSILs or carcinomas (84,79%), including the 5 carcinomas of the series. In 39 of the lesions (26,90%) we found coinfection with more than one HPV type. The agreement between cytology and biopsy was 87.6%, with a complete and partial agreement of 34.3 and 53.3%, respectively

## References

The agreement between both technologies is appropriate with rates higher than 90%. It means that both are valid and the election will depend on the characteristics and the organization of each service.

Gardasil 9® could avoid 2/3 HPV infections and around 85% of the HSILs and carcinomas.

The HPV coinfection is a relative feature more than an exceptional phenomenon.

There is a relative good agreement between cytology and biopsy genotyping.

00348

Prevention of HPV-associated recurrence of CIN3: the experience of vaccination against HPV

## **05. HPV prophylactic vaccines**

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### **Background / Objectives**

Improvement of the treatment results of HPV CIN III after the initial treatment through vaccination against HPV considering the proven etiological role of the papillomavirus in cervical oncogenesis.

### **Results**

The research is based on the analysis of clinicomorphological data and the results of the HPV test performed on 50 HPV CIN III infected persons from 22 to 46 years of age. High-risk HPV genotypes were identified with Hybrid Capture II method in all clinical observations whereby types 16/18 had the highest S.G. (more than 70%), in all other cases - types 45 (7%), type 33 (4,5%), type 35 (3,8%), type 56 (2,6%), and type 58 (0,2%). Suprathreshold concentration of the HPV DNA (concentration 100 k genocopies/ml or 1mg/ml) was identified in all the research samples. During the first stage of treatment surgical and radio-wave cervical cone biopsy was performed on all patients; at the second stage in order to eradicate HPV photodynamic therapy of the cervical stump was performed with simultaneous vaccination (Gardasil) to prevent recontamination and development of HPV CIN III exacerbation. Cytological cervical stump swabs were performed within the established times set for the given incidence of tumorous changes in the cervical area. HPV test was performed once a year.

### **Conclusion**

In all clinical test the HPV test turned out to be negative during the whole period of observation, cytological and colposcopic tests showed absence of atypical changes in the cervical epithelial tissue, subclinical and clinical evidence of HPV infection.

### **References**

All clinical laboratory negative tests received upon check-up of women infected with HPV CIN III after the initial treatment show that ,considering the proven etiological role of HPV in cervical oncogenesis among the women of sexually active age and the promiscuous men, it is necessary to have activities directed at prevention of recontamination of high-risk HPV. Vaccination after the initial HPV treatment prevents HPV associated CIN III exacerbation. This approach is scientifically justified and is recommended for practical application in the health care services system.

00488

## PREVALENCE AND GENOTYPING OF HPV IN A PORTUGUESE VACCINATED POPULATION

### 05. HPV prophylactic vaccines

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#### Background / Objectives

HPV is the most common sexually transmitted disease and is responsible for 99.7% of cervical cancer. Cervical cancer is the fourth most frequent cancer in women and is the second most frequent cancer among portuguese young women aged 15 to 44 years old, making prevention a major objective of Public Portuguese Health.

Prophylactic HPV vaccines protect against a majority of HPV infection, which are associated with anogenital pre-malignant diseases and cancers in women and anogenital intraepithelial neoplasia and cancers in men and a subset of head and neck cancers.

Quadrivalent HPV vaccine is included in the Portuguese National Immunization Program (PNV) since 2008 and a vaccination rate higher than 85% has been achieved.

The main objective of this study is to characterize HPV genotypes in a vaccinated young woman who were referred to our department for abnormal pap smear.

#### Results

Retrospective study of women born after 1991, vaccinated with quadrivalent vaccine and referred to our department for abnormal cervical cytology and/or genital warts between 2013 and 2018.

#### Conclusion



Sixty-two women with mean of age was  $21.1 \pm 2.0$  [16-25] years old were included.

In our sample, 58.9% of women had started their sexual life prior to vaccination and 72.3% had have more than one sexual partners. Ten women (16.1%) had already become pregnant at least once, and 2 had an abortion. Twenty-eight women were smokers (45.2%).

Two women had genital warts and the remaining 60 had abnormal results in cervical cytology: 51.6% LSIL; 38.7% ASCUS; 3.2% HSIL; 1.6% AGC; 1.6% AIS.

HPV genotyping was performed using COBAS test. None were positive for HPV 16 or 18. Twenty women were positive for other high-risk HPV (HR-HPV) and of whom 47.1% were positive for p16/Ki 67 identified by the CIntec PLUS test.

## References

This study highlights the importance of cervical cancer screening in vaccinated women since not all HR-HPV genotypes are covered by the vaccine. It is therefore important to detect and surveil anogenital pre-malignant lesions in this population.

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00496

## A SYSTEMATIC LITERATURE REVIEW UPDATE OF THE IMPACT AND EFFECTIVENESS OF THE QUADRIVALENT HUMAN PAPILLOMAVIRUS VACCINE ON CERVICAL ABNORMALITIES

### 05. HPV prophylactic vaccines

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#### Background / Objectives

The real-world impact and effectiveness (VE) of quadrivalent HPV (4vHPV) vaccination on HPV infection and disease was previously assessed in 2016, ten years after 4vHPV licensure [1]. Our aim was to update the evidence related to impact and VE of 4vHPV on cervical abnormalities, including cytological abnormalities and histologically-confirmed cervical intraepithelial neoplasia.

#### Results

Medline and Embase were searched for observational studies evaluating real-life benefits of 4vHPV (March 1, 2016 – April 12, 2018). Reviews, conference, disease-burden, modeling, awareness, clinical trial studies and studies with mixed 4vHPV and 2vHPV were excluded. Impact was defined as population prevented fraction of abnormalities by comparing population before and after vaccination program or trends over time and VE as proportion of prevented abnormalities comparing vaccinated and unvaccinated individuals.

#### Conclusion

Of 1533 publications identified in the last 2 years, 8 studies (5 impact, 3 VE) from Australia, Canada and US provided data related to 4vHPV and cervical abnormalities, compared to 16 studies in previous review. Significant impact on high-grade (HG) cervical abnormalities in vaccine era were observed especially among females 15 to 24 years of age with estimated 6%-20% annual percentage declines. The impact of vaccination on reductions of histologically confirmed cervical lesions

among women 25-29 years, who were aged 18-26 years at vaccination, was newly reported in one study. VE of 50%-62% for HG and of 28%-73% for low-grade cytological cervical abnormalities were reported.

#### **References**

The impact and effectiveness of 4vHPV on reductions of cervical abnormalities is becoming increasingly evident, including first signs of a decline among 25-29 years old women who received catch-up vaccination.

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00538

## ASSOCIATION BETWEEN PAP ABNORMALITIES AND HPV INFECTION IN PARTICIPANTS IN HPV VACCINE CLINICAL TRIALS

### 05. HPV prophylactic vaccines

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#### Background / Objectives

Few studies have reported the burden of Pap abnormalities associated with the specific HPV types targeted by HPV vaccines. The purpose of this analysis is to estimate prevalence of HPV anogenital infection, by baseline Pap results, in participants of 3 worldwide trials of the quadrivalent HPV vaccine (placebo and vaccine groups, FUTURE I, II, III), and to estimate incidence of Pap abnormalities by HPV infection status at enrollment (placebo only, FUTURE I, III).

#### Results

Among 16,949 young women (YW) age 15-26 years (FUTURE I, II) and 3,674 adult women (AW) age 24-45 (FUTURE III), HPV prevalence (measured with PCR for 14 HPV types) was estimated at enrollment for women with:

- atypical squamous cells of undetermined significance (ASC-US) (n=781 YW, 115 AW),
- low-grade squamous intraepithelial lesion (LSIL) (n=993 YW, 115 AW), and
- high-grade squamous intraepithelial lesion or atypical squamous cells- cannot exclude HSIL (HSIL/ASC-H) (n=157 YW, 30 AW).

Cumulative incidence of high-grade Pap abnormalities (HSIL/ASC-H) over 4 years (placebo only), by baseline HPV status, was estimated for 1,481 (YW) and 1,701 (AW).

#### Conclusion

Prevalence of any 9-valent (9v) vaccine type (6/11/16/18/31/33/45/52/58) among women with ASC-US, LSIL, or ASC-H/HSIL at enrollment was 47%, 67%, and 89%, respectively (YM), and 29%, 55%, and 93%, respectively (AW). Prevalence of any non-vaccine type (35/39/51/56/59) among women with ASC-US, LSIL, or ASC-

H/HSIL was 32%, 64%, and 47%, respectively (YM), and 24%, 54%, 38%, respectively (AW). Over 48 months, cumulative incidence of high-grade Pap abnormalities (HSIL/ASC-H) among women with any high-risk 9v HPV type at enrollment was 8% (YW) and 6% (AW); cumulative incidence among women with no measured HPV infection at enrollment was 2% (YW) and 0.4% (AW).

## **References**

While the 9-valent vaccine will substantially reduce Pap abnormalities associated with HPV types that cause 90% of cervical cancers, non-vaccine HPV types also contribute to Pap abnormalities. These findings underscore the need for vaccination to protect against 9 HPV types, as well as the ongoing need for cervical cancer screening.

00029

Efficacy of HPV Vaccine in preventing of increasing cervical lesion in abnormal Pap test

## **06. HPV therapeutic vaccines**

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### **Background / Objectives**

Aggressive cervix cancer is one of the prevalent gynecologic cancers in developing countries; by conducting Pap smear tests and malignancy background tests and treatment, it could be prevented. On the other hand, the majority of Cervix cancers are the result of HPV virus. HVP virus is the most widespread virus which is transferred through intercourse. In developing countries, HPV vaccination is conducted to all women of 9 to 26 years of age, and in all men too. Nevertheless, with respect to the fact that this Vaccine is costly and limited in our country, and most studies have investigated the preventing effects of this vaccine, so we want to assess efficacy of HPV Vaccine in preventing of increasing cervical lesion in patient with abnormal Pap smear test.

### **Results**

In this study, 242 women that had various grades of infection( CIN1, 2, 3) which referred to a women's clinic for determine the effectiveness of HPV vaccine, by signing a consent were studied. Pregnant women and women with severe allergies were excluded. The control group comprised of 104 (42.7%) women which did not receive the treatment. In the Experimental group, 35 women (14.4%) received 2 vaccinations, and 103 (42.3) received 3 vaccinations. Throughout the 2 years, all patients were treated according to the protocol; in addition, the results of the cervix inspections were documented. The collected data were analyzed using SPSS, chi-square and fissure exact tests.

### **Conclusion**

The mean age of the samples were  $32.59 \pm 4.85$ , that was significantly differences ( $p=0.022$ ) between groups. At the end of the study, the total number of patients that

returned back to normal condition comprised of those who were received the Vaccinations in comparison with control group was significantly difference (p-value= 0.022, 0.033, 0.035) . Also efficacy of vaccine in group with 3 dose vaccinations was larger than group with 2 dose vaccinations in comparison with control group.

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this study showed that a quadrivalent HPV vaccine prevents of increasing cervical lesion in patient with abnormal Pap test.

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00224

## THERAPEUTIC HUMAN PAPILLOMAVIRUS VACCINES IN HEAD AND NECK CANCER: A SYSTEMATIC REVIEW OF CURRENT CLINICAL TRIALS

### **06. HPV therapeutic vaccines**

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#### **Background / Objectives**

This systematic review provides an overview of the current clinical trials investigating therapeutic vaccines in HPV+ head and neck cancer, and discusses future directions of therapeutic vaccine therapy.

#### **Results**

In PubMed, EMBASE, Cochrane and clinicaltrials.gov, a systematic search was conducted for clinical trials investigating therapeutic vaccines. We included studies initiated between 2000 and 2018 with patients diagnosed with HPV+ head and neck cancer, and extracted data on type of vaccine therapy, adverse events, immunogenicity and clinical outcome measures as tumor response, progression free survival and overall survival.

#### **Conclusion**

We identified 11 studies (n=376 patients) initiated between year 2005 and 2017. Four studies (n=34) presented temporary results, in patients with incurable, recurrent loco-regional or distant metastatic disease, indicating a positive immune response with 74 % (n=25/34 patients) having elevated antibody, IFN- $\gamma$  and/or T-cell response, respectively. Five studies presented data on the vaccines' safety profile, demonstrating predominantly grade 1 and 2 toxicity. Three studies evaluated the clinical outcome – one study showed no complete or partial response, one study demonstrated stable disease as best tumor response in 64% (9/14 patients) and one study showed a 33% overall response rate; one patient with complete response and seven patients with partial response.

#### **References**

Treatment with therapeutic vaccines is a promising and seemingly safe strategy for HPV+ head and neck cancer patients. But so far there are not enough data to make any further conclusions, and especially clinical outcome measures and tumor response to the vaccines are missing.

00043

Clinical significance of HC2 test results in Grey Zone range used in Slovenian Cancer Screening Program ZORA

## 08. HPV testing

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### Background / Objectives

The Slovenian national screening program ZORA uses Hybrid Capture 2 High-Risk HPV DNA assay (HC2, Qiagen, Hilden, Germany) as a triage method for women with low-grade cytology abnormalities and as a test of cure after CIN treatment. Due to reported limited analytical accuracy of HC2 near the cut-off value 1.0 RLU/CO, manufacturer recommends that specimens collected in standard transport medium (Qiagen) whose results fall near but below 1.0 RLU/CO should be repeated or retested with an alternate testing method. Based on these recommendations our laboratory at Oncology institute Ljubljana has implemented an internal RLU/CO range between 0.7 and 2.0 RLU/CO, called "Grey Zone", in which we repeat HC2 assay. The aim of this study was to determine the clinical relevance of the Grey Zone in our laboratory involved in the Slovenian cervical screening program.

### Results

In total 594 women (aged from 20 to 65 years, 44.3 on average) referred to colposcopy were included in the study. Biopsy was performed in case of abnormal colposcopy result and 162 CIN2+ cases were detected within one-year follow-up. We have determined the percentage of women with negative, Grey Zone and positive HC2 test results. We have compared three different Grey Zone ranges. Our internal Grey Zone (0.7-2.0 RLU/CO), Grey Zone proposed at the Institute of Microbiology and Immunology, Slovenia (0.4-4.0 RLU/CO) and by the manufacturer (Qiagen) for PreservCyt medium (1.0-2.5 RLU/CO). We have also calculated sensitivities and specificities of HC2 at different cut-off values.

### Conclusion

Four point five % of participating women had HC2 results within our Grey Zone range (0.7-2.0 RLU/CO), 10.8% within range 0.4-4.0 RLU/CO and 3.7% within range 1.0-2.5 RLU/CO. The calculated sensitivities and specificities of HC2 at different RLU/CO cut-off values are presented in Table 1.

Table 1. Sensitivity and specificity of HC2 results for CIN2+ at different cut-off (RLU/CO)		
RLU/CO	Sensitivity (95% CI)	Specificity (95% CI)
0.4	95.1% (91.4-98.1%)	56.7% (51.9-61.3%)
0.7	94.4% (90.7-97.5%)	60.6% (56.0-65.3%)
1.0	93.8% (90.1-97.5%)	63.2% (58.6-67.6%)
2.0	93.2% (89.5-96.9%)	66.4% (61.8-70.8%)
2.5	91.4% (87.0-95.7%)	67.6% (63.0-72.0%)
4.0	89.5% (84.6-93.8%)	69.4% (65.0-73.8%)

## References

Increase of cut-off value from lower to upper bound of 0.7-2.0 RLU/CO zone results in a decrease of sensitivity for 1.2% and increase of specificity for 5.8%. On the contrary in the 0.4-4.0 zone, sensitivity decreases below 90.0% with 12.7% increase of specificity. To determine the clinical meaning of Grey Zone range and the value of the cut-off additional studies are needed, which will evaluate the risk for CIN2+ in Grey Zone range on the general population.

00107

## PRELIMINARY EVALUATION OF THE HIGH+LOW PAPILLOMASTRIP ASSAY WITH COLLI-PEETM COLLECTED UCM PRESERVED URINE.

### 08. HPV testing

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#### Background / Objectives

The High+Low PapillomaStrip test (Operon) allows qualitative detection of 37 human Papillomavirus types in DNA samples from cervical smears or biopsies. The aim of this pilot study is to determine whether the PapillomaStrip assay is compatible with self-collected UCM preserved first-void urine samples.

#### Results

Twenty-two self-reported HPV positive women provided a Colli-PeeTM (Novosanis) collected first-void urine sample, on two subsequent days. Samples were collected at home. The collection tubes prefilled with UCM preservative were sent by mail to the University of Antwerp. 4 ml of urine/UCM mixture was concentrated using an ultrafiltration membrane and extracted with an easyMAG semi-automated DNA extractor (bioM  rieux). Subsequently, the DNA extract was analysed using the Riatol qPCR HPV genotyping assay (AML) and the High+Low PapillomaStrip assay. To provide sufficient volume for the Riatol qPCR assay the DNA extract was diluted (35  l DNA extract with 40  l elution buffer).

#### Conclusion

We observed a 100% agreement between the PapillomaStrip assay outcomes of the first and second day when expressed as high-risk (hr)HPV positive or negative. Five women were negative and 17 were positive for both samples. Also at the level of detected genotypes a very good agreement was observed. When comparing the hrHPV results generated by the Riatol qPCR and the PapillomaStrip assay, a kappa coefficient of 0.82 (CI 95%: 0.64–1.00) was observed. 31 and 10 samples gave respectively concordant positive and negative results. Three samples were negative

for Riatol qPCR and positive for the PapillomaStrip. These three samples also yielded a positive outcome using other commercial HPV assays.

#### **References**

These preliminary results confirm that the High+Low PapillomaStrip test is compatible with self-collected UCM preserved first-void urine. Confirmation of performance by testing larger series of first-void samples in a clinical setting is warranted.

00143

HPV and CMV infection among HIV-positive women in some countries of Eastern Europe and Central Asia

## 08. HPV testing

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### Background / Objectives

The frequency of detection of HPV varies greatly depending on the region of residence of the woman. Also HIV-infected women have a higher risk of HPV infection than HIV-negative women, and a higher risk of persistence and malignancy.

Objective: to study the prevalence of human papillomavirus of high carcinogenic risk (HPV HCR) and cytomegalovirus (CMV) in HIV-infected women in some countries of Eastern Europe and Central Asia.

### Results

647 HIV-infected women from Russia, Belorussia, Armenia, Azerbaijan, Tajikistan and Kyrgyzstan were examined from September 2017 to December 2017. All women were tested for HPV-test with the determination of 14 types of HPV HCR (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and CMV.



## **Conclusion**

Among the 647 women surveyed, mostly young people (under 40 y-s) predominated. As a result of the HPV-test, 265 (41%) of HIV-infected women were diagnosed with HPV HCR. All 14 HPV HCR genotypes were diagnosed in 28-48% HIV-positive women, depending on the region: Armenia - 39%, Azerbaijan - 43%, Belarus - 28%, Kyrgyzstan - 46.5%, Tajikistan - 37.8%, Russia (Samara) - 48%.

In 208 (32.1%) of HIV-infected women were diagnosed with CMV infection. 105 (16.2%) of HIV-infected women had co-infection with HPV + CMV. There are differences between the group of HIV-infected women with co-infection with HPV + CMV and a group of HIV-infected women with HPV without CMV ( $\chi^2 = 11.495$ ,  $p < 0.001$ ).

## **References**

HIV-infected women often have a combination of HPV infection and CMV infection. The data obtained should be used in the compilation of algorithms for cervical screening in HIV-infected women

00153

Etiopathogenesis of adenocarcinoma in situ cervicis (AIS) - is there a difference in relation to squamous intraepithelial lesions (SIL)?

## 08. HPV testing

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### Background / Objectives

AIS presents a rare histopathological form of cervical cancer. This histological type is still an enigma in the understanding of etiopathogens.

The aim of this study was to:

1. Determine whether there is a difference in the positivity of human papilloma virus (HPV), as well as the type of HPV in SIL and in AIS?

2. To determine if there is a difference in the epidemiology of SIL and AIS in relation to heredity and sexual behavior?

3. Determine whether contact and irregular vaginal bleeding is more common in SIL or AIS?

## Results

At the Clinic for gynecology and obstetrics, Clinical center Nis, Serbia, within the internal scientific project of the Faculty of Medicine, University of Nis, was conducted a prospective study which involved the first 120 patients who were operated for intraepithelial and invasive changes in the cervix since January 2017. In all of the operated patients, HPV typing was done with a PCR method, and the epidemiological data were taken with a survey, which was filled immediately prior to operative treatment.

## Conclusion

Of the 120 operated patients, AIS was present in 14 (11, 47%). In contrast to squamous lesions that were HPV negative in only 7,4% of subjects, AIS patients were more often HPV negative at 28.57%. As for positivity in relation to the type of virus in AIS, it is more often HPV 18 (14,28% : 5.5%). Of the other associated HPV types in AIS are detected HPV types: 39, 45, 51 and 56.

Epidemiological data from AIS and SIL showed that AIS patients in relation to the patients with AIS have a high percentage of overwhelming family history of gynecological localization (71,42% : 27,7%). Also, patients with AIS compared to SIL patients are more regularly smokers (71,42% : 46,29%) and have more often than one sexual partner (71,42% : 31,48%).

The anamnestic data on contact bleeding into sexual intercourse was more common in AIS patients (57.14% : 14.28%) and given that AIS cytological findings were falsely negative in 85%, it was precisely the contact bleeding that helped in diagnosing.

## References

Adenocarcinoma of the cervix differs in ethiepidemiology from squamous lesions of the same localization. This histological type is more often HPV negative or positive for HPV 18 and some of the HPV types that are more commonly encountered in squamous lesions (39, 45, 51, 56). In relation to epidemiology, this histological type is more often associated with the inheritance, smoking and having more sex partners.

Anamnestic, in relation to squamous lesions, domination of contact bleeding data could be of great diagnostic benefit, given the fact that cytology is low sensitivity in the diagnosis of cervical adenocarcinoma.

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00262

## HPV TEST AND IMPROVEMENT IN CYTOLOGY EFFICENCY IN WESTERN HUESCA (SPAIN)

### 08. HPV testing

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#### Background / Objectives

Human Papilloma Virus (HPV) is the most common sexually transmitted disease. There are more than 100 HPV types, 14 of them with a high oncogenic potential and relationship with cervical cancer.

HPV screening is based on its detection with a high sensitivity. It reduces the number of Papanicolaou smears (Pap smears) avoid them if HPV test is negative.

We show the differences in cytology between cytology, cotesting and HPV test screening protocols

#### Results

The target population were women from 25 to 65 years old in our regional health area. Cytologies were collected with Thinprep®. HPV was performed with COBAS 4800®. Between 2010 to 2013 the screening was based on cytology, beginning at 21. From January 2014 to January 2016 the screening was based on cotesting, beginning at 25 with cytology and cotesting with HPV from 30 to 65. From February 2016 to June 2018 the screening has been based on HPV, beginning at 25 with cytology and with HPV test from 30 to 65, the Pap smear was only performed after an HPV positive test.

#### Conclusion

From January 2010 to June 2018 has been performed 33293 Pap smears with 1986 (5.96%) atypias and 122 (0.37%) HSIL. It means that 16.76 and 272.89 Pap smears

were necessary to get one atypia or HSIL, respectively. At the same time were performed 1068 biopsies, being 377 (35.30%) positive to HSIL. It implies that 88.31 Pap smears were necessary to diagnose one histological HSIL.

In the cytology period there were 19482 Pap smears with 972 (4.99%) atypias and 38 (0.19%) HSIL. It supposes that 20.04 and 512.68 Pap smears were necessary to get one atypia or HSIL, respectively. At the same time were performed 233 biopsies, being 69 (29.61%) positive to HSIL. It means that 282.35 Pap smears were necessary to diagnose one histological HSIL.

In the cotesting time there were 10046 Pap smears with 480 (4.78%) atypias and 28 (0.28%) HSIL. It supposes that 20.93 and 358.79 Pap smears were necessary to get one atypia or HSIL, respectively. At the same time were performed 314 biopsies, being 119 (37.90%) positive to HSIL. It means that 84.42 Pap smears were necessary to diagnose one histological HSIL.

In the HPV screening has been performed 3765 Pap smears with 534 (14.18%) atypias and 56 (1.49%) HSIL. It supposes that 7.05 and 67.23 Pap smears has been necessary to get one atypia or HSIL, respectively. At the same time were performed 521 biopsies, being 189 (36.27%) positive to HSIL. It means that 19.92 Pap smears were necessary to diagnose one histological HSIL

## References

HPV has improved the cytological efficiency in cervical cancer screening:

Reducing the absolute number of Pap smears by 70%.

Decreasing the number of Pap smears to diagnose a HSIL in colposcopy biopsies.

00268

## VALIDATION OF A COMMERCIAL HPV TESTING ASSAY TO DETECT HPV16 IN ANAL CYTOLOGY SPECIMENS FROM A HIGH-RISK PATIENT POPULATION

### 08. HPV testing

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#### **Background / Objectives**

Detection of human papillomavirus type 16 (HPV16) in anal cytology specimens may be used for risk profiling for high-grade anal dysplasia and carcinoma. To validate the Cervista HPV16/18 assay, a commercial available HPV assay, in detecting anal HPV16 infection, we compared Cervista HPV16/18 and HPV16 type-specific PCR assays in 77 anal specimens collected from high-risk patients.

#### **Results**

We retrospectively searched our institutional databases for patients with anal HSIL or carcinoma or cervical intraepithelial neoplasm grade 2 or 3 (CIN2/3) and carcinoma who underwent anal cytology and anal Cervista HPV16/18 testing at our institution from 2016 to 2017. The Cervista HPV16/18 assay (Hologic Inc., Bedford, MA, USA) was performed followed by HPV16 PCR testing. Patients underwent anal cytology, high-resolution anoscopy and/or biopsy at the time of HPV testing and/or at subsequent visits. Results for the Cervista HPV16/18 and HPV16 PCR were compared and correlated with the concurrent/follow-up anal cytology and biopsy results. The kappa efficiency test and the Fisher exact test were used to compare the variables.

#### **Conclusion**

A total of 77 patients met inclusion criteria for the study and included 12 men and 65 women. The mean age was 53 years (range, 27-87). Of the 77 patients, 28 (36%) had history of anal dysplasia/carcinoma (1 HSIL, 5 AIN2, 15 AIN3, 7 carcinoma). The concurrent or follow up anal cytology and biopsy results showed 17% (13/77) of patients had high-grade anal dysplasia/carcinoma (7 HSIL, 5 AIN3, 1 carcinoma).

The concordance between Cervista HPV16/18 and HPV16 PCR results was 78% (60/77) with moderate agreement ( $\kappa=0.461$ ) (Table 1). HPV16-positive rate was higher by the HPV16 PCR (34%, 26/77) than by the Cervista HPV16/18 (21%, 17/77), but the difference was not significant ( $P=0.150$ ). The significantly higher HPV16-positive rate by HPV16 PCR was observed only in patients with a negative anal Pap cytology/biopsy result (Table 2). The Cervista HPV16/18 assay showed the same sensitivity (50%) as the HPV16 PCR but higher specificity (84.1% vs. 69.8%) for anal HSIL/AIN2/3/carcinoma.

## References

The Cervista HPV16/18 assay is comparable to HPV16 PCR assay and a valid testing assay to detect HPV16 in anal cytology specimens from high-risk patients for risk profiling. In patients with negative anal cytology/biopsy results, the clinical significance of the higher HPV16-positive rate by HPV16 PCR that is mostly due to low HPV16 copy numbers is unclear. Further studies are needed to delineate the risk and recommended follow-up of patients with positive HPV16 testing.



**Table 1. HPV16 testing by Cervista HPV16/18 and HPV16 type-specific PCR assay in anal Pap specimens (n=77)**

	<i>PCR HPV16 +</i>	<i>PCR HPV16 -</i>	<i>Total</i>
<i>Cervista HPV16 +</i>	13	4	17
<i>Cervista HPV16 -</i>	13	47	60
<i>Total</i>	26	51	77

*Kappa=0.461; Agreement: 77.9%, (60/77)*

**Table 2. HPV16 test results by Cervista HPV16/18 and HPV16 type-specific PCR assay stratified by anal Pap cytology/biopsy result (n=77)**

Anal Pap/Biopsy (Case No.)	Cervista HPV16/18+ (%)	HPV16 Type- specific PCR+ (%)	P-value
NILM (38)	4 (11)	13 (34)	0.026
ASCUS/ASC-H/ LSIL/AIN1 (25)	6 (24)	6 (24)	1.000
HSIL/AIN2/3/ Carcinoma (14)	7 (50)	7 (50)	1.000
Total (77)	17 (22)	26 (34)	0.150

NILM: no intraepithelial lesion or malignancy; ASCUS: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion

00298

## EVALUATION OF P16INK4A AND KI-67 EXPRESSION AND ASSOCIATION WITH HUMAN PAPILLOMAVIRUS INFECTION IN CARRIERS OF PENILE CANCER IN BRAZIL

### 08. HPV testing

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#### Background / Objectives

Penile carcinoma (PC) is a rare disease that affects people of all ages and is considered a public health problem. Poor hygiene, phimosis in adult life, and human papillomavirus (HPV) infection are risk factors for penile carcinoma. The lack of specific predictors that identify inguinal micrometastases could decrease the indication of a large number of unnecessary inguinal lymphadenectomies. The search for possible markers to identify the increased risk of inguinal metastasis has been the subject of research. **Objetives:** This study aims to determine the prevalence and genotypes of HPV in cases of penile carcinoma in Central Brazil, to evaluate the expression of the proteins associated with tumor suppression (p16<sup>INK4a</sup>) and cell proliferation (Ki-67) in these neoplasms and to associate with clinicopathological findings of PC cases in Central Brazil - Goiás.

#### Results

**Methods:** Retrospective study of 183 individuals with Penile carcinoma undergoing treatment at Hospital Araújo Jorge, Goiânia-GO in Central Brazil, from 2003 to 2015. The paraffin-embedded samples had DNA-HPV detected and genotyped by the INNO-LiPA kit, specific for paraffin-embedded tissues. Expression of p16INK4a and Ki-67 was evaluated by immunochemical (IHC) using the immunoperoxidase method.

#### Conclusion

**Results:** The prevalence of HPV-DNA in penile carcinoma was 30.6% (95% CI: 24.4-37.6), high-risk HPV 24.9% (95% CI: 18.9-31.3), that 62.5% of the samples were HPV-16. HPV status did not change the SCE in five years. Expression of p16<sup>INK4a</sup> was associated with HPV infection (p = 0.001), Ki-67 immunolabeling was associated with inguinal metastasis (p = 0.0002) and lymphovascular invasion (p <0.0001). There was no difference in SCE over five years between individuals with high and low risk HPV infection and between expression of p16<sup>INK4a</sup> (p = 0.07) and Ki-67 (p = 0.542).

## References

**Conclusion:** The study showed that 80.3% of the genotypes identified in CP are immunopreventable by the quadrivalent or nonvalent anti-HPV vaccine. Expression of p16INK4a by IHC in penile tumors can be used as a marker of high-risk HPV infection. Ki-67 expression was associated with the characteristics of greater tumor aggressiveness, although there was no association with worse survival

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00351

## HPV COLONIZATION IN CERVICAL SECRETIONS VERSUS INFECTION IN CERVICAL CANCER TISSUE

### 08. HPV testing

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#### Background / Objectives

Despite of HPV colonization in cervical secretions persistent HPV infection with cervical cancer risk develops to a small percentage of women. With increased certain HPV type persistence in organism, chances for virus to be cleared out spontaneously decreases, and chances to diagnose precancerous condition increases.

Objective. To determine and compare high risk HPV types in cervical secretions and tissue in women with cervical cancer.

#### Results

In this study 29 women with diagnosed cervical cancer (CA) during 2017 - 2018 years in hospital of Lithuanian University of Health Sciences were involved. For all women 2 samples for Human Papillomavirus (HPV) genotyping were taken: liquid based cytology medium (LBCM) and tissue biopsy. HPV DNA was isolated using commercial QIAamp DNA (Qiagen, USA) kit. For DNA amplification polymerase chain reaction method was used. HPV genotype was detected using hybridization and visualisation AmpliSens PCR kit (Bratislava, Slovakia).

#### Conclusion

High risk (HR) HPV was detected in 96.6% (n=28) of tested women with CA in both samples groups. One HR HPV type was detected in 58.6% (n=17), two in 27.6% (n=8) and three in 10.3% (n=3) cases in LBCM. In tissue biopsy one HR HPV type was detected in 89.7% (n=26) and two in 6.9% (n=2) cases. More than one HR HPV type was found in LBCM 39.3% (n=11), in tissue biopsy, respectively 6.9% (n=2). We

have found trends that more often HPV16 and HPV18 types alone were detected in tissue biopsy compare to LBCM (82.8%, n=24 and respectively 58.6%, n=17;  $p=0.054$ ). HPV16 and/or HPV18 with other HR HPV type were detected in 31.0% (n=9) in LBCM and only 3.4% (n=1) in tissue biopsy samples ( $p=0.056$ ). Only other HR HPV types were found in 6.9% (n=2) of LBCM and 10.3% (n=3) of tissue samples of tested women ( $p>0.05$ ).

## **References**

A lot of HR HPV types can colonize vagina and cervix, but often HPV16 or HPV18 alone or together penetrate in the tissue and cause cervical cancer.

00379

# EVALUATION OF A CUSTOM CONFIGURED TECAN EVO FREEDOM WORKTABLE SET UP FOR PRE-ANALYTIC PROCESSING OF PRESERVCYT LBC SAMPLES FOR HIGH RISK HPV DNA TESTING IN PRIMARY SCREENING AND TRIAGE

## 08. HPV testing

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### Background / Objectives

Pre-analytic processing of liquid-based cytology (LBC) samples is an important step preceding high risk (hr) HPV detection and should ensure adequate mixing of this per se inhomogeneous sample type. Automation of this process is desirable, particularly in the light of increasing demand expected with the introduction of primary HPV screening.

### Results

A worktable set up of the TECAN EVO Freedom liquid handler was custom configured to automate the entire process of aliquot preparation from original Preservcyt LBC vials, including matching barcodes with secondary tubes, mixing and opening LBC vials, liquid transfer, closing LBC vials, identification and sorting of vials that are not suitable for further processing and process documentation. LBC vials sent in for hrHPV DNA testing (primary screening [P], N = 324; triage [T] N = 183) were anonymized and used for this evaluation. Matched pairs of manually and automatically preprocessed samples were tested in parallel with RealTime High Risk HPV (Abbott), a qualitative, clinically validated multiplex real-time PCR test for the detection of DNA from 14 hrHPV types, distinguishing HPV 16 and HPV 18 from non-HPV 16/18 hrHPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), and detection of a human  $\beta$ -globin ensuring sample adequacy. LBC vials were mixed manually for 15-20 seconds, while automated mixing was set for 15 seconds. The concordance of assay results obtained from matched pairs and corresponding kappa values were calculated. Average turn-around-time and hands-on-time of both pre-processing methods were estimated.

### Conclusion

The TECAN processed 503/507 LBC vials. A total of 56 (17.4%) primary (P) and 167 (92.3%) triage (T) vials tested positive for hrHPV DNA after manual and automated aliquot preparation. Agreement of overall hrHPV results from matched pairs was 99.1% ( $k=0.97$ ) for P-vials and 99.4% ( $k=0.96$ ) for T-vials, while partial genotype pattern agreement was 99.1% ( $k=1.00$ ) for P-vials and 98.9% ( $k=0.97$ ) for T-vials, respectively. The TECAN aliquoted 48 LBC vials in 70 minutes without user intervention, while a minimum hands-on-time of 45 minutes was required for manual aliquot preparation.

## References

The excellent agreement between hrHPV DNA test results from manually and automatically (TECAN Evo Freedom) aliquoted matched samples in conjunction with significant savings of labor time, full process documentation and a low rate of non-processed LBC vials demonstrates that the custom configured worktable configuration of the TECAN instrument evaluated in this study is well suited for improving the workflow of HPV testing LBC samples in high throughput settings.

00422

## ATYPICAL GLANDULAR CELLS ON PAP TESTING AND ITS CORRELATIONS TO HPV

### 08. HPV testing

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#### Background / Objectives

The cytological diagnosis of atypical glandular cell (AGC) denotes cellular features beyond reactive changes that lack criteria for glandular neoplasia. AGC has a low incidence (0.2% of all cytologies) and as a result the diagnosis is challenging. Although a high percentage of reevaluation identify benign conditions, a significant subset of AGC cases also reflect significant lesions like endocervical adenocarcinoma and uterine or adnexal carcinoma. The prevalence of high risk HPV is 93% but a negative test does not exclude cervical pathology. The aim of this study is to evaluate the prevalence, clinical findings, HPV correlation and follow-up of AGC results between 2008 and 2017 in a central Hospital.

#### Results

Pap smears classified as AGC between January 2008 and December 2017 were retrieved from an institutional cytopathological database. Clinicopathologic variables and follow-up were collected from medical records retrospectively.

#### Conclusion

A total of 84 cases were diagnosed in the 10 year time period. The mean age of the patients was 48.1 [22-83] years. The majority of women were multiparous and half of the sample (n=42) were combined contraceptive pill users. Only 12 patients were obese and 26 overweight; also, a small number (n=11) were smokers. HPV tests (COBAS test) were performed in 70 cases and the rate of high risk HPV was 61.4%. Other high risk HPV was the most common type (n=28), followed by HPV 16 (n=12) and HPV 18 (n=3). Positivity for p16/Ki-67 were found in 24 of all hrHPV cases. Colposcopy lesions were biopsied in 40 patients, 14 showed cervical intraepithelial



neoplasia (CIN) II/III and 15 low-grade lesions. A high number of these (n=27/29) tested positive for HPV and 20 were also p16/Ki-67 positive. 76 Seventy six transvaginal ultrasound were performed, four of them revealed benign endometrial pathology. Cervical sampling was collected in 50 cases; one of them was compatible with adenocarcinoma. Regarding treatment and follow up, 37 women were kept under surveillance, 7 were targeted for vaccination, 32 underwent cervix conization and 8 were submitted to hysterectomy, one in context of cervical adenocarcinoma. Only 3 women persist with AGC after 12 month follow up and there were no cases of further malignant pathology.

## **References**

Despite published literature correlating AGC with high incidence of neoplasia, in our department this association was not confirmed. However HPV is increasingly associated with these cytological abnormalities. The results of our study, although limited by the small size of the sample, are in favour of a less aggressive therapeutical approach at AGS diagnosis in Pap smears.

00463

## PERFORMANCE OF THE SEMI-QUANTITATIVE HUMAN PAPILLOMAVIRUS GENOTYPING TEST ANYPLEX II HPV28 IN A REFERRAL POPULATION

### 08. HPV testing

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#### Background / Objectives

We compared by HPV type and viral load, the diagnostic performance of Anyplex II HPV28, a semi-quantitative DNA PCR (genotyping 14 high risk (hr)HPV types in set A and 5 hrHPV and 9 low risk (lr)HPV types in set B), to the gold standard HPV DNA test Hybrid Capture 2 (HC2) and to the mRNA test PreTect HPV-Proofer ('Proofer'), in detecting cervical intraepithelial neoplasia grade 2 or worse (CIN2+). We looked particularly at four specific groups of HPV types: 1) the five hrHPV types in Proofer, 2) the seven hrHPV types in the 9-valent HPV vaccine Gardasil9, 3) the HPV types in Anyplex II HPV28 set A, and 4) the HPV types in Anyplex II HPV28 set A and B, according to viral loads.

#### Results

In this cross sectional study, 296 women referred to follow-up with abnormal cervical cytology and/or persistent HPV infection, were included. The women had all three HPV tests performed from liquid based cytology samples.

#### Conclusion

The sensitivity of Anyplex II HPV28 to detect CIN2+ was 99% (95% CI 96-100) and specificity 43% (95% CI 34-53). Restricting to medium and high viral loads in Anyplex II HPV28 set A, sensitivity and specificity were 97% (95% CI 94-99) and 60% (95% CI 50-69) with positive (PPV) and negative predictive value (NPV) 75% and 93%, respectively, comparable to HC2. In a screening population, calculated PPV and NPV was 20% and 100%, respectively. Analyzing Anyplex II HPV28 by the five HPV types in Proofer (HPV16, 18, 31, 33, and 45), sensitivity and specificity were

comparable to Proofer`s according to CIN2+. Adding HPV52 and 58 raised sensitivity of CIN3+ to the level of Anyplex II HPV28 set A.

## **References**

The clinical performance of medium and high viral loads in Anyplex II HPV28 set A was comparable to HC2, with additional benefit of genotype and viral load information useful in risk stratification. The detection of CIN2+ was not increased by adding set B of Anyplex II HPV28. Anyplex II HPV28 including all 28 HPV types is not suitable in primary cervical cancer screening.

00478

## CO-TEST VALUE AFTER LOOP ELECTROSURGICAL EXCISION IN HIGH GRADE CERVICAL LESIONS

### 08. HPV testing

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#### **Background / Objectives**

Co-test (liquid-based cytology and HPV DNA testing) has been performed in the follow up of patients submitted to loop electrosurgical excision procedure (LEEP) for several years. The aim of this study was to evaluate the outcome of patients with high grade cervical lesions treated with LEEP, concerning middle follow up co-test results.

#### **Results**

We performed a retrospective 4-year analysis of patients who underwent LEEP with histological high grade cervical lesions (January 2012 to December 2015). Follow up was scheduled at 6-month intervals for 24 months after LEEP, and we reviewed the middle follow up co-test results. Cytology was considered as abnormal when the result was at least atypical squamous cells of undetermined significance (ASC-US). Patient outcomes were evaluated concerning the middle follow up co-test results.

#### **Conclusion**

124 patients were included, and co-test evaluation was performed between 9 and 20th months after LEEP (mean 13,8 months).

34 had a positive HPV DNA test – Group 1 (27,4%), with cytology abnormality in 12 of these patients (35,3%). The prevalence of mono-infection was higher than co-infection (79,4% vs. 20,6%). 32 patients test positive for high risk HPV (94,1%), and the most prevalent genotype was HPV-16 (32,4%).

The remaining 90 patients had a negative HPV DNA test (Group 2), 7 of which with cytology abnormalities in the co-test (7,7%).

Group 1 presented a superior mean age (41,5 vs. 36,9 years), and both groups had a similar percentage of Portuguese women (79,4% vs. 76,6%). Regarding positive LEEP margin status, Group 2 presented the lower percentage (15,9% vs. 34,2%).

At the 24th month of follow up Group 1 outcomes included: 2nd LEEP (n=3), hysterectomy (n=6), prolonged follow up (n=6) and return to screening program (n=17). For the same period of time, Group 2 outcomes included 73 returns to screening program and 1 prolonged follow up. 2 patients of Group 1 and 16 of Group 2 were lost during follow up.

## **References**

In patients with high grade cervical lesions submitted to LEEP, positive HPV DNA co-test appears to be an important factor that can help predict less favourable, long term, outcomes.

00593

Pitfalls in HPV68a detection

## 08. HPV testing

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### Background / Objectives

Despite the proved oncogenic potential, HPV68 genotype may be excluded from HPV screening tests and from newly developed vaccines due to its rarity in cervical cancer. HPV68 may exist in two subtypes (a and b), differing in 6% *E6*, 5% *E7* and 7% *L1* ORF sequence, and the HPV68a subtype is usually not detectable by primers targeting *L1* gene. The aim of the study was to evaluate the efficacy of routinely used cobas® 4800 HPV Test (targeting *L1* gene) in HPV68a detection.

### Results

Cervical swabs (n=2198) obtained by physicians and self-sampled cervicovaginal swabs (n=217) were analyzed for the presence of HPV by cobas® 4800 HPV Test (cobas, Roche) and PapilloCheck® HPV-Screening test (PapilloCheck, Greiner Bio-One). For viral load assessment, HPV68 positive samples were further analyzed by a quantitative multiplex real-time PCR (qPCR) detecting of *E6* HPV68 gene and human *GAPDH* gene (internal control) using specific TaqMan® probes. Real-time PCR followed by high resolution melting (HRM) curve analysis and Sanger sequencing of *E6* PCR products was used for HPV68a/b subtyping.

### Conclusion

HPV68 was detected in 39 of 2198 (1.77%) cervical swabs and 4 of 217 (1.84%) cervicovaginal swabs using PapilloCheck, with 33 single-type positive cases altogether. Cobas gave false negative result in 20 of 33 (60.6%) HPV68+ cases despite the median viral load of HPV68 was 1548 (1-320175) *E6*/ng DNA. The median viral load of HPV68 was 281 (9-17229) *E6*/ng DNA in true positive cases median viral load. HPV68a subtype was detected in all (20/20) false negative cases by HRM analysis as well as by Sanger sequencing. HPV68a subtype was detected in 3 of 13 (23.1%) and HPV68b in 8 of 13 (76.9%) of true positive cases, respectively.

### References

Though cobas is routinely used HPV screening test, the false negative result was detected in 60.6% of HPV68 single-type infection cases due to its lower sensitivity for HPV68a. Prevalence of HPV68 genotype reported from the screening could be therefore underestimated. Due to low prevalence in cervical cancer, and given a substantial loss of specificity adding more HPV types in screening assays, HPV 68 may be excluded from newly developed screening tests or HPV vaccines. If the nonavalent vaccine successfully reduces the prevalence of its target genotypes, the prevalence of HPV68 may significantly increase. In this situation, the HPV genotyping assays not able to efficiently target both HPV68 subtypes will miss a clinically relevant proportion of cervical lesion in future.

00600

## HPV INFECTION IN WOMEN AND MEN FROM INFERTILE COUPLES AND GAMETE DONORS

### 08. HPV testing

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#### Background / Objectives

Sexually transmitted infections (STI) are believed to cause fertility alternations. However, the exact impact of human papillomavirus (HPV) infection, the most prevalent STI, remains uncertain. The aim of the present study was to systematically investigate the prevalence of HPV infection in gamete donors and women and men from infertile couples and to find out if there is any relation to reproductive outcomes.

#### Results

Cervical swabs were prospectively collected from oocyte donors (OD, n=207) and women treated for infertility (IW, n=945). Semen samples and penile swabs were prospectively collected from sperm donors (SD, n=97) and men treated for infertility (IM, n=328). Cervical swabs and semen samples were tested for the presence of 14 hrHPV genotypes by cobas 4800 HPV system (Roche), then genotyped using PapilloCheck HPV-Screening system (Greiner Bio-One). Penile swabs were analysed by PapilloCheck HPV-Screening system only. The association between hrHPV positivity and fertility outcome or socio-behavioral and health characteristics was assessed using a statistical software R. All participants signed informed consent and filled in a questionnaire focused on their health status and sexual behaviour.

#### Conclusion

hrHPV prevalence was significantly higher in OD than in IW (28.0% vs. 16.1%,  $P < 0.001$ ). Interestingly, women who became pregnant spontaneously (19.6%) and women not treated with in vitro fertilization (IVF, 18.1%) were more frequently



hrHPV+ than women treated with IVF (12.7%,  $P=0.077$ ). No associations between hrHPV+ of OD or IW and pregnancy or abortion rates were found. Overall, the hrHPV prevalence in penile swab or semen sample was 28.9% in SD compared to 35.1% in IM ( $P=0.312$ ). Penile swabs were more frequently hrHPV+ than semen samples in both IM (32.3% vs. 11.9%,  $P<0.001$ ) and SD (26.8% vs. 6.2%,  $P=0.006$ ). IM with hrHPV+ semen sample had lower semen volume (median 2 ml vs. 3 ml,  $P=0.002$ ), sperm concentration (median  $13\times 10^6/\text{ml}$  vs.  $26\times 10^6/\text{ml}$ ,  $P=0.020$ ) and total sperm count (median  $33\times 10^6$  vs.  $71.8\times 10^6$ ,  $P=0.004$ ). No association between penile hrHPV positivity and semen parameters was found.

## References

Despite high prevalence of hrHPV in both OD and IW, no associations between hrHPV positive status and pregnancy or abortion rates were found. IM were more frequently hrHPV positive than SD and the higher hrHPV positivity was found in semen samples compared to penile swabs. hrHPV positivity significantly influenced the sperm parameters in IM and could be therefore one of the cause of men infertility.

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00603

## LUMINEX AND MULTIPLEX QPCR FOR DETECTING HPV GENOTYPES IN URINE SAMPLES.

### 08. HPV testing

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#### Background / Objectives

Norwegian girls have been invited to the HPV-vaccination program since 2009. As part of the national HPV-surveillance programme, urine samples from 17 and 21 years old girls have been collected before and at several timepoints after vaccination and used as a measure of HPV prevalence in the population. These data also provide a measure of the effect of the vaccine on the prevalence of HPV genotypes in the population.

#### Results

The original method for HPV genotyping in DNA from these urine samples was Luminex, where a mix of primers should amplify a region of L1 that can hybridize to specific oligos from 37 distinct HPV genotypes. The oligonucleotides are coupled to specific detectable beads, enabling multiplex detection of the HPV genotypes.

However, we question the performance of this method when vaccination results in a change in prevalence of HPV16 and/or HPV18, which may lead to variations in our ability to detect certain other genotypes. We therefore validate the performance of Luminex to detect specific genotypes in the presents or absents of HPV 16/18. We have also established an alternative multiplex qPCR assay, where a total of 14 HPV genotypes can be detected with specific Taqman probes against E6 or E7. We have combined detection of 4 targets in each reaction by choosing specific, compatible fluorescent probes.

#### References

We have compared the strengths and weaknesses of Luminex and multiplex qPCR for HPV detection from urine samples, and will share data illustrating the strengths and weaknesses of both methods.

## 09. HPV screening

00016

## HPV SCREENING AMONG HIV-POSITIVE WOMEN IN ALBANIA

### 09. HPV screening

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#### **Background / Objectives**

Albanian National Statistics reflect a total of 312 HIV infected women. Naturally they face a much higher risk of HPV infection and cervical cancer than HIV uninfected ones, but there is no coverage of cervical cancer screening in the country at present. And as a consequence, there is a lack of data on the association between cervical cancer and HIV. The aim of this study was to determine the prevalence of HPV infection of high- and low-risk types and to analyze how HPV infection manifests itself in HIV-infected women in Albania.

#### **Results**

A number of 105 HIV positive women aged from 30 to 65 years, who attended the Outpatient Clinic of Infectious Diseases Unit of Mother Theresa Hospital Center, responded in the year 2017 to the invitation for a routine HPV cervical infection screening. Sampling was done by self-collection using digene Female Swab Specimen Collection Kit (Qiagen, Gaithersburg, MD). Samples were analyzed with Hybrid Capture 2 (HC2) Assay (Qiagen, Gaithersburg, MD). Demographic, epidemiological, and behavioral data were obtained from all responders through a standardized questionnaire.

#### **Conclusion**

At the time of HPV screening: 91% (96/105) of women were on antiretroviral therapy and 31.4% (33/105) of them were on AIDS stage (A3, B3 and C1- C3). The median CD4+ T-cell count was 400 cell/mm<sup>3</sup>. Only 8 women (7.6%) have undergone, once in their lifetime, a cytological screening for cervical cancer, and this with normal results. 65% of women presented a "not detected"(ND) or low viral load. The overall prevalence of HPV cervical infection in HIV-positive women in Albania was 44.7%. Oncogenic hr-HPV was detected in 41.9% (44/105) of them, whereas 22.85% (24/105) were infected with lr-HPV. In a percentage of 16% multiple infections with hr-HPV and lr-HPV types, were detected. From these, the majority (71.4%) were

found in women of  $\geq 41$  years. Lower CD4 counts ( $<200$  cell/mm<sup>3</sup>) were associated with 40% of hr-HPV positivity, and were not associated with marital status, no. of lifetime or recent partners, no. of pregnancies, use of contraceptives.

## **References**

For Albania, a country with low endemic rates of cervical cancer, a high HPV DNA infection prevalence signalizes an increased risk of cervical cancer among HIV-positive women. Therefore it is of a great importance to actively screen HIV infected women for cervical infection with the objective of finding differences with regard to persistence of high risk HPV infection or progression of CIN among highly active antiretroviral therapy (HAART) treated versus non treated women in Albania.

00019

## URINE DETECTION OF HPV ONCOPROTEIN: IS IT A NEW ALTERNATIVE FOR CERVICAL CANCER SCREENING?

### 09. HPV screening

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#### Background / Objectives

Background: Cervical cancer (CxCa) is a significant public health problem, especially in low- and middle-income countries, where women have little access to CxCa screening; consequently, 80% of CxCa related mortality occurs in these regions. The development of screening methods that need less infrastructure thus represents an urgent medical need.

Objectives: To evaluate the detection of HPV16 and HPV18 E6 oncoprotein in urine samples from women attending at Barretos Cancer Hospital.

#### Results

Methods: Between January 2017 and September 2017, 124 women, aged 25 to 64 years, who attended at the Prevention or Gynecologic Oncology Departments of Barretos Cancer Hospital (HCB), Barretos, Sao Paulo, Brazil were recruited. At study enrollment, each woman provided urine and self-collected vaginal samples; then the physician collected a cervical sample. 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.

## **Conclusion**

Results: The median age of participants was 40 years. Cervical disease status was categorized as positive (CIN2+) in 63.7% of women. Of those, 44/79 (55.7%) had a histological diagnosis of invasive carcinoma, 26/79 (32.9%) of CIN3 and 9/79 (11.4%) of CIN2. High-risk (HR) HPV-DNA test was positive in 66% of the physician and 65% of the vaginal self-collected samples and 50.0% of the urine samples. HPV16 was the most frequent type detected (51.2%) of the physician collected samples. The HPV16/18-E6 test was positive in 31% of the physician-collected samples, 20% of the vaginal self-collected and 21% of the urine samples. Considering only HPV16 or HPV18 positive samples on Cobas test, the HPV16/18-E6 detection rate was 73.1% (38/52) for the physician sampling, 50% (25/50) for the self-collected vaginal sample and 53.5% (23/43) for the urine specimens. The sensitivity and specificity for CIN2+ detection using urine was: 30%% and 95%, respectively for HPV16/18-E6 test and 72% and 89%, respectively for HPV-DNA test.

## **References**

Conclusions: Our results suggest that using urine could be an acceptable option to perform HPV16/18-E6 test since the HPV16/18-E6 test positivity rates for self-collected vaginal sample and urine were similar. However, the urine-based E6 test needs further protocol development and standardization.



00120

## NON-16/18 HPV GENOTYPES PREDOMINANT IN BIOPSY SAMPLES WITH HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESIONS IN WOMEN WITH PRECEDING NEGATIVE HPV TESTS

### 09. HPV screening

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#### Background / Objectives

High-risk human papillomavirus (hrHPV) testing has been increasingly used in clinical practice in recent years for triaging equivocal cytology or co-testing with cytology. Recent studies indicated that a considerable portion of patients with high grade cervical lesions ( $\geq$ HSIL) had preceding negative hrHPV tests. The study attempted to elucidate the factors potentially contributing to the finding by testing biopsy samples from these patients.

#### Results

We retrospectively reviewed the correlation of cytology, histology and hrHPV testing from our Cytology Laboratory database of 130,648 Papanicolaou (Pap) tests between March 1, 2013 and June 30, 2014. Patients with negative hrHPV tests and  $\geq$ HSIL on follow-up biopsy were identified, and the corresponding paraffin blocks were tested for HPV on Cobas system, DNA microarray against 40 HPV genotypes, and DNA sequencing.

#### Conclusion

Twenty-one (8.3%) of 252 women with  $\geq$ HSIL on biopsies had preceding negative HPV tests. A wide range of HPV genotypes were detected in 20 of the 21 (95%) biopsy samples, including non-16/18 hrHPV (50%), non-hrHPV (30%), and HPV16/18 (20%). HPV59 and 45 were most commonly detected HPV genotypes (50% and 29%, respectively). One sample was negative for all three tests (1/21, 5%).

#### References

HPV was detected in vast majority (95%) of the biopsy samples with HSIL in women who had preceding negative HPV tests, including hrHPV (70%) and non-hrHPV (30%). In addition to those infected by non-hrHPV genotypes or possible non-HPV related dysplasia (5%), multiple factors might have contributed to the prior false-negative HPV tests, including inadequate sampling, interference material, technical errors, and reduced L1 gene expression in high-grade lesions. We noticed that non-16/18 hrHPV genotypes were most commonly detected in the cohort with HPV59 and HPV45 predominance, a genotypic prevalence pattern markedly different from that in general population in US. This may suggest these genotypes are relatively less sensitive on Cobas test or have lower expression level of L1 gene. Additional studies will help to validate the findings and elucidate the contributing factors and underline mechanisms.

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00135

## Distribution of genotypes in a mRNA HPV-positive screening population

### 09. HPV screening

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#### Background / Objectives

In Sweden, updated recommendations for cervical cancer screening was released in 2015 (1). In the new program, women between 30-64 years, is screened primary with HPV followed by cytology on positive samples. In Örebro county, the new program was introduced in 2016 and for HPV screening, a test detecting mRNA in 14 hrHPV genotypes is used. This test is however not providing genotype information. The aim of this study was to investigate the distribution of genotypes in a HPV-positive screening population in Örebro, Sweden.

#### Results

HPV positive samples (HPV Aptima, Hologic) between November 2016 and April 2017 (n=530) were included in the study. Genotyping was performed at the same sample as the screening test by extracting DNA directly from residual material in the Aptima specimen transfer tube and genotype using Anyplex II (Seegene).

#### Conclusion

More than half of the study population were in the age group 30-39 years (n=274, 52%), 32% were 40-49 years (n=173) and 16% 50-58 years (n=83). Despite of a positive screening result, 46 samples tested negative in the genotyping (9%). The most common genotypes were HPV31 (n=93), HPV16 (n=76), HPV52 (n=57) and HPV68 (n=49) and of 28 detectable genotypes, 27 was present in at least one sample. Single infections (n=281) were more common than multiple infections (n=203) and the distribution was similar between the three age groups (Fisher's Exact Test, p=0.867).

#### References

A wide repertoire of HPV genotypes was present without differences between age groups. Also, a distinctive portion of mRNA positive samples were found to be negative when DNA tested. Negative samples will be further evaluated with a second HPV test, using different viral target genes.

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00144

Countries of Eastern Europe and Central Asia: the situation of HPV infection in HIV-positive women

## **09. HPV screening**

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### **Background / Objectives**

The frequency of detection of HPV varies greatly depending on the region of residence of the woman. Also HIV-infected women have a higher risk of HPV infection than HIV-negative women, and a higher risk of persistence and malignancy.

The aim of the study was: to study the prevalence of human papillomavirus of high carcinogenic risk (HPV HCR) in HIV-infected women in some countries of Eastern Europe and Central Asia.

### **Results**

647 HIV-infected women from Russia, Belorussia, Armenia, Azerbaijan, Tajikistan and Kyrgyzstan were examined from September 2017 to December 2017 . All women underwent HPV-test with the determination of 14 types of HPV HCR (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68).

### **Conclusion**

Among the 647 women surveyed, mostly young people (under 40 y-s) predominated. As a result of the HPV-test, 265 (41%) of HIV-infected women were diagnosed with HPV HCR. The percentage of HPV detection ranged from 28 to 48%: Armenia - 39%, Azerbaijan - 43%, Belarus - 28%, Kyrgyzstan - 46.5%, Tajikistan - 37.8%, Russia (Samara) - 48%. All 14 HPV HCR genotypes were diagnosed in HIV-positive women in the region.

The distribution of HPV genotypes is different for these countries: 16 and 68 HPV genotypes are registered in Armenia (33.3% and 23%, respectively), in Azerbaijan 16, 18 and 56 HPV genotypes (32.6%, 20.9%, 20.9%), in the Republic of Belarus - 16 (28.6%) and 56 (28.6%), in the Republic of Kirghizia - 16, 31 and 68 (23.9%,



21.7% and 21.7% respectively), in Tajikistan - 16.31, 56 (29.7%, 21.6% , 21.6% ), in Samara (Russian Federation) - 52 and 16 (23.6% and 22.2%).

HPV infection was caused by a combination of several genotypes in 49,1%/. The leading genotypes among 265 HIV-infected women with HPV were: 16 genotype - 26.4%, 31 genotype - 13.6% and 18 genotype - 9.4%.

#### **References**

There is a high incidence of HPV infection in HIV-infected women.

#### **References**

Given the high risk of developing cervical cancer and the wide spectrum of detectable genotypes of HPV HCR in this group, it is necessary to use a test system to diagnose the 14 genotypes of HPV. The results should be taken into account when planning vaccination in the region.

00190

## INCIDENCE OF HUMAN PAPILOVIRUS INFECTION IN RIVERSIDE WOMEN OF AFLUENTES AMAZONAS RIVER - BRAZIL

### 09. HPV screening

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#### Background / Objectives

Introduction: The relationship between cervical intraepithelial lesions and malignant potential associated with human papillomavirus (HPV) infection is already well established. The identification of HR HPV types is very important to the prevention of this neoplasm and in evaluating the vaccine efficacy. Low-income populations with low socioeconomic status, especially in developing countries, are the most disadvantaged due to get access to public policies for prevention and diagnosis, viral dynamics and the evolution of neoplasia, as is the case with river populations in Rio Amazonas. Objective: This study aims to identify the presence of HPV-DNA in cervical intraepithelial lesion of riverside women from the tributary communities of the Amazon River.

#### Results

Method: A total of 123 cervical samples were collected in BLM (Cellpreserv) from riverside Negro and Madeira rivers. The cytopathological material was automatically processed in the KLP 2000 (Kolplast) and classified according to the Bethesda System (2011). The residual material was submitted to DNA-HPV genotyping by the MicroArray methodology (Euroimmun). The tests were performed in duplicate, using a positive and negative HPV control. This project has been approved by the Ethics Committee of the Santo Amaro University - SP (Brazilian Plataform - CAAE: 61414216.4.0000.0081).

#### Conclusion

Results: 123 cell samples submitted to cytology analyze, 12 (9.7%) samples presented cervical intraepithelial lesions, 2 (1.6%) samples were classified by ASCUS, 5 (4.1%) samples were diagnosed as LSIL, 5 (4.1%) samples classified as

HSIL. 4 (3.2%) samples presented cervical lesion had HPV infection. 2 (1.6%) samples were from the Negro River, and 1 (0.8%) sample presented a cytologic picture as LSIL and had HPV 43,53,72 types. 1 (0.8%) sample showed a cytologic picture as HSIL, and HPV 51 and 39 infection. 2 (1.6%) samples were from the region of the Madeira River. 1 (0.8%) sample showed a cytologic picture of LSIL and 1 (0.8%) sample was classified as HSIL, both showed HPV 61 infection. The women from the Negro and Madeira Rivers regions exhibited 56% and 66.7% of HR HPV infections, respectively, and 43.75% and 33.33% of LR HPV infection in the Negro and Madeira Rivers, respectively. 25 (20.3%) samples with cytologically diagnosed as reactive status had HPV infection. The most prevalent types were HPV 16 (20%) and 45 (16%). 2 (1.6%) samples classified as normal status had HPV 16 (50%) infection.

## References

Conclusion: The study leads us to consider that according to the relationship between the social, behavioral factors in the cytological and molecular study of the population of riverside women in the of the Amazon River have a high risk to develop cervical cancers associated a HSIL and HR HPV types found in both regions.

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00225

Organization of in-depth examination for diagnostic cervical cancer of Rostov region female population

## **09. HPV screening**

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### **Background / Objectives**

Cervical cancer in Russian Federation ranks as the 5th most frequent cancer among women and the 2nd most frequent cancer among women between 15 and 44

years of age. Liquid-based cytology with partial automation in which a glass slide is pre-read by the robot improves the accuracy of cytological examination and results in fewer unsatisfactory smears. The HPV test is needed to triage of indeterminate Cytology results, which accounted for around half of abnormal results. The efficiency of these methods demonstrated in a programme of organized screening.

Objective: to evaluate co-testing in the opportunistic screening for cervical cancer

### **Results**

Since 2014, in Rostov-on-Don and the Rostov region a regional program of cytological cervical cancer screening with parallel HPV testing has been implemented. Liquid-based cytology BD TriPath and clinically validated test COBAS RealTime High Risk HPV that provides qualitative detection of 14 high risk HPV types, three results per test reported corresponding to HPV 16, HPV 18, and 12 other high risk HPV, single and mixed infections) are used for screening purposes in the specialized laboratory of the Regional Consultative and Diagnostic Center. The performance of all tests is automated

### **Conclusion**

As a result of 4 years work, on the basis of Rostov Region Regional Counseling and Diagnostic Centre, an algorithm for screening and in-depth diagnostics of cervical pathology has been developed. This algorithm is optimal for the region in the absence of a national cervical screening programs.

During the period of 2014-2017 cytology is conducted for 267 510 women. HPV testing is conducted for 65 993 females. Pathological changes in the epithelium identified by cytology are detected in 45 477 women (17%): LSIL – 43 131 (95,7%), ASC-US/ASC-H - 819 (1,8%), HSIL – 909 (2%), the cancer is detected in 213 women (0,5%). Positive results in HPV testing contribute to 9551 (21%).

#### **References**

Complex approach to the diagnosis of cervical lesions and coordinated working process of medical institutions can significantly shorten the patients examination process and help to timely use high-tech testing and treatment methods in frames of one multi-disciplinary diagnostic center.

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00385

High prevalence and concordance of anal and cervical HPV genotype detection

## 09. HPV screening

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### Background / Objectives

The same high-risk (HR) Human Papilloma Virus (HPV) types associated with cervical cancer can also cause anal cancer and its precursors (AIN)<sup>1</sup>. The incidence of anal cancer is increasing by 2% each year in women and men<sup>2</sup>. Women with history of CIN have a 10 fold increased risk of anal dysplasia. Aim was to explore the correlation between cervical and anal HPV infection as well as the development of cervical and anal dysplasia.

### Results

Women with gynecological clinical indication (HPV/cytology/histology) were recruited. Initially, anal cells were collected using swabs (PAPCone) for HPV testing and cytological examination followed by a cervical smear (Cervex-brush and Cytobrush) to prevent contamination between the smears taken. All women with an anal HR-HPV positive finding were followed up. Anal HR-HPV positive women underwent rectal examination and standard anoscopy. Anal and cervical smear test are repeated after 12 months to document possible changes or clearance in HPV genotypes. The anal HR-HPV positive women are followed up for 48 months.

### Conclusion

This interim analysis includes 66 women. Cervical examination demonstrated CIN I (9), CIN II (14), CIN III (8), in addition, VIN I (1), VIN II (1), VAIN I (1), VAIN II (1), VAIN III (2), metaplasia (2) and condylomas (1) were found. In six cases, the histology showed no abnormality. 53 women (80.3%) had  $\geq 1$  anal HPV type (3 anal smears unsatisfactory DNA amount) and HR-HPV was found in 48 women (72.7%). From these 46 (95.8%) were also positive at the cervix, 41 (85.4%) women had at

least one identical cervical HPV type. The anal smear tests showed no clinical indication. 26 women underwent rectal examination and anoscopy. In 25 cases, no AIN was diagnosed and one case had a non-valid result. Findings were healed fissures (2), hemorrhoids (6), condylomas (1), colon irritable (1) and a fibroid (1). Fourteen anoscopies demonstrated no findings. Nine women attended the 12-month follow up so far. Six underwent surgical intervention at the cervix in the past year. In 4/6 cases, cervical and anal HPV type changes after surgery were observed. In 2/6 cases, the surgical removal of one particular cervical HPV type also lead to the clearance of the identical HPV type in the anal smear test.

## **References**

HPV infection in the anus in women with cervical clinical indication is common. The concordance between cervical and anal HPV is prominent (69.7%). Despite this very high anal infection rate, the rate of anal dysplasia is low as compared to cervical sites. Recruitment is ongoing.

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00400

## NEW MOLECULAR MARKERS IN HPV INFECTION RISK STRATIFICATION: SYSTEMATIC REVIEW

### 09. HPV screening

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#### **Background / Objectives**

Cervical cancer is one of the most common oncologic diseases in women worldwide, with its highest mortality rates in countries without a well established mass screening program, which is why we can safely say that screening is critical in reducing morbidity and mortality from this cancer. It is also well established that human papillomavirus (HPV) infection is associated with cervical cancer, however, since most lesions caused by HPV regress and only some progress to cancer, new biological markers able to identify clinically relevant HPV infection and avoid needless colposcopy referral are still needed, and that is the aim of this review.

#### **Results**

A search was conducted using the Pubmed database, restricted from 2008 onwards, using the keyword “papillomaviridae” in combination with “RNA, messenger” or “E6/E7”, “p16” and “ki-67”, “methylation”, “microRNAs”, “BIRC5”, and “DNA Topoisomerases, Type II” or “MCM2, and the Web of Science Core Collection, restricted to the same time frame, using the keywords “HPV” and “cervical cancer” in combination with “mRNA” or “E6/E7”, “p16” and “ki-67”, “methylation”, “microRNA” or “miRNA”, “BIRC5”, and “TOP2A” or “MCM2”. Reference lists of relevant articles were also included. Preference was given to experimental and observational studies with comparison groups.

#### **References**

E6/E7 mRNA and p16/ki67 dual-stained cytology are the most studied new molecular biomarkers, with evidence in regards to the benefit they would provide to screening, particularly as a triage test before referral to colposcopy, however, more robust studies and a cost benefit analysis are still needed if they are to be included in mass screening protocols. Assessment of methylation levels also appears to be an interesting way to identify clinically relevant HPV infection, once agreement pertaining to the genes associated with the highest diagnostic accuracy is achieved.



Lastly, microRNAs, BIRC5, TOP2A and MCM2 are novel targets that also show great promise and should be the focus of future studies.

00442

## ACCEPTABILITY OF POINT OF CARE HPV-BASED CERVICAL SCREENING: A QUALITATIVE SYSTEMATIC REVIEW

### 09. HPV screening

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#### Background / Objectives

The World Health Organization (WHO) recently recommended high risk HPV (hrHPV) testing for primary cervical screening in all countries, including low- and middle-income settings. HPV testing has high sensitivity and specificity compared to conventional cervical cytology, allows for less frequent screening, and when used at the point-of-care (POC), bypasses the need for sophisticated laboratories. Testing for hrHPV at POC means that results can be provided in a shorter timeframe (60 minutes compared to up to 2 weeks for Pap smears in high-income settings and much longer in other settings) allowing for quicker turnaround of results, faster access to treatment, a reduction in patient loss to follow-up and, in time, reduced morbidity and mortality. Such new technologies can however place additional stress on women, health care workers (HCW) and clinic workflow. It is critical to better understand the impact on different end-users to develop better communication messages that will make this user-friendly technology more acceptable. This qualitative systematic review will explore women's and health care providers' experiences and perceptions of cervical cancer screening and the socio-cultural factors influencing the acceptability of HPV-based cervical screening in all settings.

#### Results

The review will be conducted in accordance with the PRISMA guidelines. Two independent reviewers will screen all titles and abstracts to confirm the relevance of the studies according to the inclusion criteria of qualitative studies that explore acceptability of HPV-based cervical screening, from the perspective of women ('patients') and healthcare providers in any setting. Data extracted will include title, authors, country, study design, research aim, intervention, data collection method, theory used, outcomes and results. Studies will be analysed using the meta-aggregation approach. The quality of the included studies will be assessed using the

Critical Appraisal Skills Programme tool. Findings from the review will be available for presentation at the conference.

## **References**

With advancements in cervical screening methods being rolled out around the world, it is critical to explore how these technologies are understood by women and their health care providers across settings. Qualitative research is important to provide contextual information about how to prepare women and health facilities for HPV-based testing. The findings of this qualitative systematic review will provide guidance for action and inform future health education programs and interventions to increase uptake of HPV-based cervical screening globally.

00530

## EVALUATION OF THE EFFICACY OF THE TREATMENT OF VAGINAL INTRAEPITHELIAL NEOPLASIA IN PUBLIC HOSPITAL IN SAO PAULO, BRAZIL

### 09. HPV screening

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#### Background / Objectives

Vaginal intraepithelial neoplasia (NIVA) is a premalignant lesion marked by the presence of squamous cells with atypia and no invasion. It has an incidence of 0.3 per 100,000 women in the United States and corresponds to 0.4-0.5% of intraepithelial lesions of the lower genital tract (1, 2). and high grade categories ( 2 and 3): NIVA 1 involves the lower third of the epithelium, NIVA 2 and 3 more than two-thirds to the total thickness. NIVA of high grade precursor of the carcinoma of the vagina, in analogy to the Cervical Intraepithelial neoplasia (NIC).The aim of study was the clinical evaluation of NIVA treatment and therapeutic measures, comparing the treatment of the laser with the immunological treatment and the rates of relapse in both treatments.

#### Results

Series of cases sent to the Clinical Hospital of the University of São Paulo with biopsy diagnosed with NIVA, through electronic medical record research. Evaluation of the type of treatment: laser, imiquimode or both and the result, regression, corresponds to a normal colposcopy or biopsy or biopsy with the functions of less than or equal to a NIVA 1; persistence, corresponds to patients who are submitted to biopsy treatment with NIVA 2 or 3; or evolution to carcinoma. In addition, the patients with regression and continued in follow-up, evaluating the occurrence of relapse. Statistical analysis was performed as Student's test for continuous variables and chi-square test for categorical variables. The tests were performed in the Numbers® Version 5.0.1 for Mac OS program.

#### Conclusion

148 patients had NIVA diagnostic, 59 had NIVA 1, and an expectant management was performed. The remaining 89 patients were diagnosed with high grade NIVA (2 or 3). Thus, 56 patients with high-grade NIVA evaluated in this study, 76.8% underwent laser / megapulse treatment ; 21.4% to imiquimode and 1.8% to imiquimode + laser. Of the 43 patients submitted to laser, 58.1% presented improvement, 30.2% persistence, 4.7% progressed to carcinoma and 7 are still in follow-up, with no results yet after treatment and 5 presented relapse. Of the patients submitted to imiquimode, 66.7% presented improvement and 33.3% presented persistence of the lesion. And of the 8 patients with improvement, 3 presented recurrence. When comparing the recurrence rate of each treatment, both laser and imiquimod treatment had a 25% relapse after regression in the initial total - 5 cases of 25 with regression after laser and 2 cases of 8 with regression with imiquimode.

## **References**

Imiquimode presents as a possible alternative treatment for high-grade NIVA. However, further studies are needed to complete the treatment of HPV, with the objective of returning to the lesions and clarifying the HPV, in order to reduce relapses, to follow a minimum number of patients evaluated.

00608

## HPV INFECTION AMONG HIV-POSITIVE MEN: A FIVE-YEAR REVISED EXPERIENCE OF A DIAGNOSIS LABORATORY.

### 09. HPV screening

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**Centro Medicina Laboratorial Germano de Sousa - Lisbon (Portugal)**

#### **Background / Objectives**

The spread of HIV epidemics globally has increasingly drawn attention to the interaction between HIV and the “classic” sexually transmitted infections (STIs). A consensus has grown that other STIs increase the spread of HIV, following on from the early epidemiologic studies that explored the epidemiologic synergy between STIs and HIV. However, the interaction of the many STIs with HIV is potentially complex, with the possibility of reciprocal influences on susceptibility, infectiousness, and the natural history of infections. There is growing evidence of a significant burden of human papillomavirus (HPV) infection and associated disease in men. HIV infection increases HPV prevalence, incidence, and persistence and is strongly associated with the development of anogenital warts as well as anal, penile, head and neck cancers in men. Despite increasing access to antiretroviral therapy, there appears to be little benefit in preventing the development of these cancers in HIV-positive men, making prevention of infection by vaccination and information, a priority.

The authors present 5 years revised casuistic as a reference laboratory center in sexually transmitted infectious diseases diagnosis.

#### **Results**

Male samples were tested by HPV-molecular and conventional-cytology methods. HPV molecular methods used where: Hybrid Capture 2 (hc2, Digene); Clart human papillomavirus 2 (Genomica) and PapilloCheck. The cytological results were registered with comprehensive classification system, multi-axial nomenclature SNOMED. The diagnosis of “classic” sexually transmitted infections (STIs) as Herpes Simplex virus 1 and 2, Syphilis, Gonorrhea, Chlamydia trachomatis, Ureaplasma and Mycoplasma infections statistics were used for data analysis; the Fisher exact test was employed to assess the association between categorical variables. P-values (2-sided test) less than 0.05 were considered significant.

## **Conclusion**

The results obtained for the incidence of most frequent HPV genotypes in men and MSM are in agreement with several studies [Dunne EF, 2006]. The most common anogenital HPV types detected in men varied by study but were similar to the types commonly detected in women.

Type 16 was consistently found among the most common; however, other types were also reported (types 6, 11, 18, 31, 33, 42, 52, 53, 54, 59, and 84) [Dunne EF, 2006], but a shift possibility can occur with universalization of the vaccine.

## **References**

This study will contribute to a better understanding of the wide spectrum of male HPV infection.

On genotyping tests multiple infections decreased by the severity of the cytological interpretation, revealing that persistent and relapsing HPV infections are at higher risk for anal dysplasia development and malignant transformation. HPV infection appears to occur early in MSM.

## 10. Self-sampling



00003

## PREVALENCE OF HUMAN PAPILLOMAVIRUS ON THE HANDS OF HEALTH PROFESSIONALS

### 10. Self-sampling

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#### Background / Objectives

The HPV infection can lead to a variety of lesions both benign and malign. [1] The main route of transmission is sexual intercourse but there is evidence that supports other routes such as self and heteroinoculation through the hands. [2-9] The objective of this work was to determine the HPV prevalence on the hands of health professionals – doctors, nurses and medical auxiliaries.

#### Results

Health professionals from four specialties – dermatology (DERMA), otolaryngology (ORL), gastroenterology (GASTRO) and obstetrics&gynecology (GINECO) – working in Hospital de Santa Maria in Lisbon, participated voluntarily in the study by signing an informed consent, completing a questionnaire and allowing the sampling of their dominant hand (through the use of the kit “LINEAR ARRAY HPV Genotyping Test”®, a spiral brush and a scraping with a scalpel blade) to search for DNA of different HPV genotypes: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84(MM8), IS39 and CP6108.

#### Conclusion

There were 102 participants in the study and the distribution by specialty was: DERMA - 26; ORL - 25; GASTRO - 19; GINECO - 32. Among the total were 32 doctors (31.37%), 44 nurses (43.14%) and 26 medical auxiliaries (25.49%). The mean age was 40.2 years. Regarding sexual orientation: 100 heterosexual participants and 2 men who have sex with men. On average, the studied population had 1.02 sexual partners in the last 6 months. In total, 37 of the 102 study participants were positive for HPV on the hands. The most frequently found genotype was 6 (n = 14), followed by 51 (n = 11) and 66 (n = 10). The

distribution of positive specimens by specialty and their respective genotypes is shown in the table:

Specialty	Total of participants	Total HPV+ samples (%)	Detected genotypes (n)	High-risk	Low-risk
<b>DERMA</b>	26	8 (30.77)	6 (3), 51 (3), 66 (2)	51, 66	6
<b>ORL</b>	25	10 (40)	6 (3), 11, 51 (4), 66 (2)	51, 66	6, 11
<b>GASTRO</b>	19	8 (42.11)	6 (2), 11, 51 (3), 51+66, 66	51, 66	6, 11
<b>GINECO</b>	32	11 (34.38)	6 (6), 56+66, 16+83, 66 (3)	16, 56, 66	6, 83
<b>Total</b>	102	37 (36.27)	6 (14), 11 (2), 51 (10), 66 (8), 16+83, 51+66, 56+66	16, 51, 56, 66	6, 11, 83

## References

In the present study, a high prevalence of health professionals with positive samples for HPV on the hands was observed - 36.27%. In 56.76% of these samples high-risk HPV subtypes were identified making appropriate the discussion about the risk of virus transmission to patients and devices which will later be used in patients.

We thank Professor Eduardo Franco for the contribution to the accomplishment of this work.

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00290

## WILL HPV SELF-SAMPLING DIMINISH THE SOCIAL INEQUALITIES IN THE FLEMISH CERVICAL CANCER SCREENING PROGRAM?

### 10. Self-sampling

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#### Background / Objectives

This study aimed to study the social inequalities in the uptake of cervical cancer screening, when self-sampling kits were offered to Flemish non-attenders of the Flemish cervical screening program.

#### Results

The study was based on data that were obtained from a randomized controlled trial (n=16,660) measuring how offering HPV self-sampling affected screening participation. Women either received a kit to perform a self-sample for HPV testing or a letter offering the opportunity to order a kit. Socioeconomic data on the study population were derived by linkage to the Crossroads Bank for Social Security, using the National Social Security Number. Descriptive analyses were conducted to assess the distribution of socio- demographic (current and nationality of birth ) and SES

variables (work intensity at household level and preferential tariff as a proxy for poverty).

## **Conclusion**

Women with a preferential tariff, have a significant higher risk of not returning a self-sampler (OR: 1.66 (95% CI: 1.47-1.86)). There was a positive association between work intensity at household level and participation. Women living in (quasi-)jobless households, were more likely not to participate (OR: 1.36 (95% CI: 1.21; 1.54)) than those not living in a jobless household. Participation in the study was 18.1% for women who had Belgium as their nationality at birth and 20.2% who have Belgium as their present nationality. Participation in the study was 10.8 % for women born in Eastern Europe and 4.8% for women born in Maghreb or Turkey. The crude OR for not being screening with a self-sample, for having a nationality at birth other than Belgium or being from the Netherlands was 2.36 (95% CI: 2.13- 2.62). The OR for not participating for having a present nationality other than Belgium or being from the Netherlands was 1.86 (95% CI: 1.63- 2.11).

## **References**

Here we show that low SES, as measured by preferential income and household work intensity, was strongly associated with low participation in being screening with self-sampling, and that immigrants were less likely to participate. Our results demonstrate that cervical screening with a self-sampling kit does not socially balance cervical cancer screening. Social inequalities in the uptake of cervical cancer screening, when self-sampling kits are offered to Flemish non-attenders, remained and other routes need to be explored.

The research was funded by the Flemish government.

00342

Performance of 6 methylation markers tested on self-collected dry samples – feasibility study

## 10. Self-sampling

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### Background / Objectives

In high-income countries, a high proportion of cervical cancers are diagnosed in screening non-attendees. One approach to improve screening coverage is to offer vaginal self-sampling for HPV testing. To improve the rather low specificity of HPV testing additional markers such as HPV-specific DNA methylation can be used. Aim of this feasibility study is to determine the performance of the methylation marker assay GynTect®, comprising 6 methylation markers, on dry self-collected samples of different storage age.

### Results

100 samples collected during an earlier study 2013/14 were stored in PreservCyt at room temperature. Furthermore, we aim to collect up to 100 fresh self-samples among patients from our colposcopy clinic. For the fresh samples, the Evalyn Brushes are stored at room temperature after self-sampling and after one week, cells are transferred to ThinPrep PreservCyt solution for analyses. GynTect® and HPV-testing (Abbott RealTime HighRisk HPV Test) were performed on both, old and fresh samples.

### Conclusion

Preliminary data show a good performance of GynTect® on fresh samples, whereas older (>4 years ) samples seem to have insufficient DNA quality to reliably determine the methylation status of the samples.

### References

This feasibility study has just started and only few preliminary data is available up to now. At Eurogin, we will have data for the methylation assay on about 100 older and 100 fresh samples and both, HPV assay and the methylation assay, can be

evaluated regarding the performance, reproducibility and general handling on self-collected dry cervical samples.

00372

## EVALUATION OF THE INNO-LiPA HPV GENOTYPING EXTRA II ON UCM PRESERVED FIRST-VOID URINE

### 10. Self-sampling

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#### **Background / Objectives**

Self-sampling opens opportunities to increase the participation rates in screening programs and may improve today's cervical cancer prevention. Non-invasive urine collection is considered the preferred way of self-sampling compared to vaginal/cervical swabs.

The INNO-LiPA HPV Genotyping Extra II allows identification of 32 HPV genotypes and excellent performance has been shown using cervical scrapes. Our study aim was to develop a protocol for first-void urine and to demonstrate equivalent performance of the INNO-LiPA HPV Genotyping Extra II in comparison to cervical samples.

#### **Results**

Samples: UCM-preserved first-void urine samples collected between 2010-2012 and stored at -20°C until analysis. HPV DNA results for genotype 16 and 18 were available based on real-time PCR data on urine and cervical samples.

INNO-LiPA HPV Genotyping Extra II testing: DNA was extracted using the QIAamp DNA mini kit with a starting volume of 200 µL and a final elution volume of 50 µL. Amplification and LiPA hybridization was performed according to the manufacturer's instructions.

#### **Conclusion**

The INNO-LiPA HPV Genotyping Extra II showed HPV DNA positive results on all samples with a historical positive reference result on first-void urine. In comparison to



the reference data which used 4 mL UCM-preserved urine and included a concentration step, these data indicate that it is possible to test directly on a reduced amount of first-void urine.

Concordant HPV genotyping results for HPV 16 and 18 were obtained on first-void urine in comparison to the HPV genotyping result on the corresponding cervical sample (collected on the same day or another day) in more than 90% of the samples tested. Additional HPV genotypes were identified using the INNO-LiPA HPV Genotyping Extra II.

## **References**

A testing protocol was developed for the INNO-LiPA HPV Genotyping Extra II enabling the use of self-collected first-void urine as input sample. The high analytical sensitivity of the SPF10 Plus-based amplification allowed the use of a limited amount of sample volume. A good concordance between HPV genotyping results in urine and cervical scrapes were obtained. Further investigation by direct comparison of first-void urine and cervical scrapes with the INNO-LiPA HPV Genotyping Extra II is ongoing.

00383

## USABILITY OF THE COLLI-PEE: A FIRST-VOID URINE SELF-SAMPLING DEVICE

### 10. Self-sampling

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#### Background / Objectives

The aim of this study was to compare the usability of the previous version (CP2000) of the Colli-Pee to the current improved device (FV5000), in which the funnel material was changed from cardboard to polypropylene and the number of components was reduced from 8 to 4.

#### Results

Usability data collected from five clinical studies was used. For each study, a questionnaire on usability consisting of yes-no, open and 5-point Likert scale (very unclear to very clear) questions was completed by the participants using one of both devices. Fisher Exact Test was used to compare usability data from both versions of the Colli-Pee.

#### Conclusion

Among a total of 371 participants (185 male; 186 female), 46% used CP2000 and 54% FV5000. Colli-Pee was found very easy (70%) and easy (21%) to use, no significant difference between versions was observed ( $p=0.445$ ). The FV5000 version had less urine spillage during collection: 8% compared to 23% for the CP2000 ( $p=0.070$ ). Some spillage after collection was present in both devices (11%,  $p=1.000$ ). The instructions for use were significantly clearer and easier for FV5000 compared to CP2000 (90% vs 65%,  $p=0.006$ ). 92% of the subjects would like to use Colli-Pee as sample method for

screening purposes (92%,  $p=1.000$ ) and would recommend it to others (CP2000: 92%, FV5000: 100%;  $p=0.175$ ). Packaging and recyclability raised a few concerns for both device versions, but the Colli-Pee exists of polypropylene and high-density polyethylene which can be recycled or incinerated into energy. The CP2000 was generally described as hygienic, convenient, women friendly and a good urine collection system with no need to interrupt the urine flow. The improved device (FV5000) was also found suitable for home use and for transportation by regular mail.

## **References**

Usability of the Colli-Pee device was valued as very good and design updates were shown effective through easier assembly of the device before use and reduced spillage of urine during collection. In addition, the current Colli-Pee can be sent by regular mail, making it an easy device suitable for screening purposes.

00392

## ACCURACY OF URINARY HUMAN PAPILLOMAVIRUS TESTING AMONG WOMEN REFERRED FOR TREATMENT OF CERVICAL LESIONS: A TECHNICAL PILOT STUDY

### 10. Self-sampling

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#### Background / Objectives

Acceptability of urine sampling is very high for which urinary hrHPV testing could be an alternative to reach previous screening non-responders. We assessed if first void urine specimens are suitable for hrHPV testing and provide reasonable accuracy to detect hrHPV and underlying disease with a given HPV assay as cervical specimens.

#### Results

We included 10 patients (3 carcinoma, 4 CIN3 and 2 CIN2) referred for treatment of cervical lesion. They completed self-collection using two vaginal devices: a brush (Evalyn®Brush, Rovers Medical Devices, The Netherlands) and a swab (FLOQSwabs™, COPAN, Brescia, Italy) and collected 20ml first void urine using a Colli-Pee™ (Novosanis, Belgium) before their appointment at the hospital where a physician took a cervical specimen. Vaginal and cervical specimens were preprocessed and tested using Anyplex™ II HPV28 (Seegene, Korea), Cobas®4800 HPV test (Roche Molecular Diagnostics, USA) and Xpert®HPV (Cepheid, USA)<sup>1</sup>. Urine specimens were collected without preservative to avoid any interference of the preservative with specimens subject to biobank. Urine was stored at +4°C for couple of days before 10ml of urine was centrifuged and rest urine was aliquoted. Aliquots were then kept first at -20°C and later on at -80°C. Urine aliquots were chosen to have concordant and discordant pairs in cervical and vaginal hrHPV testing. 4.5 ml urine was thawed, mixed with 2.25 ml UCM buffer and aliquoted for

three forementioned assays and for Cobas®6800 HPV test (Roche). Amicon filtration, EasyMag DNA extraction and in-house human DNA quantification (GAPDH) preceded Anyplex™ II HPV28 testing.

## **Conclusion**

Detection of hrHPV in urine tended to be inferior to hrHPV detection in physician-and self-collected cervical cells. Detection of hrHPV in urine varied substantially according to the sensitivity of hrHPV assay being lowest at 40% for Xpert®HPV and highest at 80% for Anyplex™ II HPV28. Viral load in urine usually was lower than in physician-specimen. A brush showed highest concordance with a physician-collected specimen in partial genotyping using all HPV assays. HPV type distribution in different specimen types was similar except for one cancer case which was HPV51 positive in urine and HPV16 positive in cervical and vaginal cells. Furthermore, HPV52 and HPV56 not detected with other collection methods were present as additional HPV types in urine.

## **References**

This pilot study using frozen urine aliquots without preservative does not support full implementation of urinary hrHPV testing. To achieve the most optimal performance, first-void urine is preferred. It should be immediately mixed with preservative to prevent DNA degradation during storage and extraction and, thus, to maximize viral load. Assays with high analytical sensitivity may result in better performance.

## **References**

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00475

## COMPARISON BETWEEN THE SELF-SAMPLING AND URINE FOR THE DETERMINATION OF HPV IN HIGH RISK POPULATION OF OUR MEDIUM

### 10. Self-sampling

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#### Background / Objectives

To compare our results in the determination of HPV between spontaneous urine samples versus self-sampling in women who practice prostitution in the province of Almería

#### Results

Prospective study, approved by the Ethics Committee's H. Torrecárdenas, is divided into two phases, the results of the first phase are shown.

Displacements are made to the different centers where the prostitutes work, we had the help of non governmental organization, after explaining the procedure, they are given an informed consent, a questionnaire is carried out, and they are given a bottle of urine and a swab to perform a vaginal and cervical self-sampling . Subsequently, the determination of HPV in urine is performed, and with the Self-sampling, a study of sexual infection agents, agents related to genital ulcers and determination of the human papillomavirus is carried out, the 14 genotypes studied both in the urine and by the Self-sampling are: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. The medium used belongs to Werfen and the culture media are: STI essential assay and Genital ulcer Assay. The extraction of HPV DNA has been carried out with the QIASIMPHONI® from Qiagen,

#### Conclusion

In 51 women who have participated in this first phase of the study, we have seen a prevalence of women with infection of some type of HPV in 25/51(49%), being collected in urine 14/51(27.4%), and in Self-sampling 20/51(39.2%).

The patients who had positive results for urine and Self-sampling at the same time were: 12/51(23.5%). The coincidence in the determination of genotypes between both samples was in: 7/51(13.7%). Patients with genotypes detected in urine and not in Self-sampling: 4/25(16%)

The most frequent genotype in the urine sample was: 16, with 3/51 (5.8%), followed by 51, 58, 33 with 2/51(3.9%) each. The most frequent genotype in the sample by Self-sampling was: 68, with 6/51(11.6%), followed by 16, 66, with 4/51(7.8%), 35, with 2/51(3, 9%).

Patients with infection of more than one genotype were: in Self-sampling, with two 3/20 (15%), with three 2/20 (10%), with four 1/20 (5%). In urine: infection with two: 3/14 (21.4%) with 3 0/20, with 4 0/20.

The viral load was determined qualitatively from + to +++, being generally greater in the Self-sampling than in the sample collected by urine. Self-sampling with broomstick: +++ 3/20 (15%), ++ 13/20 (65%). Viral load in the urine sample: +++ 0/14, ++ 4/14 (28.5%).

## References

The prevalence in our sample is higher than that reported in the literature for high-risk population, 24% compared to 49% in our country. There seems to be no high correlation between urine and self-sampling. The Self-sampling does not include all the genotypes that the patient presents. The viral load and the number of genotypes detected by self-sampling is greater than with urine.

00535

Social economic determinants for cervical cancer screening non-attenders offered HPV self-sampling as an alternative to ordinary screening: Who accepted and who did not?

## 10. Self-sampling

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### Background / Objectives

The Copenhagen Self-Sampling Initiative (CSi) offered a HPV self-sampling test as an alternative to cervical screening non-attenders (unscreened > 4 years). In total, 4824 screening non-attenders residing in the Capital region of Denmark accepted and returned a self-sampling brush out of 23.632 invited. To allow for future optimization of self-sampling strategies to recruit screening non-attenders, we assessed socioeconomic determinants of response to self-sampling invitations. Here, we characterize women accepting self-sampling versus women who preferred their own general practitioner (GP) after invitation or remained non-responders.

### Results

All women invited for CSi were linked to Statistics Denmark. Logistic regression was used to analyse associations between the socio-demographic information retrieved. The main outcome was divided into: women accepting self-sampling, women screened by GP after invitation to CSi and women not responding at all. Moreover, we dichotomised the group of women who accepted the self-sampling into those returning the brush and those who didn't.

### Conclusion



Presented with the invitation for self-sampling, women with non-Danish origin both born (OR=2.07) in and outside (OR=2.05) Denmark were more likely to go to their own GP for a regular screening sample, or ignoring the self-sampling invitation letter (OR=2.17, OR=2.17 respectively) compared women of Danish origin.

For employment and educational level, we found that that women with low educational level (OR=1.29), unskilled workers (OR=1.99), early retirement (OR=2.79) and women on social welfare (OR=2.21) more often ignored the invitation for self-sampling using medium educational level as reference group. In contrast, women with high educational level (OR=1.27), self-employed (OR=1.28), students or women on maternity leave (OR=1.44) more often went to their GP after a self-sampling invitation. Lower income was a determinant of no-reply to the self-sampling invitation (OR=1.82, 1.23 respectively). Women with lower household income were less likely to return the brush despite active acceptance of the self-sampling offer (OR=0.86, 0.89 respectively).

### **References**

Women with low education and/or lower income as well as women of non-Danish origin were the most challenging to recruit for cervical screening for the HPV self-sampling. Initiatives to enhance participation in these groups could include addition of language differentiated information material to the current self-sampling activity.

## 11. Genotyping

00210

## PREVALENCE OF HUMAN PAPILLOMA VIRUS IN VULNERABLE WOMEN FROM BUCARAMANGA COLOMBIA

### 11. Genotyping

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#### Background / Objectives

Introduction: Annually more than five hundred thousand women worldwide are diagnosed with cervical cancer<sup>1</sup>. A persistent infection with high-risk Human Papilloma Virus (HR-HPV) is the necessary cause for this cancer<sup>2</sup>.

Objective: To determine the prevalence of the HPV infection in vulnerable women living at the northern district of Bucaramanga, Colombia.

#### Results

Methods: a survey study was conducted in women between 35 and 65 years old residing in low income areas of the city. Women with moderate or high risk ( $\geq 3$  points) to develop cervical cancer, determined by a standardized epidemiological survey, were asked to take their own cervico-vaginal samples. In these samples molecular tests were performed by polymerase chain reaction and reverse dot-blot hybridization using HPV CHIP Direct Flow system. Undetermined genotypes were processed again by performing a salting out DNA extraction.

#### Conclusion

Results: 874 women were surveyed; of these, 469 (53.6%) had moderate or high risk of cervical cancer and took their own samples. The median age was 46 years (RIQ 40-52 years), the median educational attainment was 5 years (RIQ 4-9 years) and the median pregnancy rate was 4 (RIQ 3-5 pregnancies). The prevalence of HPV infection was 11.3% (95% CI: 8.6 - 14.5, n = 53), for HR-HPV was 4.3% (95% CI: 2.6 - 6, 5; n=20), for low-risk HPV (LR-HPV) was 4.1% (95% CI: 2.5-6.3, n=19), for undetermined genotypes was 1.3% (CI 95 %: 0.5 - 2.8, n=6) and for coinfections was 1.7% (95% CI:

0.7-3.3, n=8). The most common HR-HPV genotype was HPV-59 (n=5) and for LR-HPV was HPV62/81 (n=8). We found coinfection with HR-HPV and LR-HPV in six women. Coinfection with two LR-HPV genotypes and with three LR-HPV genotypes occurred in one woman each.

## References

Conclusion: The frequency of HR-HPV genotypes found in this study is different from what have been commonly reported by other similar studies. HPV-16 was not the most prevalent genotype and HPV-18 was not found in any of the samples analyzed.

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00331

# ASSESSMENT OF ISOTHERMAL AMPLIFICATION AMPFIRE ASSAY FOR DETECTION AND GENOTYPING OF HPV IN FORMALIN-FIXED PARAFFIN-EMBEDDED HEAD AND NECK CANCER SAMPLES

## 11. Genotyping

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### Background / Objectives

Infection with high risk HPV is etiologically linked to a group of oropharyngeal squamous cell carcinomas (OPSCCs) that show better clinical outcome and response to treatment. The identification of HPV-related tumours in the clinical setting is mainly based on the detection of p16<sup>INK4A</sup> overexpression. However, the combination of p16<sup>INK4A</sup> overexpression and HPV DNA testing increases diagnostic accuracy and have a better prognostic value. HPV-DNA detection platforms are usually expensive, require well trained staff and have been designed for a high-throughput workflow, hampering its implementation for OPSCC diagnosis and raising the need for rapid and cheap molecular methods for low and middle income countries. The aim of this study was to assess the AmpFire HPV Test (Atila Biosystems), a simple, fast and low cost HPV DNA assay, for formalin-fixed paraffin-embedded (FFPE) samples.

### Results

Two batches of samples were assayed using the AmpFire test. The first batch comprised 56 OPSCC samples from the head and neck ICO international collection (1950-2009). Longstanding remaining DNAs extracted from these samples by Proteinase K digestion, had previously been used to perform a SPF10/DEIA/LiPA25 HPV test. Also, 105 head and neck tumour FFPE samples collected during 2016-2018 at Hospital de Bellvitge, were extracted by Maxwell 16 FFPE Plus LEV DNA Purification kit and tested using the Roche Linear Array HPV Genotyping test. Viral DNA was amplified and detected in a CFX96 real time qPCR instrument using a 60°C isothermal reaction for 74 min. The AmpFire Test allows real time fluorescent detection of 15 high risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68) and individual genotyping of HPV16 and 18.

## **Conclusion**

Out of 56 samples from the ICO international collection, 5 were HPV positive, 29 were negative and 23 were invalid (fail in  $\beta$ -globin gene amplification). This data represents a 93% agreement with SPF10/DEIA/LiPA25 results. To avoid the high rate of invalid tests, probably due to aged samples and DNA degradation, we analysed 105 recent FFPE samples. HPV-DNA was found in 30 out of 105 samples, 74 were negative and 1 was invalid, showing a 96% agreement with Linear Array HPV Test and a kappa index of 0.923 and 100% concordance for the presence of HPV16 and 18.

## **References**

Preliminary data have shown that AmpFire HPV Test is a sample-to-answer and low cost assay for detection and genotyping of HPV in FFPE oropharyngeal samples recently collected that exhibits high agreement with data assayed by SPF10/DEIA/LiPA25 and Linear Array HPV tests.

00451

## PERFORMANCE OF THREE HPV GENOTYPING ASSAYS ON FORMALIN-FIXED PARAFFIN-EMBEDDED CERVICAL SAMPLES

### 11. Genotyping

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#### Background / Objectives

HPV genotyping of formalin-fixed paraffin-embedded (FFPE) cervical tissue material is important for epidemiological research, vaccine surveillance and for clinical purposes. In this study, the performance of three HPV genotyping assays will be evaluated.

#### Results

A total of 80 cervical FFPE samples from 61 patients with high-grade (CIN2 n=10; CIN3 n=38, with biopsy vs cone material from 19 patients; ACIS n=9) and cancer lesions (SCC n=12; AC n=11) were included in the study. Four sections of 5 µm from each paraffin block were prepared for DNA extraction, with hematoxylin and eosin (HE) sections before and after. DNA extraction was performed by using the QIA Symphony DSP DNA Mini Kit. Sample adequacy was evaluated by beta-globin PCR. The presence of HPV was assessed by PCR using modified general primers (MGP) followed by type-specific hybridization by Luminex technology, detecting and genotyping 37 HPV types, and Anyplex HPV28 (Seegene), detecting and genotyping 28 HPV types. HPV negative samples were analyzed with in-house E6/E7 type-specific PCR for the 14 high-risk HPV types. This assay will also be used for verification of discordant genotype results. Illumina next generation sequencing of MGP amplicons will be performed on all samples.

## **Conclusion**

In total, 72 of 80 samples (90%) were found HPV positive with Luminex (66/72) and/or Anyplex HPV28 (69/72); eight samples (10%) were negative/invalid by both methods. Multiple infections were found in 14 samples, of which six were reported as single infections with Luminex and one as negative with Anyplex. Single infections identified by both assays (n=50) showed full concordance. Altogether, 26 of the 38 genotypes covered by the two assays were identified. High-risk HPV types were detected in 68 samples, representing all high-risk types except HPV56. Three patients were found positive for genotypes other than the 14 high-risk types (HPV40, AC; HPV42/53, CIN3; HPV70, SCC). Of the eight samples negative/invalid by both assays, six were found positive with E6/E7 type-specific PCR; findings on HR sections may explain the two triple negative samples.

## **References**

This study shows that both Luminex and Anyplex HPV28 reliably detect HPV in FFPE samples, with Anyplex HPV28 showing a slightly higher sensitivity. We further recognize the value of applying extended genotyping to the high-risk types, although this primarily will contribute in finding multiple infections. Discrepancy in high-risk HPV sensitivity and specificity (n=14) will be verified with E6/E7 type-specific PCR. Correlations including data from next generation sequencing will be presented.



00509

## RETROSPECTIVE ANALYSIS OF THE HPV GENOTYPE AND ESTIMATED IMPACT OF THE VACCINE AGAINST HPV IN SOUTHERN SPAIN

### 11. Genotyping

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#### **Background / Objectives**

Persistent infection of high-risk human papilloma virus (HR-HPV) is thought to be responsible for about 100% of cervical cancer cases. To reduce the incidence of these pathologies, mainly of cervical cancer, several vaccines against HPV have been developed: a bivalent vaccine against HPV 16 and 18 (with some cross-protection against HPV 31, 33 and 45) and a quadrivalent vaccine against genotypes HPV 6, 11, 16 and 18. In Andalucia (Spain), the bivalent vaccine was introduced in the official immunization schedule for girls at the end of 2008. Three doses (0–1–6 months) were administered to girls born after the 1st of January of 1994 when they were 14 years old. The objective of this study was to estimate the impact of HPV vaccine through retrospective analysis of the distribution of HPV genotypes in local area of Cadiz, southwest of Andalucía.

#### **Results**

We studied 1272 women in the period 2009-2018, stratified by age in two groups: 17 to 23 years (489) and 24 to 48 years (823). The patients, included in an opportunistic protocol for cervical cancer screening, were studied retrospectively in two time periods: from 2009 to 2013 (819) and from 2014 to July 2018 (453). Only samples with a positive result for at least one HPV genotype were included in the study. HPV genotype was determined with CLART HPV2 (Genomica) until 2013 and Anyplex II HPV28 (Seegene) in 2014.

#### **Conclusion**

HPV 16 was detected between 2009 and 2013 in 125 women under 23 years of age (33.9%). This proportion has been reduced to 7.5% (9 women) in the 2009-2013 period. In women with 24-28 years of age, the proportion remains constant in both

periods (26.7%). There are also significant reductions in the detection of HPV 18 (11.1% vs 2.5%) in women under 23 years of age. The detection of HPV genotypes 31, 33, 45, 51, 52 and 58 have remained without significant changes. Unlike what happens with high-risk genotypes, an increase in the detection of low-risk genotypes can be observed, such as HPV 6 (34.2% vs. 14.6%) and HPV 42 (26.7% vs. 11.4%).

## **References**

In summary, the retrospective analysis of samples studied in the last ten years seems to indicate a significant decrease in the genotypes included in the vaccine administered since 2008. However, this effect is not contemplated in high-risk genotypes not included in the vaccine and there is a significant increase in low risk genotypes, associated with an increase in the appearance of other sexually transmitted infections in a younger population. The study has the limitation of its retrospective character and the opportunistic obtaining of the sample, but it can indicate an interesting tendency that it is necessary to confirm with studies designed for that purpose.

## 12. Molecular markers

00051

Detection of potential biomarkers in the high grade anal intraepithelial neoplasia from HIV/HPV co-infected MSM

## 12. Molecular markers

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### Background / Objectives

MCL-1, Exportin-5, and Importin-beta expression in anal intraepithelial neoplasia (AIN) in Man who have sex with man (MSM) that are HIV-infected is poorly characterized. The aim of this pilot study was to analyze the expression of these proteins as well as in situ HPV DNA and E6/7 RNA in high grade AIN.

### Results

Immunohistochemistry was used to analyze the expression of MCL-1, Exportin-5, and Importin-beta. HPV DNA was detected with the Enzo polybiotin assay and HPV E6/7 RNA detected in situ via the ACD assay. A total of 10 anal biopsy specimens from HIV/HPV co- infected MSM were analyzed

### Conclusion

Each lesion showed AIN 2-3. HPV DNA was detected in all layers of the dysplastic lesion, though higher copy towards the surface. HPV RNA localized primarily to the cells in the upper (differentiated) layer of dysplastic lesion. MCL1, importin-beta, and exportin-5 localized primarily to the more basal (undifferentiated) layer of the AIN.

### References

AIN2-3 lesions show low copy HPV DNA that co-localizes with MCL1, importin-beta, and exportin-5 in the more basal, less differentiated area of AIN. As the cells differentiate towards the surface, they show higher copy HPV DNA, HPV E6/7 RNA,

and lose the expression of MCL1, exportin-5, and importin-beta. The data suggests that MCL1, exportin-5, and importin-beta proteins may have an important role and may be useful biomarkers of high grade AIN.

00055

Meta-analysis of the accuracy of p16 or p16/Ki-67 immunocytochemistry versus HPV testing for the detection of CIN2+/CIN3+ in triage of women with minor abnormal cytology

## 12. Molecular markers

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### Background / Objectives

Consistent evidence shows that women with atypical squamous cells of undetermined significance (ASC-US) can be triaged appropriately with a high-risk (hr)HPV test. However, triage of low-grade intraepithelial lesions (LSIL) with hrHPV testing is problematic due to its very low specificity. Overexpression of p16, with or without Ki-67 and identified through immunocytochemistry, indicates neoplastic transformation of HPV-infected cervical cells and may predict more accurately underlying cervical intraepithelial neoplasia of grade 2 or worse (CIN2+).

### Results

A literature search was conducted in three bibliographic databases. Studies were selected if they included women with ASC-US or LSIL who were triaged with dual staining (p16/Ki-67) and/or p16 staining and, if available, with a comparator hrHPV test to detect CIN2+.

### Conclusion

Thirty-eight studies published between 2005 and 2017 were found eligible. The pooled sensitivity of p16 staining to detect CIN2+ was 82% [95% CI, 76-87%] and 83% [76-88%] in triage of ASC-US and LSIL, respectively, whereas the pooled specificity was 71% [65-76%] and 62% [52-71%], respectively. The pooled sensitivity of dual staining was 84% [77-89%] and 86% [82-89%] for ASC-US and LSIL, respectively, and the pooled specificity was 77% [70-82%] and 66% [59-72%], respectively. Six hrHPV DNA tests, used as comparator tests, did not show inter-test

heterogeneity. The sensitivity of p16 staining was significantly lower compared to hrHPV DNA testing (ratio=0.90 [0.84-0.96] and ratio=0.84 [0.80-0.89] in case of ASC-US and LSIL, respectively). In contrast, the specificity of p16 staining was substantially higher with a relative specificity of 1.60 [1.35-1.88] and 2.29 [2.05-2.56], in case of ASC-US or LSIL, respectively. Dual staining was also significantly less sensitive than hrHPV DNA testing (ratio=0.90 [0.84-0.97] and ratio=0.90 [0.87-0.94] for ASC-US and LSIL, respectively), but more specific (ratio=1.65 [1.42-1.92] and ratio=2.45 [2.17-2.77] for ASC-US and LSIL, respectively). Dual staining and p16 staining were equally sensitive and specific with regard to LSIL triage. For ASC-US triage, p16 showed similar sensitivity but was significantly less specific (ratio=0.87 [0.76-0.99]) than dual staining.

## References

This meta-analysis has demonstrated that there is a loss in sensitivity with dual staining and p16 compared to hrHPV DNA testing in triage of ASC-US and LSIL, but there is a gain in specificity, especially in LSIL triage. Among the staining methods there was no difference in accuracy, except for the specificity of p16 alone, which was lower in the triage of ASC-US.

00186

## **P16 IN THE HISTOLOGICAL DIAGNOSIS OF THE INTRAEPITHELIAL NEOPLASIAS OF THE CERVIX: EVALUATION AND PROPOSALS FOR USE.**

### **12. Molecular markers**

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#### **Background / Objectives**

It is known the low agreement between pathologists when diagnosing grade 2 cervical intraepithelial neoplasia (CIN2), an important diagnosis, given that its clinical management is very often an excisional treatment. The LAST project proposes the application of a binary classification (high or low grade lesions) to diagnose cervical pre-neoplastic lesions and facilitate their clinical management, follow-up (low grade) versus treatment (high grade). LAST proposes the use of the p16 protein to improve the agreement between pathologists and as an aid in difficult cases, with diagnostic doubt low / high grade, frequently assigned to CIN2. As published in the literature, p16 is a technique that is easily performed and interpreted, with good agreement between pathologists. It is true? Do pathologists need specific training to adequately capitalize on the use of p16 under this indication?

#### **Results**

Three general pathologists with more than twenty years of experience were asked, one of them with special dedication to the field of gynaecology, who evaluated 132 biopsies stained with p16, from haematoxylin eosin (HE) of biopsies previously diagnosed as CIN2. The pathologists were only given the cuts stained with p16, without their HE. In each case, a cut dyed with p16 and its corresponding internal control. The sections stained with p16 were accompanied by an explanatory document (Bergeron) on how to evaluate the biopsies: positive those cases with intense and diffuse staining that affects the entire thickness of the epithelium, and negative the rest of the cases.

#### **Conclusion**



The percentage of positivity for each pathologist was variable:Pathologist(P)1:76,3%  
P2:76,6% P3 61,5%

The agreement between them: P1/P2 0,82(Kappa:K) P1/ P3: 0,649 P2/P3 0,625

When comparing these results, with important series of the literature:

Meserve:0,73 Galgano:0,87 Bergeron:0,89(K)

It is verified that in the comparisons that involve the pathologist 3, we move away much from the expected results. These differences may be due to the difficulty in interpreting the positivity, or to the different criterion of the pathologist 3, since it is markedly different from the other two. We have considered only two options, positive or negative, but there are diverse proposals, frequently used, that provide intermediate positivity criteria based on the percentage of positive cells, and others that consider the intensity of positivity. In short, there is no consensus in the reading and subsequent report of p16 stains

## References

It is necessary to establish standardized criteria of positivity of the technique, and to provide pathologists with previous training that has an impact on the correct interpretation of the biopsy and, ultimately, on a better clinical management of the patients.

## References

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00208

## XRCC1 rs1799782 and ERCC2 rs13181 POLYMORPHISMS AS POTENTIAL PROGNOSTIC AND PREDICTIVE FACTORS IN CERVICAL CANCER PATIENTS

### 12. Molecular markers

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#### Background / Objectives

Cancer cells efficiently repair treatment-induced DNA damage, exhibiting greater resistance to radiation or DNA damaging agents. Cervical cancer is commonly treated by platinum-based chemoradiotherapy, and the inactivation of DNA repair may increase the efficacy of treatments. Given that genetic polymorphisms seem to influence the repair capacity of tumor cells and can be identified by using blood samples, they are promising biomarkers in the clinical decision-making process for cancer patients. Thus, the aim of present study was to assess the prognostic and predictive values of XRCC1 rs1799782 and ERCC2 rs13181 polymorphisms in cervical cancer patients.

## Results

This retrospective hospital-based study includes a total of 260 Caucasians patients with histologically confirmed cervical carcinoma (FIGO stage IB2-IVA). The patients were recruited between February 2002 and October 2009 and treated with cisplatin-based concomitant chemoradiotherapy in Portuguese Institute of Oncology of Porto. The genotyping was performed using Taqman™ Allelic Discrimination methodology by Real-Time PCR. Difference in frequencies of the genotype between the different therapy responses groups were evaluated by  $\chi^2$  test. Overall survival (OS) and disease-free survival (DFS) were estimated by Kaplan-Meier method and log-rank test. A level of  $P < 0.05$  was considered statistically significant.

## Conclusion

There were no significant statistical differences between the different genotypes of the XRCC1 rs1799782 and ERCC2 rs13181 polymorphisms and treatment response ( $P = 0.738$  and  $P = 0.805$ , respectively). Concerning the OS, we observed that patients with advanced disease, negative lymph nodes metastasis (LNM) and carriers of ERCC2 CC genotypes present a higher survival when compared with carriers at least one A allele (AA/AC genotypes) ( $P = 0.020$ ). Additionally, we verified that carriers ERCC2 CA/AA genotypes carrier patients present a risk of death of approximately 9 times higher than patients with the CC genotype, adjusted for LNM prognostic factor ( $P = 0.030$ ;  $P = 0.029$ , bootstrap analysis). The results also showed that patients group with stage IIb or higher, age above 39 years old and carriers of ERCC2 CC genotypes present a statistically significant lower risk of developing relapse than CA/AA genotypes carrier patients ( $P = 0.040$ ).

## References

In conclusion, we demonstrated the clinical significance of polymorphisms in DNA repair genes in cervical cancer patients. The ERCC2 rs13181 polymorphism might be used as a prognostic marker for patients undergoing cisplatin-based chemoradiotherapy. However, additional studies are required for validation these results.

00281

## IS RNA EXTRACTION METHOD CRUCIAL FOR HUMAN PAPILLOMAVIRUS E6/E7 ONCOGENES DETECTION?

### 12. Molecular markers

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#### Background / Objectives

The aim of the present study was to study the relationship between HPV E6/E7 oncogenes mRNA expression and cervical cancer development in women population in Basque Country (Spain) and compared three RNA extraction methods to evaluate its impact in mRNA expression.

#### Results

Samples were genotyped by Linear Array genotyping Test. RNA extraction was performed using three RNA extraction methods: NucliSENS kit (bioMérieux), High Pure Viral RNA kit (Roche) and RNeasy Plus Mini kit (Qiagen). HPV16, 18, 31, 33 and 45 high-risk genotypes E6/E7 mRNA was detected by NucliSens® EasyQ® HPV v1 Test (Biomérieux). Finally, E6/E7 mRNA and pathology were studied over time. Pathology was classified in three groups: 1) normal, 2) ASCUS (Atypical squamous cells of undetermined significance) and low-grade cervical intraepithelial neoplasia (CIN1) and 3) High-grade cervical intraepithelial neoplasia (CIN2/CIN3).

#### Conclusion

mRNA E6/E7 positivity rate was: 62% with NucliSENS kit, 24% with High Pure Viral RNA kit and 6% with RNeasy Plus Mini kit. Because of low positivity rate with RNeasy Plus Mini kit, this was discarded for further analysis. Genotype 33 was the most expressed followed by 18.

mRNA expression was higher in patients with lesion and progression of the lesion than the group that not presented lesion or persistence of the lesion.

NucliSENS and High Pure Viral RNA extraction kits showed similar negative predictive value (73.68% and 65.79%, respectively), but positive predictive value was higher with High Pure Viral RNA extraction kit (75%). NucliSENS extraction kit

showed lower specificity than High Pure Viral RNA extraction kit (89.28% vs. 50%) but higher sensitivity (77.27% vs. 40.90%).

## **References**

NucliSENS extraction method was more concordant to detect women with lesion and seems to be more suitable with progression of lesion. High Pure Viral RNA extraction method was more appropriate to discard women without progression of lesion and was more appropriate for screening of cervical cancer due to its high specificity.

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00289

## COULD METHYLATION ASSAYS FOR EARLY DETECTION OF CIN2+ LEAD TO OVERTREATMENT? – CASE REPORT

### 12. Molecular markers

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#### Background / Objectives

The connection between persistent HPV infection and cervical cancer is well established. The most recent challenge is to make an early differential diagnosis between transient infections from those that will develop into CIN 3 or cervical cancer. Tests were developed based on knowledge underlying molecular mechanisms of oncogenic expression that allow the clinician to distinguish benign HPV infections from those that progress to precancer, making an early identification of patients who could benefit from treatment from those who are better off with no intervention.

The authors present a case of a G1 P1 45 year-old-woman referred to consultation due to a positive oncogenic HPV33 test result and a liquid-based cytology (LBC) NILM, with a T3 transformation zone (TZ) colposcopy.

#### Results

This patient had a previous history of loop excision biopsy technique (LEEP) 8 years before due to CIN2. She was submitted to a second LEEP one year after the first one due to persisting positive oncogenic HPV (not 16/18 – hybrid capture II) and T3 TZ colposcopy. Histology revealed CIN 1. She was discharged two years after the second LEEP with negative oncogenic HPV test results (hybrid capture II) and NILM LBC. When she was referred to our consultation with a positive oncogenic HPV result (HPV genotyping seegene) and NILM LBC, a colposcopy was performed which was unsatisfactory (T3 TZ); a methylation specific real-time PCR assay (GinTect®) was requested in order to ascertain whether or not methylation was present in six different DNA regions that correlate with the presence of pre-cancerous or cancerous cervical lesions. This test was positive, showing alterations in 4 out of 6 DNA regions.

#### References

The clinician was facing a rather anxious woman who demanded immediate treatment. So, within this context, she was submitted to a new LEEP, which confirmed a CIN 1. The authors are aware that expectant treatment would have been the best approach in this scenario, due to the fact that this was probably a new contact with a positive oncogenic HPV test result with NILM LBC. Nevertheless, in face of her previous history, a methylation assay was considered in order to convey an additional advantage on the detection of a CIN2+ cervical lesion. This is an interesting case because both LBC (NILM result) and methylation assay (positive test) showed a discordant result from the definitive histological result. The authors present it in order to expose the complex management of these patients and the importance of the clinician's awareness of the positive and negative predictive values of these tests.



00337

## DETECTION OF HIGH RISK HPV TYPES AND RISK PREDICTION OF CERVICAL CANCER

### 12. Molecular markers

**G Fu, T. Dafa'alla**

**GeneFirst Limited - Abingdon (United kingdom)**

#### **Background / Objectives**

Most hrHPV infections are transient and regress at a very high frequency within 1-2 years. The malignant progression of hrHPV infections to detectable tumours is often a slow process that can arise in few years to decades after the original infection. In general, the detection of hrHPV virus (DNA) in cervical cellular samples allows earlier diagnosis before the development of cervical cancer (CC) as compared to cytology, but it does not allow the distinction between transient and risky persistent infections leading to CC. Thus, using such a test as a standalone diagnostic tool in CC screening would result in overdiagnosis and overtreatment as transient hrHPV infections are extremely common in sexually active individuals. Hence, the presence of hrHPV DNA alone is not enough to determine the risk of developing CC; the integration of the virus genome within the host genome appears to be the main driver in triggering the induction of viral oncogenes (E6/E7) leading to instability of the host cells and oncogenesis. GeneFirst has already developed a first line screening assay that detects and differentiates DNA of 14 hrHPV types. In this study, we are developing an RNA-based triage assay for the assessment of the risk of CC development due to hrHPV infections.

#### **Results**

The predictive triage IVD is secondary qualitative genotype-specific diagnostic assay. The assay is designed to detect specific-transcripts of viral E6/E7 oncogenes, biomarkers for the onset and progression of host cell instability and oncogenesis associated with hrHPV infections. The assay can specifically distinguish transcripts of the oncogenes for all the 14 hrHPV types identifiable by the Papilloplex® Kit. The triage IVD is a single tube PCR assay based on the MPA technology. All individuals who test positive by this assay are recommended to undertake thorough medical investigations checking for changes in the appearance of cervical squamous and epithelial cells. This assay includes internal controls for sample cellularity and PCR inhibition.

## References

The combination of a primary DNA screening and a triage to detect viral-specific oncogenic biomarkers will allow earlier detection of CC as compared to cytology (Pap smear) and will help in avoiding overdiagnosis as compared to current DNA-based diagnostic tools. In addition, the use of our combined devices will significantly reduce costs to health systems; for unnecessary overtreatment and follow up investigations in women with harmless infections. Hence the designed IVDs allow for reliable implementation of self-collected cervical cellular samples, this will improve the CC screening coverage, by reducing refusal to participation due to embarrassment associated with the involvement of medical staff in collecting the samples.

## References

00397

Inter-observer agreement on interpretation of dual stained p16/Ki-67 samples in a HPV positive primary screening population

## 12. Molecular markers

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### Background / Objectives

Dual staining for p16/Ki-67 is recognised as a potential triage test for women with a HPV positive primary screening test. This study examines the reproducibility of p16/Ki-67 dual stain interpretation among three slide reviewers.

### Results

In partnership with CervicalCheck, The National Cervical Screening programme, CERVIVA are undertaking a longitudinal observational HPV primary screening study which is evaluating different triage strategies for management of a HPV-positive primary screening test. As part of this ongoing study dual staining for p16/Ki-67 (CINtec PLUS) was performed on 841 primary screening samples that tested positive for HPV (cobas 4800 HPV test). Three reviewers, including two cytopathologists and one individual with advanced training and experience with p16/Ki-67, independently reviewed a subset of 245 cases. A sample was deemed positive by the presence of one or more dual stained cells in a specimen. The results were compared to determine inter-observer agreement.

### Conclusion

The proportion of cases interpreted as positive by each reviewer ranged from 29.1%-43.8%. There was consensus agreement between all three reviewers for a positive or

negative result in 77.0% of cases. Agreement between reviewer 1 and 2 was 79.7% (Kappa 0.538; 0.420-0.685), reviewer 1 and 3 was 78.7% (Kappa 0.558; 0.431-0.685) and reviewer 2 and 3 was 81.6% (Kappa 0.605; 0.478-0.733). Disagreement amongst reviewers was most commonly seen in cases with weak staining intensity for p16 or Ki-67 and samples with a very low number, 1-2 cells, of dual positive cells present on the entire slide. Discordant results will be subject to pathologist review.

## **References**

These preliminary findings show that the reproducibility of interpreting p16/Ki-67 dual stained slides is moderate between three reviewers. These findings suggest that with adequate training dual staining for p16/Ki-67 can be incorporated in to routine cytology laboratories.

00431

## CERVICAL CANCER CELL LINES MEMBRANE PROTEOMICS OFFER NEW INSIGHTS IN THE DISEASE MECHANISMS

### 12. Molecular markers

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#### Background / Objectives

Cervical cancer is the fourth most common malignancy in women worldwide and while its incidence and mortality are declining in developing countries, the available therapeutic approaches can seriously affect the fertility of the patients. Thus, there is a pressing need for less toxic and targeted therapies. The membrane proteome is a potential source of therapeutic targets; however, despite the significance of membrane proteins in cancer, proteomic analysis has been a challenging task due to their unique biochemical properties. The aim of this study was to develop an efficient membrane protein enrichment protocol and to compare the expression pattern of membrane proteins of one normal (HCK1T) and three cervical cancer cell lines, C33A (HPV-), SiHa (HPV16+), and HeLa (HPV18+), in order to discover proteins which are involved in cervical carcinogenesis and may constitute novel drug targets.

#### Results

A novel reproducible protocol for membrane protein isolation and enrichment was developed, involving differential ultracentrifugation and detergent-based solubilization. LC/MS-MS proteomics and bioinformatics analysis were performed in the membrane fraction of the cervical cell lines.

## Conclusion

The percentages of membrane and transmembrane proteins in our enrichment protocol were significantly higher compared to the corresponding data derived from total cell extract analysis. Differentially expressed proteins were detected by the comparison of cervical cancer cell lines with the normal cell line. Among these, the most notable membrane proteins include thioredoxin-related transmembrane protein 2, constitutive coactivator of PPAR- $\gamma$ -like protein 1, cleft lip and palate transmembrane protein 1, nicastrin and cytoskeleton-associated protein 5. While these membrane proteins have not been reported in previous cervical cancer studies, published reports support their involvement in other cancer types. Bioinformatics analysis revealed that the differentially expressed proteins participate in biological pathways relevant to malignancy, such as HIPPO signaling, PI3K/AKT Signaling, Cell Cycle: G2/M DNA Damage Checkpoint Regulation and EIF2 Signaling.

## References

An efficient and reproducible protocol for membrane proteomics analysis was developed, resulting in the identification of a significant number of unique membrane proteins relevant to cervical cancer. These unique membrane protein identifications, offer insights on a previously inaccessible part of the cervical cancer proteome and may represent putative diagnostic and prognostic markers, and eventually therapeutic targets.

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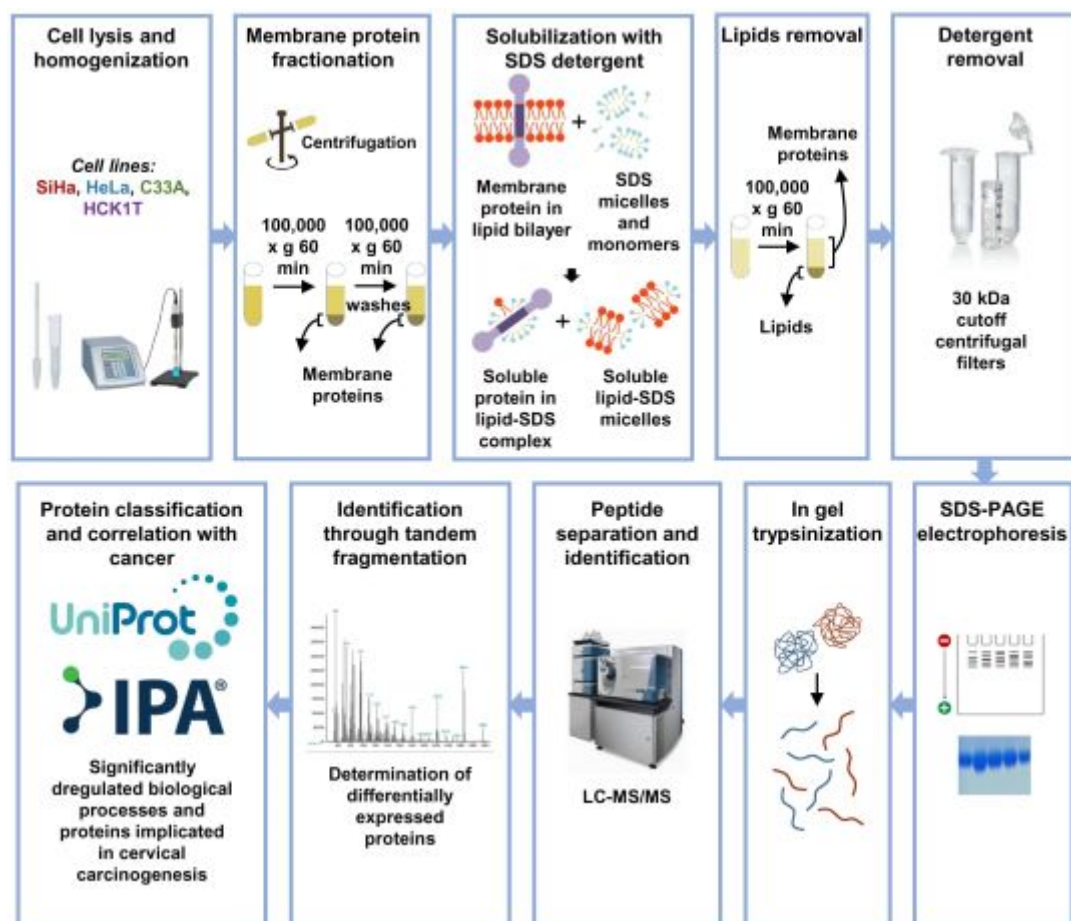
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00471

## EXPRESSION AND CLINICAL SIGNIFICANCE OF ANGIOPOIETIN-1, ANGIOPOIETIN-2, TIE2 RECEPTOR AND HPV STATUS IN PATIENTS WITH PENILE CARCINOMAS

### 12. Molecular markers

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#### Background / Objectives

Background/objectives. Penile carcinoma (PC) is a rare cancer and HPV infection is one of the risk factors for this disease. Little is known about the prognostic markers for PC. The aim of this study was to evaluate whether angiopoietin/Tie2 gene expression influence clinical outcome, HPV status and survival rates in patients with penile carcinoma.

#### Results

Methods. Quantitative real time polymerase chain reaction (qPCR) was performed to evaluate Angiopoietin-1, Angiopoietin 2 and Tie2 receptor gene expression in a group of 53 penile carcinoma specimens. The commercial kit INNO-LiPA (Fujerebio) was used to detect and genotype HPV. Gene expression was investigated in association with clinical-pathological parameters, HPV status and patient's survival rates.

#### Conclusion

Results. Lower expression of angiopoietin 1 was associated to Jackson stage III ( $p=0.001$ ) and inguinal relapse after surgery ( $p=0.019$ ). Patients with higher Ang1 expression presented higher survival rates ( $p=0.037$ ). Ang2 expression was lower in patients with deep tumor invasion ( $p=0.011$ ). Tie2 was not associated to any of the evaluated clinical parameters. The prevalence of HPV in the group was 35.9% and 71.4% of the positive cases were HPV 16 or 18. HPV infection was associated with high Ang1 expression ( $p=0.029$ )

## References

Conclusions. In the current study we have shown that Angiopoietin 1 is a biomarker for better prognosis in penile cancer. High risk HPV 16 and 18 were found in most of the infected samples, showing its importance in the development of PC. Further studies, are necessary to evaluate the exact role of angiopoietin/tie2 pathway associated to HPV status in penile carcinoma. These findings could possibly guide more precisely surgical treatment and target therapies in penile cancer.

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## **13. Screening methods**

00025

## COVERAGE OF PAP TEST AND ADHERENCE TO ALTERNATIVE METHODS OF CERVICAL CANCER PREVENTION IN BARRETOS CITY, SÃO PAULO: PRELIMINARY RESULTS FORM A POPULATION-BASED STUDY

### 13. Screening methods

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#### Background / Objectives

Cervical Cancer (CC) is a serious public health problem in Brazil and the coverage rates of the Pap test, the incidence and mortality due to the disease remain high, and non-adherence to screening and periodic screening is involved in late diagnosis.

Objective: To estimate the prevalence and adherence to the Pap test in women aged between 25 and 64, to verify the proportion of women adherent to the program and offer 5 options of cervical cancer screening exams.

#### Results

Methods: A population-based study, where the Census Sectors were selected, comprising the urban area of the Municipality of Barretos and the sample plan by cluster. We divided the proportion of heads of households into income and literacy and the census tracts were divided into 9 strata, named "A" to "I". This stratification was made to represent the individuals belonging to different socioeconomic strata of the population to be included in the study. In this sense, 2,400 women between 25 and 59 years old will be included, with 1920 who never did or did not have the Pap test for more than 3 years, randomized into 5 groups of alternative exams (384 women in each group): Group 1- women invited to a Pap test and HPV screening at the Barretos Cancer Hospital (BCH) Prevention Department; Group 2- to the women were invited to a Pap test and HPV screening in the Mobile Unit; Group 3- women were asked to collect urine at home for HPV screening; Group 4- in this group women self- collected samples at home for HPV test; Group 5- women choose

between Pap test in the BCH Prevention Department, Pap test in the Mobile Unit, urine collection or self-collecting in the home. For groups 3 and 4 will was also be offered the Pap test that is the official recommendation of the Ministry of Health of Brazil.

## **Conclusion**

Preliminary Results: To date, 333 residences were visited, 175 women were included in the study. 82 (46.8%) did not know what a Pap smear was, 43 (25.7%) never did or did not take the Pap test for more than 3 years, 8 women had never taken the exam in their lives and reported that they did not do it out of ignorance, carelessness, did not feel sick and out of shame. Of these, 38 (88.4%) accepted one of the alternative methods of prevention.

## **References**

Conclusion: This ongoing study is evaluating noncompliance with the Pap test and proposing alternatives to overcome the cultural, moral, education and access to health barriers by comparing adherence to other collection methods for CCU prevention.

00044

Suitability of the training program for evaluation of p16/Ki-67 staining for laboratory staff without skills in gynecological cytology and immunocytochemistry

## 13. Screening methods

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### Background / Objectives

For the purpose of the Slovenian Cancer Screening Program ZORA we developed a training program for the evaluation of p16/Ki-67 immunocytochemical staining (ICS) on conventional PAP smears. We aimed to test if this program is also suitable for teaching laboratory staff without skills in gynecological cytology and ICS.

### Results

The training program was tested on two students (Ss), biologist (S1) and medical doctor (S2) who had no prior knowledge in gynecological cytology and ICS. The p16/Ki-67 ICS slides and staining results (reference, R) used for training were from the L3-5512 project. The training program consisted of two parts: 1. lectures, teaching slides and evaluation of 118 p16/Ki-67 ICS slides; 2. discussion of 118 p16/Ki-67 ICS slides with discordant results on multihead microscope. After the training qualification the Ss were tested with 383 p16/Ki-67 ICS slides. All slides were reevaluated by senior cytotechnologist (SC). Exact agreement, Kappa statistics, sensitivity and specificity for CIN2+ were calculated for the Ss and SC and compared to R.

### Conclusion

The agreements of pairs of evaluators (S1/SC; S2/SC; S2/R) for p16/Ki-67 ICS interpretation increased from 82.2%, 83.1% and 83.8% (Kappa 0.57, 0.57, 0.61) to 85.4%, 85.9% and 85.4% (Kappa 0.71, 0.70, 0.68) after the second part of the training, respectively. The agreement between S1 and R decreased from 81.0% to 75.5% (Kappa from 0.55 to 0.52). Sensitivity for CIN2+ decreased for both Ss (from 97.1% and 95.7% to 87.0% and 84.4%) with the increase of specificity (from 36.7 and 42.9% to 63.7% and 78.8%). However, the sensitivity (87.0%) and specificity

(80.4%) of R were still better than those of both Ss. The difference was not significant.

#### **References**

Ability of Ss for p16/Ki-67 ICS interpretation improved during training. Their specificity for detection CIN2+ has substantially increased with reasonable decrease of sensitivity. Due the suboptimal specificity further supervision by SC is recommended.

00518

Diagnostic concordance between cytology, colposcopy and biopsy in cervical pathology in Funchal, Portugal

## 13. Screening methods

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### Background / Objectives

The aim of the study is to determine the diagnostic concordance between cytology, colposcopy and biopsy in patients with cervical abnormalities.

### Results

A retrospective study was performed in the period between September/December 2017. Inclusion criteria was patients that have been submitted to cervical biopsy due to abnormal cytological findings in Hospital Dr. Nélío Mendonça's Cervical Pathology Department (HDNMCPD). All the cytologies and biopsies were interpreted in the Hospital's Pathology Service; the colposcopies performed by two experienced colposcopists; the biopsies were obtained from the suspected areas of the cervix during colposcopy. Data was collected from individual patients hospital records.

### Conclusion

In the study period (Set 2017-Dec 2017), in HDNMCPD, 75 women have been submitted to a cervical biopsy in consequence of an altered cytology result that motivates subsequent colposcopy examination prior to biopsy. Mean age was 42.6 years (min. 22, max. 87) with a mean Body Mass Index (BMI) of 27.3. The elapsed time between cytology and biopsy was 174 days, on average. Eight cases were excluded due to inconclusive colposcopy (N=2) or insufficient or inconclusive biopsy sample (N=6). The results of the cytologies that led to the biopsies were: 10.4% atypical squamous cells of undetermined significance (ASC-US), 32.8% atypical squamous cells cannot exclude HSIL (ASC-H), 29.9% low-grade squamous intraepithelial lesions (LSIL), 22.4% high-grade squamous intraepithelial lesions (HSIL) and 4.5% atypical glandular cells not otherwise specified (AGC-NOS) according to the Bethesda classification. Colposcopies were classified with the International Federation of Cervical Pathology and Colposcopy (IFCPC) terminology: 68.7% for grade 1 (minor), 28.3% for grade 2 (major) and 3.0% for suspicious for



invasion. Biopsies results were classified in: 34.3% without dysplasia, 29.9% LSIL, 32.8% HSIL and 3.0% superficially invasive squamous cell carcinoma (SISCCA), according to the Lower Anogenital Squamous Terminology (LAST) classification. We found an incidence of cervical dysplasia of 65.7% ( 45.5% LSIL; 50.0% HSIL; 4.55% SISCCA). In the biopsy group classified as LSIL a concordance of 50.0% was found between cytology-colposcopy-biopsy. In the biopsy group classified as HSIL the triple-test concordance was higher with a percentage of 72.7. For the SISCCA group there was no concordance. Global agreement cytology-colposcopy-biopsy was 38.8%. The concordance found between colposcopy-biopsy was 55.2%, higher than the 44.8% found between cytology-biopsy.

## **References**

The concordance found between cytology-colposcopy-biopsy was moderate (38.8%).

00591

Evaluation of an immunoassay for high-grade cervical pre-cancer screening among women presenting for colposcopy in Brazil

### 13. Screening methods

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#### **Background / Objectives**

Invasive cervical Cancer (ICC) affects women without sufficient access to care, with higher rates among minority groups in higher-income countries and women in low-resource regions of the world. Early diagnosis of high-grade cervical intraepithelial neoplasia (CIN2/3) is vital for effective prevention of cervical cancer. Expression of the biomarkers P16INK4A and KRT7 has been associated with a higher risk of squamous intraepithelial lesions. The development of a highly sensitive and specific Point of Care (POC) screening test could dramatically increase access to screening for women with difficulties obtaining in-clinic cervical screening.

Our objective was to evaluate the expression of P16INK4A and KRT7 and assess the clinical performance of an investigational POC assay in an enriched population of women presenting for colposcopy following an abnormal cytology result.

#### **Results**

Women were enrolled if they were aged 25-50 years, not pregnant, and referred to colposcopy following an abnormal cytology test within the last 6 months. Women able and willing to give informed consent provided cervical specimens for testing by a new investigational POC test, and for an HPV test (Roche Cobas® HPV Test). Blood samples were also taken for HIV serological testing. The expression of the biomarkers P16INK4A and KRT7 were evaluated using a particle-based sandwich immunoassay with the signal based on Surface Enhanced Raman Scattering (SERS). We specifically investigated the correlation between the investigational POC assay and the diagnostic test results collected during this trial (cytology, high-risk HPV status and histology/pathology). Receiver Operator Characteristic (ROC) curves

were generated using a Proper Binormal Model (PBM). Unique threshold levels were used for each assay in the multiplex. Optimal positive/negative cutoff values were determined by calculating the Youden Index Point.

## **Conclusion**

To date, a total of 95 women participated, with a median age of 38 years and all of whom were HIV negative. The high-risk HPV DNA test showed HPV positivity in 60 (63%) of the subjects with 1 invalid result (1%). During the colposcopy, 64 patients had an indication of biopsy: squamous cell carcinoma (n=1), CIN3 (n=20) CIN2/3, (n=10) CIN2 (n=4), CIN1 (n=11) and normal (n=18). Considering the end point as CIN2+, P16INK4A sensitivity was 56.2% (95%CI: 40.9, 71.5) and specificity 86.5% (95%CI: 71.1, 97.8). The sensitivity and specificity of the P16INK4A/KRT7 ratio were 70.2% (95%CI: 55.3, 85.1) and specificity 81.8% (95%CI: 65.6, 94.3).

## **References**

Conclusion: The P16INK4A biomarker provided the strongest correlation with the histopathology result. This Initial data also showed improved performance when the KRT7 biomarker is also considered in the algorithm.

## **14. Liquid based cytology**

00253

## THE EVALUATION OF p16/Ki-67 DUAL STAIN CYTOLOGY AS AN ADJUNCTIVE TOOL TRIAGING WOMEN WITH ASCUS AND LSIL CYTOLOGY

### 14. Liquid based cytology

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#### Background / Objectives

The detection of HGSIL (CIN2+) among women with ASCUS and LSIL cytology is challenging. The purpose of this study is to assess the diagnostic performance of p16/Ki-67 dual stain cytology and Hr-HPV test in women with ASCUS and LSIL cytological abnormalities.

#### Results

Consecutive liquid-based cytological samples (ThinPrep pap tests) were collected from 146 women diagnosed with ASCUS (n=67) and LSIL (n=77). Both, Hr-HPV test (HC2) and p16/Ki-67 dual stain immunochemistry (CINtec Plus test) were performed by the residual material of the vial of liquid-based cytology. Two cases were excluded from the study (2 ASCUS) due to inadequate material into the vial. All women were referred to colposcopy for the detection of CIN2+ lesions, with or without diagnostic biopsies.

#### Conclusion

Four cases of histologically confirmed HSIL were detected by the group of ASCUS diagnoses (prevalence rate 5.97%), as well as 18 cases of HSIL were also detected by the group of LSIL cytological abnormalities (prevalence 23.37%). Furthermore, in the LSIL study cases, 17 cases out of the 18 histologically confirmed CIN2+ were identified, by using the dual stain cytology (94.44%) comparable to 15 cases obtained by the HPV testing (88.23%). Finally, both dual staining and HPV testing

had the same results (4 to 4 cases) in predicting HSIL abnormalities, among women with ASCUS cytology.

### **References**

The accuracy of dual staining was higher concerning the diagnosis of CIN2+, in women with LSIL (94.44%) comparable to HPV testing (88.28%). Nevertheless, additional studies would be conducted to further assess of p16/Ki-67 dual stain cytology, as an adjunct tool to other tests, for improving the triage of LSIL cytology.

## **15. Automation in cytology**

00419

## Automated High-Throughput Cytology using Rapid Evaporative Ionization Mass Spectrometry (REIMS)

### 15. Automation in cytology

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#### **Background / Objectives**

Introduction: One in ten women screened for cervical changes will have an abnormal result. Cytological abnormalities can be difficult to manage and often require repeat visits with increased patient anxiety and risk of non-compliance. Furthermore, there is great variability in cytology reporting across cytotechnicians. Here, we investigate whether Rapid Evaporative Ionization Mass Spectrometry (REIMS) could be an automated alternative to assess the presence and grade of cytological abnormality at the bedside.

#### **Results**

Material & Methods: We recruited women with cytological abnormalities and normal controls and collected a liquid based cytology (LBC) cervical smear. Prior to REIMS analysis, 2 mL of sample underwent centrifugation to pellet cells, which were then heated using a carbon dioxide laser and the generated aerosol aspirated to a mass spectrometer. The resulting mass spectra underwent standard processing, with multivariate statistics completed using MetaboAnalyst.

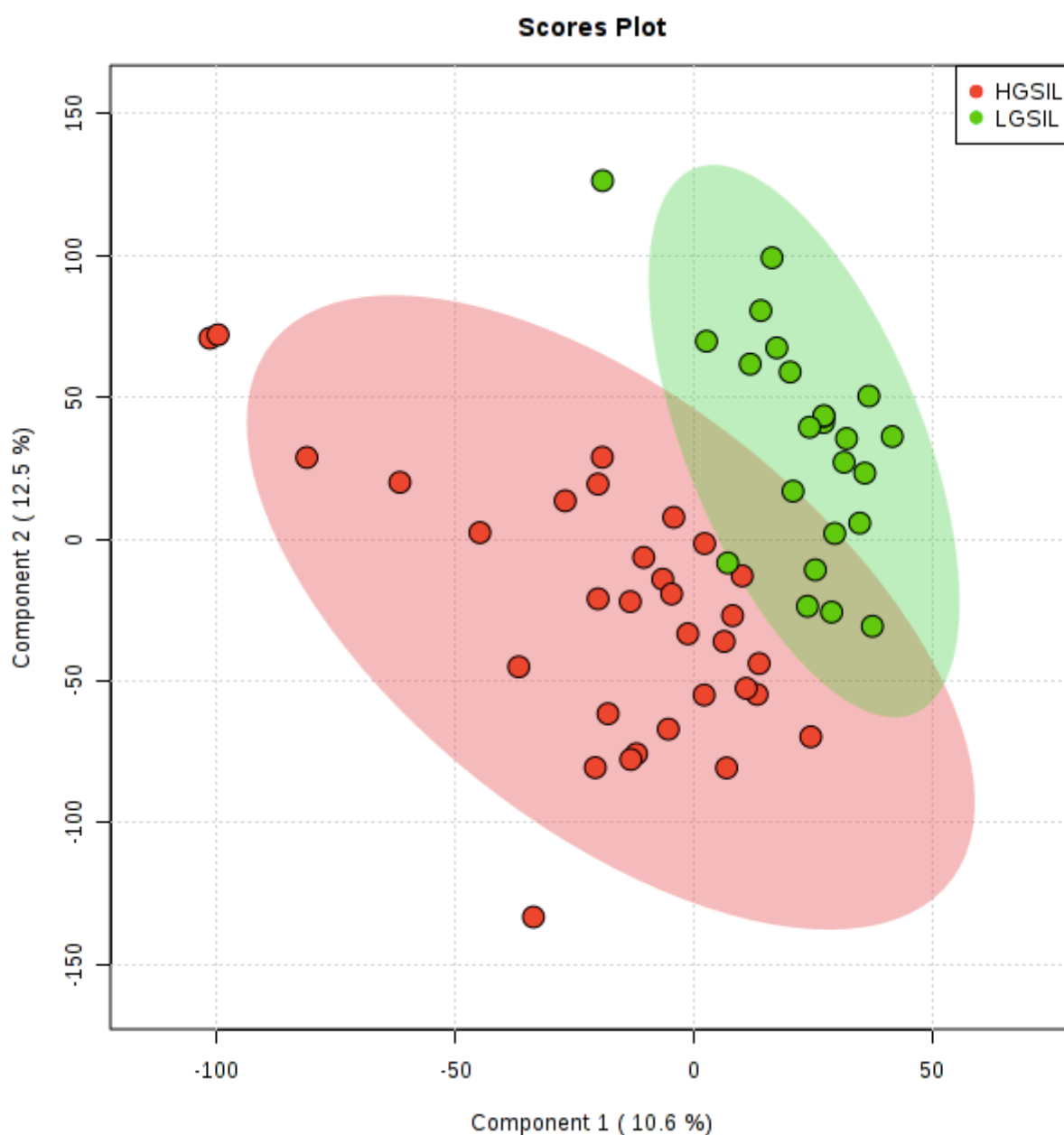
#### **Conclusion**

Results: A total of 94 LBC samples underwent the REIMS testing. 19 samples did not produce adequate spectra leaving 75 LBCs (four normal, 14 HPV changes only, 14 CIN1, 16 CIN2, 12 CIN3 and 15 cervical cancer) for analysis. Promising classification of LBC pellets by smear result using discriminant analysis was demonstrated - providing a platform for developing a classification model for larger cohorts. Further successful results included separation of samples positive and negative for HPV infection including a subanalysis of borderline/mild samples, classification of 'normal/HPV changes/CIN 1 vs CIN2/3/cancer', 'HGSIL vs LGSIL' and 'normal vs LGSIL vs HGSIL vs cancer'.



## References

Conclusion: This novel approach signifies an exciting step in translating laboratory-based diagnostics to the clinical setting as a bedside test with instant results. If the use of REIMS in cervical cytology proves to be as accurate in larger cohorts, this has the potential for use in the primary care setting as a rapid low-cost bedside screening tool for cervical cancer that could replace cervical cytology and/or a HPV DNA test.



## 16. Methylation

00449

Clinical performance of a quantitative specific methylation PCR test on a cohort of HPV positive women aged  $\geq 30$  years

## 16. Methylation

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### Background / Objectives

HPV-based cervical cancer screening should be supported by efficient triage. In this study we evaluated efficacy of a quantitative multiplex methylation specific PCR, as a triage test in HPV positive women aged  $\geq 30$ . The test is based on DNA methylation analysis of the host tumor suppressor genes *FAM19A4* and *miR124-2*, potential biomarkers for detection of progression of cervical cancer.

### Results

Cervical specimens were obtained from the representative Slovenian population cohort attending national cytology-based cervical screening during 2009-2014. Cervical samples were collected in ThinPrep PreservCyt medium, aliquoted and tested for HPV using Hybrid Capture 2 (Qiagen) and RealTime High Risk HPV (Abbott). Cytology examination results were interpreted by certified cytologists who were blinded to HPV results. DNA from cervical specimens was extracted using QIAamp DNA Investigator Kit, BioRobot EZ1 or STARMag Cartridge Kit according to manufacturers' instructions. DNA with concentration above cut-off value of 0.8 ng/mL was bisulphite treated using EZ DNA Methylation Kit and the methylation of *FAM19A4* and *miR124-2* host cell genes determined using a quantitative multiplex methylation specific PCR (Qiagen) on Rotor-Gene Q MDx instrument.

### Conclusion

A total of 950 samples obtained from HPV positive women aged  $\geq 30$  were included in the study. Overall methylation positivity rate was 28.3% (269/950) and was highest among women with HSIL (70.8%, 51/72) and ASC-H (60.0%, 3/5). Positivity rate according to histology was as follows: all samples obtained from women with invasive cancer were methylation positive (4/4), 79.2% (42/53) of women with CIN3 were positive, as well as 52.5% (21/40) women with CIN2, and 23.7% women with  $\leq$  CIN1 lesions (202/853). Clinical sensitivity of the methylation test for CIN2+ was

69.1% (67/97; 95% confidence interval (CI), 58.9-78.1%) and clinical specificity 76.3% (651/853; 95% CI, 73.3-79.1%), while sensitivity and specificity for CIN3+ were 80.7% (46/57; 95% CI, 68.1-90.0%) and 75.0% (670/893; 95% CI, 72.1-77.8%), respectively.

## **References**

The methylation PCR test used has shown good clinical sensitivity and specificity for CIN3+ lesions in population of women aged  $\geq 30$  and can be considered as possible triage test for HPV positive women.

00480

## DNA Methylation Panel for the Triage of HPV Positive Women in a Primary Screening Population.

### 16. Methylation

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#### Background / Objectives

Triage of HPV positive women is one of the key challenges facing HPV primary screening. Specific triage tests to avoid large numbers of unnecessary referrals are required. Host methylation factors have been repeatedly shown to be hypermethylated in cervical cancer/pre-cancer and have the potential to triage HPV positive women at high risk of cervical cancer. This study aims to investigate methylation of a specific panel of three markers [CADM1-M18, MALM1 and hsa-mir124-2] in HPV positive women. This study is part of a larger CERVIVA HPV Primary Screening Study.

#### Results

In partnership with CervicalCheck, The National Cervical Screening Programme in Ireland, CERVIVA are undertaking a longitudinal HPV primary screening pilot study evaluating triage strategies for managing HPV-positive primary screening tests. In total, 13,496 women attending for routine screening have been enrolled. HPV testing is performed using the Cobas HPV DNA test. HPV positive samples are tested for a panel of methylation specific biomarkers [CADM1-M18, MAL-M1, hsa-mir-124-2] via Quantitative Methylation-specific PCR. Here we present the pre-validation and optimisation work for the Methylation Panel for the use in a HPV Primary Screening Population.

## Conclusion

Initial optimisation of the qMSP was carried out on methylation positive SiHa cells and HPV negative/cytology no abnormality detected (NAD) smear samples. The SiHa cells showed detectable qMSP product for all markers and NAD samples remained undetected through qMSP. Serially diluted bisulfite converted SiHa DNA showed detectable methylation of at least 500pg/μl and no greater than 5pg/μl. To show that methylation is raised in high grade cervical lesions a small cohort of smears with CIN3 follow up histology (n=15), HPV negative cytology NAD (n=15) and HPV positive cytology NAD (n=15) were tested for the CAD M1-M18, MAL M1 and hsa-mir-124-2. CIN3 samples showed detectable qMSP product compared to both HPV negative/NAD and HPV positive/NAD samples (p=0.082, 0.045, 0.016 & p=0.083, 0.044, 0.016). Use of a Total Methylation Score (CAD+MAL+mir) showed statistically significant values (p=0.033 for both).

## References

From our research the use of CADM1-M18, MAL-M1 and hsa-mir-124-2 in a primary screening population is feasible. The approximate LOD of methylated DNA in a qMSP is <70 methylated cells and the small cohort of CIN3 samples showed significant differences when compared to normal controls. The use of a Total Methylation Score also shows promise in aiding differentiation of high grade, relevant lesions from normal cervical smears and hopefully provide a more stable and understandable result for scientists and clinicians if adopted into the National Screening Programme. A larger validation panel is set to determine the utility of this methylation panel.

## **19. New technologies**

00141

## Comparison of visual and cytology cervical cancer screening in Maharashtra, India

### 19. New technologies

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#### Background / Objectives

**Objective:** To assess the concordance between EVA and Pap in an Indian clinic.

**Background:** India has the most deaths from cervical cancer in the world. When available, cytology is the primary method of screening for cervical cancer. Because cytology requires a laboratory infrastructure, visual screening - digital cervicography using a smartphone-based colposcope (Enhanced Visual Assessment (EVA) System) - was proposed. In this study, visual screening using the EVA System is compared to cytology.

#### Results

Altogether, N=327 women, 20-50 years old from the Mumbai region of India were enrolled in the study. XXX patients enrolled in Hirnandani Hospital, YYY patients enrolled in screening camps. Patients were screened using both the EVA System and cytology. Patients positive with either method were referred to colposcopy with biopsy, following the standard of care.

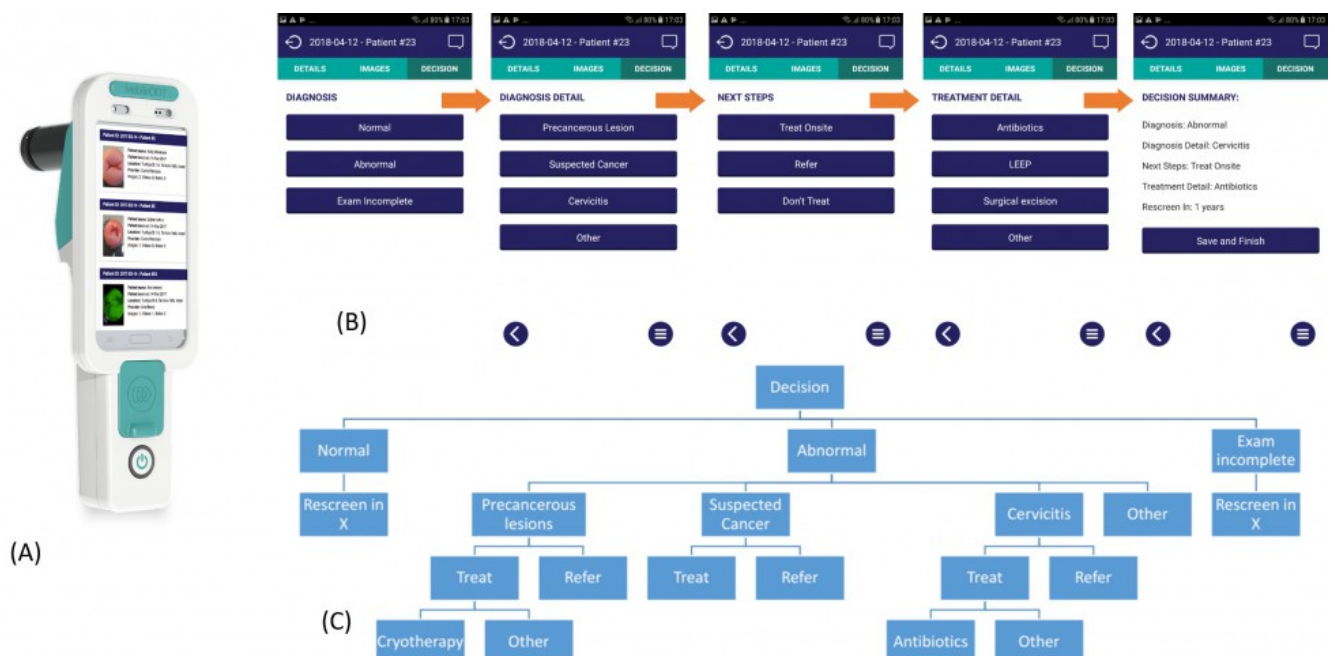
#### Conclusion

Of the N=327 patients, 304 patients were EVA-/Pap-, 19 patients were EVA+/Pap-, 0 Patients were EVA-/Pap+, and 4 patients were EVA+/Pap+. The Pap+ rate (1.2%) appears to be much lower than one would expect in India; the EVA+ rate (7.0%) is more believable. Of the 16 biopsies in total, 8 were positive. Of these 8 positive biopsies, 5 were EVA+, only 1 was Pap+. Although the sample size is very low, these results suggest cytology is missing many positive patients (7 of 8). In comparison, EVA caught most of the positive patients (5 of 8).



## References

These results suggest that cytology screening results had false negatives, some of which were caught by the EVA System. Feasibility of visual cervical cancer screening with a mobile colposcope was demonstrated. Additional research is needed to find a way to mitigate the frequency of loss to follow up, which was significant in this study.



**Fig. 1:** (A) EVA System. (B) Screenshots of Decision Support Job Aid on the EVA System app. (C) Full decision tree of job aid.

00269

## A NOVEL APPROACH TO IDENTIFY PROTEINS BY GEL-BASED PROTEOMICS IN RESIDUAL CERVICAL CYTOLOGY SAMPLES DURING CERVICAL CARCINOGENESIS

### 19. New technologies

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#### Background / Objectives

The introduction of HPV-DNA test improved the effectiveness of Cervical Cancer (CC) Screening Programs, but its high sensitivity frequently leads to unnecessary follow-up and over-treatments [1]. Besides unregulated HPV E6/E7 genes expression represents a key event, other factors seem to be implicated in the progression of HPV-related lesions. Proteomics is a growing technology for the study of proteome. An omic sentence says "The study of proteome is useful to identify novel biomarkers. If genome carrying the genetic information suggests what can happen, only the proteins, being the final workers of life events, indicates what makes it really happen". Thus, to screen for proteins expression during cervical cancerogenesis would signify to better understand and predict which mechanisms triggered by HPV are able to drive cells toward cancer.

In this retrospective study we firstly assessed the feasibility of residual cytological samples stored in PreservCyt medium (Hologic, USA) for 2D gel-based proteomics combined with MALDI-TOF Mass Spectrometry. Then, we compared proteome from specimens showing different diagnosis regarding HPV infection.

#### Results

In our Institution is placed a Tissue and Cell Biobank (Adriatic Biobank) holding about 50,000 residual PreservCyt specimens collected from women participating in the Regional Cervical Cancer Screening Program. For our purposes, we retrieved 12 samples related to patients grouped as follows: HPV-negative (reference negative group), HPV-DNA positive/E6-E7 negative (+/-), HPV-DNA positive/E6-E7 positive (+/+), CC. A 5 mL aliquot from each sample was removed to evaluate proteins yield

using the Coomassie protein assay (Better Bradford Pierce, USA). Then we proceeded with 2-DE analyses, combined with MALDI-TOF MS/MS. All samples were electrophoretically run three times as technical and biological replicates.

## **Conclusion**

We estimated an average total protein content of about 1.5 mg. For each biological replicated, 2DE resolved a total number of  $745 \pm 115$  protein spots. Comparison of all 2D maps demonstrated different protein profilings. In particular, when compared with negative group, a low matching percentage of 47 and 37 were detected in +/+ and carcinoma groups, respectively. Gene Ontology analysis (Panther) classifies the MS/MS assigned proteins in biological categories as follows: protein biosynthesis, cellular transport and immunity.

## **References**

Residual methanol-based cytological specimens are suitable for proteomic analysis. Proteomics demonstrated to be precious to selectively target differences in proteins expression during the different steps of cervical carcinogenesis [2,3].

## **References**

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00489

## EVALUATION OF A NOVEL ISOTHERMAL AMPLIFICATION ASSAY FOR RAPID HPV DNA DETECTION AND GENOTYPING

### 19. New technologies

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#### Background / Objectives

Methods to determine human papillomavirus (HPV) genotypes, especially of HPV16 and HPV18, are regarded as useful for the prevention of cervical cancer. A large number of assays designed for HPV genotyping have been developed in recent decades. They have variable analytical sensitivity and specificity for different HPV genotypes and may be used for routine clinical diagnosis, epidemiological studies, and evaluation of vaccine efficacy and monitoring. The objective of our study was to assess the performance of the new AmpFire HPV assay, comparing it to results obtained by other HPV genotyping tests, including from Roche, Becton Dickinson, Abbott, and InnoGenetics.

#### Results

AmpFire HPV detection technology (Atila BioSystems) is an isothermal amplification assay, with a simple sample processing protocol. The test can detect fifteen high risk HPV types in a single tube reaction and also genotype HPV16 and HPV18 using real-time fluorescent detection. The assay does not require extraction of DNA. Raw samples are heated and lysed prior to isothermal amplification. The new HPV test can be completed within an hour, including hands-on time. Eight samples were chosen for repeat testing and dilutions of 1:5 and 1:50. HPV16 plasmid DNA was diluted to assess assay analytical sensitivity. 60 fresh samples stored in PreservCyt medium were tested with the cocktail HPV assay; 45 samples with multiple infections (HPV16, HPV18, plus other types) were analyzed with the genotyping assay. An additional 20 archived samples (stored in PreservCyt at -20C for 10+ years) were also tested.

#### Conclusion

AmpFire had excellent reproducibility (>95%), with an analytical sensitivity of less than 10 copies of HPV16 DNA and worked well on 1:50 dilutions of clinical samples. On the fresh samples we observed almost identical results to commercial tests (Abbott n=60, 98% agreement; InnoGenetics SPF10 n=58, 97% agreement). We also compared the performance of AmpFire genotyping results to SPF10-LiPA25 in 45 samples with multiple HPV types. 44.4% showed identical results and 33.3% showed partial agreement. Overall, the two assays mostly agreed with each other. We tested 20 samples archived in PreservCyt for more than ten years, comparing our results to the original data on the Abbott, Roche and, Becton Dickinson tests. Seventeen of 20 samples showed identical results between AmpFire and the other HPV tests.

## **References**

The AmpFire assay is promising as a new routine HPV DNA detection and genotyping test. It has an isothermal microplate-based format and features minimal instrumentation and a rapid simple procedure that can produce accurate results in about one hour.

00584

## HUMAN UTERINE CERVIX-ON-A-CHIP: ESTABLISHING THE FIRST IN VITRO MODEL TO STUDY THE DEVELOPMENT OF CERVICAL CARCINOMA AND HUMAN PAPILOMA VIRUS MECHANISM OF ACTION

### 19. New technologies

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#### Background / Objectives

Predicting the effects of drugs before human clinical trials is at the heart of drug screening and discovery processes. The cost of drug discovery is steadily increasing owing to the limited predictability of two-dimensional (2D) cell culture and animal models. The conjunction of microfabrication and tissue engineering led to organ-on-a-chip technologies, which offer an alternative to conventional preclinical models for drug screening. Organ-on-a-chip devices can imitate key aspects of human physiology fundamental for the understanding of drug effects, improving preclinical safety and efficacy testing. This technique recently allowed creating microfluidic chips that can partially mimic organ function such as liver, lungs, gut and even tumors on chip.

The objective of this study was to develop a microfluidic 'uterine cervix-on-a-chip' platform that let the cultured cells to take characteristic positions similar to those observed in native human uterine cervix. This platform would be able to be used as an in vitro model to study the transformation zone of cervix during Human Papilloma virus (HPV) infection and cervical cancer developing (figure 1).

#### Results

A microfluidic device prepared by demolding cured polydimethylsiloxane (PDMS). On the chip, we carried out cell culture and co-culture of ectocervical epithelial cells (Ect1/E6E7) and endocervical epithelial cells (End1/E6E7) cell lines. Endocervical epithelial cell has been marked by AAV-GFP control viruses to provide a convenient

way to measure transduction efficiency into endocervix cells via fluorescence and to differentiate them from ectocervix epithelial cells. Numbers of experiments have been designed to check the functionality of the chip, such as live/dead assay, prestoBlue cell viability, 2D migration of cells (scratch tests) and 3D migration of cells by the use of 3D printers.

## **Conclusion**

We found that both cell lines can grow from both sides of the chip to reach each other in order to make the transformation zone and prepare the squamo-columnar junction. This multilayer-cell junction contains both types of epithelial cells and can mimic the transformation zone of cervix practically.

## **References**

Uterine cervix-on-a-chip may provide a powerful alternative in vitro model for studies on uterine physiology, real-time, high-resolution imaging, and analysis of biological responses in the cervix, as well as drug development. This established uterine cervix-on-a-chip is simple, effective, and easy to operate. It is expected to have important applications in personalized treatment of HPV infection lesions and cervical cancer and to play a potential role in other clinical treatments and tissue engineering.





## **20. Diagnostic procedures / management**

00126

## CYTOMORPHOLOGICAL PARALLELS OF PATOLOGY OF GLANDULAR EPITHELIUM OF CERVIX AMONG REPRODUCTIVE AGE WOMEN

### **20. Diagnostic procedures / management**

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#### **Background / Objectives**

The incidence of glandular cervical cancer has increased in Western countries from 5 to 25% of all cases of malignant neoplasms of the cervix. Cytological and histological criteria of cervical adenocarcinoma are developed much later than squamous cell carcinoma, in addition, the study material obtained from the cervical canal is often less informative.

#### **Results**

The results of cytology and cervical scraping of 86 women with different pathology of glandular epithelium were analyzed. Mean age was  $32.1 \pm 8.4$ .

#### **Conclusion**

Among 58 patients were found signs of chronic inflammation of different degree of severity and variable in activity of inflammation. 2 patients had chronic follicular cervicitis. The data were obtained from morphological and cytological investigation. 26 patients of inflammatory infiltrates were not detected in traditional or liquid cytology, histological examination revealed scanty lymphohistiocytic infiltrates in the submucosal layer. In 49 cases we detected signs of endocervical glandular hyperplasia, 2 endometrioid heterotopia, 27 reactive glandular changes, in 7 AGC and 1 AIS confirmed by immunohistochemistry (Ki67, CEA, p16). In traditional cytology, evaluation of the condition of the glandular epithelium was somewhat difficult due to application of the material (in 28 the glandular epithelium of 86 traditional smears was not visible due to thickness and presence of erythrocytes), in liquid cytology the glandular cells in the structures are well cytological signs of hyperplasia, atypia, reactive changes were noted (in 6 it was not possible to evaluate glandular epithelium due to high density of location of glandular structures). Hyperplasia of glandular epithelium of cervix was represented mainly by micro-glandular hyperplasia of endocervix, micropapillary formations, in some cases with

foci of squamous metaplasia, glandular and glandular-cystic hyperplasia. In 1 case intestinal metaplasia was detected and confirmed by additional stains for mucin. Reactive changes are a fairly common group of various changes in glandular epithelium, including atypia, as a result of inflammation. These changes were also found in cytological and morphological material in 35 cases. In cytological study, the degree of change was more pronounced, which provoked the overdiagnosis of atypical changes (in 16). In morphological study of biopsy the presence of a massive inflammatory infiltrate in stroma and more relaxed histological pattern in the glandular epithelium allowed to reduce the degree of severity of pathological process in morphological conclusion.

## **References**

A comprehensive study of cervical problem will increase level of early detection and reduce incidence in women of reproductive period.

00220

COLPOSCOPIC AND HISTOLOGICAL FINDINGS IN WOMEN  
WITH LOW GRADE INTRAEPITHELIAL LESION (LSIL)  
SUBMITTED TO ZT EXCISION

## **20. Diagnostic procedures / management**

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### **Background / Objectives**

Low-grade intraepithelial lesions occur at 2.9% of cervical cytologies and are usually associated to HPV cellular changes and mild dysplasia that can regress with no treatment. It is a cytological finding that carries a risk of 25% to high-grade development within 2 years.

The aim of this study is to correlate the colposcopic and histological findings in women with LSIL cytology.

### **Results**

We conducted an observational, descriptive, retrospective study. The sample included all women with LSIL cytology who were submitted to ZT excision, during their follow up at cervical pathology unit of Centro Hospitalar e Universitário do Algarve – Unidade de Faro, between 2012- 2016. The data was analysed with SPSS 21.

### **Conclusion**

From the 572 women with LSIL cytology referred to our cervical pathology unit, we studied 125 women (21,85%) that were submitted to ZT excision during their follow up. The mean age of the women was 39 years old (range 24 to 66 years old). The mean age of first sexual intercourse was 17 years old and the mean number of sexual partners was 4. 12,5% of the women were postmenopausal. About 56% had smoking habits and only 4% had HPV vaccination.

We found that 60% of the women presented grade 2 and 35% grade 1 colposcopic findings. Only 5% had normal colposcopy. 84% of the lesions visualized at colposcopy examination were biopsied and 64,8% of them revealed high-grade

lesions. The pathology exam of ZT excision revealed 53,6% HSIL, 34,4% LSIL and 11,2% no alterations. Women with grade 1 colposcopy findings had high-grade lesions in 54,8% of the cases. Only 27,8% of the women with grade 2 findings revealed a ZT excision without lesion or with a low-grade lesion.

## **References**

LSIL cytology is associated with transitory HPV infection, however sometimes they can have underlying high-grade lesions. In the present study we verified that the rate of high-grade lesions at ZT excision was 53,6%, showing that women with LSIL cytology must have surveillance with HPV test and colposcopy. These women follow-up is essential and the decision between treatment and an expectant approach should consider the concordance between results of cytology, HPV test, colposcopy, biopsy, age and parity.

00265

## HUMAN PAPILLOMA VIRUS IN URINE AND BLADDER CANCER RISK: A SYSTEMATIC REVIEW AND META-ANALYSIS.

### 20. Diagnostic procedures / management

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#### Background / Objectives

Association between human papilloma virus (HPV) infection and risk of bladder cancer (BICa) remain inconclusive. We carried out a systematic review with meta-analysis of the available case-control studies in order to verify possible differences in the occurrence of HPV infection in urine samples in patients with BICa and normal subjects.

#### Results

PubMed was used to search for articles published from January 1965 to August 2018 using the key words "bladder cancer" and "HPV". No restrictions to date, language, or article type were applied. Case-control studies reporting Odds Ratio (OR) for HPV infection in urine samples in patients with BICa and normal subjects were analyzed. The quality of the studies was evaluated by the New Castle Ottawa scale. Data were combined using random effect models. The Cochrane Chi-square (Cochrane Q) statistic and the I-square test were used to analyze heterogeneity. The publication bias was graphically explored through funnel plot, and Duval and Tweedie's "trim-and-fill" test was used to correct possible publication bias.

#### Conclusion

The selection process yielded only three studies with eligible criteria for analysis, that gave information on 278 patients with HPV infection in urine and 903 patients without HPV infection in urine. The pooled OR estimated showed that patients with HPV infection in urine exhibit a significantly higher prevalence in BICa than patients without HPV infection (OR = 2.602, 95%CI: 1.484, 4.56; P = 0.001). We obtained a heterogeneity chi-squared value  $Q_{exp}=1.573$  (p=0.456) (I-square = 0%) . Funnel

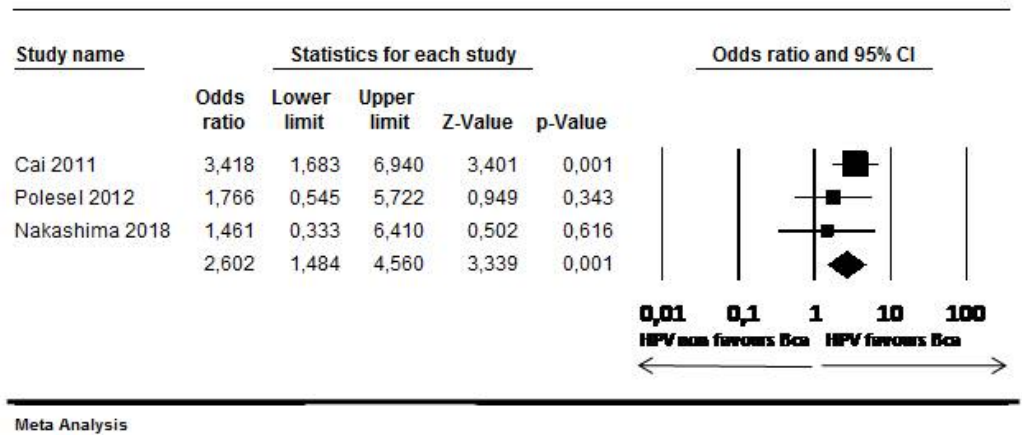
plot non suggested a possible publication bias in the analysis. Only one study compared the incidence of HPV infection in urine with HPV infection at the tissue level. A higher incidence of HPV infection was observed in the urine of patients with bladder cancer than in tumor tissue.

## References

The pooled OR value showed a moderate relationship between urinary HPV infection and BICa. HPV infection in the urine may have a role in carcinogenesis of the bladder tumor. Further well-conducted studies could be useful to confirm this conclusion, and thus be able to identify if the determination of HPV in urine can be considered useful in clinical practice for its use in the diagnosis and follow-up of patients with BICa.

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00428

## COMPARATIVE PERFORMANCE OF HPV DNA AND MESSENGER RNA TESTS IN THE POST-LEEP TEST-OF-CURE SETTING

### 20. Diagnostic procedures / management

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#### Background / Objectives

In British Columbia (BC), women undergoing loop electrosurgical excision procedure (LEEP) for CIN2+ are tested approximately 6 months post-LEEP with the hybrid capture 2 HPV DNA test (HC2). Women HC2 negative with negative histopathology are discharged to routine screening, while those HC2 positive continue follow-up within the provincial colposcopy program. Since various HPV screening tests may have different performance characteristics in the test-of-cure setting, our objective was to evaluate the performance of other HPV DNA and mRNA tests compared to the standard of care (HC2).

#### Results

Three hundred one (301) women attending their first post-LEEP visit were enrolled from December 2016-May 2017 and were tested by HC2. Follow-up management was based on HC2 results and histopathology findings. Residual post-LEEP samples were then tested by the cobas 4800 HPV DNA test (Roche), RealTime High Risk HPV DNA test (Abbott) and the Aptima HPV mRNA assay (Hologic). Overall, positive and negative agreement with HC2 were calculated. All CIN outcomes following the post-LEEP HC2 test were obtained from the centralized BC cervix screening program database.

#### Conclusion

Valid results were obtained for 301 samples with the HC2, cobas and Aptima tests, while the RealTime test had three invalid results (HPV negative by all the other tests). Percent positive was 30.6% (91/301) for HC2, 29.6% (89/301) for cobas,

31.5% (94/298) for RealTime and 23.6% (71/301) for Aptima. Overall, positive and negative agreement is shown in the Table. Agreement between HPV DNA tests was higher than for all DNA tests vs. Aptima. Analysis of CIN outcomes by HPV test result is in progress.

Summary of Agreement between HPV Tests			
	Overall Agreement (95%CI)	Positive Agreement (95%CI)	Negative Agreement (95%CI)
<i>HPV DNA vs. DNA</i>			
HC2 vs. cobas	96.7% (94.0-98.2)	88.4% (82.9-92.3)	95.0% (92.5-96.7)
HC2 vs. RealTime	94.6% (91.5-96.7)	91.4% (86.5-94.6)	96.1% (93.8-97.6)
cobas vs. RealTime	94.3% (91.1-96.4)	90.7% (85.6-94.1)	96.1% (93.8-97.6)
<i>HPV DNA vs. mRNA</i>			
HC2 vs. Aptima	90.4% (86.5-93.2)	82.2% (75.6-87.3)	93.4% (90.7-95.4)
cobas vs. Aptima	87.4% (83.2-90.7)	76.3% (69.1-82.2)	91.4% (88.4-93.7)
RealTime vs. Aptima	87.6% (83.4-90.9)	77.6% (70.6-83.3)	91.4% (88.4-93.7)

## References

The HPV DNA tests all had similar performance vs. the other DNA tests. All DNA tests had lower agreement with Aptima, primarily due to the smaller number of positive Aptima tests. If Aptima is shown to detect statistically similar rates of residual CIN2+ disease compared to the DNA tests, it would increase the number of women returned to routine screening vs. extended follow-up within the colposcopy program.

00437

## CONNECTION BETWEEN THE FREQUENCY OF PREVENTIVE GYNECOLOGICAL EXAMINATIONS AND CERVICAL CANCER DETECTION: A CASE-CONTROL STUDY

### 20. Diagnostic procedures / management

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#### Background / Objectives

The task of organized screening of cervical cancer has not been solved in Russia currently. In practice, the examination of patients to identify cervical disease includes isolated or combined use of the following methods: gynecological examination, colposcopy, cytology and HPV tests. The aim of our study was to establish a connection between the frequency of preventive gynecological examinations and detection of cervical cancer.

#### Results

The study included 115 patients with diagnosis of cervical cancer at various stages of the pathological process: Cr in situ – 13.0%; IA1 – 13.9%; IA2 – 3.5%; IB1 – 35.7%; IB2 – 2.6%, IIA – 12.2%; IIB – 7.0%; IIIB – 3.5%; IV – 1.7%; without an exact stage – 7.0%. The average age of diagnosis was 42.6 years. According to the medical documentation, data was received on the passage of preventive examinations at the gynecologist and screening for cervical cancer in the last 5 years before the diagnosis was established.

#### Conclusion

Patients who visited the gynecologist more than once a year, in the majority had concomitant pathology from the side of the reproductive system, which caused an increased frequency of gynecological examinations. Among those who did not undergo screening for cervical cancer or who had undergone it on detection of the disease, the risk of diagnosing in stage II-IV was 5.2 times higher than among those who underwent

cervical screening two years ago, or once a year in over the past five years (OR=5.2; 95% CI: 1.1–24.5).

## **References**

The current opportunistic nature of screening for cervical cancer in Russia can not lead to a significant reduction in morbidity. It is necessary to ensure the regularity and frequency of preventive examinations.

00458

## HSIL MANAGEMENT IN YOUNG WOMEN

### **20. Diagnostic procedures / management**

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#### **Background / Objectives**

Human papilloma virus (HPV) infection is most common in teenagers and women in their early 20s. Most young women have an effective immune response that, on average, clears the virus within the first 8 months. With the regression of the infection, most cervical neoplasia will spontaneously resolve in this population.

Although the risk of invasive cervical cancer in women with a finding of high-grade squamous intraepithelial lesion (HSIL) is substantial, HSIL are among the least common cytological findings. Younger women, under the age of 25, with HSIL have lower risk of invasive cervical cancer when compared with women at least 25 years old. For these reasons, and considering the risk of obstetric complications after cervical conization, observational management is recommended in this population, with adequate colposcopy and cytology every 6 months for 24 months. Persistence of CIN 2 or 3 over 24 months requires excisional treatment.

The primary objective of this study is to document the outcome of women under 25 years-old with HSIL managed in our colposcopy unit.

#### **Results**

Retrospective study of the cases of HSIL in women under 25 years-old managed in Hospital Prof. Doutor Fernando Fonseca (HFF) between January 2013 and December 2017. Data analyses – Microsoft® Excel version 2010

#### **References**

HSIL is an uncommon cytological finding particularly in young women. Observational management was preferred in cases of CIN 2 or lower anomalies as recommended in the literature, with favorable outcomes.



00469

## VALUES OF HPV STATUS, SAMPLE MARGINS, AND ENDOCERVICAL CURETTEMENT AFTER LLETZ CONIZATION AS PROGNOSTIC FACTORS FOR SUCCESSFUL TREATMENT

### 20. Diagnostic procedures / management

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#### Background / Objectives

Control Pap smear test and HPV typization were performed six months after LLETZ conization as a part of continuous monitoring of patients who underwent LLETZ treatment. Pre-treatment HPV status, cone margins status, and endocervical curettement of the residual cervical canal were used as prognostic factors of success of the treatment of cervical intraepithelial lesions with LLETZ conization.

#### Results

The study included 34 female patients treated at Sestre Milosrdnice University Hospital Centre in 2017. In all patients LLETZ conization was performed. Patients were grouped according to Pap smear test and HPV typization six months after LLETZ conization.

#### Conclusion

The patient mean age was 43.32 years; there were 13 women (38.24%) in 27-39 age group, and 21 women (61.76%) in 40-68 age group. The first group was made up of 22 women (64.71%) whose Pap smear tests, performed six months after the treatment, were normal, and whose HPV typization was negative. Within this group 20 women (90.91%) had positive pre-conization HPV tests, and in two women

(9.09%) HPV tests were negative. HPV types 16 and 18 were confirmed in 10 women in this group (50%) whose HPV tests were positive. Positive margins were identified in five women (22.73%). Endocervical curettage was negative in all women in this group. In the second group abnormal Pap smear tests and/or positive HPV statuses were identified in 12 women (35.29%) six months post-conization. Prior to the treatment 11 women (91.67%) were HPV positive, whereby types 16 and 18 were present in 50% of women. Within this group margins were positive in 8 women (66.67%). In two women (16.67%) positive endocervical margins were detected, as well as a positive endocervical curettage, HSIL changes in the Pap smear test, and positive HPV status six months after the treatment. Positive HPV status six months after the treatment was confirmed in 9 women (75%) within this group. A Pap smear test performed six months after the treatment showed abnormalities in 6 women (50%) within this group; in three HSIL changes were observed.

## **References**

The status of cone margins is the best prognostic factor for successful LLETZ treatment. We have to note that we have excluded multi-LLETZ treatments which would significantly increase the representation of positive margins. Its prognostic value increases with a positive test result of endocervical curettage and the residual HPV infection.



## **21. Colposcopy**

00502

The accuracy of colposcopy utilizing RCI or Swede score combined with adjunct hrHPV for prediction of high-grade intraepithelial neoplasia (CIN2+) in the patients with ASC-US, ASC-H, LSIL and HSIL Pap smears on Bethesda classification

## 21. Colposcopy

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### Background / Objectives

To determine the prevalence of cervical intraepithelial neoplasia CIN2+ (high-grade) among women with all spectra of squamous lesions on Bethesda classification. The assessment of colposcopy (Reid index and Swede score) with adjunct hrHPV test to predict CIN2+.

### Results

A retrospective medical record review of 150 women attending colposcopic examination at the Clinic of Obstetrics and Gynaecology JMF CU in Martin in 2016. A reason for expert colposcopic evaluation was abnormal screening result on Bethesda classification. All women underwent colposcopy and the findings were scored by both Reid colposcopic index (RCI) and Swede score, biopsy was taken from all abnormal areas and adjunct HPV test was either conducted or reviewed from previous test done not more than 3 months prior expert colposcopy.

### Conclusion

A 139 of initially enrolled 150 patients were reviewed based on all required entry data. Women aged 20 to 62 (mean 37.8 yrs.) were divided into subgroups accordingly smear results: AGC NOS (n=7), ASC-US (n=30), ASC-H (n=13), LSIL (n=64) and HSIL (n=25). Only squamous lesions have been evaluated in this study. A total of 40 (30.3%) CIN2+ lesions were detected. Prevalence of high-grade lesions

in women with ASC-US, ASC-H, LSIL and HSIL was 13.3% (4/30), 30.8% (4/13), 25.0% (16/64) and 64.0% (16/25), respectively. Reid colposcopic index at a cut-off of 2 showed sensitivity, specificity, positive likelihood ratio and negative likelihood ratio for detection of CIN2+ lesions of 84.6%, 77.6%, 3.75 and 0.2 (AUC=0.863,  $p<0.0001$ ). Using Swede score at a cut-off of 2 were data as follows: sensitivity 84.6%, specificity 72.6%, +LR 3.79, -LR 0.2 (AUC=0.822,  $p<0.0001$ ). There was a significant correlation between RCI and histology ( $r=0.5932$ ,  $p<0.0001$ ), as well as for Swede score ( $r=0.5924$ ,  $p<0.0001$ ).

## References

The expert colposcopy utilizing either Reid colposcopic index or Swede score with adjunct hrHPV test can be used flexibly depending on the setting and have high clinical diagnostic value for the detection of CIN2+ lesions. The lower threshold scores can be used for screening and higher for screen-and-treat selection to decrease the over/under-treatment rate.

00506

PRACTICE OF COLOPOSCOPY AT THE GYNECOLOGICAL AND OBSTETRICAL CLINIC OF CHU ARISTIDE DANTEC OF DAKAR (SENEGAL) FROM 2005 TO 2017 (SENEGAL): ABOUT 1559 CASES.

## 21. Colposcopy

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**University, Obstetrics and Gynecology Clinics Aristide Le Dantec Hospital, Dakar, Sénégal - Dakar (Senegal)**

### Background / Objectives

**OBJECTIVE:** To specify the indications, the colposcopic and therapeutic aspects of the patients received at the Cervico-Vaginal Colposcopy and Pathology Unit of the Dantec Hospital for cervical cytological lesions according to the Bethesda classification.

### Results

**MATERIALS AND METHODS:** This was a retrospective and descriptive study ranging from January 31, 2005 to January 31, 2017. All patients with pathological smear were included in the study. The studied parameters concerned epidemiological data, indications, colposcopic aspects, the results of the colposcopy biopsy in case of lesion, the therapeutic aspects, the results of the histology of the operative specimen and the follow-up. The collection was carried out thanks to the data collection sheet and the colposcopy register. The analysis was done by Epi version info 3.5.

### Conclusion

**RESULTS:** During the study period, we performed 1559 colposcopies. Women in active periods predominated (67.5%) followed by menopausal women (24.5%). The mean age at first intercourse was 20.4 years with extremes of 10 and 46 years. The

mean gestational age was 4.9 with extremes of 0 and 19 and mean parity of 4.4 with extremes of 1 and 13. Patients used contraceptive products in 44.4% of cases. Indications for colposcopy were dominated by low-grade lesions (41.5%) followed by squamous cell abnormalities of indeterminate significance (14.9%). Colposcopy resulted in atypical Grade 2 transformation in 22.7%, atypical Grade 1 transformation in 19.6%. After 367 biopsies under colposcopy, the histology found a micro-invasive squamous cell carcinoma in 4.6%, a CIN 3 in 6.5%, a CIN 2 in 8.4%, a CIN 1 in 16.3%, and squamous metaplasia in 39.8%.

We performed 49 conizations and 42 hysterectomies. The postoperative course was simple in all our patients.

After surgical treatment, pathology examination showed CIN3 in 34% of cases, CIN2 in 22% of cases, CIN1 in 11% of cases, microinvasive squamous cell carcinoma in 17% of cases and was normal. in 17% of cases.

## **References**

**CONCLUSION:** Colposcopy plays a key role in the management of cervical dysplasia

**Keywords:** Cervico-vaginal smear, Dysplasia, Colposcopy, Conization, Hysterectomy, Dakar, Senegal

## **22. Cervical neoplasia**

00097

USE AND RESULTS OF A CORIOLUS VERSICOLOR-BASED  
VAGINAL GEL IN WOMEN HPV+ AND/OR ABNORMAL PAP  
SMEAR ATTENDED IN A REGIONAL SPANISH HOSPITAL.  
PRELIMINARY ANALYSIS

## **22. Cervical neoplasia**

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### **Background / Objectives**

A Coriolus versicolor-based (CV) vaginal gel is recently available In Spain to prevent and treat the HPV-dependent low grade cervical lesions. Recommended dose: 1 cannula/day for 1 month + 1 cannula/alternate days for 5 months (except menstrual days).

To analyze how CV vaginal gel is being used in our hospital and to evaluate the treatment results in our patients.

### **Results**

A retrospective, observational study. Medical records of patients who completed 3 or 6 months treatment period during 2017 were analyzed. Baseline characteristics of CV vaginal gel users were described.

Pre and post treatment number of patients with ASCUS/LSIL, positive-HPV and high-risk positive HPV were assessed.

### **Conclusion**

A total of 86 medical records were analyzed. Most of them (84%) were treated for 6 months. Mean age was 38.4 years (from 18 to 72 years), 43.5% were vaccinated before treatment with CV vaginal gel, 32,5% were smokers and 42% used condoms regularly in all their sexual relationships. Baseline pap smear: Normal 11(13%), ASCUS 3 (3.5%), LSIL 65 (75.5%) and HSIL 7 (8%). HPV test was performed in 68 patients of which 57 (89%) were high risk HPV.

After treatment, reductions of 54% (from 68 to 31;  $p \leq 0.0001$  Chi-Square test), 57% (from 68 to 29;  $p \leq 0.0001$ ) and 58% (from 57 to 24;  $p \leq 0.0001$ ) were observed in number of patients with ASCUS/LSIL, positive-HPV and high risk positive HPV, respectively vs baseline.

## **References**

In our hospital, presence of LSIL is the main reason to prescribe the CV vaginal gel. In this preliminary analysis, significant reductions of patients with pap smear alterations and high-risk HPV were observed after 3-6 months application of CV vaginal gel.



00098

## EFFICACY OF A CORIOLUS VERSICOLOR-BASED VAGINAL GEL IN HIGH RISK HPV+ WOMEN. PRELIMINARY RESULTS.

### 22. Cervical neoplasia

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#### Background / Objectives

A Coriolus versicolor-based vaginal gel (Papilocare®) is recently available In Spain to prevent and treat the HPV-dependent low grade cervical lesions.

To evaluate the efficacy of the Coriolus versicolor-based vaginal gel to clear HPV and to normalize pap smear in high risk HPV+ women.

#### Results

An exploratory, prospective, observational non-controlled study. High risk HPV+ vaccinated and unvaccinated women older than 24 years were included during routine follow-up visits and treated with the recommended dose of Papilocare®: 1 cannula/day for 1 month + 1 cannula/alternate days for 5 months (except menstrual days).

Primary endpoint: composite efficacy variable consists of percentage of patients with normal pap smear and/or HPV clearance at month 6 vs baseline. Secondary variable: percentage of patients clearing HPV 16-18 vs baseline.

#### Conclusion

A total of 86 patients, mean age 42.1 years (24 to 81) were included. At 6 months, 53% of women negativized pap smear and/or cleared HPV and were classified as responders to treatment. A total of 25 patients were positive to HPV 16-18 at baseline (12 and 13 with positive and negative pap smear, respectively). Overall, at 6 months, 48% of these patients cleared HPV 16-18 (50% and 46% of patients with positive and negative pap smear, respectively).

#### References

In these preliminary analyses, Papilocare® shows positive trend to improve pap smear alterations and HPV clearance in women infected by high risk HPV, after 6 months; these findings need to be confirmed upon analyses completion.

00099

## CORIOLUS VERSICOLOR VAGINAL GEL IN THE TREATMENT OF HIGH-RISK POSITIVE HPV PATIENTS.

### 22. Cervical neoplasia

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#### Background / Objectives

In our center, about 25% of patients with high-risk HPV (HR+HPV) clear the virus at 12 months with a wait and see approach. Evaluate the efficacy of a Coriolus versicolor vaginal gel in HR+HPV patients.

#### Results

This was a retrospective, descriptive, observational study following the protocols of the ICO (Institut Català d'Oncologia – Hospitalet de Llobregat - Barcelona). Data of HR+HPV patients between 20 and 65 years with ASCUS, L-SIL (Group A) or normal cytology (Group B) treated with a Coriolus versicolor-based vaginal gel for 3 weeks and then alternating days until 6 months were included in a database. Both vaccinated and pregnant patients were excluded. Recruitment period: December 2016 to October 2017. Primary endpoint: composite efficacy variable consists of percentage of patients with normal cytology and/or HPV clearance at month 6 vs baseline. HR+HPV evaluation was performed by hybrid capture.

#### Conclusion

A total of 91 HR+HPV patients were included (Group A: 46 (50.5%) and Group B: 45 (49.5%)). 72.5% of patients negativized cytology and/or cleared HPV. In group A, 63% of patients normalize their situation (59% and 66.6% of patients with initial ASCUS and LSIL, respectively), no effect was seen in 24% and 13% worsened. In group B, 82.2% cleared or reduced viral load, 13.3% increased it and 4% (2 patients) were lost of follow-up.

#### References

Coriolus versicolor-based vaginal gel appears to be effective against ASCUS and L-SIL lesions caused by high-risk HPV. In HR+HPV patients with normal cytology, there is a clearance of the virus at 6 months of treatment. Further prospective studies are needed to confirm these exciting results.

00119

## EVALUATING ErbB RECEPTOR PROFILE IN YOUNG PATIENTS WITH CERVICAL CANCER

### 22. Cervical neoplasia

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#### Background / Objectives

The aim of our study was to assess the expression and clinical significance of ErbB receptors in severe cervical dysplasia and cervical cancer specimens. The human EGF system plays an important role in cell proliferation, differentiation and apoptosis during embryogenesis and postnatal development. It is present in several tissues and has four receptors (ErbB-1, -2, -3 and -4) and numerous ligands. ErbB receptors are trans-membrane glycoproteins with an extracellular region containing two ligand-binding domains, a transmembrane domain and an intracellular domain with tyrosine kinase activity.

#### Results

We evaluated retrospectively tissue specimens from 75 young women with cervical precancer or cancer, managed in our institution. Among them, 33 patients had *in situ* and 42 patients had invasive cervical cancer. These specimens were immunostained for ErbB-2, -3 and -4. Tumors were scored based on the proportion of tumor cells stained. Immunostaining of 5% of tumor cells was considered as an optimized cut-off for tumor positivity.

#### Conclusion

Regarding ErbB-2 expression, 8 cases were positive (10.7%) and 67 cases were negative (89.3%). For ErbB-3 expression, 24 cases were positive (32%) and 51 cases were negative (68%). For ErbB-4, 37 cases were positive (49.3%) and 38 cases were negative (50.7%). A statistically significant correlation between ErbB-2 expression and invasive cervical cancer emerged in our study. All ErbB-2 positive

specimens originated from patients with invasive cervical cancer. Moreover all specimens from patients with *in situ* cervical precancer were ErbB-2 negative.

## References

Dysregulation of the EGF system signaling network is implicated in various disorders. Loss of control of the cell functions mediated by this system is a hallmark of oncogenesis, being found in several types of human cancers where it becomes hyperactivated with various mechanisms (ligand overproduction, receptor overproduction, constitutive receptor activation).

Overexpression and structural alterations of ErbB-1 are associated with higher grade, disease progression, poor survival and resistance to radiotherapy and chemotherapy. Overexpression of ErbB-2 is an indicator of a more aggressive clinical behavior. As for ErbB-3, despite its overexpression is related with ErbB-2 positivity and lymph node involvement, a definitive relationship with poor survival has not been established. Finally, overexpression of ErbB-4 is related with favorable prognosis in breast and bladder cancer.

It is likely that ErbB receptor overexpression indicates of a more aggressive clinical behavior in cervical cancer patients. Future studies will further elucidate the clinical significance of ErbB receptors in this neoplasm.

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00125

## RHABDOMYOSARCOMA OF THE CERVIX – CASE REPORT

### 22. Cervical neoplasia

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#### Background / Objectives

Rhabdomyosarcoma is defined as a malignant neoplasm which derives from embryonic muscle cells. In children, it is the most commonly discovered soft tissue tumor, whereas in adult, rhabdomyosarcomas are rare, representing less than 5% of soft tissue tumors and less than 1% of all malignant cancers. Histopathologically, rhabdomyosarcoma was classified by the Intergroup Rhabdomyosarcoma Study Groups into 3 major subtypes: embryonal, alveolar and undifferentiated. The surgical treatment for cervical rhabdomyosarcoma could be conservative or radical surgery with survival rates of vaginal and cervical lesions being reported to be of 96% and 60%, respectively.

#### Results

We present the case of a 46-year-old woman which addressed the clinic in mid 2017 due to an abnormal vaginal discharge. Gynecologic examination revealed a large 5/6 cm cervical mass with grape- like feature protruding into the vagina. A biopsy was performed and pathologic examination was consistent with embryonal type rhabdomyosarcoma. The patient refused further investigations and opted for local resection. She has since came back 2 times with recurrence of the mass, each time refusing radical surgery.

#### References

The case is particularly interesting due to the uncommon site of the rhabdomyosarcoma and the patients age.





00155

## POSITIVE MARGINS AFTER LOOP ELECTROSURGICAL EXCISION PROCEDURE FOR CERVICAL INTRAEPITHELIAL NEOPLASIA: EXPECTANT MANAGEMENT AND FOLLOW-UP

### 22. Cervical neoplasia

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#### Background / Objectives

Early detection and treatment of Cervical Intraepithelial Neoplasia(CIN) has proven to be effective in reducing the incidence and mortality of cervical cancer. Loop Electrosurgical Excision Procedure(LEEP) is a less invasive treatment, associated with minor complications. Positive margins after LEEP(5-40%) is the most important predictive factor for CIN persistence/recurrence. However, management of these cases remain controversial, since often no CIN is observed during follow-up. Evidence supports individualized management: some patients profit from close follow-up while others benefit from a second LEEP or even hysterectomy as a definitive treatment. We studied 181 patients that underwent LEEP for CIN in our center, in order to identify persistent/recurrent cases and to determine if close follow-up and expectant management were advantageous, refraining more invasive procedures.

#### Results

We performed a retrospective study with descriptive and bivariable analysis, using SPSS Statistics. Data was based on clinical reports of patients treated between 2013-2017.

#### Conclusion

The average age of patients was 35,89±9,46 years-old. The majority came from primary care screening(56,35%;n=102). 45,86%(n=83) were multiparous, 54,1%(n=98) were using oral contraception and 33,7%(n=61) were smokers. Colposcopy was satisfactory and biopsy was performed in 97,8%(n=177) of patients. As for histologic definitive diagnosis, after LEEP, CIN1 was encountered in 13,8%(n=25), CIN2 in 33,1%(n=6), CIN 3 in 52,5%(n=95) and 0,6%(n=1) showed

chronic cervicitis. 26,5%(n=48) had positive margins. We did an expectant management in every patient (surveillance mean time of 31,22±15,14 months). From the positive margins group, 31,3%(n=15) had persistent/recurrent disease during follow-up, whereas only 6,48%(n=7) with free margins recurred, with statistical significant difference between groups (p<0,001; OR=8,27). In patients with positive margins, a positive Human Papillomavirus (HPV) during follow-up had significant association with persistent/recurrent disease (p<0,001; OR=23,7). Patients with persistent/recurrent CIN underwent a second LEEP (54,5%; n=12) or total hysterectomy (45,5%; n=10), depending on their characteristics, among them age (respectively 33,92±7,14 vs 43,50±9,38; p=0,01).

## References

Positive margins after LEEP are associated with persistence/recurrence of CIN. In our sample the majority of patients didn't recur, which confirms that, in accordance with American and European guidelines, expectant management is acceptable, as long as follow-up is guaranteed. For patients with persistent disease any treatment option is acceptable, based on patient's risk factors and desire for future fertility.

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00343

## GENITAL PROLAPSE ASSOCIATED WITH CERVICAL CANCER – A CASE REPORT

### 22. Cervical neoplasia

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#### **Background / Objectives**

Genital prolapse and carcinoma of the cervix are common entities but their association is rare. The displacement of the uterine cervix from the natural environment of the vagina may decrease the neoplastic process of viral infection which can explain the lower risk of cervical cancer in uterine prolapse. The best treatment approach in this clinical setting is not clearly defined and vary considerably among authors.

#### **Results**

We present a case of an ulcerated prolapsed uterus that presented in our department.

#### **Conclusion**

A 74-year-old woman, G3 P3, with a 9 years history of an uterine prolapse without prior cervical cancer screening. The reasons for medical visit were the onset of vaginal bleeding and genital prolapse increasing size. Physical examination revealed a complete and irreducible fourth-degree urogenital prolapse with induration of the entire vaginal mucosa, inflammatory signs and ulcerated lesions that made impossible to clearly identify the cervix (figure 1). No evidence of bladder or rectum involvement were present. Biopsies were performed and histopathology revealed squamous-cell carcinoma. A pelvic MRI revealed bilateral parametrial invasion and hydronephrosis. Computed tomography of the thorax and abdomen revealed no distant metastasis. She was staged according to the 2009 International Federation of Gynecology and Obstetrics staging system as at least FIGO IIIA. After a multidisciplinary board meeting, given the locally advanced stage and the volume of externalized prolapse, the patient was referred to end-life care measures, regarding her comfort, and died 1 month after the initial presentation.

## References

This case highlights an uncommon association between cervical cancer and uterine prolapse with few published reports in the literature. The optimal treatment needs to be individualized in order to improve prognosis and quality of life.

00416

Cervical cancer in Catalonia. A systematic survey of new cases in a general hospital.

## 22. Cervical neoplasia

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### Background / Objectives

It is known that most cervical cancers in Catalonia are diagnosed in women not attending screening programs. Monitoring of cervical cancer patients from Pathology departments can identify errors in prevention, diagnosis, follow-up and patient management.

### Results

Since 2010 we have systematically collected clinical and pathological data from patients with a new diagnosis of cervical cancer, including age, screening history, symptoms, stage, cytology, histology and high-risk Human papillomavirus status (HPV).

### Conclusion

From 1/2010 to 6/2018 one hundred and six cervical carcinomas were diagnosed in our department, which included 81 (76,4%) squamous cell carcinomas (SCC), 22 (20,8%) adenocarcinomas (ADC), 2 (1,9 %) adenosquamous carcinomas (ADSC) and 1 (0,9 %) clear cell carcinoma (CCC). In 41 cases (51,9%) there was no cervical cytology in the previous 3 years. In 63 patients there was cytology, of which 27 (54%) were screening-based: 5 (7,9%) negative and 58 (92,1%) positive. HPV test (HC2 or cobas) was performed in 45 cytology samples with a positive result in 93.3%. Regarding biopsies, HPV was present in 87 (82.1%) cases. The negative samples corresponded to 6/22 (27.2%) ADC, 6/81 (7.4%) SCC, 1 (50%) ADSC and 1 (100%) CCC. Clinical stage (FIGO 2009) was: 5 (4.7%) IA, 38 (35.8%) IB, 2 (1.9%) IIA, 37 (34.9%) IIB, 1 (0.9%) IIIA, 10 (9.4%) IVA, 2 (1.9%) IVB and in 9 (8.5%) cases it was

not found. In 82,9% IA/IB stages there was a recent cytology, while only 45.1% of higher stages ( $\geq$ II) had it. HPV+ cases were younger (mean age =54,7y) than HPV - (mean age=62,1y).

## **References**

Systematic collection of clinical and pathological data from cases with a diagnosis of cervical cancer in Pathology departments is manageable and procures monitoring. The detection of errors can improve the prevention and diagnosis of cervical cancer. Differences between HPV positive and negative cases were related to age and histological type. Patients with cytology detected cases presented with less advanced stage compared to symptomatic cases. HPV negative cervical cancer cases deserve further analysis in the forthcoming era of HPV based screening programs.



00497

## THE ROLE OF HPV E6/E7 ONCOPROTEINS IN EARLY DIAGNOSTIC OF CERVICAL PRECANCEROUS LESIONS.

### 22. Cervical neoplasia

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#### Background / Objectives

The role of High Risk (HR) HPV mRNA E6/E7 expression as a predictive marker of high grade cervical precancerous lesions is shown in some studies (Fontecha 2016). ). There are no studies in Latvia on E6/E7 mRNA expression in patients with cervical intraepithelial neoplasia.

#### Results

108 women aged 18-65 with abnormal cytology referred for colposcopy during their first visit to Reference Colposcopy Centre in Riga East Clinical University Hospital in July 2016 - July 2017 were included in the study. For each patient material from cervix for HR HPV E6/E7 common RNA was taken and under colposcopy control punch biopsy with a following histological examination was performed. HPV mRNA E6/E7 were identified by real time PCR test.

#### Conclusion

29 patients with low grade squamous intraepithelial lesions (LSIL), 70 with high grade squamous intraepithelial lesions (HSIL), 8 patients with atypical squamous cells of undetermined significance (ASCUS) and 1 patient with atypical glandular cells of undetermined significance (AGUS) were included in the study.

HPV E6/E7 RNA presence was found in 89/108 cases: significantly highest proportion of E6/E7RNA expression - 90.0% (63/70) was found in patients from HSIL cytology group; 72.4% (21/29) in LSIL group and 62.5% (5/8) in ASCUS group ( $p < 0.05$ ). CIN2+ histology report was strongly associated with positive HPV E6/E7

RNA – 89.6% (69/77) cases compared with 64.3% in CIN1. In patients with HSIL cytology, punch biopsy CIN 2+ results were detected in 89.6% (67/70 cases), 64.3% (18/28) in LSIL group ( $p<0.05$ ). Punch biopsy histology results CIN 2+ in ASCUS group were detected in 3/8 cases and in patients with LSIL cytology in 9/29 cases. HPV E6/E7 RNA was positive in all of these 12 cases.

## **References**

Our findings suggest that detection of HR HPV E6/E7 RNA simultaneously with cytology test may be a possible positive prognostic factor in early high grade cervical precancerous lesions diagnostic. More detailed studies for method standardizing are required.

## 22. Cervical neoplasia

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### Background / Objectives

Malignancy risk is increased in transplant recipients. Risk of HPV related lesions is particularly relevant given the high prevalence of HPV infection in the general population. We aimed to assess the prevalence of HPV related lesions and possible predictive factors in transplant patients.

### Results

Retrospective analysis of clinical data of transplant recipients observed in our institution between 2013 and 2017. Patients not under immunosuppressive therapy were excluded, as well as those who underwent bone marrow transplant or hysterectomy. Statistical analysis performed using Microsoft Office Excel® and IBM SPSS-Statistics®22.0.

### Conclusion

A total of 171 women were included. Over two thirds (70,2%) were renal transplant recipients, while 22,8%, 5,3%, 0,6% and 0,6% had undergone liver, heart, lung and intestinal transplants, respectively. One patient had a double transplant (kidney and liver). Around 9% were carriers of a second graft. The majority of patients were parous (48,5%) and over half (52,63%) were postmenopausal.

An abnormal cervical smear result was present in 34 women (19,9%); 41,2% (n=14) had a negative HPV test and 50,0% (n=17) a positive one. In 17,6% of women (n=3) HPV16 was detected, while in 64,7% (n=11) other high risk (HR) types were present; both HPV16 and other HR types were present in 2 cases, and in 1 woman both HPV18 and other HR types were identified. HPV positive smears were as follows: 29,4% ASC-US, 52,9% LSIL, 11,8% HSIL and 5,9% concomitant HSIL and AGC. Staining with p16/Ki67 was positive in 29,4% (n=5).

Among women with abnormal smears, 6 (3,5%) underwent excision of the transformation zone (ETZ); 2 were ultimately classified as LSIL and 4 as HSIL. There were no cases of invasive carcinoma. After ETZ, one third maintained LSIL, one third resolved (NILM, negative for HR-HPV) and one third had not yet performed a control smear. Among women subject to expectant management, 6 (3 ASC-US and 3 LSIL) experienced regression of lesions, 3 remained stable (1 ASC-US and 2 LSIL) and 2 did not perform a follow-up smear.

Several factors seemed to influence the incidence of HPV related lesions, namely: age, age of first sexual intercourse, smoking, nulliparity, premenopausal status, history of condylomatosis and of a second graft (p value 0,001, 0,007, 0,016, 0,011, 0,028, 0,001, 0,007, respectively).

### **References**

HPV related lesions were present in a fifth of transplant patients, with 3,5% having undergone EZT. HR-HPV types other than 16 or 18 were the most frequently encountered. Various factors seemed to influence the incidence of altered smears. These results highlight the need for closer gynecological surveillance in the transplant population.

00616

## CERVICAL CANCER SCREENING PROGRAM IN LITHUANIA

### 22. Cervical neoplasia

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#### **Background / Objectives**

Background:

Cervical cancer in Lithuania is the fourth commonest cancer among women with malignancies. Since 1992 Lithuania have the increasing rates

in incidence of cervical cancer among the Northern Europe countries and the highest in Baltic sea region countries. 2004 according Vilnius

Institute of Oncology data the cervical cancer incidence was 25.2/ 100 000, the mortality-12.8/ 100 000. In the middle of 2004 Lithuanian

Ministry of Health started cervical cancer screening programme.

#### **Results**

Guidelines for Screening and Policy Recommendations:

The programme includes screening of women in the age of 25-60 years. There are approximately 764 000 women of this age.

Screening interval - 3 years.

Cost of screening test for the women – free.

Primary test screening - conventional Papanicolaou test with Bethesda 2014 for reporting.

Pap test is investigated in 14 Pathology departments according quality control requirements.

438 primary health care centers are reimbursed for invitation of women and Pap smear taking.

The programme is coordinated by Coordinating Committee .

## **Conclusion**

During 2004 - 2017 year were screened approximately 57 % of invited target age group women. Funds absorption rate was - 70 percent of the program budget.

Number of invitations from 2010 - 2017 period growing from 143 551 to 211 733. Number of screened women was 126 790 in 2017 year in comparison to 2010 (101646).

Cytological abnormalities in 2017 according "Sveidra" patient data base was: 1004 HSIL cases (0,79). LSIL cases - 1254 (0,99), ASC-US ratio was - 3,41% , inadequate ratio was - 3,03 % (3837). 9 squamous cell carcinoma cases was diagnosed in 2017.

According histological records CIS cases from the beginning of programme 2004-2015 was changed dramatically from 60 to 634. Invasive cervical carcinoma cases statistics was from 468 to 364.

We did not observed significant decrease in cervical cancer death (208 death in 2007 and 200 death in 2012) and incidence (451 cases in 2007 and 487 cases in 2012).

## **References**

Analysis of the data and the first experience shows opportunistic screening feature, ineffective decentralized invitation system, the high

quality management of women with cytological abnormalities and indicates the need to improve the programme quality assurance,

creation of data base.

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## **23. Vaginal neoplasia**

00194

## MELANOMA MALIGNO DE VAGINA

### 23. Vaginal neoplasia

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#### Background / Objectives

O melanoma primário da vagina é um tumor extremamente raro, com menos de 250 casos publicados ao redor do mundo<sup>1</sup>. Corresponde a 3% de todos os tumores vaginais e 0,3% a 1% de todos os melanomas malignos, sendo mais agressivo se comparado a neoplasia cutânea, com altas taxas de recorrência e metástases<sup>2</sup>. Seu diagnóstico ocorre normalmente em fases avançadas. Assim, em virtude do mal prognóstico, exige abordagem terapêutica interdisciplinar planejada<sup>1</sup>. O presente trabalho possui como objetivo relatar caso de paciente com melanoma primário da vagina atendida no Hospital Universitário Professor Alberto Antunes (HUPAA), Maceió-AL.

#### Results

Estudo descritivo e observacional de relato de caso de paciente, M.U.S., 74 anos, sexo feminino, G21P18A3, natural e procedente de Campo Alegre-AL, portadora de melanoma primário de vagina encaminhada do interior do estado para serviço de oncologia do HUPAA. Para confecção do trabalho, os dados foram colhidos no prontuário da mesma.

#### Conclusion

Paciente com quadro de leucorreia e sangramento vaginal, associado a dor pélvica a cerca de 3 meses. Procurou assistência médica, onde foi evidenciada tumoração em região vaginal. Procedeu-se com biópsia local evidenciando pela imunohistoquímica melanoma maligno invasivo. Realizou então ressonância magnética (RNM) e PET/CT que demonstraram formação nodular heterogênea (medindo cerca de 5cm em seu maior diâmetro) situada na topografia vaginal/colo uterino em íntimo contato com parede anterior do reto, bem como com uretra. Programou-se então



exenteração pélvica. Contudo, ao realizar novo PET/CT 6 meses após o primeiro, evidenciou-se metástase pulmonar. Foi então programada terapia adjuvante com Decarbazina (DTIC). Apesar do tratamento paliativo, nova RNM mostrou aumento da massa tumoral (medido agora cerca de 13cm em seu maior diâmetro) com invasão de bexiga. Paciente realizou 4 ciclos de quimioterapia, vindo a falecer em seguida.

## References

A sobrevida dos pacientes com metástase a distância é de aproximadamente 14 meses na maioria dos casos. Sendo assim, o estágio de apresentação o principal fator prognóstico nesse tipo de neoplasia<sup>2</sup>. Com isso, apesar das modalidades terapêuticas disponíveis (cirurgia, radioterapia, quimioterapia e imunoterapia) em virtude do diagnóstico geralmente tardio, seu prognóstico é sombrio<sup>3</sup>. Demonstrando, desse modo, a importância do acompanhamento primário com diagnóstico precoce, fato não observado em nossa paciente, que chegou ao nosso serviço com doença já avançada. Contudo, há anos DTIC tem sido a terapia padrão para o melanoma maligno cutâneo<sup>3</sup>.

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00341

## HIGH-GRADE VAGINAL INTRAEPITHELIAL NEOPLASIA – A RETROSPECTIVE STUDY

### 23. Vaginal neoplasia

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#### Background / Objectives

Vaginal intraepithelial neoplasia (VaIN) is a rare disease, not completely characterized, corresponding to 0,4% of the intraepithelial neoplasias of the lower genital tract. Evidence supports that risk factors for the development of VaIN are similar to those found in cervical intraepithelial neoplasia. The prevalence of HPV infection ranges from 93% for VAIN 2/3 to 99% for VAIN 1. HPV 16 is the most frequently identified, followed by HPV 18. It is generally asymptomatic and the diagnosis is histologic. Excision and CO2 laser ablation are the mainstays of treatment. The aim of this study is to describe and analyse the diagnosis and treatment of high grade vaginal intraepithelial neoplasia (VaIN 2 or VaIN 3) in a secondary care hospital.

#### Results

Data from patients with diagnosis of VaIN 2 and VaIN 3 managed in our hospital between january 2012 and may 2018 were retrospectively collected. Demographic characteristics, general and gynaecologic medical history, methods of diagnosis, lesion characteristics, treatment procedures and outcomes were analysed.

#### Conclusion

Five patients presenting with high grade VaIN (3 VaIN 2 and 2 VaIN 3) were managed at our department. Mean age at diagnosis was 63,4 years ranging from 51 to 71 years. Only one had potential immunosuppression. All the patients had previous hysterectomy with associated cervical disease (CIN 3 or cervical cancer). The mean time between hysterectomy and diagnosis of VaIN was 34 months (ranging from 9 months to 8 years). VaIN was detected by an abnormal Pap smear in all cases (3

HSIL, 1 LSIL and 1 squamous cell carcinoma). White epithelium and punctuation were the most frequently colposcopic findings. All patients presented focal lesions in the upper third of the vagina and one had extension to the medium third of the vaginal mucosa. Four patients were treated by laser ablation and one treated with colpectomy. All patients had follow-up with cytological examination and vaginoscopy for therapeutic response evaluation. Remission was reported after the first ablation in one patient. Two patients had persistent disease and were treated with another laser ablation and one squamous cell carcinoma was detected 70 months after laser ablation. The patient treated with colpectomy had remission of the disease. There were no reported therapeutic side effects.

## **References**

Despite the reduced sample size, the present study supports the maintenance of a long term cytologic screening after hysterectomy and a long term follow-up due to the recurrent character of VaIN.

00425

VAIN – A 7 YEARS HOSPITAL EXPERIENCE

## **23. Vaginal neoplasia**

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### **Background / Objectives**

The diagnosis of vaginal intraepithelial neoplasia (VaIN) has increased steadily over the past several decades. The true incidence of VaIN is unknown, but is estimated at 0.2-0.3 cases/100.000 women.

Nowadays we use a two-tiered nomenclature: LSIL for low-grade disease (VaIN1) and HSIL for high-grade disease (VaIN 2/3).

The prevalence of HPV in VaIN 2/3 is high (96%), with HPV 16 being the most frequently detected type (59%).

VaIN is consistently associated with prior or concurrent neoplasia elsewhere in the lower genital tract (50-90% have intraepithelial neoplasia or carcinoma of the cervix or vulva).

The purpose of this study is to review the cases of VaIN in our hospital and to compare the two groups: VaIN 1 and VaIN 2/3 in terms of demographics, patient history, hrHPV testing, therapy and follow-up.

### **Results**

A retrospective study of women diagnosed with VaIN by colposcopy directed biopsy was performed at the Obstetrics and Gynecology Entre o Douro and Vouga Hospital Center between January 1, 2011, and August 1, 2018.

All available data including demographics, history of hysterectomy, cervical or vulvar pathology, histological information, cytology, and hrHPV testing results were recorded.

We divided our sample in two groups: group A (patients with VaIN 1) and group B (patients with VaIN 2/3).

## Conclusion

During the seven years, we had 17 cases of VaIN (estimated prevalence of 2.7cases/100.000). The most common was VaIN 1 (58,8%), followed by VaIN 2 (23,5%) and VaIN 3 (17,7%).

In the group B the mean age was higher and a history of cervical or vulvar pathology was more frequent (71,5%).

The median interval between hysterectomy and VaIN diagnosis was 3,5 years , and the posthysterectomy status was more frequent in group B (57,1%).

Cytological findings: LSIL was more frequent in group A (50%) and in group B we didn't find any specific cytological pattern. The prevalence of HPV was similar in both groups (60% vs 57,2%), being the most frequently detected type hr-HPV (no 16 or 18) in group A; group B had the same percentage of hr-HPV (no 16 or 18) and HPV 16+ (28.6%).

In group A 100% of the patients didn't need treatment (just surveillance) and in group B 71,8% of patients underwent surgical treatment.

The recurrence/progression was higher in group B in the first year (57,1% vs 0%) and in the second year (14,2% vs 0%) of follow-up.

## References

Despite the study limitations (its retrospective nature, missing data and a small sample size), this study showed that we have an estimated prevalence of 2.7cases/100.000 (higher than the literature), being the VaIN 1 the most frequent.

VaIN 2/3 has worse outcomes and it is associated with higher posthysterectomy status and cervical pathology.

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## **24. Vulvar diseases and neoplasia**

00024

## Vulvar Intraepithelial Neoplasia: Retrospective Study of 10 Years

### 24. Vulvar diseases and neoplasia

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#### Background / Objectives

Intraepithelial neoplasia of the vulva (VIN) occurs in about 2.86 per 100,000 women, with a growing incidence in the younger age groups. The usual VIN is typically found in women with HPV infection, young and smoker, whereas differentiated VIN (<5% of VIN) occurs in postmenopausal women, often associated with lichen sclerosus.

#### Goals:

To analyze the characteristics of patients diagnosed with VIN by vulvar biopsy.

To verify the therapeutic approaches and the relapses after treatment.

#### Results

This is a retrospective evaluation of 42 patients diagnosed with VIN in the cervical pathology unit of the Centro Materno Infantil do Norte from July of 2008 to June of 2018. The variables analyzed were: age at diagnosis; HPV co-infection; immunosuppression status; smoking; presence of a multifocal lesion, presence of a multicentric lesion; initial treatment modality; surgical margins; follow-up time; and recurrence of VIN. Statistical analysis with SPSS® software.

#### Conclusion

In the 42 cases of VIN only 2 cases was differentiated VIN (4.8%). The mean age at diagnosis was 55 years (29-83 years), with immunocompromised patients (33.3%) and smokers (27%) being significantly younger at the time of diagnosis ( $p < 0.05$ ). 58% of immunocompromised patients had multifocal vulval lesions. 19 patients had

multicentric lesions (9 VaIN 2/3, 8 CIN 2/3, 3 AIN) the majority immunocompromised patients ( $p = 0.02$ ).

The HPV-HR test was performed in 69% of the patients, being negative in only 17.2% of the tested.

The treatments included: extended excision of the lesion (50%), laser excision (30%), simple vulvectomy (17.5%) and laser ablation (2.5%). One case refused treatment and one case is still waiting for surgery. Surgical margins had high grade lesions in 36.8% of the cases and in 7.5% of histologies there was invasive lesion.

The mean follow-up time was 28 months, with 5 patients opting for private surveillance. A recurrence of the disease occurred in 30% of the cases, with an average of 29 months. In the comparison of surgical approaches, simple vulvectomy was found to confer greater disease-free time ( $p < 0.05$ ). There were no statistically significant differences in smoking, immunosuppression or affectation of margins for risk of recurrence. During follow up occurred five cases of invasive vulvar carcinoma (12.5%).

## References

We emphasize the importance of excisional treatments for the possibility of occult carcinoma and the long-term surveillance for the probability of recurrence. We found that the more conservative methods of exeresis presented shorter times until relapse. Follow-up is particularly important in immunocompromised patients because of the greater probability of multifocal and multicentric lesions.

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00165

## CO2 LASER TREATMENT IN VIN LESIONS: WHAT IS ITS ROLE?

### 24. Vulvar diseases and neoplasia

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#### Background / Objectives

The incidence of vulvar intraepithelial neoplasia (VIN) has been increasing worldwide. This increase is more significant in young women (about 75% of cases), due to this fact it is necessary to institute conservative therapies in order to preserve the vulvar anatomy. The CO2 laser treatment has high clinical efficacy, allows microscopic precision with preservation of normal tissues, rapid healing with "ad integrum" restitution and a reduced number of complications.

To evaluate the role of CO2 laser in the treatment of VIN lesions.

#### Results

We present 2 cases of CO2 laser treatment in VIN lesions.

#### Conclusion

A 59 year old patient with persistent pruritic vulvar lesion with 6 months of evolution; had an incisional biopsy of the lesion that revealed "Bowen's Disease" (VIN III). History of total hysterectomy at age 39 due to a cervix HSIL. The examination had an extensive maculo-papular lesion, with hyperpigmented areas and hyperkeratosis from the middle 1/3 of the small left vulvar lip posteriorly to the lower 1/3 of the small right lip, involving the furcula and the entire perineum to the anus, but without vaginal involvement.

Extensive local excision of the CO2 laser lesion was performed under colposcopic control, and at the end the fulguration of the lesion margins was achieved. The histological study revealed high-grade VIN, with classic and multifocal aspects of VIN II and VIN III, with bowenoid and basaloid areas, with focal margin reach.

A 38 years old patient with vulvar lesion with 3 months of evolution had an incisional biopsy that revealed VIN II. History of cervical adenocarcinoma in situ 6 years ago in a excisional biopsy and Sjögren Syndrom with multiple vulvar ulcer. The examination revealed a leucoplastic lesion in both small lips with a condiloma in the furcula.

Cervical cytology revealed ASC-H and incisional cervix biopsy HSIL with HPV 53 e 42 positive. Extensive local excision of the CO2 laser vulvar lesion and excisional cervix biopsy was performed under colposcopic control. The cervix biopsy revealed CIN II and vulvar biopsy showed HSIL.

Both patient presented an excellent aesthetic and functional result in the subsequent consultations, with no signs of local recurrence to date. Then they was vaccinated with the nonvalent vaccine.

## **References**

CO2 laser surgery allows the treatment of VIN lesions on an outpatient basis, under local anesthesia with excellent cosmetic and functional results. The treatment can also be adjusted to the specific needs of each patient, with the possibility of calibrating the depth of the vaporized and removed tissues. Excisional treatment is the preferred method because it allows the histological evaluation of excised tissue and the detection of possible occult early invasion.

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## **25. Anal neoplasia**

00251

## DNA-HPV PCR: A TOOL TO INDICATE ANAL CYTOLOGY IN IMUNOSSUPRESSED WOMEN.

### 25. Anal neoplasia

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#### Background / Objectives

Anal cytology is a problem to implementation of cancer anal screening due to a lack of well-trained professionals, especially in immunosuppressed people. The objective of this study was to evaluate whether PCR for DNA-HPV screening could help identify cases with greater possibility of anal cytology with atypia to refer to specialized centers the positive cases.

#### Results

This cross-sectional study examined 31 kidney-transplanted women receiving immunosuppressive therapy at the General Hospital of Fortaleza in Brazil. Anal samples were collected and preserved in order to perform liquid-based cytology and a real-time PCR assay (Cobas 4800 [Roche Molecular Systems, Alameda, CA]) detecting high-risk HPV. The relative risk for atypical cytology was calculated for a confidence interval of 95%. The project was approved by the ethics committee of General Hospital of Fortaleza, Fortaleza, Brazil.

#### Conclusion

The patients ages ranged from 31 to 70 years old (mean: 42.6+10.4). Anal cytology was atypical in 35.4% of cases (ASC-US in 1[3.2%] and LSIL in 7[21.7%]). The presence of HPV was confirmed in 48.8% of the cases. a positive anal HPV test did correlate with a higher risk of atypical anal cytology ( $p=0.0197$ ), yielding a relative risk of 10.18 (95% CI: 1.45–71.54).



## References

The presence of HPV in an anal sample correlates with an increased risk of atypical anal cytology. HPV tests could be useful tools for identifying patients who require anal cytology.

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00291

Anal intraepithelial lesions in women with high-grade cervical intraepithelial neoplasia

## 25. Anal neoplasia

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### Background / Objectives

Anal intraepithelial neoplasia is believed to be a precursor of anal cancer and it appears to be related to high-risk human papillomavirus. Women with genital neoplasia have been shown to be at increased risk for anal cancer.

The aim of this study is to describe the prevalence of abnormal anal cytology in women with high-grade cervical intraepithelial neoplasia and identify risk factors for abnormal anal cytology in this population.

### Results

A cross-sectional study of 323 women from 2012 to 2017 with histopathological diagnosis of high-grade cervical intraepithelial neoplasia underwent anal cytology and completed a questionnaire detailing medical history and sexual behavior. All abnormal anal cytologies were submitted to anoscopy followed by a biopsy if pertinent. The hypothetical risk factors analyzed were: immunosuppression, tobacco use, lifetime number of sexual partners and history of anal intercourse.

### Conclusion

Of total of 323 patients, 29 had a abnormal anal cytology (8,98%) which was: 3 with viral cytopathic effect (10,34%); 20 with ASCUS (68,97%); 3 with LSIL (10,34%); 1 with ASC-H (3,45%) and 2 with HSIL (6,90%). Of the 29 patients sent to anoscopy, 9 have missed the appointment (31%). The remaining was submitted to anoscopy: 7 had no alteration related to HPV and had no biopsy (35%); 4 had negative biopsies (25%); 9 had positive biopsies(45%): 1 revealed papilomatosis (5%); 1 a rectal adenoma (5%), 6 revealed condylomas (30%) and 1 revealed LSIL (5%).

From the risk factors analyzed, tobacco use was significantly associated with an abnormal anal cytology ( $p<0.01$ ).

## References

Tobacco use was significantly associated with an abnormal anal cytology and should be considered a risk factor. However, larger studies are needed to identify risk factors associated with abnormal cytology and anal intraepithelial neoplasia. We also need clarity to delineate the best screening method, screening frequency and appropriate treatment for anal intraepithelial neoplasia.

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00322

Does sampling strategy influence cytological and genetic findings in anal cancer screening of patients at risk?

## 25. Anal neoplasia

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### Background / Objectives

Anal cancer (AC) screening of high-risk population can decrease the morbidity and mortality of this disease. High-resolution anoscopy (HRA) is a recommended method for AC screening when available. In case of the absence of HRA or a proctologist experienced in HRA, different strategies are considered. Especially those tests used in cervical cancer screening are of note for AC screening because of an analogy of the natural history of both diseases. Therefore, anal cytology and hrHPV mRNA detection might be effective tests for the detection of severe anal lesions. However, sampling strategy could affect the value of test's results, in terms of adequate cell collection. We have focused on how cytological and HPV finding could differ in specimens sampled by a skilled nurse which is a routine practice, and by a proctologist during an ordinary anoscopic examination.

### Results

120 anal smears from 60 Czech men having sex with men (MSM)/HIV positive MSM were collected into a liquid-based cytological vial using a damp dacron swab. One sampling was carried out by a proctologist during an ordinary anoscopic examination (AS) and another by a nurse during a routine check (RS). All samples were processed for cytology and tested for mRNA expression of 14 hrHPV types, with separate identification of HPV types 16,18, and 45, and finally genotyped.

### Conclusion

In our study group, cytological finding ranging from NILM to HSIL were described. 19 % of samples had not sufficient cellularity for a credible cytological diagnosis. Cytology results of RS and AS differed in half of the patients. 50 % of cytological samples were not influenced by sampling strategy and routine and anal samples revealed the same diagnosis.

HPV mRNA test results differed between RS and AS to a lesser extent, namely in 38 % of patients. Moreover, when considering partial genotyping, HPV results of RS and AS differed in 51.6 % of patients. RS and AS usually shared identical HPV genotypes with a few exceptions.

## **References**

According to our data both sampling strategies, i.e. routine and anoscopic, represent “blind” collection of cells without targeting the lesional tissue of the anal canal. An anal smear taken during an ordinary anoscopic examination does not seem to bring any improvement of cytological based AC screening under given circumstances, however, another sampling device is going to be tested for an increase of cellularity of samples. Using cytology and HPV tests for anal cancer screening, high-grade lesions could be detected in a high-risk population. Nevertheless, repeat sampling would probably increase the sensitivity of such a method.

## **References**

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00403

## ANAL CANCER SCREENING COMPLIANCE AND KNOWLEDGE AMONG HIV POSITIVE MSM IN THE CZECH REPUBLIC

### 25. Anal neoplasia

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#### **Background / Objectives**

HIV-positive men who have sex with men (MSM) are at increased risk of anal cancer. Prior to our study no regular screening program for anal cancer in the Czech Republic was available.

#### **Results**

Anal cancer screening was proposed to the HIV positive MSM attending Dermatovenereology and Infectious Disease Department Na Bulovce Hospital, Prague, the Czech Republic from July 2017 to June 2018. The blind rectal swab for anal cytology, DNA methylation and HPV testing was done immediately, anoscopy examination for each patient was scheduled.

#### **Conclusion**

During the one year period, anal cancer screening was proposed to 183 patients of whom 127 (69.4 %) agreed to participate. The age range was 18 to 63 years of age (mean 33 years). Prior to our study anal cancer screening has been proposed or performed in 14 (11.2 %) of the patients who entered the study. Only 17 (13.4 %) patients were aware of the increased risk of anal cancer. The blind rectal swab was performed in all of the patients, but the scheduled anoscopy examination underwent only 47 (37.0 %) patients. Only 2 (1.6 %) of the patients have been vaccinated against HPV.

## References

The knowledge regarding anal cancer and its screening among HIV positive MSM in the Czech Republic seems to be very low. Compliance with blind rectal swabs was significantly higher than with anoscopy examination. The low compliance with anoscopy examination shows the need for sensitive diagnostic tests / biomarkers for triage of high-risk patients.

00588

## Anal Cancer Risk Among People With HIV Infection in the United States

### 25. Anal neoplasia

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#### Background / Objectives

People with HIV infection have an elevated risk of anal cancer. However, recent calendar trends are incompletely described, and which population subgroups might benefit from cancer screening is unknown. With this study, we aim to quantified the risk of anal cancer among people with HIV infection in the United States during 1996 to 2012, by examining the associations of anal cancer incidence with demographic characteristics and prior AIDS diagnosis, and we assessed temporal trends. We also provide estimates of the cumulative incidence of anal cancer among subgroups of individuals with HIV with and without AIDS.

#### Results

We used linked data from HIV and cancer registries in nine US areas (1996 to 2012). We calculated standardized incidence ratios to compare anal cancer incidence in people with HIV infection with the general population, used Poisson regression to evaluate anal cancer incidence among subgroups of people with HIV and to assess temporal trends, and estimated the cumulative incidence of anal cancer to measure absolute risk.

#### Conclusion

Among 447,953 people with HIV infection, anal cancer incidence was much higher than in the general population (standardized incidence ratio, 19.1; 95% CI, 18.1 to 20.0). Anal cancer incidence was highest among men who have sex with men (MSM), increased with age, and was higher in people with AIDS than in those without



AIDS (ie, HIV only; adjusted incidence rate ratio, 3.82; 95% CI, 3.27 to 4.46). Incidence among people with HIV increased steeply during 1996 to 2000 (annual percentage change, 32.8%; 95% CI, -1.0% to 78.2%), reached a plateau during 2001 to 2008, and declined during 2008 to 2012 (annual percentage change, -7.2%; 95% CI, -14.4% to 0.6%). Cumulative incidence after a 5-year period was high for MSM with HIV only age 45 to 59 or  $\geq 60$  years (0.32% to 0.33%) and MSM with AIDS age 30 to 44, 45 to 59, or  $\geq 60$  years (0.29% to 0.65%).

## References

Anal cancer incidence is markedly elevated among people with HIV infection, especially in MSM, older individuals, and people with AIDS. Recent declines may reflect delayed benefits of HIV treatment. Groups with high cumulative incidence of anal cancer may benefit from screening.

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## **26. Oral HPV infection**

00233

## PREVALENCE OF ORAL HPV INFECTION IN A PROSPECTIVE COHORT OF HIV-INFECTED AND UNINFECTED MEN WHO HAVE SEX WITH MEN: THE OHMAR PROJECT

### 26. Oral HPV infection

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#### Background / Objectives

Human Papillomavirus (HPV) plays a role in the development of head and neck squamous cell carcinoma (HNSCC). Oral HPV infection is more frequent in individuals with risky sexual behavior, such as men who have sex with men (MSM), than in the general population. We investigated the presence of HPV-DNA and high-risk (HR) HPV E6/E7 mRNA in oral rinse-and-gargles of HIV-infected and uninfected MSM enrolled in a longitudinal study (Oral HPV in Men At Risk, OHMAR Project).

#### Results

Participants were recruited among attendees of an STI/HIV Unit. They were thoroughly examined for the presence of lesions clinically suspicious for HNSCC and all clinically healthy subjects were enrolled. Oral samples were collected by using a commercial mouthwash for a 30 second rinse-and-gargle. Specimens were tested for the presence of HPV-DNA and HR-HPV E6/E7 mRNA by using the Linear Array HPV Genotyping Test and APTIMA HPV assay, respectively.

#### Conclusion

Overall, 310 MSM were enrolled, of which 117 HIV-infected (37.7%). These were mostly under cART (94.0%), and had undetectable HIV-1 RNA load (93.6%). HPV-DNA (any HPV type) was detected in 28/117 (23.9%) and 33/193 (17.1%) HIV-infected and uninfected subjects, respectively ( $p=0.14$ ). HR-HPVs were found in 12/117 (10.3%) and 21/193 (10.9%) HIV-infected and uninfected MSM, respectively ( $p=0.86$ ). Of the 33 HR-HPV positive MSM, 3 (9.1%) harbored HPV16 only, 8

(24.2%) HPV16 together with other HR types, and 22 (66.7%) only HR-HPVs other than HPV16. All the 33 HR-HPV DNA positive and 9 HR-HPV DNA negative random samples were tested with APTIMA HPV assay. Overall, 2/42 samples (4.8%), which were among those HR-HPV DNA positive, gave an invalid APTIMA result. All the HR-HPV DNA negative specimens tested APTIMA negative. HR-HPV E6/E7 mRNA was detected in 4 of the remaining 31 HR-HPV DNA positive oral rinses with a valid APTIMA test (12.9%). HR-HPV E6/E7 mRNA positivity was more frequent among the HPV16-positive participants than among those harboring only other HR types (2/11, 18.2% vs. 2/20, 10.0%,  $p=0.52$ ).

## References

Oral HPV infection is 2- to 3-fold more common among MSM than in the general population. Oral HPV prevalence is higher in HIV-infected MSM, although not significantly compared to the HIV-uninfected counterparts. Oral rinse-and-gargles from cancer-free MSM have detectable levels of HR-HPV E6/E7 mRNA. Although only a minor fraction of those harboring HR-HPV DNA were also positive for HR-HPV mRNA, HR-HPV E6/E7 mRNA testing of samples simply and non-invasively collected may help identify, among individuals without clinically evident lesions, those with transforming HPV infections, thus at greater risk for HPV-associated HNSCC.

00446

## HPV IN ORAL CAVITY OF PATIENTS WITH BREAST CANCER

### 26. Oral HPV infection

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#### Background / Objectives

The Human Papillomavirus (HPV) is proven to be the main risk factor for cervical cancer, as well as for benign oral lesions. Recently, its role in the pathogenesis of breast and oral cancers has been investigated. Breast cancer is the second most prevalent type of neoplasm among women in Brazil, however, studies for detecting and genotyping HPV in these tumors are still scarce. Thus, the overall objective was to investigate the frequency of HPV and genotype in the oral cavity in patients with breast carcinoma.

#### Results

The research was conducted in patients attending in the mastology service of the Group of Education and Oncology Studies (GEEON) - Faculty of Medicine of the Federal University of Ceará (UFC), Brazil. The pilot project was conducted in 17 patients with breast cancer (case group) and 16 patients without breast cancer (control group). The HPV DNA was extracted from samples of the scrapings of the jugal mucosa and oropharyngeal swab. HPV detection and genotyping were performed through the multiplex nested PCR technique, through a set of primers that amplify the HPV E6/E7 consensus region. HPV genotyping amplified the E6/E7 region of the genome, followed by region-specific amplification for each viral type. Ten types of HPV were investigated: 6/11, 16, 18, 31, 33, 45, 52, 56, and 58. To statistical significance was used the t Student test and Fisher exact test to confidence interval of 95%. The project was approved by the Ethic Committee of UFC, nº 2.396.348-2017.

#### Conclusion

The patients in the group of breast cancer had mean age of 47.8 ( $\pm 12.83$ ) and in the control group was 49 ( $\pm 11,40$ ;  $p=0.49$ ). In the study group HPV 6/11 was identified in oropharynx and jugal mucosa in two out of seventeen tested participants (11,76%). In the control group was identified HPV 6/11 in oropharynx and jugal mucosa in three

of sixteen participants (18.76%), and two (12.5%) participant in jugal mucosa with HPV 52 and other with HPV 33 (6.25%). In total it was detected HPV in 56.25% of the control group and 41.17% in the case group ( $p=0.49$ ).

## References

Despite the small number of cases studied, it was possible to observe that HPV is very frequent in the oral cavity, both in patients with breast cancer and without breast cancer. It will be necessary to study more cases for a more adequate conclusion.

## **27. HPV and oropharynx / Head and neck cancer**

00100

## DIFFERENCES IN THE PROGNOSIS OF HPV16 POSITIVE PATIENTS WITH SQUAMOUS CELL CARCINOMA OF HEAD AND NECK ACCORDING TO VIRAL LOAD AND EXPRESSION OF P16

### 27. HPV and oropharynx / Head and neck cancer

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#### Background / Objectives

Patients with squamous cell carcinomas (SCCs) of head and neck cancers (HN) infected with HPV have more favorable prognosis than for those without infection (Ragin et al. 2007; Dayyani et al. 2010; O'Rourke et al. 2012). However, in more than 40% of HPV infected HNSCC patients progression of cancer disease is observed. Their identification is particularly important nowadays, because of ongoing trials concerning de-escalation of anticancer treatment in patients with HPV positive HNSCCs (Mirghani et al. 2015). Therefore, the aim of the study was to evaluate the impact of HPV16 load (VL-the number of virus genome copies per cell) and P16 expression on prognosis of patients with HNSCC.

#### Results

HPV16 presence was assessed in the group of 109 patients with HNSCCs by quantitative polymerase chain reaction (qPCR). VL (assessed by qPCR) and P16 expression (evaluated by immunohistochemistry) were analysed only in the subgroup of HPV16-positive tumours. These features were correlated with 5-year overall survival (OS) and disease-free survival (DFS).



## Conclusion

HPV16 infection was found in 36 tumours (33.0%). Virus-positive patients had better OS and DFS than those without infection ( $P = 0.041$  and  $0.005$ ). Among HPV16-positive HNSCCs, 18 (50.0%) had higher VL (median value  $> 6764.3$  copies/cell) and 25 (73.5%) P16 over expression. The significant differences in OS and DFS ( $P = 0.008$  and  $0.004$ ) were noticed according to VL, wherein 100% DFS was found for patients with higher VL. According to P16 expression, significant difference was found only for OS ( $P = 0.020$ ). In multivariate analysis, VL ( $P = 0.045$ ; HR = 2.795; CI 0.121–1.060) and the level of smoking ( $P = 0.023$ , HR = 2.253; CI 1.124–4.514) were independent factors affecting DFS of HPV16-positive patients.

## References

On the basis of viral load, it is possible to differentiate prognosis of patients with HPV16-positive HNSCCs. In this subgroup, viral load has stronger prognostic potential than P16 expression.

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00312

## HUMAN PAPILLOMAVIRUS INFECTION IN SINO-NASAL INVERTED PAPILLOMA: AN ITALIAN EPIDEMIOLOGICAL STUDY

### 27. HPV and oropharynx / Head and neck cancer

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#### Background / Objectives

Inverted Papillomas (IP) are benign epithelial tumor of the ciliated epithelium located innasal cavity and paranasal sinuses. Natural history usually includes dysplasia, in situ, and invasive squamous cell carcinoma(SCC).Local and distant invasivenessand high recurrence rateare the three distinctive features. Several etiologies have been investigated, from chronic inflammations,to allergy, environmental pollution, tobacco and/or occupational exposure, infections. Detection of HPV has raised the issue of its etio-pathogenic role in IP occurrence, as well as in malignancy progression. Aim of the study was to assessthe HPV prevalence in IP lesions and its potential heterogeneity in Italy.

#### Results

A total of 77 patients withIPwere recruited: they underwent endoscopic surgical therapyin 2 university Italian hospitals.Samples were collected from 62 and 15 patients with IPonly and IP with SCC, respectively. Moreover, a control group of 31 patients with normal mucosa was enrolled. DNA extraction was carried out using DNAeasy Blood & Tissue Kits (QIAGEN) and HPV detection and genotyping were performed using the Anyplex II HPV-28 kit (Seegene).

The HPV prevalence in patients with IPwas 11.7%, with 6 samples positive for HPV-6, one for HPV-11, and 2 for HR-HPV (HPV-39 and -56, respectively). Only one (6.7%) SCC was HPV positive. HPV was not found in the control group. No significant differences in prevalence and genotype distribution were found by gender,agegroups, and geographic areas.

#### References

Our study suggests a marginal role of HPV in IP. Further studies are needed to understand the prevalence of LR-HPV genotypes and their priming action on cell replication. RNA detection using E6 and E7 transcripts can help further elucidate the IP pathogenesis.

00319

## PSYCHOLOGICAL IMPACT OF PATIENTS LIVING WITH HEAD AND NECK CANCER: A SYSTEMATIC LITERATURE REVIEW

### 27. HPV and oropharynx / Head and neck cancer

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#### Background / Objectives

Head and Neck Cancer (HNC) includes malignant tumors that develop in the mouth, pharynx, larynx, salivary glands, noses and sinuses. Worldwide, HNC accounts for more than 550,000 cases and 380,000 deaths annually.<sup>1</sup> A meta-analysis estimated the HPV-attributable proportion of HNC to be 45.8% for the oropharyngeal cavity, 24.2% for the oral cavity, and 22.1% for the larynx.<sup>2</sup> The aim of this study was to investigate the psychological impact of HNC.

#### Results

A systematic literature review (SLR) of health related quality of life in patients with HNC was conducted in MEDLINE, EMBASE, Cochrane CENTRAL and PsycInfo. Studies in English and published in the last ten years were selected when pre-set eligibility criteria were met. Studies in patients with HNC that reported patient-related outcomes from questionnaires specific to psychological impact, such as the Hospital Anxiety and Depression Scale (HADS) and Beck Depression Inventory (BDI), were included. When the HADS-Anxiety score was >7, HADS-Depression score was >7, HADS-Total score was >14 or BDI score was >10, patients were considered psychologically distressed.

#### Conclusion

Twenty-four studies reporting HADS or BDI scores in HNC patients were identified. Eight of the 24 studies reported psychological distress in patients with HNC from Japan, The Netherlands, Canada, United Kingdom, Brazil, Germany and The United States (US). The characteristics of these eight studies are presented in Table 1.

HADS and BDI scores were measured at different time points: at baseline, at the end of a treatment (radiotherapy, chemotherapy or surgery) and during follow-up visits (3 weeks to 5 years). Five studies reported a psychological distress for the HADS-Total score (>14) at baseline or in post-treatment follow up. All studies that reported BDI scores (n=3) showed that HNC patients presented a mild mood disturbance (score: 11-16; n=2) or a borderline clinical depression (score: 17-20; n=1). The association between HPV and HNC was not stated among the included studies.

## References

As shown in eight of the 24 studies included in our SLR, HNC can cause psychological distress in patients according to both the HADS and BDI tools and irrespective of age, country, length of follow up or gender. Moreover, a three-fold higher incidence of suicide has been reported in HNC compared with the general US population.<sup>3</sup> It is unclear whether psychological distress differs between patients with HNC caused by HPV and those with other etiologies. This warrants further evaluation.

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**Table 1: Studies on HNC psychological impact included in the systematic review**

Author & year	Country	Study time period	Patient characteristics	Age	Time point	N	HADS-D Mean (SD)	HADS-A Mean (SD)	HADS-T Mean (SD)	BDI Mean (SD)
Ichikura 2018	Japan	2013	HNC patients (90.5% male) - Group of patients smoking, drinking, seeking support, or engaging self-distraction	40-80+	Post-treatment follow-up	79	NR	NR	NR	11 (7.4) [BDI-II]
Kobayashi 2008	Japan	2005-2007	Patients who were scheduled for surgery for HNC - Low self-esteem based on Rosenberg self-esteem scale	62	Preoperatively	20	5.8 (2.2)	6 (1.7)	11.8	NR
					Post-treatment follow-up (6 months)	20	11.8 (4.3)	4.4 (3.9)	16	NR
					Preoperatively	38	3.6 (3.1)	4.4 (1.9)	8	NR
					Post-treatment follow-up (6 months)	38	3.2 (2.7)	2.5 (2.0)	5.7	NR
Krebber 2016	The Netherlands	2009-2012	HNC treated patients who scored positive for psychological distress (HADS Total >14, Anxiety >7 or Depression >7)	61.7	Post-treatment follow-up (time frame of 37 months)	135	8.77 (3.81)	8.84 (3.84)	17.39 (6.27)	NR
Moubayed 2015	Canada	2011-2012	HNC patients diagnosed at least 12 months prior to clinic visit that had completed initial treatment (pre-treatment predictors of depression symptoms: >3 medications, current smoking, > 14 drinks per week or T3/T4 disease stage)	<60 for n=112 and >60 for n=97	Post-treatment follow up: 38.7 months	209	0.8 (0 predictors)	NR	NR	NR
					Post-treatment follow up: 38.7 months		3.5 (1 predictor)	NR	NR	NR
					Post-treatment follow up: 38.7 months		3.9 (2 predictors)	NR	NR	NR
					Post-treatment follow up: 38.7 months		5.6 (3 predictors)	NR	NR	NR
					Post-treatment follow up: 38.7 months		8.3 (4 predictors)	NR	NR	NR
					Post-treatment follow up: 38.7 months					
Clarke 2014	United Kingdom	2007-2008	Patients aged ≥18 with HNC at least 6 months post-treatment	60.47	Baseline	49	5.88 (3.8)	6.69 (4.7)	12.57	NR
					Baseline (female)	18	6.61 (3.56)	8.17 (5.28)	14.78	NR
					Baseline (male)	31	5.45 (3.93)	5.84 (4.18)	11.29	NR
					Post-treatment follow-up (9 months)	20	4.8 (2.96)	6.8 (4.69)	11.6	NR
Sawada 2012	Brazil	2009-2010	HNC patients undergoing radiotherapy	30-90	Start of treatment	41	NR	NR	NR	9.44 [BDI]
					End of treatment	41	NR	NR	NR	12.52 [BDI]
Singer 2012	Germany	NR	HNC patients from the radiation-oncology department	58	First day in hospital	113	NR	NR	14.22	NR
					6 months after admission	56	NR	NR	14.95	NR
Chen 2009	USA	2006-2007	Patients with a primary diagnosis of non-metastatic HNC undergoing radiotherapy	55 (median)	Before treatment	40	8.4 (5.5)	7.5 (4.3)	16.9	12.6 (10.0) [BDI-II]
					End of treatment	40	11.2 (5.5)	6.9 (5.0)	18.1	18 (12.2) [BDI-II]

BDI: Beck's Depression Inventory; HADS-A: Hospital Anxiety and Depression Score anxiety scale; HADS-D: Hospital Anxiety and Depression Score depression scale; HADS-T: Hospital Anxiety and Depression Score total; HNC: head and neck cancer; NR: Not reported

Notes: HADS scoring (either for Depression or Anxiety): Total score: 0-7 = Normal, 8-10 = Borderline abnormal (borderline case), 11-21 = Abnormal (case); BDI scoring: 1-10: These ups and downs are considered normal, 11-16: Mild mood disturbance, 17-20: Borderline clinical depression, 21-30: Moderate depression, 31-40: Severe depression, over 40: Extreme depression. Scores showing psychological distress are in bold.

00378

## ABSENCE OF DISRUPTIVE TP53 MUTATIONS IN HIGH-RISK HUMAN PAPILLOMAVIRUS-DRIVEN NECK SQUAMOUS CELL CARCINOMA FROM UNKNOWN PRIMARY

### 27. HPV and oropharynx / Head and neck cancer

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#### Background / Objectives

Patients with head and neck squamous cell carcinoma (SCC) may not rarely present with neck metastases without evidence for a primary tumor. Similarly to oropharyngeal SCC (OPSCC), a substantial proportion of neck SCC from unknown primary (NSCCUP) contains transcriptionally active high-risk human papillomaviruses (hrHPVs) infections and show better overall survival rates. p53 oncosuppressor mediates radiation-induced apoptosis and *TP53* mutations have been linked with increased resistance to ionizing radiation. hrHPV-driven OPSCCs are considered to contain sufficient intact p53 to preserve a radiosensitive phenotype. To enforce the evidence for causality between hrHPV infections and NSCCUP and provide biological basis for treatment de-intensification trials in this clinical entity, we searched for *TP53* mutations by analyzing exons 4 to 10 in NSCCUP.

#### Results

FFPE tissue were available from 70 NSCCUP patients from Germany, Italy, and Spain. HPV-driven cases were defined by presence of E6\*I mRNA together with at least one additional marker (HPV-DNA and p16<sup>high</sup>). *TP53* mutations were searched by analyzing exons 4 to 10. PCR products were sequenced by fluorescent capillary electrophoresis.

## Conclusion

Of the 70 NSCCUP samples, 26 (37%) harbored a transforming HPV infection. Exons 4 to 8 of the TP53 gene were screened for mutations in all patients, while exons 9 and 10 were additionally sequenced in 57 patients. Sequencing success ranged from 77% for exon 4 to 91% for exon 9. TP53 sequencing resulted in the identification of 19 single mutations. These included 16 disruptive alterations (84%), comprising missense (n=7) located within the DNA binding domain (amino acids 102-292), frameshift (n=4, resulting in nonsense mutations at a later amino acid position), and nonsense mutations (n=5, four within the DNA binding domain). When comparing NSCCUP patients with disruptive *TP53* mutation in any exon (n=16) with patients without disruptive mutation but with complete sequence of exon 4-9 (n=32), all disruptive mutations were found in patients with non-HPV-driven NSCCUP (16/31; 52%) and in none of the 17 patients with HPV-driven NSCCUP (P=0.0002).

## References

In a relevant fraction of cases, NSCCUP is a HPV-driven entity showing transcriptionally active hrHPV infection and harboring a wild-type *TP53* sequence. HPV-driven NSCCUP may benefit from treatment de-intensification obtained not only by restricting the prophylactic irradiation of the upper aero-digestive tract to the OP and its corresponding lymphatic pathways but also, perspective, by including these patients in clinical trials evaluating dose de-escalation strategies for HPV-driven OPSCC.

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00417

## THREE CASES OF HPV-RELATED OROPHARYNGEAL CANCER WITH GOOD COURSE DESPITE NONCOMPLETION OF (CHEMO)RADIOTHERAPY

### 27. HPV and oropharynx / Head and neck cancer

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#### Background / Objectives

HPV status is an important factor in the treatment of oropharyngeal cancer. Recently, the establishment of minimally invasive treatment for HPV-related cancers is one of the urgent issues in the treatment of head and neck cancers. We report three cases of HPV- positive oropharyngeal cancer with good course despite noncompletion of radiotherapy.

#### Results

We reviewed 113 cases of oropharyngeal squamous cell carcinoma treated at Osaka University Hospital and Osaka Rosai Hospital between 2013 and 2015. (Chemo) radiotherapy was used to treat 87 cases, with noncompletion of the radiotherapy in three cases, which were all HPV-DNA positive and p16 positive. We analyzed the records of these three cases for the reasons for noncompletion of treatment, the effect of primary treatment, and the subsequent course. UICC 7th edition was used for staging.

#### Conclusion

Case 1, Tonsillar carcinoma, T4aN3M0, 61-year-old man, PS1

The planned treatment was 70Gy radiation and six courses of weekly chemotherapy, but due to severe pneumonia, the treatment was changed to 45Gy radiation and three courses of chemotherapy. At the end of the treatment, the cancer remained clearly in both the primary and the lymph node in the CT, but after 12 weeks, the lesion was not detected by PET-CT. The patient is currently alive without relapse for 39 months.

Case 2, Tonsillar carcinoma, T4bN2cM0, 71-year-old woman, PS2

Treatment was completed with 56Gy radiation therapy and five courses of chemotherapy due to multiple compression fractures of the thoracic spine and lumbar vertebra caused by falls. The tumor was not detectable by PET-CT after 12 weeks of treatment. The patient is currently alive without relapse for 53 months.

Case 3, Tonsillar carcinoma, T2N2bM0, 53-year-old man, PS1

The planned treatment was 70Gy radiation therapy, but treatment was terminated at 48Gy due to delirium and gastrointestinal bleeding. After radiotherapy, tonsillectomy and neck dissection were performed, but pathologically no cancer was detected. The patient is currently alive without relapse for 58 months.

## References

We examined three cases of HPV-positive oropharyngeal cancer with good course, despite noncompletion of the planned treatment. The patients, who are currently alive with relapse, had been given irradiation doses of 45Gy, 48Gy or 56Gy. These cases may offer suggestions for minimally invasive treatment of HPV-related oropharyngeal cancer.

Case	Age	Sex	Primary site	TNM	Treatment	Outcome
1	61	Male	Tonsil	T4aN3M0	CCRT RT <del>45</del> Gy CDDP60mg/m <sup>2</sup>	39 M NED
2	71	Female	Tonsil	T4bN2cM0	CCRT RT <del>56</del> Gy CDDP100mg/m <sup>2</sup>	53 M NED
3	53	Male	Tonsil	T2N2bM0	IMRT RT <del>48</del> Gy	58 M NED

00467

Droplet digital PCR quantification suggests that higher viral load correlates with improved survival in HPV-positive oropharyngeal tumours.

## **27. HPV and oropharynx / Head and neck cancer**

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### **Background / Objectives**

Previous studies in small cohorts have shown an association of HPV viral load with improved survival rates in oropharyngeal cancer (OPC). Although HPV-positive OPC patients have a better prognosis, there is still an HPV-positive group who have poor outcomes. Therefore, a single test that can better delineate patient outcomes would be desirable. Our objective was to analyse viral load by a highly accurate technique, droplet digital absolute PCR quantification (ddPCR), in a cohort of 134 cases of HPV-positive oropharyngeal tumours on whom clinical outcome data, including survival data, were available.

### **Results**

The present analysis was performed on a subset of a population based patient cohort diagnosed with OPC in 2013, who were the subject of a previous audit on HPV status and survival outcomes. 121 samples were designated as positive for HPV16 using the Diamex Optiplex genotyping test. Viral load was determined for these samples, using ddPCR assays specific for HPV16 L1 and E6.

Viral load was classed as "high" if more than 5 copies of the L1 portion of HPV16 genome per cell were detected. Analysis of the association between high versus low viral load and overall survival in addition to hazard of death & disease progression was performed; adjustments for sex, age, deprivation, smoking, alcohol consumption and stage (TMN and ICON) were carried out.

## **Conclusion**

Of the the 121 HPV16-positive samples, 100/121 samples were positive using the ddPCR assays. The range of viral load (VL) was from 0.0014 to 304 genome copies per cell with a mean of 30.9 (SD 51.1).

In the univariate analysis those with a high viral load have a lower hazard of death and recurrence ( $p=0.027$ ). The majority of samples had an E6 to L1 ratio  $\sim 1$ . However, 10 samples had a ratio  $>1$  (range=1.3 to 2.3) and 9 samples had a ratio  $<1$  (range=0.3 to 0.7), suggesting variation in episomal versus integrated genome numbers. Full data in addition to adjusted analyses according to demographic and behavioural attributes will be presented.

## **References**

These data suggest that HPV viral load may be informative in determining patient outcomes.

00477

High risk human papillomavirus genotypes detected in recurrent respiratory papillomatosis by Linear array and NSG.

## **27. HPV and oropharynx / Head and neck cancer**

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### **Background / Objectives**

Recurrent Respiratory Papillomatosis (RRP) is a benign illness, mostly associated with low risk papillomavirus types 6 and/or 11. This papillomatosis is a public health issue representing an economic burden both to the public health system and the patients. The objective of this work was to identify any other HPV types in larynx biopsies, besides the formerly reported types 6 and 11.

### **Results**

We collected 30 larynx biopsy samples from adult patients in the Otorhinolaryngology Service at the Hospital de Especialidades del CMN SXXI, Mexico City. Virus genotyping was done on DNA from each sample, by two techniques: 1) Sanger's sequencing of PCR amplimers from MY09/MY11 and/or GP5+/GP6+ primers; and 2) Roche Linear Array HPV genotyping kit. Also, in 20 out of the 30 samples we used the next generation sequencing analysis in a DNA sequencing platform (NSG).

### **Conclusion**

By Sanger's sequencing we identified HPV-6 in 18 samples and HPV-11 in 12 samples. By Linear Array we got the same results, except that four of the samples were co-infected: one with HPV types 6 and 11; two with HPV 6 and 16, and one with HPV 11 and 16. In the 20 samples sequenced by NSG, we found that nine samples were infected with high risk HPV types: six samples with HPV-16, five samples with HPV-58 and four samples with HPV-31; all 20 of them were co-infected with other high and low risk HPV types.

## **References**

We identified some high risk HPVs (HPV types: 16, 58 and 31) in addition to HPV6/11 prototypes in larynx biopsies. The prevalence of the HPV-6 and/or HPV11 in all the RRP samples analyzed, as well as the coinfection with HPV-16 in some cases, allows us to suggest that the administration of the quadrivalent prophylactic vaccine would be useful at least in the patients with aggressive and recurrent forms of laryngeal papillomatosis in their therapy.

## **28. HPV and associated skin diseases**



00621

Development of G-quadruplex mediated HPV antiviral drugs

## 28. HPV and associated skin diseases

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### **Background / Objectives**

Human papillomaviruses (HPVs) are causative agents of benign and malignant lesions of the genital tract [1]. Most HPV-related cancers are due to oncogenic HPVs namely types 16, 18, 31, 33 and 45 [1]. Until now, several G-rich sequences were found in the HPV genome that can form stable G-quadruplexes (G4). Some of them include high-risk HPV types, responsible for the majority of cases of cervical cancer [1]. Viral G4s are usually located in regulatory regions of the genome and are implicated in the control of key viral processes [1]. G4 ligands have mediated mechanisms of action at the genome and transcript level in latent infections promoting a way for cutting-edge therapeutic approaches in the treatment of HPV related cancer [2]. Therefore, we report the screening of ligands in terms of their ability to recognize G4 found in HPV genomes. The ligand C<sub>8</sub> was evaluated in terms of transcription, replication and viral proteins production.

### **Results**

Human cervical keratinocyte lines stably maintaining HPV DNA (HCK16-5 and HCK18C) were grown in monolayer culture using E medium in the presence of mitomycin C-treated J2 3T3 feeder cells. J2 3T3 feeder cells were grown in DMEM medium supplemented with 10% FBS and gentamycin. HPV organotypic raft cultures were then established. The raft cultures were then fed by diffusion from below with E medium supplemented with 100, 250 and 500 nM C<sub>8</sub> or DMSO alone as a solvent control. The raft cultures were allowed to stratify and differentiate for 20 days.

### **Conclusion**

HPV G4s were shown to possess a high degree of polymorphism upon ligand binding and stabilization which may have impact on transcription, replication and viral proteins production. We evaluated the antiviral effect of C<sub>8</sub> in raft epithelial cultures infected with episomal high-risk HPVs 16 and 18. Treatment with increasing concentrations of C<sub>8</sub> had a pronounced thinning of the raft of HPV18 comparing to the control. The

quantification of total viral genomes showed a decreasing number of HPV18 genome copies, from  $4 \times 10^5$  to  $7.6 \times 10^3$  genomes/ $\mu$ L at 250 nM of C<sub>8</sub>. The exposure of cervical cells to C<sub>8</sub> concentration of 250 nM resulted in a 10-fold decrease in HPV18 viral titers. This data suggests that while C<sub>8</sub> has a lower effect on viral genome amplification, it may play a role in viral genome encapsidation.

## References

In summary, our results propose original binding modes of several ligands towards high- and low- risk HPV G4s structures which have never been reported before, using a variety of different techniques. These G4 structures were found within regions involved in transcription, replication and viral proteins production. The antiviral effect of C<sub>8</sub> was confirmed on organotypic raft cultures containing replicating HPV18 and C<sub>8</sub> was able to decrease the viral load by several orders of magnitude.

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## **29. Genital warts**

00407

## ANOGENITAL WARTS IN PREGNANCY

### 29. Genital warts

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#### Background / Objectives

Anogenital warts are benign proliferative lesions caused by human papillomavirus (HPV) infection. Approximately 90% are associated with HPV types 6 and 11. During pregnancy, genital warts tend to rapidly enlarge and multiply. Eradication or reduction of lesions is the primary goal of treatment, mainly to prevent physical obstruction to vaginal delivery and reduce the transmission to the sexual partners and fetus.

#### Results

Descriptive study of 61 medical files of pregnant patients with macroscopic genital warts followed and treated at Obstetrics A Department of CHUC between 2001 and 2017.

#### Conclusion

The average age of our women was  $28.9 \pm 5.6$  years. 4.9% (n=3) of patients were referred in the first, 41.0% (n=25) in the second and 54.1% (n=33) in the third trimester of pregnancy. 32.1% of the pregnant patients were multiparous and 28.0% were smokers. The average number of previous sexual partners were  $2.8 \pm 1.8$ . Reasons for referral were: genital warts in 80.3% (n=49), vulvar itching in 4.9% (n=3), abnormal cervical cytology in 4.9% (n=3) and cervical lesion in 9.8% (n=6).

A histological study was obtained from 52 patients, confirming condylomata acuminata.

In the 9 remaining patients, the evaluation was deferred until after delivery, due to advanced gestational age. As for the treatment (n=44), surgical excision was performed in 50.0% (n=22), trichloroacetic acid (TCA) in 47.7% (n=21),

electrocautery in 36.4% (n=16) and silver nitrate in 9.1% (n=4). 18 patients were submitted to more than one different procedure. 3 patients were submitted to cervical loop electrosurgical excision procedure due to extensive cervical condylomatosis, in the first trimester of pregnancy. Of the 61 patients, 4 were submitted to cesarean section due to extended genital warts (3 with cervical and 1 with vulvar warts) obstructing the birth canal, without any previous treatment (all referred at the end of pregnancy).

Over the studied years, our report showed no trend in the incidence of anogenital warts in pregnancy.

## **References**

Surgical excision was the preferred treatment for genital warts. As vaginal delivery does not seem to increase the risk of laryngeal papillomatosis, cesarean section was reserved for cases of large genital warts that might obstruct the birth canal or result in heavy bleeding.

## **30. Sexually transmitted diseases and HIV infection**

00132

RETROSPECTIVE COHORT STUDY OF MEN WHO HAVE SEX WITH MEN THAT ARE INFECTED BY THE HUMAN IMMUNODEFICIENCY VIRUS AND WERE SUBMITTED TO HUMAN PAPILLOMAVIRUS SCREENING IN THE ANAL CANAL.

### **30. Sexually transmitted diseases and HIV infection**

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#### **Background / Objectives**

Human papillomavirus (HPV) infection is the most prevalent sexually transmitted infection in European and North American countries. It causes significant morbidity in women, men who have sex with men (MSM) and immunocompromised. Squamous cell carcinoma (SCC) of the anus is the most common non-AIDS-defining epithelial neoplasia in human immunodeficiency virus (HIV)-positive patients, which is associated with HPV infection. The aim of the study was to characterize anogenital HPV morbidity in HIV-infected patients through polymerase chain reaction (PCR) and/or anatomopathological examination of associated anal canal lesions.

#### **Results**

Retrospective 8-year study of HIV-infected male patients submitted to HPV DNA analysis on the anal canal by PCR. Population was characterized according to the following parameters: demographics, antiretroviral therapy (ART), HIV viral load and CD4+ T cells count at the moment of specimen collection, subtypes of identified HPV, biopsy result when performed and prescribed treatment. Data were obtained consulting the patients' clinical process and were treated statistically using Microsoft Excel 2016®.

#### **Conclusion**

Of the 71 patients undergoing screening, HPV was identified in 85.9% (n=61). The average age was  $41.8 \pm 15.8$  years old. 88.5% (n=54) were under ART and 73.7% (n=45) had undetectable HIV viral load. The mean CD4+ T cells count was 559 cells/mm<sup>3</sup>. The most frequent subtype was HPV-16, identified in 29.5% (n=18), followed by HPV-6, HPV-11 and HPV-53 found in 27.9% (n=17), 24.6% (n=15) and 23% (n=14), respectively. In 24.6% (n=15) of the screened patients only one HPV subtype was identified and in 19.7% (n=12) more than 5 HPV subtypes were identified in the same patient. Biopsy was performed in 41% (n=25): in 24% (n=6) condylomas were identified, 20% (n=5) had no changes and in 20% (n=5) had evidence of SCC. There were 32.8% (n=20) of patients submitted to treatment, and of these 50% (n=10) did topical application of imiquimod cream, 30% (n=6) electrocautery and 20% (n=4) had surgical excision. Tumor-related mortality was of 3.3% (n=2).

## References

Our results emphasize the importance of screening the presence of HPV infection in HIV-infected patients, especially in MSM. However, we highlight the small number of patients in whom the presence of HPV in anal canal was investigated, which reinforces the importance of creating an internal referral protocol and a systematic investigation of the virus in this population. In this way, it is intended to reduce the incidence of neoplasia and consequently associated morbidity and mortality.



00145

## HIV INFECTED PREGNANT WOMEN DATA COMPLETENESS FROM STATE OF ESPIRITO SANTO - BRAZIL PUBLIC DATABASE

### **30. Sexually transmitted diseases and HIV infection**

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#### **Background / Objectives**

The completeness of HIV infected pregnant women data registered in Notifiable Diseases Information System (SINAN) is important to provide accurate information for a better definition of their epidemiological characteristics. Thus, it becomes possible to offer action planning subsidies in order to control mother to children HIV transmission. This study, therefore, aimed at assessing the completeness of HIV infected pregnant women data reported in Espírito Santo, southeastern Brazil between 2007 and 2012.

#### **Results**

It is a descriptive analytic study based on secondary data of HIV infected pregnant women registered in Notifiable Diseases Information System (SINAN), in Espírito Santo State, southeastern Brazil between 2007 and 2012. The scores used were: excellent (variable shows less than 5% of incomplete coverage), good (5% to 10%), fair (10% to 20%), poor (20% to 50%) and very poor (50% or higher). The linear trend equations were calculated for incompleteness over time, with a 5% statistical significance. Data were grouped according to the information type: information in the notification, pregnant woman, residence, prenatal, delivery and newborn.

#### **Conclusion**

495 HIV infected pregnant women were reported in the SINAN. There were serious gaps in the completeness of the SINAN database of HIV-infected pregnant women in the state of Espírito Santo. There was higher frequency completeness as fair, poor and very poor in prenatal care, delivery, and newborn information. There were two variables with a downward incompleteness trend: municipality where prenatal took place (value of  $R^2 = 0.697$ ;  $p = 0.039$ ) and the Unified Health System card number (value of  $R^2 = 0.916$ ;  $p = 0.003$ ). On the other hand, the delivery health unit ( $R^2$  value =  $0.761$ ;  $p = 0.023$ ) had an upward incompleteness trend.

## References

The results of this study indicated an essential data high non-completeness regarding prevention information on mother-to-child transmission of HIV. We suggest continuing education and training for the notifier professional to make them able to record all data and accurate information in order to support the control of mother-to-child transmission of HIV.

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**Table 1. Non – completeness frequency by field and classification according to Romero & Cunha regarding data on HIV infected pregnant women notified between 2007 and 2012 in the State of Espírito Santo - Brazil.**

<b>Fill fields (n = 495)</b>	<b>N</b>	<b>%</b>	<b>Classification</b>
<b>NOTIFICATION</b>			
Notification date	0	0,0	Excellent
State of notification	0	0,0	Excellent
City of notification	0	0,0	Excellent
District	0	0,0	Excellent
Health Unit of notification	0	0,0	Excellent
Gestational trimester of notification	40	8,1	Good
<b>PREGNANT WOMAN</b>			
Name	0	0,0	Excellent
Birth date	16	3,2	Excellent
Pregnant Woman Mother's name	10	2,0	Excellent
Age	17	3,4	Excellent
Race	17	3,4	Excellent
Schooling	121	24,4	Poor
Employment	378	76,4	Very poor
Telephone number	186	37,6	Poor
National Health Card	391	79,0	Very poor
<b>RESIDENCE</b>			
State of residence	0	0	Excellent
City of residence	0	0,0	Excellent
District of residence	0	0,0	Excellent
Neighborhood	17	3,4	Excellent
Street	15	3,0	Excellent
Postal Address Code	378	76,4	Very Poor
<b>PRENATAL</b>			
Held prenatal	11	2,2	Excellent
Pregnant Woman on SISPRENATAL	375	75,8	Very Poor
State of prenatal assistance	107	21,6	Poor
City of prenatal assistance	46	9,3	Good
Health Unit of prenatal assistance	120	24,2	Poor
HIV laboratory evidence	11	2,2	Excellent
Date of HIV diagnosis	11	2,2	Excellent
ARV use during prenatal assistance	62	12,5	Fair
Initial date of ARV use during prenatal assistance	157	31,7	Poor
<b>DELIVERY</b>			
Delivery date	0	0,0	Excellent
Delivery city	118	23,8	Poor
Delivery health unit	249	50,3	Very Poor
Delivery type	131	26,5	Poor
AZT use during delivery	128	25,9	Poor
<b>NEWBORN</b>			
Reproductive Outcome	124	25,1	Poor
AZT prophylaxis in newborn (hours)	153	30,9	Poor

N: Absolute Frequency; %: Relative Frequency.

00315

Epidemiology of HPV anal infection in person with HIV attending a Sexually Transmitted Infection Clinic in Brazil

### **30. Sexually transmitted diseases and HIV infection**

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#### **Background / Objectives**

Background: HIV-positive patients have significantly more anal cancer than the general population. It is estimated that 75 to 80 percent of sexually active people will acquire a genital tract infection by HPV before age 50. Objective: To estimate the prevalence and persistence of anal infection, genotype distribution and correlates with anal HPV infection in HIV-infected patients attending a Sexually Transmitted Infections (STI) clinic in Vitoria, ES.

#### **Results**

Methods: Cross section was performed including demographic and behavioral assessment. Anal specimens were collected for cytology and for HPV testing by PCR followed by reverse line blot analysis for genotyping and sequence alignments for HPV16 variants determination.

#### **Conclusion**

Results: A total of 223 patients were enrolled for the study, 143 females and 80 males. Mean age was 40.6 years and the average education was 9.1 years. A total of 31.8% initiated sexual activity before 15 years old and 73.1% reported anal sexual activity. Previous STI was reported by 67.9% patients; 61.8% had undetectable viral load and 81.5% were taking antiretroviral therapy (ART). The prevalence of anal abnormal cytology was 29.3% and 20.8% had abnormal anoscopy. The prevalence of HPV infection of any type was 68.0 %, and high-risk HPV types were 50.2%, among then, HPV 16, 51 and 52 were the most frequent. A total of 38.8% individuals had multiple HPV types. The HPV16 European variant was the most frequent (71.4%). There was persistence of 52.3% of anal HPV infection of the same type in patients who have the collection in three consecutive visits. Twenty-nine patients had positive biopsy for anal intraepithelial neoplasia (AIN) (18.3%).

## References

Anal HPV infection was common among HIV-infected persons. Anal cytology screening for HIV-infected, particularly for those with anal HPV infection and history of STI, will increase the likelihood of detecting anal intraepithelial neoplasia.

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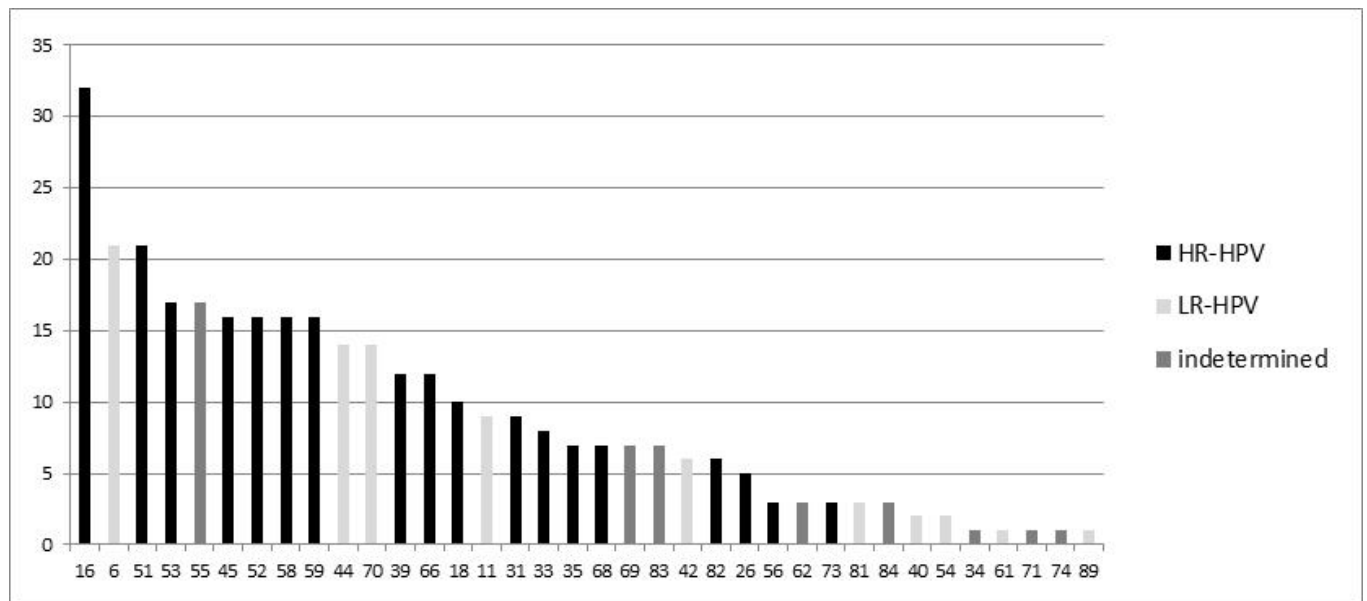
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00483

Papanicolaou (PAP) smear changes and cervical HPV prevalence in women living with Human Immunodeficiency Virus

### **30. Sexually transmitted diseases and HIV infection**

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#### **Background / Objectives**

Women living with Human Immunodeficiency Virus (WLHIV) currently experience longer life expectancy due to antiretroviral therapy. They are at greater risk for human papillomavirus (HPV) infection and persistence, increasing the risk of abnormalities in the cervical cells and invasive cervical cancer. Although the incidence of HIV-defining cancers has declined, cervical cancer has remained high among WLHIV. Our goal is to characterize PAP smear results and HPV prevalence in HIV-positive women in follow-up on Gynecology appointment of Centro Hospitalar e Universitário de Coimbra between 2013 and 2017.

#### **Results**

Analysis of clinical records. All women who had at least one appointment and one PAP smear between 2013 and 2017 were included. HPV test was performed by COBAS System. The statistical analysis was made with SPSS.

#### **Conclusion**

There were 232 WLHIV observed between 2013 and 2017. Among these, 11,6% (n=27) had co-infection with hepatitis C virus (HCV), 1,3% (n=3) with hepatitis B virus (HBV) and 0,4% (n=1) had a triple infection (HIV, HCV and HBV). They were in average 46,6 [18-80] years-old on the first visit and 61,2% (n=142) were pre-menopausal. Prevalence of HPV infection was 15,5% (n=36). Other types of high-risk HPV including 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (OHRHPV) were presented in 91,7% (n=33) of women; among these, 15,2% (n=5) were co-infected

with HPV16 and 9,1% (n=3) with HPV18. Only 8,3% (n=3) of women tested positive only for HPV16.

Cervical smear was NILM in 67,2% (n=156) of women; 3,2% (n=5) tested positive for HPV [1,9% (n=3) for OHRHPV and 1,3% (n=2) for HPV16]. ASC-US were presented in 15,1% (n=35) of women. In this group, 34,3% (n=12) were HPV positive: 25,7% (n=9) for OHRHPV, 5,7% (n=2) for HPV16 and OHRHPV and 2,9% (n=1) for HPV16 alone. PAP smear revealed LSIL in about 16,4% (n=38), 47,4% of which (n=18) were HPV positive. All of them had OHRHPV and 33,3% (n=6) were co-infected with HPV16 or 18. HSIL at pap smear was present in only 0,9% (n=2) of women and only one was positive for OHRHPV. In this period one woman had a diagnose of epidermoid carcinoma of the cervix.

Logistic regression analysis showed HPV infection to be associated with smoking and a higher number of sexual partners ( $p<0,01$ ).

## References

The prevalence of HPV in our population of WLHIV is lower than in other studies reported in literature. Almost all women with positive HPV had OHRHPV serotypes and the serotype less prevalent was HPV18. The prevalence of HPV is increased in higher grade lesions and HPV infection is associated with smoking and a higher number of sexual partners in this population.

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## **31. Conventional therapies**

00158

## PLACENTAL GROWTH RESTRICTION IN HIV-INFECTED WOMEN AS A SIGN OF EARLY PREGNANCY AGGRESSION

### 31. Conventional therapies

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#### Background / Objectives

There are few studies on placental growth disorders including HIV-infected pregnant women. Most of them use less than optimal growth assessment and are confined to one or two placental measures at a time, mainly to placental weight and placental-to-fetal-weight ratio. It is important to search for growth disorders in a comprehensive set of placental measures in HIV-infected women to improve the quality of care. This study aimed to determine the occurrence of placental growth disorders in low income, antiretroviral users, HIV-infected pregnant women attending a public hospital in Brazil.

#### Results

Out of 250 cases of HIV-infected pregnant women with terminations between 2001 and 2012 in a public tertiary University Hospital in Vitória City, Espírito Santo State, Brazil, we selected singleton pregnancy cases with ultrasound validated gestational age (GA) in which there was a comprehensive set of at birth measures: fetal weight (FW), placental weight (PW), placental-to fetal weight ratio (PFR), chorionic plate area (PA), and mean thickness (PT). These dimensions were converted to z score for GA and categorized as small (s), adequate (a) and large (l) for GA by the  $-1,28 < z < +1,28$  usual criteria. The frequency of growth disorders was calculated for each individual measure and for all possible combination.

#### Conclusion

109 cases met the inclusion criteria. Some growth disorders were observed in 51 (58,6%) cases: FW, PW, PFR, PA, and PT were SGA in 20,2%, 22,9%, 10,1%, 34,9% and 5,5%, and LGA in 4,6%, 0%, 42,2%, 0% and 11,9%, respectively. SFW only occurred in 9,17% and small placental growth disorders only (any combination) in 26,6%. Out of 22 SFW, there were 54,5% SPW, 63,6% SPA, and 9,1% PT, but

among 87 non-SFW there was an additional 14,9% SPW, 27,6% SPA, and 4,6% SPT.

## References

In this HIV-Infected Pregnant women casuistry, growth restriction occurred more often in the placenta than in the fetus and mostly in the form of a small placental area than from small placental thickness, pointing to an early (I and II trimester) aggression.

Small Gestational Age Placental Measurements in SGA Fetal Weight Distribution



SGA – Small for Gestational Age; SFW – Small Fetal Weight; SPW – Small Placental Weight;  
SPA – Small Placental Area; SPT – Small Placental Thickness

00424

## OBSTETRIC OUTCOMES AFTER EXCISION OF THE CERVICAL TRANSFORMATION ZONE - A RETROSPECTIVE 8-YEAR STUDY

### 31. Conventional therapies

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#### **Background / Objectives**

Most of the pre-invasive cervical disease and early cervical cancer occur in women at the childbearing age, and the local conservative therapies, usually the excision of the cervical transformation zone (ETZ), result in sequels that are a concern for future pregnancy. As a consequence of this technic the cervix becomes shorter, which may lead to a higher risk of obstetric complications, such as preterm delivery.

Objectives: To evaluate the impact on the obstetric outcomes in women submitted to cervical ETZ due to pre-invasive cervical disease and early cervical cancer, in Madeira Islands.

#### **Results**

Descriptive retrospective longitudinal study of the obstetric outcomes in women with previous cervical ETZ, between 2009 and 2017, in Hospital Dr. Nélio Mendonça (HNM). Several variables were collected from the patient files: age, reason of the procedure, histological result and size of the cone, parity, number of abortions, pregnancy complications, weeks of pregnancy and method of delivery. All the ETZ were carried out with diathermic loop or by the classical method.

#### **Conclusion**

There were 29 women with a previous cervical ETZ that became pregnant, counting a total of 31 pregnancies. The mean age at ETZ was 32 years old and 33 years old during pregnancy. From the sample 37% were nulliparous. The main reason for the procedure was a cervical cytology with a high-grade squamous intraepithelial lesion. Regarding obstetric outcomes, 45% had a delivery at term, 10% a pre-term delivery, 13% a spontaneous abortion, 13% a voluntary termination of pregnancy, 3% an ectopic pregnancy and 16% were still pregnant at the end of the study. The rate of preterm birth was 18%. The mean cone length in women with birth at term was 9 mm



and 13 mm in the pre-term group. Lastly, 76% had a vaginal delivery and 24% had a caesarean delivery.

## **References**

As already described in other studies, women with this type of procedure have an increased risk of pre-term delivery, and therefore should be considered as a high-risk pregnancy, mostly if the ETZ cone is longer. Our results are consistent with the literature, since the overall rate of preterm deliveries on our hospital is 6%, and in women with ETZ we demonstrated a higher rate. Therefore is important to assess the cervical length at the beginning of the pregnancy to stratify the risk of pre-term delivery in these women.

00426

## 3-YEAR STUDY OF EXCISION OF THE CERVICAL TRANSFORMATION ZONE IN WOMEN IN MADEIRA ISLAND – ARE WE MEETING THE QUALITY INDICATORS?

### 31. Conventional therapies

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#### **Background / Objectives**

Excision of the transformation zone (ETZ) is indicated in a variety of reasons, that can range from an excisional procedure of a malignant lesion, to discordance between the cytology and the colposcopy results. This procedure has not only a diagnostic purpose, as in some cases it consist on the treatment. As an invasive procedure it is associated with some complications, the most frequent is haemorrhage from mild to severe, and also can lead to stenosis or cervical insufficiency.

Objectives: Characterize the woman submitted to cervical ETZ on Hospital Dr. Nélio Mendonça (HNM), in Funchal, from 2015 to 2018.

#### **Results**

Descriptive retrospective longitudinal study of woman submitted to cervical ETZ from July 2015 to July 2018, in the HNM. Several variables where collected from the patient files: age, risk factors for HPV (smoker status, sexual partners and age of first intercourse), the reason for the procedure, vaccination status, cytology, colposcopy findings, histological result, margins and size of the cone, and follow-up.

#### **Conclusion**

The total number of procedures was 151, and mean age was 38 years old. All the ETZ were carried out with diathermic loop or in some cases by the classical method. From the sample 13% were nulliparous, 25% were smokers or ex-smokers, the mean age of the first sexual intercourse was 18 years old, and in mean each women had a history of 3 sexual partners. The main reason for referral to the cervical pathology appointment was an abnormal result in the cervical cytology (15% LSIL, 37% HSIL and 21% ASC-H) and all of these women were submitted to a colposcopy previously to any treatment.

In most cases a biopsy was performed before ETZ (81%) and the main results were 46% cervical intraepithelial neoplasia (CIN) grade 2, 7% CIN grade 2/3 and 21% CIN grade 3. There was no registry of early complications from the ETZ. The main histological results from the cones were 35% CIN 2, 10% CIN 2/3 and 31% CIN 3. Also, were diagnosed 5 invasive cervical carcinoma (3%). 78% of the cone margins were free, and just 15% of the cones had no dysplasia. Lastly, 75% of these women initiated the vaccine against HPV.

## **References**

Cervical ETZ is an effective, simple and safe method for the diagnosis and treatment of premalignant lesions and early stage cervical cancers. It's important to have algorithms for the referral to this procedure, to minimize unnecessary ETZ and maximize the benefits for the women.

## **32. Economics and modelling**

00154

## **COST-EFFECTIVENESS OF THE NEWLY IMPLEMENTED HPV-BASED SCREENING PROGRAMME IN THE NETHERLANDS**

### **32. Economics and modelling**

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#### **Background / Objectives**

In 2017 the Dutch cervical cancer screening programme has switched from cytology to the HPV-test as the primary screening test, using five instead of seven lifetime screens. The aim of this study was to quantify both costs and the effects of this programme change.

#### **Results**

The microsimulation model MISCAN was used to calculate the number of screening tests, colposcopies, CIN/cancer diagnoses, cancer deaths, life years, QALYs gained and costs for the old and the new screening programme. Costs and effects were discounted annually by 4% and 1.5% respectively. Univariate sensitivity analyses were performed adjusting test characteristics of cytology, costs of screening tests, participation in HPV self-sampling and utility losses for false positive referrals.

#### **Conclusion**

The new programme reduces the cervical cancer mortality from 217 to 184 per 100.000 women simulated (-15%) and the incidence from 577 to 503 (-13%) compared to the old programme combined with a cost reduction of 20%, mainly caused by the reduction in number of screening rounds. Although the new programme results in 214% more referrals to colposcopy of women without CIN2+ (from 938 to 2,943 per 100.000 women simulated), it is still more cost effective (€3,497/QALY) than the old programme (€5,741/QALY). The new programme remained more cost-effective in all sensitivity analyses.

#### **References**

Although the new programme increases the amount of unnecessary referrals substantially, causing negative consequences for individual women, it decreases the cervical cancer incidence and mortality and reduces costs on the population level, making it a more cost-effective programme compared with the cytology based programme.

00166

## THE EFFECT OF AN HPV GENDER-NEUTRAL VACCINATION PROGRAM ON VACCINE HESITANCY

### 32. Economics and modelling

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#### Background / Objectives

In Denmark, access to public financed HPV (Human Papilloma Virus) vaccination for 12-years old females has been an option since 2009. Boys are not included in the program. From 2009 to 2013 vaccination rates were high (>70%) but in 2014, 2015 and 2016 the vaccination rates were low (34%-57%) which may reinforce the argument for inclusion of boys in the program.

In addition, the nine-valent (HPV9) vaccine is now the applied vaccine in the program. Therefore, we present updated modelling analyses of vaccination which take the reduced vaccination rates and the improved protection from the HPV9 vaccine into account.

#### Results

A dynamic HPV transmission cost-effectiveness model was calibrated to a Danish setting and used to estimate the incremental costs and effects associated with different vaccination strategies compared to screening only. In the modelling, the actual vaccination rates for the first 9 years (vaccination of 12-years old females only) were applied. That is, gender-neutral strategies with vaccination of 12-years old boys via a public financed program (i.e. vaccination rates higher than 5%) were included from year 10 and onwards. Among others, the incremental effects were estimated as number of avoided HPV-related cancers, pre-cancers and deaths.

#### Conclusion

The updated model simulations show that this recent observed vaccine hesitancy in the long run (100 years) markedly affect the number of avoided HPV-related cancers.

If the 2016 female vaccination rate of 34% is maintained, the number of avoided HPV-related cancers will decline with 33% compared to a scenario where the future

vaccination rate is higher (60%).

In the scenario with maintained low vaccination rates (34%), inclusion of boys in the vaccination program will increase the number of avoided HPV-related cancers with almost 17%. In the scenario with future vaccination rates of 60%, gender-neutral vaccination will increase the number of avoided HPV-related cancers with 12%.

The gender-neutral vaccination program will improve the protection in females and males as the number of avoided HPV-related cancers and deaths increase, especially for anal, penile and head & neck cancer.

## **References**

From a public health perspective, attention to the low vaccine coverage should be paid as it leads to an increasing number of HPV-related cancers and deaths. Initiatives that increase the vaccine confidence should be supported. In addition, gender-neutral HPV-vaccination should be considered as vaccination of boys increase the number of avoided HPV-related cancers, pre-cancers and deaths – especially when the female vaccination rates are low.



00485

## **COSTS AND HEALTHCARE RESOURCE UTILIZATION FOR CERVICAL CONIZATION IN MID-ADULT WOMEN IN THE UNITED STATES**

### **32. Economics and modelling**

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#### **Background / Objectives**

Cervical conization with Loop Electrical Excision Procedure (LEEP) or cold knife conization (CKC) is a standard treatment option for pre-cancerous cervical lesions caused by human papillomavirus (HPV). The estimated incidence for conization in mid-adult women is 1.7-1.8/1000, and the costs associated with these procedures are not well described and potentially preventable by HPV vaccination. We aimed to estimate healthcare resource utilization (HCRU) and costs of conization in the US.

#### **Results**

We performed a retrospective cohort study using the Truven MarketScan® database, a large healthcare claims database in the US. All mid-adult women (27-45 years old) who were treated from 2012-2016 by LEEP or CKC (CPT code 57522, 57520) or both LEEP and CKC within 30 days were included if they had continuous enrollment ≥6 months before and 1-month after the procedure. Exclusion criteria included pregnancy, cancer diagnosis, or immunocompromised status and invalid cost data (i.e. cost ≤ \$1 or 99th %ile of top costs). All-cause (i.e., medical claims for inpatient/outpatient visits and/or admissions and prescriptions irrespective of disease or procedure) and conization-specific HCRU and costs were estimated for the 30-days post-conization. Conization-specific costs included costs of admissions, outpatient visits, follow-up tests (pap smear, HPV test, colposcopy), and post-operative complications (hemorrhage, vaginal bleeding/discharge, fever, post-

procedural pain, scarring of the cervix, and dysmenorrhea). Costs were converted to 2017 US\$ using the medical consumer price index.

## **Conclusion**

Of 30,978 eligible mid-adult women with conization, 81.0% had LEEP, 17.1% had CKC, and 2.0% had both CKC and LEEP). Few women were hospitalized (0.10%), with mean length of stay of 1.5 (0.80) days. The mean (sd) number of outpatient visits were 1.1 (0.4) for LEEP and 1.2 (0.4) for CKC. Post-operative complications occurred in 5.9% women overall (8.4% (CKC) and 5.3% (LEEP)). Mean all-cause costs were \$3381, \$5169, \$2954, and \$5443 for patients with any conization, CKC, LEEP, and both CKC and LEEP, respectively. Mean conization-specific costs were \$1586, \$2344, \$1399, and \$2671 for patients with any conization, CKC, LEEP, and both CKC and LEEP, respectively.

## **References**

CKC is less frequent, more resource-intensive and expensive compared to LEEP. However, differences in patient characteristics could be related to type of conization procedure or predisposition for complications. HPV vaccination will reduce the incidence of pre-cancerous cervical lesions and should reduce the incidence and economic burden of conization.



00492

## INCIDENCE AND TREATMENT-RELATED ECONOMIC BURDEN OF HPV-RELATED CERVICAL, VULVAR, VAGINAL, AND ANAL CANCERS IN THE US

### 32. Economics and modelling

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#### Background / Objectives

Human papillomavirus (HPV) infection is the most common sexually transmitted infection in the US. Persistent infection with high-risk types can progress to cancer. Recent estimates of the economic burden of HPV in the US are not available. The objective of this study was to estimate the incidence and treatment-related direct medical costs of cervical, vaginal, vulvar, and anal cancers attributable to high-risk types targeted by the 9-valent (9v) HPV vaccine (16/18/31/33/45/52/58) in US.

#### Results

We identified newly diagnosed cervical (ICD-O-3 histology codes 8010–8671, 8940–8941), vaginal (ICD-O-3 site code C52.9), vulvar (ICD-O-3 site codes C51.0–C51.9), and anal cancer cases (ICD-O-3 site code C21.0–C21.9) in all persons age 9 and above from the National Cancer Institute's SEER (Surveillance, Epidemiology, End Results) cancer registry in 2015. Annualized incidence rates were estimated from SEER\*Stat software. Cancer sites were limited to squamous cell carcinomas (ICD-O-3 histology codes 8050–8084, 8120–8131). We applied published estimates of the proportion of each cancer site attributable to the 9vHPV vaccine-types to estimate incident cases attributable these types. Published lifetime costs of treatment for the cancers (two-year costs for cervical, vaginal, and vulvar cancer, and lifetime costs for anal cancer) were applied to estimate direct medical costs associated with the incident HPV-related cancer. Costs were inflated to 2015 dollars using US urban medical care consumer price index.

## **Conclusion**

The 2015 annualized incidence rate per 100,000 person-years of cervical, vaginal, vulvar, anal (female), and anal (male) cases was 5.85, 0.29, 1.42, 1.78, and 0.95, respectively (Table 1). The economic burden (2015 dollars) of cervical, vaginal, vulvar, anal (female), and anal (male) cancer was \$671 million, \$50 million, \$88 million, \$129 million, and \$71 million, respectively. Cervical cancer treatment costs accounted for 63% of the overall treatment costs of the 4 types of cancers. The economic burden of these cancers attributed to the 9vHPV vaccine types in 2015 dollars was over \$1.0 billion.

## **References**

Cancers attributable to 9vHPV vaccine types inflict substantial health and economic burden on society. The estimate of economic burden is conservative, as it does not include costs of prevalent infection (other than newly diagnosed cases), costs associated with screening and treatment of pre-cancers, indirect costs such as productivity losses due to cancer morbidity and mortality, and the burden of other HPV-related cancers (e.g., penile, oropharyngeal). Screening/HPV vaccination can help reduce the burden of these HPV-related cancers.

**Table 1: Incidence and economic burden of HPV-related cancers**

Age group	Incidence rate per 100,000 population for 9vHPV-related (16/18/31/33/45/52/58) cancers (2015)				
	Cervical cancer	Vaginal cancer	Vulvar cancer	Anal cancer - Female	Anal cancer - Male
09-15years	0.02	0.00	0.00	0.00	0.00
16-20years	0.03	0.00	0.00	0.00	0.00
21-25years	1.00	0.00	0.04	0.00	0.02
26-30years	5.47	0.00	0.09	0.00	0.17
31-35years	8.39	0.13	0.26	0.16	0.29
36-40years	10.28	0.05	0.46	0.35	0.41
41-45years	12.31	0.19	0.93	0.87	0.99
46-50years	10.86	0.36	1.73	2.41	1.47
51-55years	9.40	0.46	2.45	4.09	2.02
56-60years	9.58	0.68	2.52	5.97	2.63
61-65years	9.89	0.49	3.53	6.61	2.70
66-70years	8.21	0.91	4.04	7.06	2.75
71-75years	7.96	1.32	5.54	6.99	2.79
>75years	5.90	1.63	8.39	5.03	3.31
All ages	5.85	0.29	1.42	1.78	0.95
<b>2015 incident count for 9vHPV-related cancers (16/18/31/33/45/52/58)</b>					
All ages	8,554	359	1,552	2,509	1,377
Economic burden for each cancer (million \$; 2015 dollars)	\$671.5	\$49.5	\$88.2	\$129.2	\$70.9
Total economic burden all cancers (million \$; 2015 dollars)	<b>\$1,009.1</b>				

### **33. Advocacy, acceptability and psychology**

00081

## HPV KNOWLEDGE AMONG EUROPEAN ADOLESCENTS AND THEIR PARENTS: RESULTS FROM A SYSTEMATIC LITERATURE REVIEW

### 33. Advocacy, acceptability and psychology

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#### Background / Objectives

The implementation of an HPV vaccination program has been recognized as a priority by Public Health Authorities. However, parental and adolescent attitudes towards HPV vaccination seem to have a major role to succeed and achieve a significant reduction of HPV-related burden of disease. Despite vaccines have shown to be efficacious, effective and with an acceptable safety profile, their level of acceptance and the vaccination coverage rates of the national HPV immunization programs differ widely across European countries (between 17-84%). To our knowledge, there is not a complete European systematic literature review summarizing factors influencing HPV knowledge and vaccine acceptability among adolescents and their parents.

The aim of this systematic review is to identify factors associated to parental and adolescents' HPV knowledge in European countries where HPV vaccine is licensed. In addition, we summarize the results of studies evaluating parental and adolescents HPV knowledge and compile the measurement tools used in the published research studies in those countries.

#### Results

A systematic literature review was conducted through Pubmed, The Cochrane Library, Medline, EMBASE, Popline and the World Bank Library Network. Time period studied: January 1st 2006 to December 31st 2017



## **Conclusion**

2,118 publications were identified. After excluding duplicates and studies not fulfilling inclusion/exclusion criteria, a subset of 70 studies were finally included in the systematic review. These studies have been performed in 17 European countries: United Kingdom: 27.1% Italy: 14.3%; Sweden: 11.4%, Netherlands 10.0%; Germany (5.7%); Greece, Hungary, Romania and Spain (4.3%); Denmark and France (2.9%) and Iceland, Finland, Belgium and Austria (1.4%). 18 of them reported data results on HPV knowledge. The number of respondents to any of the items related to knowledge in the surveys used across the studies was 161,483 adolescents and 75,830 parents. Overall, 53.1% of adolescents and 54.2%% of parents had heard about HPV; 34.0% of adolescents and 63.5% of parents knew diseases related to this virus; and 40.1% of adolescents and 77.3% of parents knew that HPV is a sexual transmitted infection.

Main socio-demographic factors associated to higher knowledge of HPV were gender (being female) in four 4 studies and vaccination status in 3 studies. No matter how investigators asked about HPV knowledge, it is consistently observed that the percentage of female adolescents (16.4%-57.54%) knowing about HPV is higher than the percentage of males (8.1%-48.08%)

## **References**

HPV knowledge in parents and adolescents differs widely across regions and needs to be improved in order to increase vaccination coverage rates across Europe.

## **References**

This study has been funded by Merck Sharp & Dohme Spain.

00083

## HPV VACCINE ACCEPTANCE AMONG EUROPEAN ADOLESCENTS AND THEIR PARENTS: RESULTS FROM A SYSTEMATIC LITERATURE REVIEW

### 33. Advocacy, acceptability and psychology

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#### Background / Objectives

HPV is recognized as one of the major causes of infection-related cancer worldwide. Nowadays 3 HPV vaccines are available. Worldwide, HPV vaccination is part of the national vaccination programs of at least 80 countries. Moreover, HPV vaccination is recognized by WHO as a primary preventive intervention and recommends all countries to proceed with nationwide introduction of HPV vaccination. However, vaccine coverage rates differ widely within Europe and efforts to increase coverage rates and vaccine acceptability are still necessary.

The aim of this systematic review is to identify factors associated to parental and adolescents' HPV vaccine acceptance and summarize the results of these studies, in European countries where HPV vaccine is licensed.

#### Results

A systematic literature review was conducted through Pubmed, The Cochrane Library, Medline, EMBASE, Popline and the World Bank Library Network. Time period studied: January 1st 2006 to December 31st 2017.

#### Conclusion

2,118 publications were identified. After excluding duplicates and studies not fulfilling inclusion/exclusion criteria, a subset of 70 studies were finally included in the systematic review. These studies have been performed in 17 European countries:

27% in UK, 14% in Italy and 11% in Sweden, 14 of them reported data results on HPV vaccine acceptance.

Up to 63 factors have been identified as having a statistically significant association with HPV vaccine acceptance in at least one of the studies: 17 factors related to “sociodemographic/family characteristics/ factual conditions” such as educational status, age of the respondent, family situation, nationality, religion, ethnicity, gender of the child and childhood vaccination. In 3 studies or more, parents and adolescents believed that vaccines were effective in preventing diseases, HPV related diseases were severe and HPV vaccination protected against cervical cancer. These beliefs were positively associated to HPV vaccine acceptance. In addition, when the source of information was a healthcare professional (specifically, a physician), the probability to accept HPV vaccination was higher. In contrast, doubts about HPV vaccine safety profile and the believe that vaccine may encourage child to have more partners or unprotected sex, were negatively associated to HPV vaccine acceptance.

#### **References**

There is still an important opportunity to improve HPV vaccine acceptance across Europe. Well balanced information offered by healthcare professionals seems to be critical to attend parents and adolescents’ hesitancy.

#### **References**

This study has been funded by Merck Sharpe & Dohme Spain.

00109

The association between maternal history of cervical cancer and HPV vaccination of children

### **33. Advocacy, acceptability and psychology**

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#### **Background / Objectives**

HPV vaccination uptake remains low, with only 54% of U.S. children receiving at least one dose of the vaccine by age 13. Prior research has demonstrated that parental health beliefs and sociodemographic factors predict a child's HPV vaccination status. Less is known, however, about the relationship between maternal history of cervical cancer and children's HPV vaccination. The purpose of this study was to explore (a) whether mothers' personal history of cervical cancer predicted HPV vaccine uptake in their children and (b) whether this association may be mediated (i.e., explained) by greater perceived benefits of HPV vaccination.

#### **Results**

Data for this cross-sectional study were collected via an online survey conducted by Survey Sampling International. The survey targeted mothers or female guardians of children aged 9-13 living in the U.S. In a logistic regression model, we first estimated the effect of mothers' history of cervical cancer on maternal report of children's HPV vaccine uptake (i.e., at least 1 dose). Next, mothers' perceived benefits of HPV vaccination was included as a potential mediator of this relationship.

#### **Conclusion**

Of the 1,155 women (aged 18-81) providing data about cancer history, 70 reported that they had ever received a cervical cancer diagnosis. Children were 59% female, 41% male, and 32% had received one or more doses of vaccine. Maternal history of cervical cancer predicted greater odds of child vaccination (OR=2.65, 95% CI=1.87-3.74). Higher levels of perceived benefits of HPV vaccination also predicted greater odds of child vaccination (OR=2.78, 95% CI=2.34-3.30). When controlling for perceived benefits of vaccination, the association between maternal history of cervical cancer and child's vaccination status remained significant (OR=2.86, 95%

CI=1.68-4.87), suggesting that perceived benefits does not mediate the association between these two variables.

## **References**

While mothers with a history of cervical cancer were more likely to vaccinate their children against HPV, this association does not appear to be due to greater perceived benefits of HPV vaccination. Mothers' HPV knowledge and/or perceived severity of cervical cancer (not measured in this study) could possibly contribute to this relationship. The current study builds on existing literature showing the link between HPV and cancer as a motivating factor in mothers' vaccination decisions. Clinicians and researchers may want to discuss HPV vaccination in the context of personal narratives about HPV-related cancers as a way of highlighting and humanizing the severe adverse effects of non-vaccination.

00557

## Building Collaborations among HPV Vaccination Stakeholders: Examining the Impact of a National HPV Roundtable

### **33. Advocacy, acceptability and psychology**

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#### **Background / Objectives**

In 2014, the National HPV Vaccination Roundtable was established by the American Cancer Society and the Centers for Disease Control and Prevention to increase the uptake and completion of the HPV vaccine in the United States. One of the primary goals of the Roundtable is to increase the collaboration between immunization and cancer prevention organizations. A detailed social network analysis is being conducted to understand collaboration among Roundtable members.

Presentation objectives include:

- Outline the methodology used to assess the current collaborations
- Review findings about existing collaborations, quality of collaborations, and where there is opportunity for development for enriching collaboration
- Recommendations for how HPV Roundtables can impact collaboration

#### **Results**

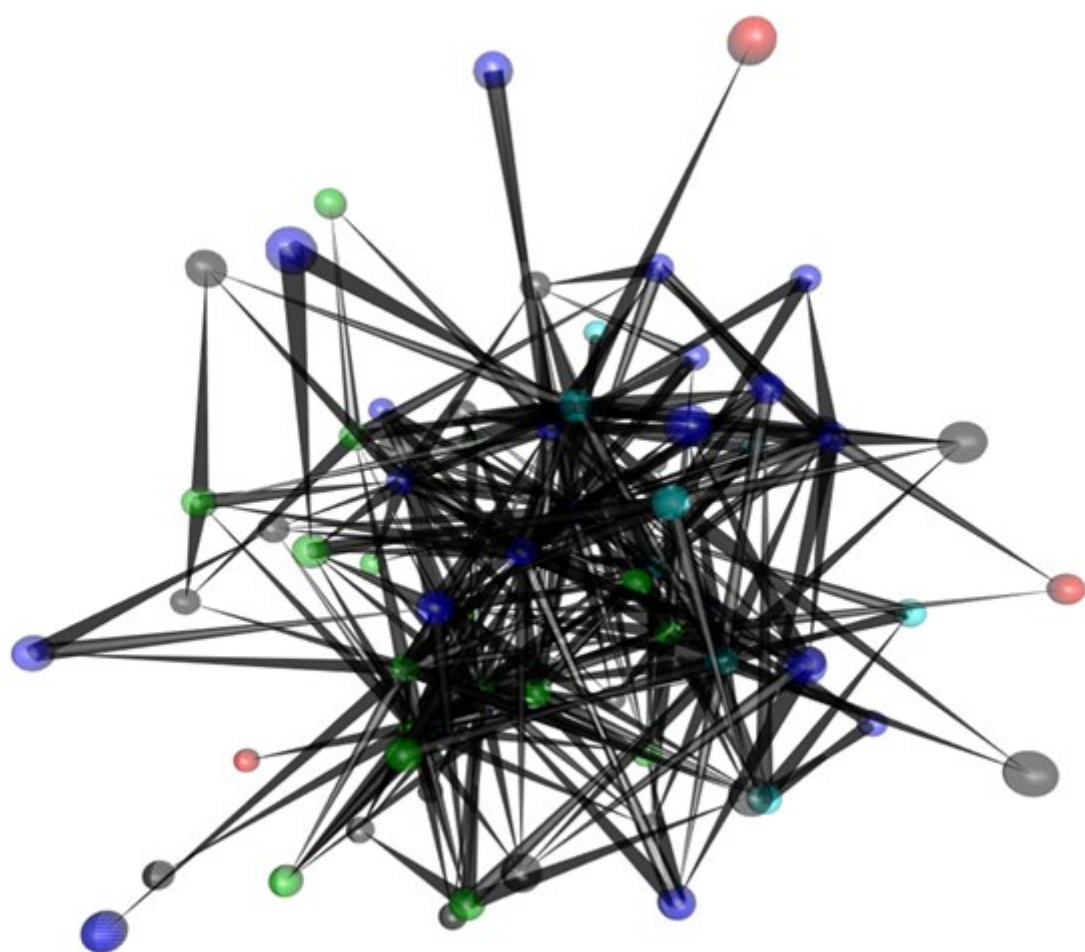
A detailed online survey was developed by a collaborative team based on existing assessments. The survey was distributed to 109 Roundtable members representing 72 organizations. Data was analyzed using SPSS, R, and NodeXL. Analysis included basic descriptive statistics and comparative analysis to examine the number of collaborations by type of organization (prevention or immunization) and by self-reported level of engagement on the roundtable. Social network analysis using R and NodeXL provided detailed information on model density, indegree, outdegree, and betweenness for each model. An overall network map was examined as well as network maps based on the depth of collaborations and the quality of collaboration scales.

#### **Conclusion**

A total of 74 respondents (67.9%) completed the survey, representing a total of 53 Roundtable organizations (73.6%). Data represented over 317 collaborative relationships. The network maps demonstrated that there were a few key organizations that had the highest degree of influence (indegree centrality) and are key information distribution points. Analysis by type of collaboration showed similar patterns of connection. Sociograms by type of collaboration found that organizations were more likely to collaborate with similar organizations than with other types. The average scale scores for quality and depth were relatively low and higher quality and deeper connections were found among similar organizations.

## **References**

This project has demonstrated that there are a high number of connections among HPV stakeholders, however there is need for expansion of collaboration across types of organizations and strengthening of existing collaborations. There are key hubs for information distribution and connection among other partners. Cancer prevention organizations have the highest levels of collaboration and are the most central in the sociogram. Immunization and advocacy organizations have the greatest potential for future development and collaboration strengthening.





## **34. Health education**

00257

## WILLINGNESS OF ORAL HEALTH STUDENTS TO TRAIN AND ADMINISTER THE HPV VACCINE IN THE DENTAL SETTING

### 34. Health education

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#### Background / Objectives

A 153-item online survey was administered to United States students in 15 oral health programs. Secondary data analyses were conducted in SAS Version 9.4. Unadjusted and multivariable logistic regression were conducted and odds ratios (OR), 95% confidence intervals (CI), and p-values ( $p < 0.05$ ) were reported.

#### Results

A 153-item online survey was administered to United States students in 15 oral health programs. Secondary data analyses were conducted in SAS Version 9.4. Unadjusted and multivariable logistic regression were conducted and odds ratios (OR), 95% confidence intervals (CI), and p-values ( $p < 0.05$ ) were reported.

#### Conclusion

Data from N=306 students were analyzed. Receiving HPV vaccination information from professional journals or publications was positively associated with WT and WA ( $p < 0.05$ ). Agreeing that HPV vaccination recommendation (OR=1.95, 95%CI=1.14-3.35,  $p=0.015$ ) and administration (OR=3.79, 95% CI=1.63-8.81,  $p=0.002$ ) is in the dental professional's scope was positively associated with WT and WA even when adjusting for other factors (not my role to recommend, not enough time to discuss, not comfortable discussing, previous patient communication about HPV). Those who saw 21 or more patients a week (OR=4.47, 95% CI=1.14-17.58,  $p=0.032$ ) and who agreed that HPV vaccine administration was in the dental professional's scope (5.9, 95% CI=2.27- 15.3,  $p < 0.001$ ) had higher odds of WA even when adjusting for other factors (not enough information, not my role, not comfortable discussing, previous communication about HPV).

## References

Engaging dental providers in HPV vaccine education and vaccine administration can reduce HPV oropharyngeal cancers, which have now surpassed cervical cancer rates in the United States. Professional guidelines and endorsement of HPV vaccination from professional organizations are needed to engage dental providers in HPV vaccination efforts.

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00408

## INSIGHTS ON HPV VACCINATION IN THE UNITED STATES FROM MOTHERS' COMMENTS ON FACEBOOK POSTS IN A RANDOMIZED TRIAL

### 34. Health education

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#### Background / Objectives

HPV vaccine uptake among adolescent girls in the USA remains below the national goal of 80%. Parent decisions to vaccinate daughters can be impeded by confusion, uncertainty, and misinformation about the HPV vaccine. This analysis summarized mothers' beliefs about vaccinating their adolescent daughters for HPV expressed in comments to posts on HPV vaccination in a social media campaign on adolescent health.

#### Results

Mothers with adolescent daughters from 34 U.S. states (n=880) were recruited to participate in a randomized controlled trial evaluating a social media campaign on adolescent health. Participants were recruited through Qualtrics survey panels or local efforts at the Tennessee study site. Eligibility criteria were: having a daughter aged 14-17, living in one of 34 states without a complete ban on indoor tanning for minors, using a Facebook account 1+ times a week, being able to read English, consenting to participate, completing the baseline survey, and willing to join the Facebook group. The campaign, implemented through Facebook private groups, included posts on HPV vaccination, as one of 7 general health topics. The experimental manipulation varied posts on indoor tanning versus prescription drug abuse prevention. Posts on HPV vaccination (n=16) and reactions and comments from mothers were extracted.

#### Conclusion

Mothers had a mean age of 43.1 years (sd=6.6); 6.5% were Hispanic and 86.6% white; and 63.1% reported that their daughter had been vaccinated for HPV (17.8%

receiving two shots and 31.5% three shots). HPV vaccination posts received on average 1.3 reactions (sd=2.8; range=0-11) and 3.3 comments (sd=8.8; range=0-35) from mothers. Comments often formed a dialogue among mothers. More than half of the comments (52.8%, n=28) were favorable, indicating that the daughter had been vaccinated and HPV vaccination reduced mothers' anxiety, HPV infection rates, and related disease risk. However, 45.3% (n=24) were unfavorable, citing safety concerns, lack of efficacy, unknown long-term consequences, inappropriate age for the vaccine, apprehension by other mothers, fears of vaccine tampering, lack of physician support, and sexual activity issues (e.g., plans to wait until daughter becomes sexually active; using vaccine to guard against unprotected sex). Some commented, mostly favorably, on the need to vaccinate boys.

## **References**

Facebook comments indicated both support for and resistance to HPV vaccination by U.S. mothers. Reasons for not vaccinating girls were similar to barriers expressed in other research and reflected negative public information on HPV vaccination. Effective strategies are needed in social media to counter misinformation on and resistance to HPV vaccines.

## **36. Public health**

00117

## Issues Arising at Launch of Anti-HPV Mass Vaccination Campaign in 2018: Case of Estonia

### 36. Public health

**T. Raud, T. Raud**

**L. Padrik - Tartu (Estonia)**

#### **Background / Objectives**

On 1st of January 2018, Estonia began an anti-HPV mass vaccination campaign at schools, using a nine-valent vaccine which had been selected and acquired through public procurement. The target group is 12-year-old girls.

Prior to starting vaccination, Estonian Health Board organized in Nov 2017 a nation-wide immunization training, at which, among other topics were the questions pertaining to the HPV vaccine and vaccination.

The state's favourable decision on vaccination (Q4 2017) and especially the launch of the vaccination program (Q1 2018) galvanised the anti-vaccination activists, who began to disseminate propaganda aimed at discrediting the HPV-vaccine through several media channels. Parents started contacting infectologists and gynecologists with concerned questions.

The Estonian Society for Infectious Diseases (EIS) and the Estonian Colposcopy Society (EKÜ) decided to support the nurses and organized nation-wide training for them between the 22nd of March and 4th of April 2018. 45% of school nurses attending these training seminars had previously participated in the training organized by the Estonian Health Board.

#### **Results**

Prior to the training, all participants were requested to submit questions which they had encountered during the launch of the HPV vaccination. As there were questions about communication with parents, the organizational aspects of the vaccination, and the vaccine itself, the questions were divided into 4 groups.

1. Safety of the vaccine
2. Necessity and efficacy of the vaccine
3. Organizational issues
4. Communication with parents/anti-vaccination activists



## **Conclusion**

Communication issues constituted 58% of all problems and were divided as follows: communication difficulties with parents and/or anti-vaccination activists amounted to 64%, and the organizational issues of the vaccination amounted to 36%.

42% of all questions were directly related to the HPV vaccine, and these were divided as follows: questions about its safety amounted to 63% and efficacy to 37%.

## **References**

1. The need for communication has substantially increased due to significant anti-vaccination movement both in the world and in Estonia.
2. Before including a new vaccine to calendar, as well as continually throughout the vaccination program, school nurses require continuous and specific training about the new calendar vaccine. At the same time it is important to raise awareness among parents, school management, media, and even the medical professionals (GPs, gynecologists) who should support the vaccination
3. It is crucial to maintain continuous communication between the school nurses performing the vaccination and the government institutions.

In order to achieve good coverage of HPV vaccination, EIS and EKÜ intend to continue organizing joint training for school nurses.

00401

## Results from Ongoing Trials of Mobile Web apps to Improve HPV Vaccine Uptake

### 36. Public health

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#### Background / Objectives

In the U.S. uptake of HPV vaccine remains significantly below the Healthy People 2020 goal of 80% series completion, and this is particularly so for the young adolescent age range, when the immunogenic response to the vaccine is stronger. While a number of factors may account for this less than desirable vaccine uptake, parental concerns and misinformation about the efficacy and safety of HPV vaccine remain barriers to reaching public health vaccination goals. Physician and clinic-based interventions have shown some limited positive effect on vaccine uptake. However, parental barriers to HPV vaccination may ideally be addressed by digital interventions (in this case, smartphone applications) that are tailored to their concerns. Specifically, research indicates there is a great deal of 1) confusion and uncertainty about HPV vaccine and 2) concomitant misinformation about HPV vaccine, who it is meant for, and the conditions under which it is maximally effective. Reported here are the developmental research results and early trial findings from two smartphone web app projects, one focused on parents and adolescent girls (ages 11-14) and the other on parents and adolescent boys in the same age range. The objective of these investigations is to develop and evaluate a mobile web app to encourage HPV vaccination in New Mexico, an ethnically-diverse U.S. state.

#### Results

With funding from the Patient Centered Outcomes Research Institute (PCORI) and the National Cancer Institute in the United States, our team systematically developed a set of mobile web app tools to prompt the informed adoption of HPV vaccination. We used Diffusion of Innovations Theory and related research on Informed Decision Making to guide the iterative development of mobile apps for parents of young female and male adolescents.

## **Conclusion**

Our presentation will review the website (Vacteens.org) and present development, baseline and preliminary efficacy trial data from the studies. Current ongoing randomized controlled efficacy trials with parents and their adolescent children in New Mexico clinics provide data to determine the impact of these mobile web apps on informed decision making and uptake for the HPV vaccine.

## **References**

The progress and initial results of these ongoing research efforts have implications for reaching HPV vaccine uptake goals set by Healthy People 2020 in the United States. The presentation will focus on how mobile web-based interventions show promise for reaching HPV vaccine uptake goals. A mobile web app can make decision-making tools widely available on popular mobile platforms such as tablet computers and smartphones as well as personal computers.

00429

# RECALL AND PATIENT NAVIGATION TO INCREASE CERVICAL CANCER SCREENING AMONG UN-/UNDER-SCREENED WOMEN IN A U.S. SAFETY NET HEALTHCARE SYSTEM: INTERIM RESULTS

## 36. Public health

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### Background / Objectives

Patient reminders are a strategy recommended by the U.S. Community Preventive Services Task Force to increase cervical cancer screening. However, the utility of this approach among women who are past-due for screening (i.e., patient recall) is mixed. The purpose of this study is to evaluate the effectiveness of a centralized telephone-based patient recall and navigation intervention to increase clinic-based cervical cancer screening among patients enrolled in a large, urban public safety net healthcare system in the U.S. that serves a predominantly low-income racial/ethnic minority population.

### Results

Women age 30-65 years who had not had a Pap test in  $\geq 5$  years (i.e., past-due for screening) were identified using the electronic medical record. A trained, bilingual (English/Spanish) patient navigator (PN) reviewed charts to assess additional eligibility criteria (i.e., no history of hysterectomy/cervical cancer, current healthcare plan coverage). Patients in the intervention group were contacted by telephone, educated about cervical cancer screening, and offered direct booking of an

appointment. Program effectiveness was assessed using an “intent-to-screen” approach by comparing Pap test screening rates between 01/18/18 and 05/31/18 among patients in the intervention group versus 220 randomly-selected historic controls.

## **Conclusion**

During the specified period, the PN reviewed charts of 2,385 of 13,914 (17%) patients identified as past-due for screening. Of these, 30% (n=729) met the eligibility criteria for the intervention. Of 729 patients in the intervention group, 60% were reached by telephone. Among those reached, 41% scheduled a Pap test with the PN. Of the scheduled appointments, 31% were attended, 52% were not attended, and 16% were used to address another health concern. The overall Pap test completion rate among women in the intervention group was 14.7% compared to 8.3% among historic controls (absolute difference = 6.4%,  $p < 0.01$ ; incidence rate ratio = 1.8,  $p < 0.001$ ).

## **References**

While Pap screening in a safety net healthcare system was increased 1.8-fold with the implementation of this telephone-based patient recall and navigation intervention, the absolute increase in Pap test screening remained small (6.4%). Of note, almost 70% of PN-scheduled Pap tests were not attended or used to address other health concerns. Further studies should explore strategies to eliminate barriers to appointment attendance among under-screened women, including potential alternatives to clinic-based screening (e.g., mailed self-sample HPV testing kits).

00510

## Evolution of gender-neutral HPV vaccination in National Immunization Programs around the world

### 36. Public health

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#### Background / Objectives

Human papillomavirus (HPV) infection carries high disease burden in both genders, accounting for 5% of total cancers for males and females, the most common being oropharyngeal and cervical cancers. More countries are expanding HPV vaccination to males in National Immunization Programs. We aim to describe the evolution and status of HPV gender-neutral vaccination (HPV-GNV) worldwide.

#### Results

A comprehensive search using official government websites was conducted, complemented by a literature review (2008-2018) including publications in English, Spanish, and Portuguese describing male HPV vaccination programs.

#### Conclusion

Twenty-six countries with HPV-GNV were identified; 10 in North/South America, 13 in Europe, and 3 in Middle-Africa/Asia-Pacific. HPV vaccination for males was first recommended as routine immunization in the US and Puerto Rico in 2011. Czech Republic was the most recent to include males for the nonavalent vaccine in early 2018. Antigua directly adopted an HPV-GNV program in 2017. The target age for

male vaccination varies from 9 to 26 years according to each country's recommendation and predominantly focuses on adolescents. Delivery locations vary by country, including schools and/or medical centers. In 2018, Germany and UK are evaluating expanding HPV vaccination to males; Sweden is awaiting final decision. In addition to HPV-GNV programs, several countries have elected to include GNV only among men who have sex with men.

## **References**

Increasingly, countries are expanding HPV vaccination to include males to improve population-level HPV infection control and directly prevent HPV-related disease in males. HPV-GNV has accelerated, with more than 50% of countries adopting such recommendations in the last 3 years.

00610

## ADHERENCE TO HPV VACCINATION IS ASSOCIATED WITH PARTICIPATION IN CERVICAL CANCER SCREENING – A DANISH NATIONAL REGISTER-BASED COHORT STUDY

### 36. Public health

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#### Background / Objectives

Since the introduction of human papillomavirus (HPV) vaccination, concerns have been raised that HPV-vaccinated women would perceive themselves as fully protected against cervical cancer (CCU) and therefore not participate in CCU screening. Non-participants in CCU screening have a higher risk of mortality caused by CCU than participants. On this background we aimed to examine the association between HPV vaccination adherence and CCU screening participation to target future interventions to high-risk groups.

#### Results

In a national register-based cohort study, we included women born in 1993, representing the first birth cohort that was offered free of charge HPV vaccination in the Danish childhood vaccination program. Participants were residents in Denmark during 1.10. 2008- 31.12. 2016.

Exposure: Minimum one dose of HPV vaccine before end of 2015. Outcome: Minimum one cervical cytology after the age of 23 years before end of follow-up 31.12. 2016.

Primary outcome: Likelihood of non-participation in CCU screening among non-HPV-vaccinated women compared with vaccinated women. Socioeconomic factors were used to adjust for confounding.

Individual level information was collected from national health registries using the unique Danish civil registration number. Simple logistic regression was used to



estimate the association between participation in HPV vaccination and screenings. Multiple logistic regression was used to adjust for confounders.

## **Conclusion**

A total of 24,841 women were included in the study population of which 21,687 (88%) were HPV-vaccinated. Among HPV-vaccinated women, 13,075 (60%) were screened compared with 1,496 (47%) of the non-vaccinated group. Non-vaccinated women were more likely not to participate in CCU screening than vaccinated women (adj. odds ratio (OR)=1.6;95% CI 1.5-1.7). This association was more outspoken among women from non-western countries (adj. OR=3.7; 95% CI 3.3-4.2) than among native Danes.

## **References**

Non-participation in CCU screening among the youngest women may be a matter more of general behaviour and attitude towards health promoting offers than of interpretation of HPV vaccination as being fully protective against CCU. In order to overcome a social gap in the prevention of CCU, health promoting initiatives addressing certain groups may be beneficial. Especially, it seems necessary to understand how to target women with lower social status and women from non-western countries.

00627

## PAPPILOMAVIRUS TRANSMISSION AND PREVENTION AND EFFECTIVE DISINFECTION

### 36. Public health

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#### **Background / Objectives**

Papillomavirus transmission studies have typically used cell-free virus prepared in organotypic raft culture, or recombinant pseudo-virus isolated from monolayer 293TT cells. Virus infection has been investigated by quantitating viral gene transcripts after infecting reporter cells. During natural in vivo infection however, virions are shed from the epithelial surface in squames, and the successful transmission/infection results in the new lesion formation.

#### **Results**

Viral transmission and survival was analysed using mouse papillomavirus (MmuPV) in a in vivo and in vitro model, in conjunction with in vitro infection of reporter cells using HPV16/18 raft virus. The output for the in vitro model for both MmuPV and HPV was quantitation of the E1<sup>E4</sup> transcript using RT-qPCR. The in vivo model utilised RNAscope to identify lesion formation microscopically and visual inspection for lesions macroscopically.

#### **Conclusion**

Similar to HPV16/18, MmuPV1 virus titre/infectivity can be quantified as virus gene transcripts by RT-pPCR (E1<sup>E4</sup>) or RNAscope (E6E7) using HaCaT cells as a reporter cell. Virus titre/infectivity can be also quantified as periods of lesion formation in vivo model with 5-6 log dynamic range. Up to 10 million virus particles can be produced from the surface layers of productively infected tissue and transmitted by direct contact, with an approximate one log drop in infectivity if transmission is mediated indirectly on fomites. Electron microscopy shows that MmuPV1 is E4 fibre-associated, and is stable following desiccation, with minimal loss of titre on fomites over 6 months. In contrast, cell-free MmuPV1 virion is not stable with loss of titre on fomites within 8 weeks.

## References

MmuPV1 in vitro and in vivo infection models using natural virus produced from productive infected lesion are powerful methods to investigate HPV as it seems to mirror the characteristics of HPV making it a useful model to investigate transmission and susceptibility of HPV. Importantly, this model has demonstrated different viral shedding patterns (cell-free or in squames) can show different virus infectivity especially following desiccation. Additionally, this model can be used to assess the safety of HPV disinfection and has been utilised to demonstrate the utility of OPA in stark contrast to other studies.