Enhancing patient management and advancing the power of HPV testing through extended genotyping and automation

BD Symposium at the EUROGIN 2019, Monaco.

Introduction by Jeff Andrews, MD, Fellow of the Royal College of Physicians and Surgeons of Canada, Worldwide Medical Director for Women's Health and Cancer, BD

"The World Health Organisation has declared that we can eliminate cervical cancer as a public health problem through intensified vaccination against HPV, screening and treatment. This has been echoed by the European Commission, which has recommended in the ECCO European Cancer Summit, 2019 that by 2030, effective strategies to eliminate cancers caused by HPV as a public health problem should be implemented in all the European countries. The WHO recommendation is expected to be made public at the IPVC conference at the end of March and voted on in April 2020.

The ultimate goal of the WHO is to bring down the death rate due to cervical cancer and to lower the incidence to 4 per 100,000 or less. Some Western European countries are close to that and the rest can achieve it. Eastern Europe has a greater challenge and Africa has a tremendous gap to fill.

When we screen women, we know that 1.2% of them have high grade cervical intraepithelial neoplasms (CIN). It is a challenge to find which woman has the disease and we don't want to do colposcopy on too many of them. For cervical cancer screening and triage, there is HPV, cytology and genotyping. Our tests are the BD Onclarity™ HPV assay and

the BD SurePath™Liquid-based Cytology. Our new instrument, the BD COR™ addresses the concerns of countries moving to primary HPV screening where there is a demand for high-throughput automation. We manage abnormal screening results with colposcopy, HPV screening, cytology and genotyping. Persistence is critical as it is persistent infection with the same HPV genotype that is the pre-requisite for the development of high-grade CIN and cervical cancer."

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The interplay of cytology and HPV testing with genotyping for disease detection and patient management

Mark H Stoler, MD, Professor of Pathology, Cytology, and Surgical Gynecology, University of Virginia Health System, USA

In his presentation, Dr. Stoler began by summarising the results of the clinical trial of the BD Onclarity™ assay for human papillomavirus (HPV) screening and extended genotyping in the US.[1] The baseline data of this trial has provided information about the demographics, cytology findings, histopathology and HPV genotype of the participants. It has also offered evidence supporting genotype-specific HPV screening. Dr. Stoler went on to describe the longitudinal data collected over the three years of the study. The data further demonstrates the clinical validity of the assay and provides more evidence of the usefulness of extended genotyping in managing patient risk.

Description of the BD Onclarity™ US clinical study

- A 3-year longitudinal study conducted at 31 sites.
- 33858 subjects enrolled.
- ASC-US triage subjects: 1960 women >=21 years of age, median age 34 years, with ASC-US cytology
- Primary screening population: 29633 women, >= 25 years of age.
- Co-testing subjects: 22383 women, ages >= 30.
- Two samples were taken from each subject – SurePath™ (liquid-based cytology and Onclarity™) and ThinPrep™ (HC2/ sequencing).
- All women with ACS-US+ or HPV+ were referred to colposcopy. Colposcopists were blinded to HPV and cytology results. A random subset of cytology-negative and HPV-negative women was referred to colposcopy for verification-bias analysis.
- Endocervical curettage (ECC) and biopsy performed for all lesions.
- Pathology review – blinded, with p16 staining in LAST-criteria cases.

Results of the study

- Baseline pathology results show that CIN2+ is seen in 1.2% of overall colposcopy results (224 CIN2 and 173 CIN3 out of 6052). Among the women of the relevant age group (age >=25) CIN2+ is seen in 6.3% and CIN3+ in 3.1% of the colposcopy results.
- The risk of CIN3+ for HPV 16+ subjects after three years is 25% higher than the baseline average risk (18.7% vs 14.2%), while that for HPV 18+ subjects is 53% higher (8% vs 5.3%). Similar increases are seen for all HPV+, HPV 16/18+ and for ASCUS/HPV+ subjects, indicative of an increased risk of CIN3+ due to the persistence of infection by a genotype.
- Compared to the baseline values, the sensitivity of the testing algorithm over the three years is seen to increase from 72.3 to 85.8 (HPV primary) and from 53.7 to 73.1 (co-testing). The HPV 16/18 screening algorithm attains the highest sensitivity (closest to the BD Onclarity™ assay sensitivity for CIN3+) over the three-year period, compared to the two other testing strategies.
- Correspondingly, an increase is also seen in the number of colposcopies/detected CIN3, between baseline and 3-year follow up values (11.2 to 18.3 in HPV primary, 11.8 to 15.1 in ASCUS triage and 11.8 to 17.7 in co-testing). This suggests that the initial baseline sensitivity drives most of the clinical decisions and renders many of the follow-ups inefficient, as more and more colposcopies detect less disease.
- The BD Onclarity™ trial data for extended genotyping shows that women testing positive for HPVs 16, 18, 31 and 33/58 have the highest baseline risk (4% or above) of developing CIN3+, irrespective of the initial cytology results. This agrees with other studies that have shown that these genotypes represent the highest odds of high-risk cytology.[2]

- HPVs 16, 18, 31, 33/58, 45 and 52 are also associated with an increased risk of cervical cancer (0.02% and above). HPVs 45 and 52 are interesting, as the baseline risk of CIN3+ associated with them is much lower than 4%, suggesting that genotypes evolve in different ways over the long term. A 20-fold CIN3+ risk discrimination is seen between HPV 16 (19.9%) and 56/59/66 (0.5%), translating to a 100-fold cervical cancer risk discrimination (0.298% for HPV 16 and 0.003% for 56/59/66)
- The 3-year follow up data has helped confirm the baseline risk of CIN3+ and the stratification of genotypes based on it. For patients with normal cytology, HPVs 16 and 31 pose the highest risk; 18, 33/58, 52 and 45 pose a moderate risk; 35/39/68, 51, and 59/56/66 pose the lowest risk. The same trend holds for patients with abnormal cytology, who are associated with higher risks of CIN3+. The cumulative risk of CIN3+ over the long term is also higher for HPVs 16, 18 and 31. Combined with cytology results, extended genotyping and 'risk banding' can help establish a better CIN3+ risk profile, enabling better clinical decisions on whether a patient should be sent for colposcopy or treatment.
- The data for the baseline sensitivity for patients >=25 years of age shows that including the HPV 31 along with 16 and 18 in the HPV primary screening increases the sensitivity of the testing algorithm (76.3% for 16/18 vs 85.6% for 16/18/31(unadjusted numbers)). As the specificity and colposcopy rates are similar, this implies that including the HPV 31 in the screening algorithm helps detect more disease without the added specificity burden. Over the long term, adding HPV 31 results in a bump in sensitivity. However, over the same time, there is only a marginal increase in the colposcopy/CIN3+ ratio, suggesting problems with specificity over the long term.
- Persistent infection, including post-treatment persistence with the same oncogenic HPV genotype, is a pre-requisite for the development of CIN2/CIN3 and cervical cancer. The BD Onclarity™ study data shows that patients with CIN2/CIN3 have a much higher genotype-specific persistence (>= 75%) within disease categories than those with CIN1 or normal cytology (approx. 15-18%). Patients who have persistence of an HPV genotype between the baseline and year 1 have a 35% progression rate to CIN2+ than those with HPV persistence (8%), new infection (7%), clearance (0.5%) or no infection(2.2%). Genotype persistence is also responsible for the increase in CIN3+ risk with the persistence of 16, 18 and 31 showing the highest levels of increase.

Key messages

- The longitudinal data reinforces the usefulness of the risk stratification of genotypes through the confirmation of the trends of baseline CIN3+ risks.
- Risk stratification through genotyping at baseline drives the tradeoff between which patient needs to go to colposcopy and which needs to have her CIN3+ found and treated. Proper triage with genotyping can help solve the problem by eliminating most of the disease at the first round of the algorithm so that genotyping can be done on follow up to reduce colposcopy that finds less and less disease.
- Genotyping combined with persistence tracking through surveillance can help improve risk discrimination and reduce the number of colposcopies/CIN3.

Risk-based primary HPV screening using genotype information – The evidence behind and the design of algorithms

Jesper Bonde, Ph.D., Dipl.Med.Sci, Dept. Pathology and Clinical Research Centre, Copenhagen University Hospital, Denmark

Dr.Jesper Bonde's presentation was about the value of using genotype information in screening for HPV. He spoke about how the different genotypes posed different risks, and on studies that stratified the genotypes into groups based on the

risks. Dr. Bonde went on to describe how genotyping could be included in screening algorithms to make cytology triages more effective. Doing so would benefit patients by reducing the number of colposcopies and biopsies.

Key points of discussion

- An important consideration in favour of genotyping is that many women today have been vaccinated by the 4V vaccine, which is effective only against 4 genotypes.[3] This means that 11 high-risk genotypes have not been included.
- Genotypes 16 and 18 are responsible for most cancers. Other genotypes present lower risk but still contribute to disease. An interesting case is that of genotype 45 which is classified as 'low risk' but is responsible for 5.9% of cervical cancers, 5.4% of squamous cell carcinomas and 11.9% of adenocarcinomas.
- The effect of the genotype is dependent on the age of the patient. Among vaccinated women above 35 years of age, the proportion of disease attributed to genotype 16 is reducing and that attributed to genotypes 31, 33, 51, 52 and 58 is doubling.[3] In Danish women over 35 years of age, HPV 31 / 33 has the same or greater 3-5 year longitudinal risk as HPV 18.[4]
- A Danish-Italian study on a referral population stratified HPV genotypes based on CIN3+ risk after a baseline HPV positive test. Genotypes 16, 18, 31, 33/58, (30- 50% CIN3+ risk), were classified as high risk; 52, 45, 51, 39_68_35, (CIN3+ risk 12-15%) were classified as medium risk and 59_56_66 as low risk.[5] A systematic review, which aimed to estimate absolute risk of disease by genotype in combination with different cytology outcomes after a high-risk HPV positive test, also revealed a similar stratification.[6] However, the important issue is with high-risk HPV infected women who have normal cytology, as it is difficult to tell whether they need to be sent for colposcopy. For this population, the study revealed that there was a huge gap in CIN3+ risk between the high-risk genotypes (16, 18, 31, 33/58) and the others, the risk being negligibly small for the non-high-risk genotypes.
- The current paradigm for testing involves HPV testing with cytology and genotyping. A positive test would result in either the return to screening or follow up depending on the result, while a negative test would be re-screened after a five-year interval.
- A new algorithm proposes changes in the current paradigms, taking genotyping information into account to optimise the requirement for colposcopy and recall/retest. The proposed algorithm[7] requires that all HPV positive patients in the HPV primary screen (post-test CIN3+ risk probability of 5%) be subjected to triage with cytology. Of these, the cases with HSIL (post-test CIN3+ risk of 40%) would be treated at evaluation and ASC-H and AGC (post-test CIN3+ risk of 15%) sent for colposcopy. Cases of ASCUS/LSIL (post-test CIN3+ risk of 7.55) and NILM would be subjected to the triage for low-grade cytology with genotyping.
- ASCUS/LSIL (low-grade cytology triage) cases testing positive for genotype 16, 18, and 31 only would be sent for colposcopy. Those testing positive for 52, 58, 45 and other low-risk genotypes would be recalled and retested after 12 months.
- In NILM cases (NILM cytology triage), only those testing positive for genotype 16 would be sent for colposcopy. Genotypes 18, 31, 33, 58, 52, 45 and other lower ones would be recalled and retested after 12 months.
- Another algorithm proposes that the cytology triage be governed by genotype information. In this, HPV+ cases in the HPV primary screen would all subjected to triage with genotyping prior to cytology. Cases testing positive for genotype 16, 18 and 31 would be subjected to colposcopy and cytology. All other genotypes would be subjected to the cytology triage, in which HSIL, AGC and ASC-H cases would be sent for colposcopy and the NLIM, ASCUS and LSIL cases would be recalled and tested after 12 months.
- Thus, the algorithms that combine cytology with genotyping information can enable better clinical decisions on which patient needs to be sent for colposcopy. Depending on how these algorithms are put together, the patients can be moved from the colposcopy group to the re-testing group, until such time that the presence or absence of the disease is established.

Key messages

- Patients and screening programs can benefit by implementing genotyping as an integrated triage step with cytology as this approach would help reduce the number of colposcopies and biopsies.
- Operationalisation of algorithms that allow for cytology triage with genotyping will depend on local country regulations.
- The importance of genotyping in the future will depend on the vaccinated cohorts that enter screening. As vaccinations may result in the systematic eradication of high-risk genotypes, HPV genotyping can allow the risk-stratification of women according to their vaccination status and the genotypes they may carry.

Questions to the panel

Q. I have a question for Mark Stoler. Could you comment on the use of p16 dual staining compared to extended genotyping and how will these options affect the number of colposcopies?

MS. If I hear correctly you want me to compare extended genotyping and dual stain. This is actually a part of the bigger

discussion of where we are in the evolution of triage technologies, all of which are designed to maximize finding those that need treatment and minimize those who don't. Part of the answer to your question depends on where you are. You may have never done any HPV testing and you are lucky to pick up a platform versus what we have in the US. A lot of us have the HPV typing system that only does 16/18 genotyping like the Cobas® system, as opposed to the BD Onclarity™ system. On the Cobas® system you are never going to do any extended genotyping as it is not set up to do that. If you want to do extended genotyping, you must change the platform, which is not easy to do in many laboratories. But there is more than 'one way to skin the cat'. Dual staining stratifies patients in a way similar to extended genotyping. We have published a paper in the International Journal of Cancer which makes a head to head comparison in terms of how many genotypes you have to use to pick out the same population that dual stain shows. What it shows is that you can use either, depending on your input HPV platform. There are choices and tradeoffs. One requires you to make a slide as opposed to getting information without doing any more work. The other gives you a little better yield in terms of the number of patients who have to have a colposcopy to find CIN3s. Eventually, we may find that the two techniques yield complementary information. For instance, in a dual staining population, 16/18 is still an important stratifier. So we are not doing any genotyping. I think at least partial genotyping is a must, looking at these risks, especially in terms of persistence.

Q. I would like to follow my colleague's question. I'm a clinician and we must manage all these complex patient flowcharts. Extended genotyping is not making it any easier. My question is whether it is not better to invest a little bit more into triage? We can triage women in a way that the risk of disease is low enough after triage so we can refer them to routine three-year screening and the example of that is dual staining. In the Scottish experience, if we triage women by partial genotyping 16/18, and on others do cytology and dual staining for those triage negatives, the triage negative women have a very low risk of the disease three years later. We can safely see them after three years, instead of re-resting an army of women every year, not to mention what it will do to colposcopies.

JB. Great question. I hear this question a lot and you can figure it out. First, it is going to be the pathology and clinical microbiology department who are going to be combining all this information. The referring physician will get the results of the test which will say either colposcopy, return to screening within x months or return after five years. The referring physicians do not care which algorithm is used and they should not because it is the task of the laboratory taking on the diagnostic task. For the laboratories, it is all about the training that they should give the staff so they can operate these algorithms. At the end of the day, you will type in the computer the outcomes of the different tests and the code should tell the referring physician what the follow up must be. So in all due respect, I don't agree that it is complicated. It is all about internal education in the department and simplifying the answer to the referring physicians.

MS. I will follow up in comment as well. If I was here ten years ago, I would never give out extended genotyping information because it would be too confusing. If you look at the discussion about processes ongoing in the US, we don't have a system of tenders where a laboratory is assigned a platform. We have several platforms available and the idea that is coming forward from the collaboration between the National Cancer Institute and several centres is to build a database to do exactly what Jesper said. If you have genotyping, dual stain or extended genotyping, you can enter the data from one patient and reference that to the database which will tell you what to do with the patient. As I said for the first question, it depends on what HPV test you are using and whether you can switch. Ultimately, no matter what the array of data you are going to get from the appropriate computer, you'll get to the same point. It doesn't mean we don't have our favourites, but right now dual stain and extended genotyping are the closest near-term replacements for cytology, which is inferior in every analysis, even though cytology has its own value.

Q. You didn't talk about persistence in your algorithm. Do you see it as something that could come along in the future?

JB. Quite naturally. We are going to see a lot of things going into the evaluation of risk. We are going to see persistence, particularly in countries where they have database systems that enable you to see the screening histories of individual women. We will also see biomarkers etc. Persistence will come but we will need a solid evidence base to be able to look at it from a clinical algorithm perspective.

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