The paradigm shift in cervical cancer diagnosis: from cytology to effective risk stratification

KEY MESSAGES

- The presence of cervical cancer in Africa and South Africa is alarmingly high.
- Cervical screening should be done in all women over the age of 30 years and in HIV positive women from the age of 25 years.
- The ideal is to introduce co-testing with HPV so that HPV testing is used in combination with an abnormal Pap smear to improve risk assessment and diagnosis.
- Where good cytology services are not available, HPV testing with the identification of HPV 16 and 18 should be used to identify high-risk women for referral for colposcopy.
- HPV testing is the test of choice for evaluating cure post LLETZ and Laser biopsy.
- An HPV negative result means that no further testing is needed for a 3-5 year period.
- Effective HPV vaccination will diminish the pool of HPV infection in South Africa and then our cervical cancer screening systems can move to using HPV testing as the prime and only screening method.

Commentary

This presentation reflects on the paradigm shift in cervical cancer prevention and diagnosis with the evolution of molecular HPV testing and its incorporation with accompanying evidence-base into cytology-based screening programmes for cervical cancer.
THE PARADIGM SHIFT IN CERVICAL CANCER DIAGNOSIS: FROM CYTOLOGY TO EFFECTIVE RISK STRATIFICATION

20 YEARS WITHOUT RCT FOR THE “PAP” TEST TO BE USED ROUTINELY IN NORTH AMERICA AND EUROPE FOR SCREENING FOR CERVICAL CANCER

- In 1928 - noticed cancer cells on vaginal smears of women using his “Pap” smear
- The understanding of the significance of these cells as a diagnostic modality was not made for many years
- Dr. Herbert Traut (O&G) collaborated with him in 1941 to develop a technique of scraping cells off the cervix and by 1943 they published their findings
- 1949/1950 First mass screening

Commentary
In 1928, Papanicolaou serendipitously noticed abnormal ‘cancer’ cells on the vaginal smears of women when he using his “Pap smear technique” which was developed in guinea pig studies of the menstrual cycle. The clinical significance of his observation was however only realised some 20 years later following collaboration with Dr Herbert Traut, an Obstetrician Gynaecologist. This collaboration, without the benefit of any randomised clinical trials, led to the clinical introduction of the pap smear. From 1943, this technique was used routinely in the United States and Europe with the first mass cervical cancer screening initiative taking place in 1949.

>80 year evolution of our knowledge

Commentary
This heralded an enormous evolution over the next 80 years in the field of cervical cancer culminating in the introduction and approval of 2 HPV Vaccines, Gardasil (Merck) and Cervix (GSK), also with availability in South Africa in 2008.
Cervical cancers in constrained countries

**Commentary**
The prevalence of cervical cancer in Africa and South Africa is alarmingly high with late presentation and poor survival being a common feature of our environment.

Components of a Cervical Screening Programme

**Commentary**
The Cervical screening programme works well if all components work well together; every part of the service must be integrated, with Cytology or HPV screening services being integrated with the other components of the service such as colposcopy and patient management.
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Screening in private practice

![Graph showing opportunistic screening in Southern Suburbs of Cape Town (2008)](image)

**Abbreviations**
- **ASCUS**: Low Grade squamous intra-epithelial lesions with atypical squamous cells
- **LSIL**: Low grade squamous intra-epithelial Lesions (similar to CIN I)
- **HSIL**: High Grade Squamous intra-epithelial Lesions (similar to CIN II and III)
- **HPV**: Human Papillomavirus detected.

**Commentary**
In my private practice where women are screened frequently, detection rates of cervical cell abnormalities and cancer are low. Yet there are unfortunate failures of screening which occur despite evaluation by dedicated technologists. This occurs because the cytology test is a subjective test without zero defects.

**Reasons for failure of screening**

![Image of reasons for failure of screening]

**Commentary**
The main reasons for failure of screening are:
1. Are not getting a Pap smear,
2. Not following up on an abnormal smear and the least common reason,
3. Is failure to either smear the lesion or misdiagnosing the changes seen on the smear.
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Pap smear: Sensitivity and specificity of a single smear

PAP SMEAR (CONVENTIONAL SMEAR): SENSITIVITY AND SPECIFICITY OF A SINGLE SMEAR

- 30% - 87% Sensitivity
- 86% - 100% Specificity

Technologist training
Screening quotas
Quality control

3 meta-analyses:
Am J Epidemiology 1995
Ann Int Med 2000
Evid Rep Technol Assess 1999

Commentary
The Pap smear started falling out of vogue in the late 1990s mainly as a result of 3 major meta-analyses that showed sensitivity varying between 30-87%. This could be ascribed to three main factors: technologist training, screening quotas and quality control. The specificity of the test has always been high: 86%-100%. In good cytology services where the above three factors are well controlled; the cytology service can work very well.

There is no zero defect but...

THERE IS NO ZERO DEFECT BUT...

- Thin Layer Cytology (LBC) to standardise cytology preparation
- HPV Testing
- Automation in Cytology
- All improve sensitivity of screening
- HPV vaccines can significantly reduce the incidence of cervical cancer

Commentary
There is no zero defect scenario in the cytology screening process but the introduction of Thin Layer Cytology (LBC), HPV testing and automation in cytology have all improved the sensitivity of screening. It is however only HPV vaccination which can significantly reduce the incidence of cervical cancer.
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Advantages of LBC

- More representative sample with random distribution of abnormal cells
- Ancillary tests-HPV possible on a single sample
- Screening area reduced with most cells in one 10x focal plane → reduced screening time
- Cells better preserved and not obscured by blood, mucus, or inflammatory cells
- Infectious organisms retained and better preserved
- Smears ideal for automated cytology

A Standardised Smear is Obtained

Commentary

The Liquid Based (LBC) smear is much easier to evaluate than a conventional smear. There are a number of advantages including a standardised sample and the ability to do ancillary tests, such as HPV, on a single sample.

NICE (National Institute for Clinical Excellence) in the UK has recommended liquid-based cytology and they are no longer using the conventional Pap smear. There has been a movement worldwide to use this technology which is ideal for automated cytology systems.

Arbyn meta-analysis lancet oncology

Commentary

A comparison in industrialised countries of the sensitivity of conventional cytology to Human papillomavirus (HPV) testing in primary cervical screening has shown that HPV testing was significantly better. There is no doubt about that. These studies compared primary high-risk HPV testing followed by cytology triage for women who were HPV DNA positive as compared to only conventional cytology screening and showed that the former strategy was clearly more effective than conventional cytology triage.

But research in the UK by Kitchener HC et al. showed that HPV testing was only marginally more sensitive than Liquid-based cytology (LBC) systems if performed in good laboratory practice environments with good training of technologists in place, the cytology load is not too heavy (about 30 slides per day) and where audit processes are in place.
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Proposed national cervical cancer prevention policy for South Africa

PROPOSED NATIONAL CERVICAL CANCER PREVENTION POLICY FOR SOUTH AFRICA - ASSUMPTIONS

- Screening older women (> 30 years) is the most cost-effective
- That the widest possible coverage of the targeted population is more important for the prevention of cervical cancer than the interval of screening
- Longer screening intervals, up to 10 years using cytology, have been shown to reduce cervical cancer by 2/3’s in well organised national programmes with built-in call and recall (IARC estimate)
- Cervical cancer is an AIDS defining illness in an HIV positive patient

Commentary
In South Africa, the proposal is to screen only older women above the age of 30 years as this is the most cost-effective strategy. Also to obtain the widest possible coverage and to screen women once every ten years as IARC estimates have shown that this will reduce the number of cervical cancer cases by two-thirds if you screen all women and call and recall appropriately.

South African national screening proposal

SOUTH AFRICAN NATIONAL SCREENING PROPOSAL

- WHERE FACILITIES ARE APPROPRIATE and cervical cytology infrastructure is functional cervical cytology will be used. Where cytology is not functional HPV testing will be used
- Asymptomatic women should be offered 3 free screens in a lifetime beginning at age 30
- If HIV status is known and is positive, screening should begin at age 25 and as long as is normal should be repeated 3 yearly.
- HPV Vaccination to be instituted in 2014 - first roll out presently to 9 year old girls through the school vaccination programme

Commentary
Furthermore where cytology services are appropriate and functional cervical screening is available, this option should be continued. “We do have relatively good cytology services in South Africa where they are available.”

Where no cervical screening facilities are available, then HPV testing and screening is the option of choice. Where HIV status is known and the women is HIV-positive, screening should start earlier, from the age of 25 years as Cervical Cancer is an AIDS defining illness.

In HIV-positive women screening should be done every three years from the age of 25 years. HPV vaccination is rolling out in South Africa and will change the whole area of cervical cancer.
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Commentary
In private practice in South Africa, just under one million women are screened and this has changed very little over the last 5 years whereas in the Public sector there has been a huge drive to screen, resulting in an enormous increase in numbers. In private practice, LBC is the smear system of choice and triage of abnormal smears with HPV is happening in all private laboratories. “In my view, the time is now right to introduce co-testing with HPV for all private sector patients”

Cytology of HPV is all encompassing

CYTOLOGY OF HPV IS ALL ENCOMPASSING!

- Bethesda system of cytology reporting:
  - Negative for intraepithelial lesion or malignancy (NILM)
  - Benign Reactive changes (Benign Cellular Changes- BCC)
  - Atypical squamous cells of undetermined significance (ASCUS)
  - Low grade squamous intraepithelial lesion (LSIL)
  - High grade squamous intraepithelial lesion (HSIL)
  - Squamous cell carcinoma (Malignant)
  - Atypical squamous cells, cannot exclude high grade lesion (ASC-H)
  - Atypical glandular cells (AGC)

Commentary
The reason for advocating this approach is that we know HPV causes abnormalities in cells. So where HPV is present on the Pap smear, we see the whole spectrum of cytological abnormalities as described in the Bethesda system of grasing.
Abbreviations
LSIL - Low grade squamous intra-epithelial Lesions (similar to CIN 1)
ASC-H -
AGC -
ASC-US - Low Grade squamous intra-epithelial lesions with atypical squamous cells
HSIL - High Grade Squamous intra-epithelial Lesions (similar to CIN II and III)

Commentary

HPV causes disease in <25% of women infected

Commentary
The problem is that there is an enormous volume of HPV infection, but the tip of the iceberg is disease. HPV is frequently present but causes disease in less than 25% of cases. This means that the presence of HPV is not a good predictor of disease and this is one of the greatest problems with HPV screening and testing because it is sensitive to the infection, but not specific for the disease.
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Appropriate evidence based utilisation of HPV testing

- Triage of Cytological abnormalities
- Primary Screening
- Test of Cure (6 months post LLETZ biopsy)
- Exit from Screening
- Management of difficult cases
- Surveillance of vaccinated population

**Commentary**

1. The evidence-base has shown that HPV testing is extremely useful in the following situations:
2. For triage of cytology particularly in cases of ASCUS where there are significant abnormalities, but not necessarily HPV related
3. As Primary screening where no cytology systems are in place, such as in Africa
4. As a test of cure, 6 months or a year after LLETZ biopsy. There is abundant evidence for this approach
5. To Exit from screening
6. Management of difficult cases and
7. Surveillance of a vaccinated population.

FDA approved tests

- **Hybrid Capture 2 (Digene)** has been the gold standard until recently but the main problem is analytical inaccuracy due to cross reactivity of the probe cocktail and lack of an internal control
- **Cervista HPV HR (Hologic)** cannot determine the specific HPV genotype and potential cross reactivity
- **Aptima HPV assay (Gen-Probe)** its advantages are it does not show cross reactivity, and is equally sensitive to HC2 but more specific. There is only a cohort of 950 pts
- **Cobas 4800 HPV (Roche)** with the recently published ATHENA trial has the largest population cohort of 47,000 pts

**Commentary**

There are at present four FDA approved tests:

The first test, Hybrid Capture 2 (Digene) was the gold standard but a problem developed with analytical inaccuracies due to cross-reactivity. This also complicated the use of Cervista HPV HR (Hologic).

Currently there are 2 tests available, Aptima mRNA test (Gen-probe), a newly available test that shows no cross-reactivity problems and is equally sensitive to Hybride Capture 2. It currently only has data on some 1000 patients.

The Cobas 4800 HPV test (Roche) has the largest patient cohort of experience, some 47000 from the ATHENA trial and is the system I am currently using in my laboratory.
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Emerging and lucrative market

ALL MANUFACTURES WILL CLAIM THEIR TEST IS BEST AS THIS IS AN EMERGING AND LUCRATIVE MARKET. AREAS OF CONCERN IN HPV TESTING:

- **SENSITIVITY**: One does not want to miss HR HPV - No test can claim 100% sensitivity, even the best methods reach the glass ceiling of 95%.
- **GENOTYPING**: HPV 16 and 18 have been shown by the ATHENA trial to be significantly more pathogenic than the other high risk positive test and therefore need to be reported separately.
- **COST**: Used as a Co-test with the pap smear the initial cost is increased but will save the patient in the long run.
- **SPECIFICITY**: How accurately does the result of HPV correlate with the pap smear / biopsy finding - The answer is poorly. All tests have specificity of these tests is 26% but the pap smear is at least 51%. That is why the HPV TEST IS NOT USED FOR SPECIFICITY, the PAP smear or biopsy (or more recently the use of p16 on cytology or histology to separate LSIL from HSIL) is the gold standard.

Commentary

All manufacturers will say their test is the best. However, the evaluation criteria are:

1. **Sensitivity**: If we are using the concept of high risk HPV evaluation, we need to be sure that the test does not miss any cases. The best HPV test has a sensitivity approaching 95%; this is much better than any cytology system.
2. **Genotyping**: We need to know if HPV 16 and 18 are present, as this increases risk and quickens the disease process.
3. **Cost**: The HPV test must be cost-effective particularly if we are going to use co-testing which in my view is the ideal.
4. **Specificity**: All HPV tests have a low specificity, as low as 26%, which is much lower than the Pap smears specificity of 51%. This means that the HPV test cannot be used for specificity and the Pap smear and biopsy remain the gold standard. There is a drive now to use biomarkers and particularly HPV 16 presence as the additive factor to improve specificity for disease and this aspect will be addressed in depth in the next talk by Professor John O’Leary.

The value of knowing if HPV 16 and 18 are positive

Commentary

What is the value of knowing if HPV 16 and 18 are positive?

In the natural history of HPV infection there are some women who move from a normal Pap smear to a high grade lesion within a two year period, a very aggressive progression. From the work of Zur Hausen, we now understand this more fully and appreciate the role of HPV 16 and HPV 18 positivity in this rapid progression to cervical cancer. This data comes primarily from the ATHENA trial and has lead to the development of a very important risk stratification algorithm.
Women at similar risk for CIN3+ should be managed similarly

Commentary
It is incredibly useful to have co-testing particularly as a risk stratifier with a Pap smear. In this risk stratification, if you have a negative Pap smear and a negative HPV test; testing need not be done for another three or five years. This underscores the value of co-testing.

If the woman has some evidence of pathology, e.g. is Pap smear negative but other types of HPV are present, then referral to colposcopy is warranted for CIN 2 or 3.

What is it that we really want by introducing HPV testing for screening?

Commentary
1. A sensitive HR HPV test is wanted as we do not want to miss any cases.
2. Must Provide HPV Genotyping-type 16 and 18.
3. An accurate Pap smear report that will tell us how far the disease has progressed.
4. An affordable HPV test is needed so that co-testing is possible.
5. Appropriate utilization of p16 and p18 positivity to assess the potential for progression from L SIL to HSIL lesions on cytology and histology.
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**Commentary**
It is the HPV E7 product that blocks the tumour suppressor gene and this negative feedback causes amplification of the p16 gene in the cell which is then picked up on testing.

**Biomarker utilization for risk stratification**

**Commentary**
Using the p16 positivity, also helps us to tease out the CIN 2 where there is a lot of inter-and intra-observer -variability and allows closer definition of LSI and HSI.
Good evidence for use of HPV as a test of cure

GOOD EVIDENCE FOR USE OF HPV AS A TEST OF CURE

Arbyn M et al 2005: Gynecol Oncol; 99:97
- Positive HPV test even in presence of no up treatment failures early & accurately

Kitchener H et al 2008: BJOG; 115:10
- Women who are cytologically normal and HPV negative can safely be returned to 3 year screening at 6 months – introduced in Sentinel sites

Horn A et al 2009: BSCC Dublin
- Edinburgh data: A single HPV test in first year after treatment is as effective as 2 HPV tests

Commentary
There is plenty of evidence for the use of HPV testing for determining cure.2,7-9

Stenosis post LLETZ / laser

STENOSIS POST LLETZ / LASER

Commentary
Also to conduct ongoing screening Post LLETZ and Laser procedures, as there is often stenosis of the cervix and evaluating encryption of CIN in these lesions can be very difficult.
Effective strategies moving forward to risk stratify and prevent cervical cancer

Commentary
This slide summarises the aspects discussed and proposes the way forward for South Africa.

"Once the pool of HPV infection diminishes, following wide-ranging and successful HPV vaccination, the use of HPV testing as the prime and only cervical cancer screening method will be feasible for all vulnerable women in both the public and private sector in South Africa," doctor Whyntaker concluded.

References

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