Comparison of current and future cervical cancer screening and impact of human papillomavirus vaccination on screening

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Cervical cancer is the fourth leading cause of cancer in women. Most of the cervical cancers are human papillomavirus (HPV) induced. It takes years for an HPV infection to develop cervical cancer, which makes screening a useful tool. This review shows an overview of the current and the renewed screening for cervical carcinoma´s in the Netherlands with attention to their relevance in the future, in vaccinated populations.

The current screening is cytological screening; it detects abnormalities in the cervical cells and has prevented many cervical cancers. Cytological screening has low sensitivity and therefor it is going to be replaced by HPV-testing. HPV screening detects the presence of viral DNA and has high sensitivity. However, not all HPV infections will lead to cervical cancer. Triage testing can reduce the low specificity. Cytology as follow up will increase the specificity but lowers the sensitivity. Therefore, new methods for primary and triage testing are of interest. In this review the methods of p16 INK4a- Ki -67 and DNA methylation are pointed out as promising. For screening to be effective high levels of participants are needed, to increase the amount of participants self-sampling is offered. This test is also interesting for developmental countries. Vaccination is as well interesting for developing and developed countries, it will prevent HPV induced cervical cancer. The current vaccination is against the two most common HPV types which count for 70% of all cervical cancers. It remains important to screen. Vaccination in combination with screening will reduce the incidence of cervical cancer. However, more research is needed to optimize cervical cancer prevention, and screening.

Keywords: HPV, cervical, carcinoma, screening, cytology, HPV-test, vaccines, prevention

Contents

HPV induced cervical cancer

Cervical cancer is the fourth leading cause of cancer in women in 2012, according to Globocan[2]. The majority of cervical cancers (99,7%) are caused by the human papilloma viruses (HPV) (Dannecker C, 2004). There are 150 types of HPV, the high-risk HPV (hrHPV) has the most potential to induce cervical cancer. The two most common HPV types in cervical cancer, are HPV16 and HPV18 and they count for three quarter of the HPV induced cervical cancers (Kirschner B, 2012).
Virus transmission occurs mostly by sexual contact. HPV will infect the squamous epithelial cells of the cervix and replicate in their nucleus. The exact entry of the HPV is not clear yet, studies point to attaching of HPV by cell surface molecules and entry via endocytosis. The virus will uncoat in the cells and transport its genome to the nucleus of the host cell where it integrates into the host genome (Graham SV, 2010).

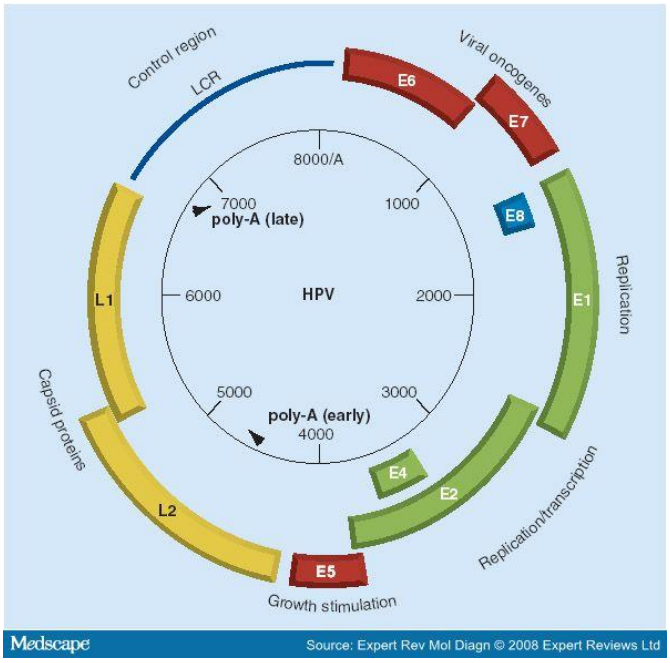

Figure 1: HPV genome (Lie AK 2008)

HPV is a small virus, but has a sophisticated design. The virus is non-enveloped with icosahedral symmetry. The genome is 8kb of double stranded circular DNA, all HPVs have the same compounds shown in figure 1 (Lie AK 2008). There are early genes (E) and late genes (L). The early genes encode proteins for interruption of the host cell cycle; by viral DNA replication and RNA transcription initiation. Replication of the viral DNA is done by proteins encoded by E1 and E2. E2 also modulates viral RNA transcription(Lie AK 2008).
E5, E6 and E7 are oncoproteins, they are involved in tumor cell growth. E5 gives disruption to the normal function of cellular growth factor receptors (Liao S.J. 2013). E6 promotes tumor progression by degradation of p53. P53 is a tumor suppressor gene, it has regulating functions in the cell cycle, for example induction of apoptosis. E7 binds and inactivates retinoblastoma (Rb) proteins. Rb is also a tumor suppressor gene, one of its functions is to inhibit the cell cycle progression. E2 decreases expression of E6/E7 and loss of E2 is the first stage in transformation (Gök M, 2010).
Preparing the viral release from the host genome is done by E4. The late genes are structural genes, which will lead to transmission and survival. L1 is the major and L2 the minor structural compound. (Zheng ZM, 2006; Lie AK, 2008).

Not all HPV infections and not all HPV types will lead to cervical cancer. Low risk types can lead for example to warts. This is because of the low risk HPV types have differences in the E6 and E7. If E6 and E7 are not efficient in interfering of p53 and pRb, invasive cancer will not be developed (Tjalma WA, 2013)

Cervical cancer

After HPV integration in the genome the cervical cells change through several steps. First the cervix has some abnormal cells in the upper layers, this is called cervical intraepithelial neoplasia 1 (CIN1). CIN1 is a low grade squamous intraepithelial lesion (LSIL). Squamous cells are flat scale like cells of the upper cell layers. Most of CIN1 lesions will not evolve in cervical cancer, because the infection of the virus is often transient (Tjalma WA; 2013). After CIN1 there is more dysplastic cells in CIN2, followed by CIN3, who has the most dysplastic cells and the biggest change to develop cancer. CIN2 and 3 are both high grade squamous intraepithelial lesions (HSIL). CIN3 is the penultimate stadium, afterwards invasive cancers can be developed (figure 2) (Petry KU, 2013).

Most of the carcinomas are squamous intraepithelial lesions, but some of them are adenocarcinomas. Adenocarcinomas are also associated with HPV infection, however they have glandular cells as origin (Chansaenroj J, 2013; Kirschner B, 2012). In the ‘50 and ‘60 of the last century 5% of the cervical cancer cases were adenocarcinomas. In the ’70 this was 20-25% of the invasive cervical cancer cases (Smith HO 2000). At this moment, adenocarcinomas count for 20% of the cervical cancers (Lee YY, 2011). The proportion of adenocarcinomas is increased in contrast to the decrease of squamous cervical cell carcinoma’s (Lee YY, 2011). Reason for the increase in proportion of adenocarcinomas is difficulties in detection of this type of cervical cancer. Therefore adenocarcinomas might have a worse survival rate (Lee YY, 2011).

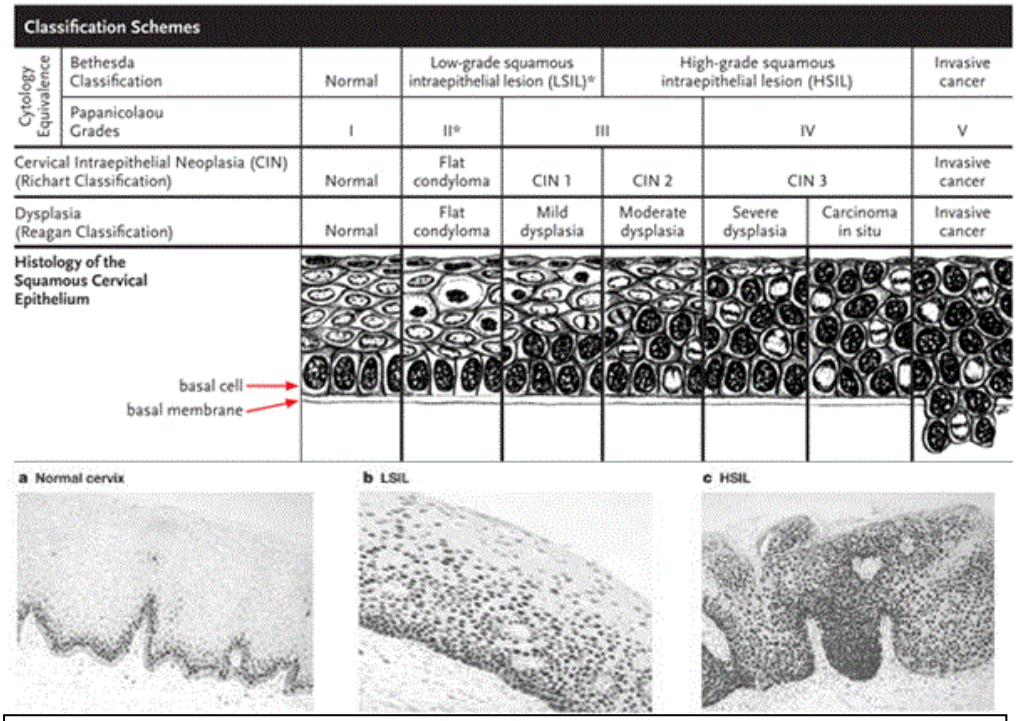

Figure 2: Different stadia of cervix from normal, proceeding to invasive cancer (Gonzalez MA, 2005; Bratcher JJ, 2008)

Screening is an effective way to prevent cervical carcinoma, because there are years of time between the start of HPV infection and the onset of cancer. Multiple countries already have screening procedures. The current screening is about to get renewed in the Netherlands, instead of detection of abnormal cells with cytology, virus DNA is going to be detected (Figure 3). Moreover an introduction of the self-test for non-attenders to test at home will be introduced. At last in the future, the screening populations will be vaccinated, since vaccine against high-risk types of HPV is in use for a couple of years now[1]. The vaccination might influence the current screening methods.
The aim of this review is to compare the current screening with the renewed screening for cervical carcinoma's, with attention to their future relevance in vaccinated populations and their application in developing countries.

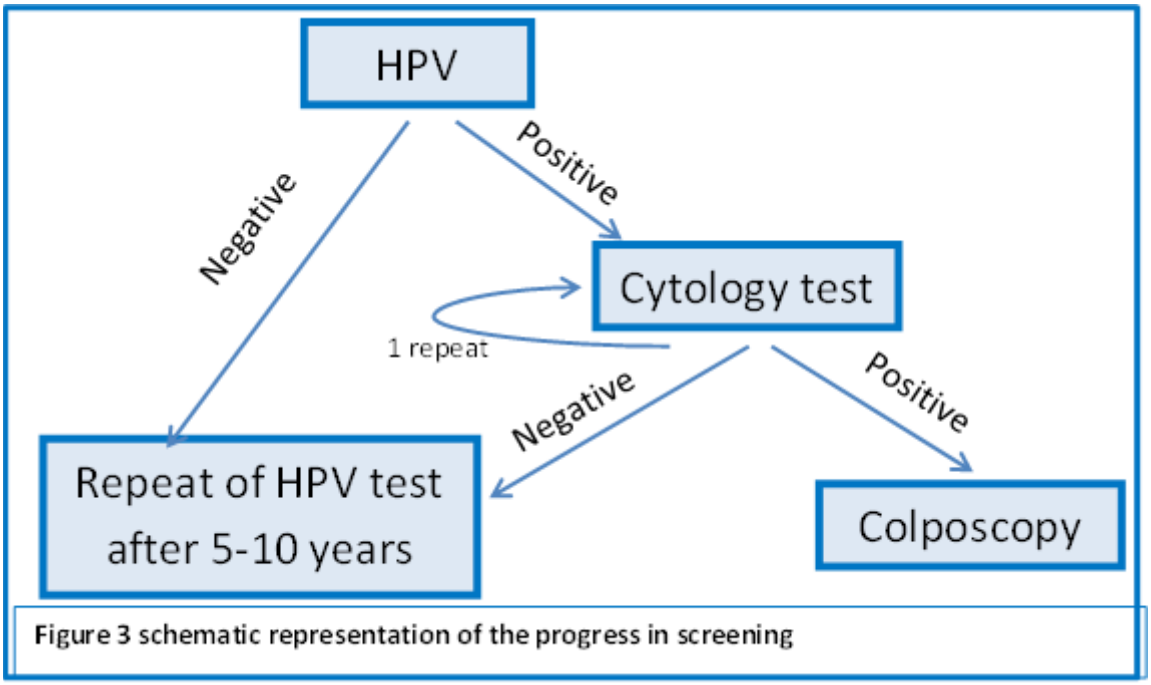

Figure 3 schematic representation of the progress in screening

Comparison of the current and renewed screening

Primary Testing

Screening gives the opportunity for early detection of cervical lesions. Therefor treatment or surgery can be started in early stages. Therefore, screening can prevent development of cervical cancer and their associated deaths (Dannecker C, 2004). Women who do not participate in cervical cancer screening have 2.5times higher risk of cervical cancer and twice the risk of dying from this cancer (Leniz J, 2013). The current most common method of cervical cancer screening is the cytology test. This method is used in multiple countries, including the Netherlands. Thanks to this screening the probability to detect and cure women with a cervical carcinoma has increased.

The cytology test examines the presence of abnormalities and lesions in the cervix by taking cells from the cervix. The examination of abnormal cells can be done in two manners, by the conventional method or the liquid cytology method. In the conventional cytology cells are smeared over a glass slide and are analyzed at the clinic. With liquid cytology method the cells are stored in liquid and analyzed in a laboratory at a later moment (Ronco G, 2007).

The cytology tests have no optimal sensitivity. The meta-analyis of Koliopoulos showed a sensitivity of 72,7% for Atypical Squamous Cells of Undetermined Significance (ASCUS) (Koliopoulos G, 2007). The sensitivity rates for cytology tests, observed by multiple researches differ from each other. This is due to the used populations but also to skills of the examiners, which makes it less reproducible and less sensitive (Wu R, 2012; Arbyn M, 2004). However, also the time between the study and the last screen has influence to the prevalence and the sensitivity, an unscreened population will have higher prevalence (Mayrand M, 2006).

In 2016, cytology will be replaced by high risk HPV-testing (hrHPV-test) in the Netherlands[1]. The HPV-test is used to show the presence or absence of specific pieces of DNA. Multiple DNA detection methods are developed. Common examples are southernblot hybridization (Arbyn M, 2004), polymerase chain reaction (PCR) and PCR in combination with a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) (Belinson JL, 2012).

The most common hrHPV test is Hybrid Capture 2 (HC2). HC2 detects 13 types of high-risk HPV without cross reaction taken into account (Terry G, 2001). Specimens are collected by the Hybrid Capture Cervical Sampler, cervical biopsies and with brushes used for cytology testing. If the sample contains HPV DNA, probes bind to this DNA and form a DNA-probe hybrid. The hybrids bind to hybrid-specific-antibodies, which are conjugated with alkaline phosphatase molecules. These antibodies are detected by chemiluminsecent substrate which are cleaved by alkaline phosphatase with light emission as result. When the amount of light passes a certain threshold this indicates the presence of HPV DNA (Qureshi MN, 2005; Eperon I, 2013).

Table 1 : Sensitivity and specificity of HPV and cytology testing in women of 18-70 years old and of self-samples in all ages.

Article	Technique	Sensitivity	Specificity
Koliopoulos G, 2007	Cytology test: PAP-test	72,7% (for ASCUS)	91,9 % (for ASCUS)
Koliopoulos G, 2007	HPV-test: HC2	90 %	86.5 %
Zhao FH 2012	Self-sampling HC2	86.1-86.2%	79.5-80.7%

HPV testing solves the problem of the low sensitivity of cytologcal testing (table 1) (Koliopoulos G, 2007). Great amount of studies show higher sensitivity for HPV-tests than for cytology tests (Cuzick J, 2008;Koliopoulos G, 2007; Zhao FH: 2012).The increased detection is for abnormal cells and pre-invasive lesions, but also earlier detection of CIN3 is observed (Zorzi M, 2013; Malila N 2013; Mayrand MH, 2006; Leinonen MK, 2012). High sensitivity and early detection lead to better screening results.

The greater sensitivity of HPV testing can be explained by the differences in methods. Cytological screens for abnormal looking cells and is done by specialists. HPV-test shows the presence or absence of HPV DNA, which is more absolute and leads to earlier diagnosis. Therefore, higher sensitivity is shown for HPV-testing compared to cytology. In addition the most effect, in gain of sensitivity, is for adenocarcinomas. Cytology tests have poor detection for adenocarcinomas since they have a different

cytology than the most frequent squamous cervical cancer (Korhonen MO,1980; Ronco G,2013). This type of cervical cancer occurs mostly in young women.

Besides adenocarcinoma, detection with HPV testing shows an overall increase of detection for young women of 25 till 34 years old (Zorzi M,2013). Young women mostly have low grade squamous intra-epithalial lesions (LSIL) which have smaller chances to become cervical cancer than HSIL. Additional recent infections are most transient. Together it results in lower specificity of HPV in compare of cytology tests (Verdoodt F, 2013; Tjalma WA 2013). A bit lowered specificity for HPV-tests is shown in table 1 (Koliopoulos G, 2007). Several studies show lower specificity for HPV-testing(Zhao FH: 2012). This review uses the article of Koliopoulos because it shows both sensitivity and specificity of both cytological and HPV screening. It also has criteria for the used articles in their meta-analyis. The selected for types of participants, type of interventions and verification of disease status (Koliopoulos G, 2007).

The low specificity of HPV testing leads to overdiagnosis (Rijkaart DC, 2011; Malila NM 2013). The overdiagnosis is unpleasant for the 'false positive' women as they might get stressed, but also for the gynecologist who has an overload of colposcopy examination. This problem is reduced by prolongation of the interval of screening which leads to less detection of recent infections. While, negative HPV tests will give longer duration of protection, because of the long interval between infection and cervical cancer (Kitchener HC, 2011).A five year HPV-screening is proven to be saver than a three year cytology screen (Ronco G, 2013; Kitchener HC,2011).

Triage

Of all women with a positive HPV-test not all will develop cervical cancer. A vast amount of women, tested positive for the virus, will not develop cervical cancer. To reduce the amount of these 'false positives' women, a follow up test is needed (Pacchiarotti A, 2013). Follow up test should not test the presence of the virus, but map out the presence of lesions and cancer in the cervix and their state, this is called triage.

Cytology can be used in triage testing as follow up of HPV-testing. The current proposed triage with cytology is shown in figure 3. If the HPV test is positive, but cytology is negative then the cytological test will be repeated after six months. If the HPV test and cytology are both positive, there is probably a high-grade lesion present. Double positive patients can be referred to a gynecologist who will perform colposcopy (Ronco G, 2006), see figure 3. Because of this extra test less false positive patients will be referred to the gynecologist (table 2) (Berkhof J, 2010; Malila NM, 2013) this is especial profitable in countries with high cytological abnormalities and high colposcopy rates (Rijkaart DC, 2011).

The sensitivity of triage is influenced by both HPV testing and cytology. Triage shows higher detection than cytology alone (Berkhof J, 2010) and will increase detection of CIN lesions (Ronco G, 2013; Kitchener HC, 2011).With reduction of cervical cancers with triage compared to cytology alone, as result (table 2)(Berkhof J, 2010**).** However, cytology has a low sensitivity which lowers the sensitivity of triage, compared to primary HPV testing. The chance for false negative screens is increased compared to primary HPV testing. Therefore repeat cytology is used after six months in the Netherlands to increase the sensitivity.
Besides, cytology testing is done by an examiner, the fact that only HPV positive women are revered can influence the outcome of the cytology test (Rijkaart DC, 2012). This has influence on the sensitivity and false positives and needs to be further investigated.

Table 2: Effect of triage or combination of HPV and cytology on the amount of cancers, the number of detected CIN2 and the CIN2 with colposcopy referral, compared with current 5 year cytology screening in the Netherlands. Data from Berkhofs meta-analysis 2010.

Screening technique	**Interval**	**Amount of cancers**	**Number of CIN2 detected**	CIN2 with colposcopy referral
HPV with cytology triage	5 year	Reduction of 23% for cancers	31% increase	21% decrease
HPV with Cytology	5 years	Reduction of 26 % for cancers	34% increase	30% decrease
Cytology with HPV triage	5 years	Reduction of 3% for cancers	1% increase	10% decrease

Renewed way to provide screening: self-sampling

Despite the reduction in mortality of cervical cancer, women still die from this cancer, also in counties with organized screening programs. This is partially due to imperfections in screening, but mainly to non-attendees. Non-attendees provide about 60% of the cases in cervical cancer (Dannecker C, 2004). An opportunity to reach out to these women is introduction of a self-sampling method. The same primary test can be used, but the sampling collection is done by the women them self.

In the Netherlands this self-test is going to be introduced for women who have trouble with being clinically tested (Meijer CJ, 2012). Reasons for not attending the screening program are for example religion, bad experience with sex or a lack of time. Taking the self-sample can be done at home without a doctor, this makes self-testing a potential solution to increase the number of participations and therefor effect of screening (Taylor D, 2013).

The sensitivity of screening, for example HPV testing, should be remained if the samples are collected by self-sampling. The samples should contain enough and representative materials, for the screening test (Wu R, 2012). Collecting enough DNA material is easier than collection of enough cells for cytological testing. Zhao et all shows high sensitivity and specificity of HPV-tests after self-sampling. Sensitivity of 86,2% for CIN2 and 86,1% for CIN 3 and a specificity of 80,7% for CIN2 and 79,5% for CIN3 is shown (table 1)(Zhao FH: 2012). Hillemans et al also showed remained high sensitivity if samples were taken with a cytobrush (Hillemanns P, 1999). This is not the case for all techniques of self-sampling, sampling with a tampon, lavage or dragon swab lower the sensitivity of the following test (Dannecker C, 2004).

Self-sampling is done by women them self, which makes usability of importance. Self-sampling is considered as easier to use and less painful than clinical testing (Dannecker C, 2004; Szarewski A, 2007). In the study of Forrest et all, every participants did not have attended screening before, but were willing to try the self-test. 65% of them would definitely use the self-test if it was offered in the national screening program (Forrest S, 2004). The efficacy of screening will be raised if self-screening is added to the program (Gök M, 2012). Anhang showed that women who were willing to go to the clinic preferred clinical testing above the self-test (68%) (Anhang R, 2005). This can indicate that self-testing is of most interest for women who do not attend clinical screening. One problem shown in

these studies is that participants were sometimes unsure if they were doing the sampling properly (Forrest S, 2004).

Self-sampling can raise the amount of attendees, but awareness has also a major impact. Women in New York were interviewed about their awareness of HPV in 2005. More than half (61%) of the participants knew about the causative role of HPV in cervical cancer (Anhang R, 2005). Recently in South Africa a study showed that 53,3% of the participatants knew about cervical cancer and the screening method and 60% had heard of HPV(Hoque MO, 2008*)*. These studies show that more awareness is needed, because awareness can increase participation for screening.

Future Screening:

Possibilities to improve primary screening and triage

There are still improvements needed for cervical cancer screening. Cytology has low sensitivity and HPV test have low specificity. Moreover the exact long term benefits of HPV testing are unknown since many studies use the same participants for testing and for control, there for no long term effects (Ronco 2006).

In the renewed screening DNA HPV testing is used, but HPV-testing can also be done with mRNA-based screening. The mRNA-based HPV-test detects the presence or absence of E6 and E7 mRNA. As told before, E6 and E7 are onco-proteins of high-risk HPVs and are only present when there is an HPV infection. This mRNA based HPV test for E6 and E7 correlates with the grade of the lesion, this in contrast to HPV-tests. (Lie AK, 2008) Not only E6 and E7 also several mRNA sequences can be tested at the same time. The 5-type tests for five mRNA sequences. The 5-type mRNA is less sensitive than the DNA HPV-tests, this can be due to the lower stability of mRNA in compare to DNA (Verdoodt F, 2013). Despite the low sensitivity this method is still interesting for improvement because it detects the functional elements of the virus and the state of lesions.

Cytology is not the only way to detect cancer factors. Alternative methods are interesting for triage or replacement of the current primary methods. One of them is P16 INK4a staining, P16ink4a is a cylcin dependent kinase inhibitor. Over expression of p16 INK4a has strong association with cervical lesions (Ikenberg H, 2013). The mechanism behind this is the inactivation of Rb by the oncoprotein E7, which results in an up-regulation of p16ink4a.
P16 INK4a is over-expressed in the HSIL states but can also occur in healthy cells, which are in cell arrest (Bergeron C, 2010). An option is to combine p16 INK4a staining with the staining of KI-67, a protein who is necessary for cellular proliferation (Cuzick J, 2012). This combination gives less false positive test, which will increase the specificity, see table 3 (Schmidt D, 2011). Moreover P16 INK4a/ Ki-67 have higher sensitivity for CIN2+. It will give less 'false positives', because it differentiates between the severities of the lesions(Ikenberg H, 2013). P16 INK4a is an interesting method for further development.
P16 INK4a staining is also more sensitive than cytology testing, see table 3 (Calvacante ML,2012). Because the staining of cells with p16 ink4a overexpression are easily detected, which gives this staining a higher reproducibility than the current cytology (Bergeron 2010). Furthermore P16 INK4a is a protein and therefore more stable than mRNA of E6 and E7 (Pacchiarotti A 2013).

Table 3 Sensitivity and specificity, for expression of P16 INK4a and Ki-67 screening in women older than 18 years(Schmidt D, 2011)

Test	Sensitivity (%)	Specificity (%)
P16 INK4a	92,9	63.4
P16 INK4a/ Ki-67	92,9	80.6

DNA methylation

Another interesting manner to screen is to identify cancer-specific methylated genes promoters in cervical cells (Eijsink JJ, 2011). DNA methylation is seen in multipe cancers (Verma M, 2013). If DNA is methylated, proteins might not be able to bind to them; this can lead to silencing of the genes. In other cases different proteins bind to the DNA with silencing as result too (Hoque MO, 2008). Methylation of especially CpG islands results in silencing of the involved genes (Cuzick J, 2012). Thus, methylation of DNA has an influence on the gene transcription without changing the DNA sequences; this is called epigenetic modification (Hoque MO, 2008).

Methylation can be tested with for example bisulfite and PCR. Cervical samples can be taken for example by self-sampling (Cuzick J, 2012). Specific parts of the genome can be replicated with designed markers and the use of PCR. Adding sodium bisulfite will convert non-methylated cytosine into urasil, but methylated cytosine remains the same. After this quantitative methylation-specific PCR (qMSP) can be used to show the differences between different genes (figure 4) (Lindsey JL, 2005).

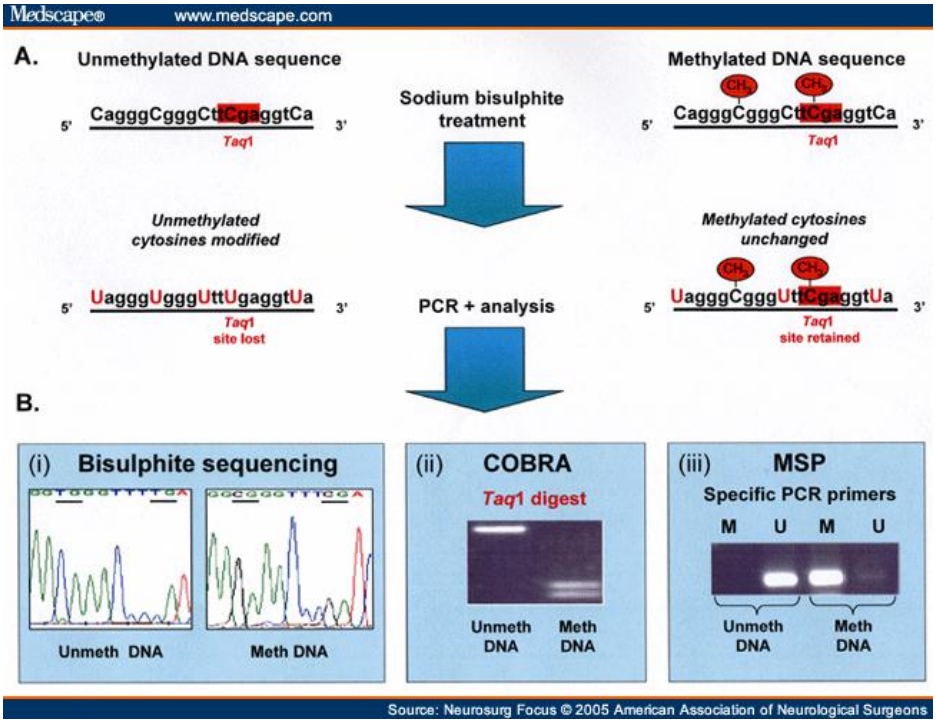

Figure 4: The method of methylation testing with sodium bisulphite (Lindsey JL, 2005)

Multiple studies looked for markers to identify methylation in cervical cancer. Markers can be tumor suppressor genes, genes that are methylated in other cancers. They can be identified in genome-wide studies (Lai HC, 2008). Some markers are mainly silenced in high-grade lesions, this has positive influence on their specificity (Lai HC, 2008). Markers can also be specific for adenocarcinomas; this is in benefit compared to cytological screening.

The level of sensitivity depends on the used markers. Different kinds of markers can be used and combined. Combinations of markers may increase the sensitivity for HSIL (Lai HC, 2008). Eijsink et all showed high sensitivity for CIN2+ and CIN3+ patients namely respectively 71% and 84% when methylation markers performed as triage (Eijsink JJH, 2011). Also the fact that new markers can be designed makes this technique of high potential for improving the current methods (Eijsink JJ, 2011).

Methylation tests are of interest for triage, because it detects later events in the carcinogenic process, it is specific for high grade lesions and therefore increases specificity. Moreover, methylation tests detect adenocarcinomas and can be used in with self-sampling, in contrast to cytological tests (Lai HC, 2008; Cuzick J, 2012).

Screening in vaccinated populations

Women will benefit from human papilloma vaccination, because it prevents development of cervical cancer. HPV vaccines that prevent infection of HPV are called prophylactic vaccines (Gertig DM 2013; Mahdavi A, 2005). Prophylactic vaccines induce the stimulation of production of neutralizing antibodies against the pathogen before the infection is present (Mahdavi A, 2006). Prophylactic vaccines usually exist of empty capsid particles. The highly conserved major capsid protein, L1, is the most common used in vaccines and can be cloned by microorganisms. When L1 particles are together they assemble to virus-like particles (VLP) (Kirnbauer R, 1992*)*. L1 VLP evokes high titers of serum-neutralizing antibodies in humans and this gives protection against viral challenges. The neutralizing antibodies are produced by the local plasma cells or are transported from the plasma into the genital secretion (Mahdavi A, 2005).

HPV vaccination has a high efficiency of almost 100%, for the types against which vaccinated. The introduced vaccination in the Netherlands is Cervarix it protects against HPV16 and HPV18. Cervarix is also offered in more than 100 countries worldwide (Malik H, 2013; Szarewski A, 2011). It reduces the amount of cervical cancers with 62% (Kitchener HC, 2011). Moreover, because vaccinated women will not develop cervical cancer, the colposcopy rates will go down.

Studies do not agree about the toleration of the vaccine. According to Mahdavi et all the vaccines are considered safe and well tolerated (Mahdavi A, 2005). However Wilyman shows negative influences on womens health. Wilyman points out to a few side-effects associated to vaccination, most of them are discomfort of performing infection and rashes (Wilyman J, 2013).
Additionally, not much is known about the long term effects of vaccination because inadequate controls are added and vaccination is introduced since a few years which is too short to see the long term effects. Finally, the duration of the effects of vaccination are not certain, the prediction is at least 5 years of protection (Wilyman J, 2013). However, duration data are limit and more data should be collected (Drolet M, 2013).

The bivalent vaccination gives protection against HPV16 and HPV18, but there are other HPV types who induce cervical cancer. HPV16 and HPV18 count for 70% of the cervical cancers, there is no protection for the remaining 30%, although there might be some cross protection. (Kitchener HC, 2011; Wilyman J, 2013). The amount of unprotected women is even higher, considering the non-attendees and the fact that prophylactic vaccines don't have a curing effect on present infections (Szarewski A ,2011).

All of this makes it hard to predict the real effects of vaccinating on screening. It is of importance to keep screening also in vaccinated populations but screening might have influences from the vaccination.

Screening has good efficiency and detects cervical cancer in an early stage. Although vaccination will reduce the efficiency of screening, with bivalent vaccination four screens remains cost efficient (Kitchener HC, 2011, Coupe VM, 2012). Another vaccination is Gardasil, this vaccination protects against HPV16, HPV18 but also HPV6 and HPV11 who are responsible for some genital warts. In the future development of broader spectrum vaccines will result in even less cost effectiveness (Coupe VM, 2012). In addition, in a vaccinated population less people will develop cervical cancer, therefor the prevalence goes down. Because of the low prevalence, abnormalities in the cervix might be interpreted as positive which can lead to a higher amount of false positives with cytological screening(Franco EL, 2006).

Next to this there might be social influences and effects. The vaccination does not influence persistent infections; therefor vaccination has to be done before onset of sexuality (Szarewski A 2011). In the Netherlands girls of 13 year are vaccinated, the vaccine works to about 18 years. This will remedy infection in those years but not the years after. Many girls are not sexual active in this period but may risk side effects of the infections and possible long term effects.
Besides parents might have troubles with sending their daughters to a vaccine for a 'sexual transmitted viral infection'. Vaccination also might have an impact on sexual and screening behavior; vaccinated women might have the feeling that they have less risk for sexual transmitted diseases (Malik H, 2013, Cuzick J, 2008). Therefore more awareness and education is needed.

Application in developing countries

In developing countries there are high rates of HPV infections, but low recourses (figure 5). In the future, a further increase of HPV infections is expected in these countries (Leniz J, 2013). In perspective almost 85% of new cases of cervical cancer worldwide occur in developing countries[2]. This leads to half of the total cervical cancer related deaths (Lorenzi AT, 2013).

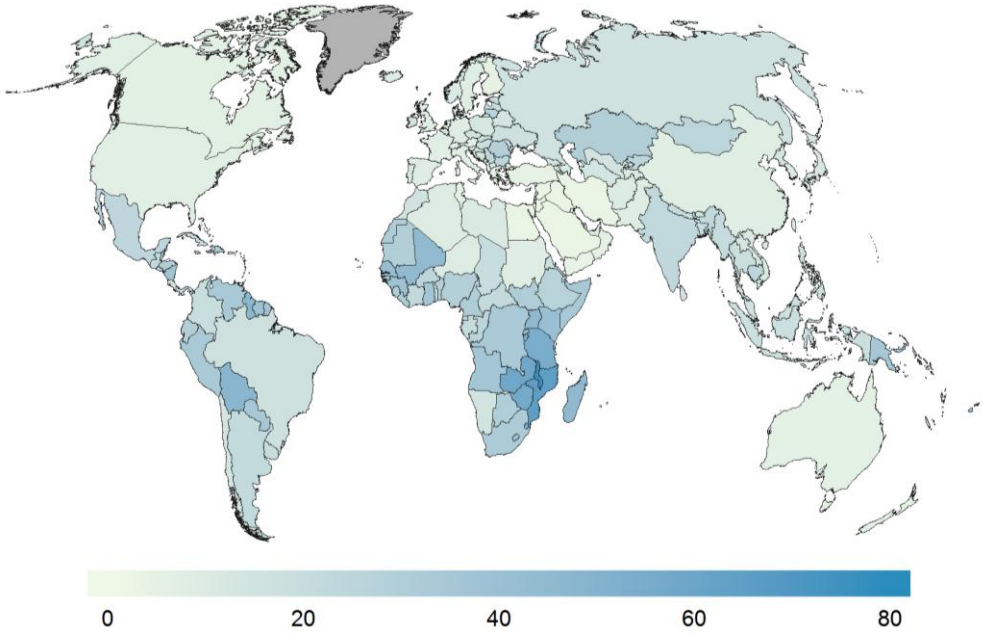

Figure 5: worldwide cervical cancer incidence in 2012. Age-standarised rates per 100.000. (Globocan 2012)

Cervical cancer prevention programs have already reduced the mortality of cervical cancer in developed countries. But in developing countries lower amounts of women attend screening, if screening is available, compared to the developed countries (Leniz J, 2013). This can be due to costs, amounts of resources, infrastructure or awareness (Cuzick J, 2008; Wu R, 2012). The self-sampler is useful for developed countries to raise the screening participation; however this method is also valuable for developing countries.
Having self-samplers in developing countries can counter some of these problems, but the actual tests and their analysis have to change. For example, HPV tests collection can be done in liquid transport media, like phosphate-buffered saline (PBS). PBS has to be kept cold; this can be problematic for low developed countries. Dry swabs might be more practical for self-sampling, they are easy to use, less expensive and dry swabs can be stored at room temperature. Eperon et al showed no significant difference in specificity or sensitivity for wet and dry sampling (Eperon I, 2013).
After the self-test, there should be an HPV-test as discussed before. In developed countries triage is recommended and if tested positive, colposcopy is used as follow up (Franco EL 2006; Ronco G, 2008). In developing countries a poor infrastructure can lead to problems. Instead of triage and colposcopy, visual inspection with acetic acid (VIA) can be used as follow up for HPV-tests. With VIA abnormal cells are painted white by the acetic acid, there for it is easy to see lesions and they can be cut out immediately. The downside is that not all tissue might be painted white, which results in remaining's of cervical cancer and low sensitivity. The measured sensitivity of VIA is between 50,3% and 55,7% for CIN 2 and 3 respectively (Cuzick J, 2008; Zhao FH, 2012).
Overall the self-sampling HPV test, with VIA as follow up has a lower sensitivity, however it it will give an increase in coverage of screening programs, which beats out the disadvantage of low sensitivity (Belinson JL, 2012).

The cervical cancer rates in developing countries are the greatest (figure 5); therefor vaccination can be a godsend. But some adaptations have to be made. The current vaccination exists of 3 injections which is disadvantageous given the poor infrastructure in developing countries.
Not all women will benefit from vaccination, because it should be injected before sexual activity, while the current vaccines are not therapeutically (Szarewski A, 2011). The exact duration is unknown for the vaccines but in the future one vaccination triplet might protect for a lifetime (Drolet M, 2013). In the current vaccines HPV16 and HPV18 are used, in the future broader vaccines might be in use. Broader vaccines will protect a higher percentage of women. Moreover, HPV types vary in different geographical areas. Chansaenroj et al recommends addition of HPV52 and HPV58 in the vaccines to improve them for Asian countries(Chansaenroj J, 2013).

Conclusion

This review showed an overview of the current screening and the renewed screening for cervical carcinoma´s with attention to their future relevance in vaccinated populations and their application in developing countries.

Vaccination will prevent HPV induced cervical cancer. Vaccination protects against the two most common HPV-types, however the remaining HPV types count for 30% of the cervical cancers, therefor screening is still needed. Screening is a useful tool because of the time in between HPV infection and onset of cancer. Unfortunately with vaccination, screening is less effective.

Cytology screening has low sensitivity, HPV-testing has higher sensitivity but has low specificity. To prevent overdiagnosis with HPV-testing follow ups are needed. Triage with cytology will decrease the

‘false positives’ but lowers the sensitivity. In my opinion research for new methods are needed. In this review the methods of p16 INK4a and methylation are pointed out as promising. Those methods are sensitive for HSIL and therefore give less 'false positives'. Moreover p16INK4a has higher sensitive than the conventional cytology and with methylation testing new markers can be designed which gives chance of improvenment.

Self-sampling will also improve the amount of participants and therefore the amount of infections. The self-sampling are interesting for developmental countries. Moreover, more awareness is needed which will also increase the participants of screening.

Vaccination in combination with screening will reduce the incidence of cervical cancer. However, it is important to keep improve the methods of screening methods and vaccination. Also research is needed of the duration of the vaccines. Screening and vaccination should be applicable in developmental countries because cervical cancer is common in those countries.

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References

1: Information obtained through the Dutch national institute for public health and environment: www.rivm.nl (visited on 19/12/2013)

2: Globosan 2012: http://globocan.iarc.fr (visited on 19/12/2013)

Anhang, R., J. A. Nelson, R. Telerant, M. A. Chiasson, and T. C. Wright Jr. 2005. "Acceptability of Self-Collection of Specimens for HPV DNA Testing in an Urban Population." *Journal of Women's Health (2002)* 14 (8): 721-728.

Arbyn M., Buntinx F., Van Ranst M., Paraskevaidis E., Martin-Hirsch P., Dillner J. 2004. ‘Virologic Versus Cytologic Triage of Women With Equivocal Pap Smears: A Meta-analysis of the Accuracy To Detect High-Grade Intraepithelial Neoplasia’ Journal of the national cancer institiute 96 (4) 280-294.

Belinson, J. L., H. Du, B. Yang, R. Wu, S. E. Belinson, X. Qu, R. G. Pretorius, X. Yi, and P. E. Castle. 2012. "Improved Sensitivity of Vaginal Self-Collection and High-Risk Human Papillomavirus Testing." *International Journal of Cancer.Journal International Du Cancer* 130 (8): 1855-1860.

Bergeron, C., J. Ordi, D. Schmidt, M. J. Trunk, T. Keller, R. Ridder,. 2010. "Conjunctive p16INK4a Testing significantly Increases Accuracy in Diagnosing High-Grade Cervical Intraepithelial Neoplasia." *American Journal of Clinical Pathology* 133 (3): 395-406.

Berkhof J., Coupe V.M., Bogaards J.A., Van Kemenade F.J., Helmerhorst T.J., Snijders P.J., Meijer C. J. 2010. ‘The health and economic effects of HPV DNA screening in the Netherlands’ International journal of cancer (127): 2147–2158

Bratcher J.,J. Palefsky. 2008. ‘Anogenital Human Papillomavirus Coinfection and Associated Neoplasia in HIV-positve men and women’ published online at www.prn.org (visited 19-12-2013)

Cavalcante, D. M., I. M. Linhares, M. M. Pompeu, P. C. Giraldo, and J. Eleuterio. 2012. "The Utility of p16 INK4a and Ki-67 to Identify High-Grade Squamous Intraepithelial Lesion in Adolescents and Young Women."*Indian Journal of Pathology & Microbiology* 55 (3): 339-342.

Chansaenroj, J., P. Junyangdikul, T. Chinchai, S. Swangvaree, A. Karalak, N. Gemma, and Y. Poovorawan. 2013. "Large Scale Study of HPV Genotypes in Cervical Cancer and Different Cytological Cervical Specimens in Thailand." *Journal of Medical Virology*.

Coupe, V. M., J. A. Bogaards, C. J. Meijer, and J. Berkhof. 2012. "Impact of Vaccine Protection Against Multiple HPV Types on the Cost-Effectiveness of Cervical Screening." *Vaccine* 30 (10): 1813-1822.

Cuzick, J., M. Arbyn, R. Sankaranarayanan, V. Tsu, G. Ronco, M. H. Mayrand, J. Dillner, and C. J. Meijer. 2008. "Overview of Human Papillomavirus-Based and Other Novel Options for Cervical Cancer Screening in Developed and Developing Countries." *Vaccine* 26 Suppl 10: K29-41.

Cuzick, J., C. Bergeron, M. von Knebel Doeberitz, P. Gravitt, J. Jeronimo, A. T. Lorincz, C. J L M Meijer, R. Sankaranarayanan, P. J F Snijders, and A. Szarewski. 2012. "New Technologies and Procedures for Cervical Cancer Screening." *Vaccine* 30 Suppl 5: F107-16.

Dannecker, C., U. Siebert, C. J. Thaler, D. Kiermeir, H. Hepp, and P. Hillemanns. 2004. "Primary Cervical Cancer Screening by Self-Sampling of Human Papillomavirus DNA in Internal Medicine Outpatient Clinics."*Annals of Oncology : Official Journal of the European Society for Medical Oncology / ESMO* 15 (6): 863-869.

Drolet, M., J. F. Laprise, M. C. Boily, E. L. Franco, and M. Brisson. 2013. "Potential Cost-Effectiveness of the Nonavalent Human Papillomavirus (HPV) Vaccine." *International Journal of Cancer.Journal International Du Cancer*.

Eperon, I., P. Vassilakos, I. Navarria, P. A. Menoud, A. Gauthier, J. C. Pache, M. Boulvain, S. Untiet, and P. Petignat. 2013. "Randomized Comparison of Vaginal Self-Sampling by Standard Vs. Dry Swabs for Human Papillomavirus Testing." *BMC Cancer* 13: 353-2407-13-353.

Eijsink, J. J., A. Lendvai, V. Deregowski, H. G. Klip, G. Verpooten, L. Dehaspe, G. H. de Bock, et al. 2012. "A Four-Gene Methylation Marker Panel as Triage Test in High-Risk Human Papillomavirus Positive Patients."*International Journal of Cancer.Journal International Du Cancer* 130 (8): 1861-1869.

Forrest, S., K. McCaffery, J. Waller, M. Desai, A. Szarewski, L. Cadman, and J. Wardle. 2004. "Attitudes to Self-Sampling for HPV among Indian, Pakistani, African-Caribbean and White British Women in Manchester, UK." *Journal of Medical Screening* 11 (2): 85-88.

Franco, E. L., J. Cuzick, A. Hildesheim, and S. de Sanjose. 2006. "Chapter 20: Issues in Planning Cervical Cancer Screening in the Era of HPV Vaccination." *Vaccine* 24 Suppl 3: S3/171-7.

Gertig, D. M., J. M. Brotherton, A. C. Budd, K. Drennan, G. Chappell, and A. M. Saville. 2013. "Impact of a Population-Based HPV Vaccination Program on Cervical Abnormalities: A Data Linkage Study." *BMC Medicine* 11 (1): 227.

Gok, M., D. A. Heideman, F. J. van Kemenade, J. Berkhof, L. Rozendaal, J. W. Spruyt, F. Voorhorst, et al. 2010. "HPV Testing on Self Collected Cervicovaginal Lavage Specimens as Screening Method for Women Who do Not Attend Cervical Screening: Cohort Study." *BMJ (Clinical Research Ed.)* 340: c1040.

Gok, M., D. A. Heideman, F. J. van Kemenade, A. L. de Vries, J. Berkhof, L. Rozendaal, J. A. Belien, et al. 2012. "Offering Self-Sampling for Human Papillomavirus Testing to Non-Attendees of the Cervical Screening Programme: Characteristics of the Responders." *European Journal of Cancer (Oxford, England : 1990)* 48 (12): 1799-1808.

Gonzalez, M. A., K. E. Tachibana, R. A. Laskey, and N. Coleman. 2005. "Control of DNA Replication and its Potential Clinical Exploitation." *Nature Reviews.Cancer* 5 (2): 135-141.

Graham, S. V. 2010. "Human Papillomavirus: Gene Expression, Regulation and Prospects for Novel Diagnostic Methods and Antiviral Therapies." *Future Microbiology* 5 (10): 1493-1506.

Hillemanns, P., R. Kimmig, U. Huttemann, C. Dannecker, and C. J. Thaler. 1999. "Screening for Cervical Neoplasia by Self-Assessment for Human Papillomavirus DNA." *Lancet* 354 (9194): 1970.

Hoque, M. O., M. S. Kim, K. L. Ostrow, J. Liu, G. B. Wisman, H. L. Park, M. L. Poeta, et al. 2008. "Genome-Wide Promoter Analysis Uncovers Portions of the Cancer Methylome." *Cancer Research* 68 (8): 2661-2670.

Ikenberg, H., C. Bergeron, D. Schmidt, H. Griesser, F. Alameda, C. Angeloni, J. Bogers, et al. 2013. "Screening for Cervical Cancer Precursors with p16/Ki-67 Dual-Stained Cytology: Results of the PALMS Study."*Journal of the National Cancer Institute* 105 (20): 1550-1557.

Kirnbauer, R., F. Booy, N. Cheng, D. R. Lowy, and J. T. Schiller. 1992. "Papillomavirus L1 Major Capsid Protein Self-Assembles into Virus-Like Particles that are Highly Immunogenic." *Proceedings of the National Academy of Sciences of the United States of America* 89 (24): 12180-12184.

Kirschner, B., J. Junge, K. Holl, M. Rosenlund, S. Collas de Souza, W. Quint, A. Molijn, D. Jenkins, and D. Schledermann. 2013. "HPV Genotypes in Invasive Cervical Cancer in Danish Women." *Acta Obstetricia Et Gynecologica Scandinavica* 92 (9): 1023-1031.

Kitchener, H. C., C. Gilham, A. Sargent, A. Bailey, R. Albrow, C. Roberts, M. Desai, et al. 2011. "A Comparison of HPV DNA Testing and Liquid Based Cytology Over Three Rounds of Primary Cervical Screening: Extended Follow Up in the ARTISTIC Trial." *European Journal of Cancer (Oxford, England : 1990)* 47 (6): 864-871.

Koliopoulos, G., M. Arbyn, P. Martin-Hirsch, M. Kyrgiou, W. Prendiville, and E. Paraskevaidis. 2007. "Diagnostic Accuracy of Human Papillomavirus Testing in Primary Cervical Screening: A Systematic Review and Meta-Analysis of Non-Randomized Studies." *Gynecologic Oncology* 104 (1): 232-246.

Korhonen, M. O. 1980. "Epidemiological Differences between Adenocarcinoma and Squamous CellCarcinoma of the Uterine Cervix." *Gynecologic Oncology* 10 (3): 312-317.

Lai, H. C., Y. W. Lin, T. H. Huang, P. Yan, R. L. Huang, H. C. Wang, J. Liu, et al. 2008. "Identification of Novel DNA Methylation Markers in Cervical Cancer." *International Journal of Cancer.Journal International Du Cancer* 123 (1): 161-167.

Lee, Y. Y., C. H. Choi, T. J. Kim, J. W. Lee, B. G. Kim, J. H. Lee, and D. S. Bae. 2011. "A Comparison of Pure Adenocarcinoma and Squamous Cell Carcinoma of the Cervix After Radical Hysterectomy in Stage IB-IIA." *Gynecologic Oncology* 120 (3): 439-443.

Leinonen, M. K., P. Nieminen, S. Lonnberg, N. Malila, M. Hakama, A. Pokhrel, P. Laurila, J. Tarkkanen, and A. Anttila. 2012. "Detection Rates of Precancerous and Cancerous Cervical Lesions within One Screening Round of Primary Human Papillomavirus DNA Testing: Prospective Randomised Trial in Finland." *BMJ (Clinical Research Ed.)* 345: e7789.

Leniz, J., M. I. Barriga, M. Lagos, C. Ibanez, K. Puschel, and C. Ferreccio. 2013. "HPV Vaginal Self-Sampling among Women Non-Adherent to Papanicolaou Screening in Chile." *Salud Publica De Mexico* 55 (2): 162-169.

Lindsey, J. C., J. A. Anderton, M. E. Lusher, and S. C. Clifford. 2005. "Epigenetic Events in Medulloblastoma Development." *Neurosurgical Focus* 19 (5): E10.

Lie, A. K. and G. Kristensen. 2008. "Human Papillomavirus E6/E7 mRNA Testing as a Predictive Marker for Cervical Carcinoma." *Expert Review of Molecular Diagnostics* 8 (4): 405-415.

Lorenzi, A. T., J. H. Fregnani, J. C. Possati-Resende, C. S. Neto, L. L. Villa, and A. Longatto-Filho. 2013. "Self-Collection for High-Risk HPV Detection in Brazilian Women using the careHPV Test." *Gynecologic Oncology* 131 (1): 131-134.

Mahdavi, A. and B. J. Monk. 2006. "Recent Advances in Human Papillomavirus Vaccines." *Current Oncology Reports* 8 (6): 465-472.

Mahdavi, A. and B. J. Monk. 2005. "Vaccines Against Human Papillomavirus and Cervical Cancer: Promises and Challenges." *The Oncologist* 10 (7): 528-538.Malik, H., F. H. Khan, and H. Ahsan. 2013. "Human Papillomavirus: Current Status and Issues of Vaccination." *Archives of Virology*.

Malik, H., F. H. Khan, and H. Ahsan. 2013. "Human Papillomavirus: Current Status and Issues of Vaccination." *Archives of Virology*.

Malila, N., M. Leinonen, L. Kotaniemi-Talonen, P. Laurila, J. Tarkkanen, and M. Hakama. 2013. "The HPV Test has Similar Sensitivity but More Overdiagnosis than the Pap Test--a Randomised Health Services Study on Cervical Cancer Screening in Finland." *International Journal of Cancer.Journal International Du Cancer* 132 (9): 2141-2147.

Mayrand, M. H., E. Duarte-Franco, F. Coutlee, I. Rodrigues, S. D. Walter, S. Ratnam, E. L. Franco,. 2006. "Randomized Controlled Trial of Human Papillomavirus Testing Versus Pap Cytology in the Primary Screening for Cervical Cancer Precursors: Design, Methods and Preliminary Accrual Results of the Canadian Cervical Cancer Screening Trial (CCCaST)." *International Journal of Cancer.Journal International Du Cancer* 119 (3): 615-623.

Meijer, C. J. and J. Berkhof. 2012. "Screening: Cervical Cancer--should we Abandon Cytology for Screening?" *Nature Reviews.Clinical Oncology* 9 (10): 558-559.

Pacchiarotti, A., F. Ferrari, P. Bellardini, F. Chini, G. Collina, P. Dalla Palma, B. Ghiringhello, et al. 2013. "Prognostic Value of p16-INK4A Protein in Women with Negative Or CIN1 Histology Result: A Follow-Up Study." *International Journal of Cancer.Journal International Du Cancer*.

Petry, K. U., F. Rinnau, G. Bohmer, B. Hollwitz, A. Luyten, N. Buttmann, M. Brunger, and T. Iftner. 2013. "Annual Papanicolaou Screening for 5 Years among Human Papillomavirus-Negative Women." *BMC Cancer*13: 379-2407-13-379.

Qureshi, M. N., D. Bolick, P. J. Ringer, F. L. Spagler, and G. Zimmerman. 2005. "HPV Testing in Liquid Cytology Specimens: Comparison of Analytic Sensitivty and Specificity for in Situ Hybridization and Chemiluminescent Nucleic Acid Testing." *Acta Cytologica* 49 (2): 120-126.

Rijkaart, D. C., J. Berkhof, F. J. van Kemenade, V. M. Coupe, A. T. Hesselink, L. Rozendaal, D. A. Heideman, et al. 2012. "Evaluation of 14 Triage Strategies for HPV DNA-Positive Women in Population-Based Cervical Screening." *International Journal of Cancer.Journal International Du Cancer* 130 (3): 602-610.

Rijkaart, D. C., J. Berkhof, F. J. van Kemenade, V. M. Coupe, L. Rozendaal, D. A. Heideman, R. H. Verheijen, et al. 2012. "HPV DNA Testing in Population-Based Cervical Screening (VUSA-Screen Study): Results and Implications." *British Journal of Cancer* 106 (5): 975-981.

Ronco, G., J. Dillner, K. M. Elfstrom, S. Tunesi, P. J. Snijders, M. Arbyn, H. Kitchener, et al. 2013. "Efficacy of HPV-Based Screening for Prevention of Invasive Cervical Cancer: Follow-Up of Four European Randomised Controlled Trials." *Lancet*.

Ronco, G., N. Segnan, P. Giorgi-Rossi, M. Zappa, G. P. Casadei, F. Carozzi, P. Dalla Palma, et al. 2006. "Human Papillomavirus Testing and Liquid-Based Cytology: Results at Recruitment from the New Technologies for Cervical Cancer Randomized Controlled Trial." *Journal of the National Cancer Institute* 98 (11): 765-774.

Ronco, G., J. Cuzick, P. Pierotti, M. P. Cariaggi, P. Dalla Palma, C. Naldoni, B. Ghiringhello, et al. 2007. "Accuracy of Liquid Based Versus Conventional Cytology: Overall Results of New Technologies for Cervical Cancer Screening: Randomised Controlled Trial." *BMJ (Clinical Research Ed.)* 335 (7609): 28.

Schmidt, D., C. Bergeron, K. J. Denton, R. Ridder,. 2011. "P16/ki-67 Dual-Stain Cytology in the Triage of ASCUS and LSIL Papanicolaou Cytology: Results from the European Equivocal Or Mildly Abnormal Papanicolaou Cytology Study." *Cancer Cytopathology* 119 (3): 158-166.

Smith, H. O., M. F. Tiffany, C. R. Qualls, and C. R. Key. 2000. "The Rising Incidence of Adenocarcinoma Relative to Squamous Cell Carcinoma of the Uterine Cervix in the United States--a 24-Year Population-Based Study." *Gynecologic Oncology* 78 (2): 97-105.

Szarewski, A., L. Cadman, S. Mallett, J. Austin, P. Londesborough, J. Waller, J. Wardle, D. G.Altman, and J. Cuzick. 2007. "Human Papillomavirus Testing by Self-Sampling: Assessment of Accuracy in an Unsupervised Clinical Setting." *Journal of Medical Screening* 14 (1): 34-42.

Szarewski, A., W. A. Poppe, S. R. Skinner, C. M. Wheeler, J. Paavonen, P. Naud, J. Salmeron, et al. 2012. "Efficacy of the Human Papillomavirus (HPV)-16/18 AS04-Adjuvanted Vaccine in Women Aged 15-25 Years with and without Serological Evidence of Previous Exposure to HPV-16/18." *International Journal of Cancer.Journal International Du Cancer* 131 (1): 106-116.

Taylor, D., C. Lunny, T. Wong, M. Gilbert, N. Li, R. Lester, M. Krajden, L. Hoang, and G. Ogilvie. 2013. "Self-Collected Versus Clinician-Collected Sampling for Sexually Transmitted Infections: A Systematic Review and Meta-Analysis Protocol." *Systematic Reviews* 2 (1): 93-4053-2-93.

Terry, G., L. Ho, P. Londesborough, J. Cuzick, I. Mielzynska-Lohnas, and A. Lorincz. 2001. "Detection of High-Risk HPV Types by the Hybrid Capture 2 Test." *Journal of Medical Virology* 65 (1): 155-162.

Tjalma, W. A. and C. E. Depuydt. 2013. "Cervical Cancer Screening: Which HPV Test should be used--L1 Or E6/E7?" *European Journal of Obstetrics, Gynecology, and Reproductive Biology* 170 (1): 45-46.

Verma, M., S. Rogers, R. L. Divi, S. D. Scully, S. Nelson, L. J. Su, S. Ross, S. Pilch, D. M. Winn, and M. J. Khoury. 2013. "Epigenetic Research in Cancer Epidemiology: Trends, Opportunities, and Challenges." *Cancer Epidemiology, Biomarkers & Prevention : A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology*.

Verdoodt, F., A. Szarewski, P. Halfon, K. Cuschieri, and M. Arbyn. 2013. "Triage of Women with Minor Abnormal Cervical Cytology: Meta-Analysis of the Accuracy of an Assay Targeting Messenger Ribonucleic Acid of 5 High-Risk Human Papillomavirus Types." *Cancer Cytopathology*.

Wilyman, J. 2013. "HPV Vaccination Programs have Not been shown to be Cost-Effective inCountries with Comprehensive Pap Screening and Surgery." *Infectious Agents and Cancer* 8 (1): 21-9378-8-21.

Wu, R., H. Du, S. E. Belinson, R. G. Pretorius, J. Zou, B. Yang, C. Wang, and J. L. Belinson. 2012. "Secondary Screening After Primary Self-Sampling for Human Papillomavirus from SHENCCAST II." *Journal of Lower Genital Tract Disease* 16 (4): 416-420.

Zhao, F. H., A. K. Lewkowitz, F. Chen, M. J. Lin, S. Y. Hu, X. Zhang, Q. J. Pan, et al. 2012. "Pooled Analysis of a Self-Sampling HPV DNA Test as a Cervical Cancer Primary Screening Method." *Journal of the National Cancer Institute* 104 (3): 178-188.

Zheng, Z. M. and C. C. Baker. 2006. "Papillomavirus Genome Structure, Expression, and Post-Transcriptional Regulation." *Frontiers in Bioscience : A Journal and Virtual Library* 11: 2286-2302.

Zorzi, M., A. Del Mistro, A. Farruggio, L. de'Bartolomeis, H. Frayle-Salamanca, L. Baboci, A. Bertazzo, et al. 2013. "Use of a High-Risk Human Papillomavirus DNA Test as the Primary Test in a Cervical Cancer Screening Programme: A Population-Based Cohort Study." *BJOG : An International Journal of Obstetrics and Gynaecology* 120 (10): 1260-7; discussion 1267-8.