HOLOGIC

SERIES 1

HPV Genotyping

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Q1 What is the difference between partial, extended and full genotyping and what is considered more important in determining appropriate triage strategies?

The definitions of partial, extended and full genotyping are internationally accepted. Partial is defined as the ability to report 16 and 18 individually, and the remaining 12 high-risk in one other group. Extended genotyping is ability to identify at least six high-risk genotypes (16, 18, 45, 31, 51 and 52) and the remaining in one group, and then full genotyping is all high-risk genotypes reported individually.

Triage strategies are based on assessment and management of risk. As I will come back to later, factors contributing to this decision are different in different screening settings.

Q2 What is the importance of full genotyping?

You can look at it from a clinical perspective and as well as an implementation perspective. It's a very exciting technology, scientifically very neat to be able to do it, but you always must ask yourself, why am I actually doing it? And the purpose of cervical screening is to identify women who have disease that requires treatment, and to reassure women who do not have disease that they are normal. To what extent is genotyping assisting with that? I'm just not sure that the benefit is there. It shouldn't have any additional benefits in terms of sensitivity. It could have benefits in terms of specificity. Colposcopy carries an emotional and financial cost, and sometimes there is limited availability. Not all colposcopy quality is equal. So a higher specificity test could have advantages.

There is also a potential advantage that people talk about with women who are poor attenders and whether they could perhaps be referred to colposcopy earlier based on extended genotyping. But you get most of the benefits of that pathway with partial genotyping because detecting HPV 16 and 18 will deliver the vast majority of women who are at higher risk.[6]

The proponents of full genotyping are partially trying to visualise what's going to be happening in the future as the vaccinated populations make up a greater proportion of women being screened, and the vaccine that they received goes from being bivalent or quadrivalent up to nine valent. It is very reasonable to be thinking about this. However, one of the things that worries me is that no country has an entirely separate and sequestrated population. For example, in Sweden, they are reporting that they have very few women now being screened in whom they're identifying certain vaccine related HPV subtypes.[7] But that's just in women being screened, and the women who are not being screened are quite likely to be the same women who weren't vaccinated. And it also is ignoring the impact of migration. Sweden has a significant number of demographic diversity, and they will bring with them their vaccine history and HPV subtypes from their previous home. If they don't attend for screening, you won't capture that in your data management.

So, I think that the long-term benefits of extended genotyping are really complicated and probably won't become quantifiable for many years, as the worldwide vaccinated cohort works its way through.

In the short term, the main benefits are around focusing colposcopy resource on women who are more likely to have disease that requires treatment, either because colposcopy resources in very short supply or just because they think that that's what should be done, but it doesn't necessarily mean that there are benefits.

> ***In my own view for each country considering using genotyping for triage, they need to do a very careful analysis of what the current and future benefits will be and whether they're able to achieve the same result with partial genotyping or no genotyping.***

There should be no massive rush to go with extended genotyping and if you are going to implement it, there are a lot of issues around pathway management, such as how countries maintain the quality of cytology and how you manage women in colposcopy, that still need a great deal of work.

Q3 What is the clinical significance of HPV extended genotyping in cervical cancer and precancerous lesions?

There is published research over many years on outcomes, in terms of risk of developing CIN 3. Perhaps less in terms of risk of developing cancer, but there is still plenty for different genotypes. This is what led to the classification of high, intermediate and low oncogenic potential in high-risk HPV types. It is important to stress that all these 14 HPV types are classified as high-risk HPV. So, HPV 6 and 11, for example, are not included. But, within that high-risk set, there are some of higher risk than others in terms of oncogenicity. The cut off is not particularly clear. There is no internationally agreed cut off, particularly between the intermediate and the lower oncogenicity high-risk types. So, for example, in the case of HPV 35, it is classified in the low-risk group in Sweden, based on evidence that there was very little CIN 3 and cancer associated with this type. However, if you look at international research it seems that in other settings it has a significant risk of oncogenicity and probably would be better off placed in the mid oncogenic group.[1-5] So, there's no 100% consensus around the middle and lower end of that, although there is obviously very strong consensus about what's in the highest oncogenic risk types (ie. 16, 18 and 45), within the high-risk cocktail group.

There are different relative risks of developing pre cancer and cervical cancer with different HPV genotypes. An additional factor now is that the HPV vaccine that was introduced in different places between about 2000 and 2010, or later in some areas. The girls who had that vaccine when they were early teenagers when programmes implemented it, are now in screening age range in most countries, and they will obviously have a lower incidence of HPV 16 and 18, which are the types that were included in those early vaccine implementations.

Therefore, part of the significance of genotyping is around monitoring vaccine effectiveness. Initially, a lot of people thought that there would be issues around HPV type replacement, but there's no evidence of that really happening now.[8-10] But in terms of the clinical significance of extended genotyping, the answer is that it depends a lot on the model of screening that the countries are using. There are probably as many models of screening as there are countries with an organised screening programme, and of course, a lot of countries do a lot of screening not in the context of an organised screening programme. The significance of extended genotyping does vary a lot depending on the model being used.

Different HPV genotypes have greater or lesser risks of oncogenicity. That's been known for a very long time but translating it into something that that can be clinically useful is more challenging, and the answer will be different depending on the model of screening that's being used.

Q4 What is the use and impact of HPV extended genotyping in national screening programmes?

Sweden has the most comprehensive implementation of an extended genotyping model for screening, but the screening programme in Sweden is not typical of most other screening programmes. Some special things about Sweden are that it's a very highly organised programme, it has high uptake, they were very early implementers of vaccination, and the vaccine also has a high uptake. So, their screened population is already quite different to most and the way the programme is delivered is highly organised and with high compliance. They also have shown particularly high compliance with early recall, which again is not replicated in other places. It is also very research and data focused, and they have very good data that has been used quite innovatively to decisively redesign their programme.[11]

There is good data showing women attending screening have low risk of abnormality associated with certain genotypes that are traditionally in the high-risk cocktail, and they have used this to produce and monitor a metric, which is the number required to screen to prevent one case of cervical cancer. They have published data showing that for some HPV genotypes that are in the general high-risk positive group, the number required to screen to prevent one case is very high, running into hundreds of thousands. This data has been used to undertake a complete transformation of screening in terms of the pathways used, but still using cytology to triage. The fundamental stated aim of this is to reduce unnecessary colposcopy. The programme has been running for a little while and it seems to be popular with clinicians and laboratory staff in Sweden.

On the positive side, it is clearly helping manage colposcopy resource and it's maintaining colposcopy expertise because the colposcopists are not having to see a lot of women with no significant disease. But I think there are also some cons and what I would particularly like to point out are that the pathways used for management require a lot of early recall and a lot of follow up for women. From the women's point of view, they're not given the reassurance of being completely negative. They're being told – "there's nothing you need to worry about at the moment, but we'd want you to come back in 12 months or 24 months so that we can keep an eye on you". And that is not an anxiety free position to be in. Women are partially reassured, but not completely reassured.

We would need to have a look after a few years of these new pathways being in place to see how much compliance there is with this early follow up. They have reported previously that compliance is good. But again, I think that wouldn't necessarily be the same in all other settings. The other issue is that the pathways that they've got using cytology as a triage do depend on high quality cytology and we know that cytology is not as sensitive as HPV primary screening for the detection of CIN2+ even in the best of hands. High quality cytology is difficult to do- it costs a lot, it requires a lot of training, a lot of quality assurance, so I do have some concerns about moving women onto longer follow-ups or even potentially back to routine recall in some cases, based entirely on their cytology results.[12-15]

Another weakness in Swedish programme, which I look forward with interest to seeing how they manage it, is that these pathways are complex. I think many countries do wrestle with implementation of complex pathways and it's important if you have a national published pathway to make sure that everybody is managed in accordance with the agreed pathway and that there are no mistakes made. The more complex the pathway is, the easier it is to accidentally put somebody on the wrong pathway. There are things that can be done about that, like IT solutions that will help, but when I look at the Swedish extended genotyping pathway, I just see a lot of places where it could go wrong.

I've used Sweden as an example, but I just want to reiterate that they have put a huge amount of work into this. They've used their excellent data and their excellent research resources, and they've produced data to support the implementation in Sweden. Many countries haven't got that degree of expertise and that degree of good quality data. But even if they did, it might show different results, and I do feel that the way you might wish to use extended genotyping is setting specific and it would look different in countries. Anybody that was wanting to implement it would need to do their own evaluation.

Q5 What is the objective of introducing extended genotyping and can RNA technology such as the Aptima® HPV assay reach same objective?

I think we can say without fear of argument that HPV 16 is the most oncogenic type of HPV, and yet most women who have HPV 16 do not, in fact have CIN 2 or worse. It varies depending on age and vaccine uptake, but it's still unlikely to exceed 20%. So, in most populations, most women who are HPV 16 positive using DNA-based HPV assays do not in fact have CIN 2 plus, i.e. disease that needs treating. I think it's just worth keeping that in mind as a baseline and HPV 16 is obviously the most extreme example, but for all subtypes most women who test positive on DNA-based HPV assays will have a self-limiting infection that was going to resolve spontaneously and not progress. So, genotyping alone doesn't achieve the objective of screening, which is to identify women who require further investigation and management and to reassure those who don't. This is where mRNA technology is helpful- if you have a positive test for HPV RNA, that is evidence that HPV DNA has been integrated into the host DNA and the infection has already progressed and is less likely to resolve spontaneously. So, what happens with RNA testing is that women with an early non-progressive infection are more likely to test negative, and this is a better outcome for women, because they get a negative result. They don't have to deal with this complex risk management type approach that we've described in the example for Sweden. It's not negative, but we want to see you again in 12 months or 24 months. It's just negative! From the woman's point of view that is a much better and less worrying result.

RNA testing avoids the anxiety of a positive test with no loss of sensitivity and no early follow up, and it also takes away a lot of the difficulties in implementation, pathway management and monitoring, in particular.

Furthermore, there is of course the longitudinal data to show that a negative RNA result is reliable[14]. It's there and it's approved in international guidance for use in HPV primary screening.

These difficulties are multiplied if you're looking at a setting where there's no organised screening programme, and where data collection or monitoring is suboptimal. Using mRNA testing also means that you aren't relying on cytology to triage the samples of these women with very early HPV infections, who would be picked up by DNA testing.

Q6 In your opinion, where should national screening programmes focus their efforts to improve risk-stratified screening strategies?

There are a lot of issues around data management. National screening programmes should ensure that they have good records about who has received which vaccine and when, and that these are linked to screening records. It is likely that vaccine status will be more predictive of individual risk, in the medium and longer term, than genotyping results of any kind.

National screening programmes should focus efforts on women who have not been vaccinated as young teenagers, and this especially includes women who were not resident in the country at that time. Both immigrants and women who did not receive vaccination when offered are at higher risk for non-attendance for screening.

In terms of risk stratification in women who attend screening there will need to be an evidence base for each screening setting. Benefits for 16/18 genotyping may be particularly favourable where there is a high rate of non-attendance at colposcopy, serious issues with colposcopy capacity or failure to comply with early follow up. If using extended genotyping or full genotyping, programmes would need to focus on optimising cytology performance and achieving excellent follow up compliance and failsafe processes.

There may also be advantages to using genotyping as a management tool, for example in women where colposcopy management is difficult, and this may give more benefits than using it on the entire primary screening population. There is good research, for example in the UK, which shows that on a population basis, partial genotyping does not impact outcomes, and this is probably due to high quality cytology and good compliance with early follow up.[15]

Q7 The WHO are evaluating evidence on the use of HPV extended genotyping for the clinical management of HPV positive women. What are your views on this and the potential inclusion in future guidelines?

The WHO has the difficult task of producing recommendations on genotyping in incredibly diverse cervical screening settings, in which populations are affected by very different and rapidly changing experiences of vaccination and previous screening.

The WHO cancer elimination strategy has had a major impact on cervical cancer prevention strategy and rightly, it is being viewed as something to work towards, not something which is not practicable in individual settings.

A major consideration though when considering implementation of extended genotyping is the role of cytology. Cytology informs colposcopy management. If good quality cytology is available, in my view it is more helpful as a triage than extended genotyping, because the positive predictive value of high-grade cytology abnormalities is very high – up to 90%. No genotyping test can come close to this. However, high quality cytology is by no means universally available, and implementing a new high quality cytology service is probably close to impossible at this time.

So, I'm hoping that the guidelines being developed will reflect the variability in different settings.

It is clear that partial genotyping is widely used and is found to be helpful in many settings. It is particularly helpful in identifying women who don't attend and would benefit from being actively encouraged to do so.

Conclusion

Extended or full genotyping do not increase the sensitivity of cervical screening, they are aimed at improving specificity, by focussing screening surveillance and colposcopy referral on women at the highest risk. But risk thresholds are difficult to calculate and are country specific, and there is potentially a major hurdle to overcome in terms of communications with screened women. There is a risk that women will procure additional screening tests from perhaps less well quality assured sources, if they don't believe that it is safe for them to wait before acting on an extended genotype result. I think it will be very difficult to explain and get public acceptance for using extended or full genotyping to give prolonged follow up intervals to women who have tested high-risk HPV positive.

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