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HPV detection as primary screening method for cervical cancer

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Cervical cancer affects women worldwide and in all ages, which makes it an extensive health problem. Fortunately, malignant transformation can be avoided if precancerous lesions are detected and cured early. Cervical cancer screening programs have been developed all over the world, screening women to detect precursors of cancer. Until recently cytology was preferred as primary screening method, however our still growing knowledge has led to new and potentially better methods.

High risk human papillomavirus (hrHPV) DNA testing has been suggested as primary screening method with cytology triage.

The focus of this thesis will be to investigate the efficacy of hrHPV DNA testing with cytology triage compared to cytology as a primary screening method.

The objective is illustrated by an investigation of results from three randomized controlled trials comparing cytology to hrHPV DNA testing. The articles are presented followed by an analysis of validity concerning recruitment procedures, trial design and data analysis.

Finally, a weighted average of the relative risk of cervical intraepithelial neoplasia grade 2 (CIN2) detection in women receiving hrHPV test compared to women receiving cytology is estimated. Individual results from the three studies combined with the weighted average (RR=1,54; 95%CI: [1,36;1,74]) suggest that hrHPV screening is more efficient than cytology, however, further prolonged investigations are needed to address a potential beneficial effect prospectively.

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Introduction

Cervical cancer affects women worldwide with 530.000 new cases and 266.000 deaths each year.[4] Despite our growing knowledge; it is the third most common cancer in the world.[5]
However, cervical cancer is preventable; a long pre-invasive phase with detectable dysplasia makes an obvious target for screening.
The goal of screening is to identify the precursors of cervical cancer, which treated, hopefully will prevent malignant transformation. The primary screening method used today is cytology; this test however, is shown to have low sensitivity. Women who get a negative result might have precursors to cancer.[5,6]
The aetiology of cervical cancer is the human papillomavirus (HPV), however the malignant transformation happens for only a small part of the infected women. High-risk HPV (hrHPV) subtypes have been identified; associated with a greater risk of cervical cancer.[5]

Question is if hrHPV detection with cytology triage is a better predictor of cervical cancer and therefore more suited as primary screening test than cytology.

Cervical cancer

Cervical cancer is defined as malignant epithelium penetrating the basement membrane, infiltrating the supporting stroma in cervix uteri. Precursor lesions are often seen in the transformation zone; the junction between the squamous epithelium at portio and the columnar epithelium in cervix.

Bethesda classification can be used to classify changes observed in cytology samples and a corresponding histologic classification categorizes biopsies as Cervical Intraepithelial Neoplasia (CIN) grade 1 to 3.[7]

Etiology of cervical cancer

Human papillomavirus

HPV is a double-stranded DNA-virus; it is circular, non-enveloped and infects both mucosal and cutaneous surfaces. The papillomavirus family consists of more than 130 genotypes; subdivided into those associated with high or low risk of cancer. The hrHPV subtypes are associated with squamous, adenosquamous cancer and adenocarcinoma, with HPV-16/18 responsible for 70%.[5]
HPV is transmitted sexually by skin to skin contact, but the infection might only be transient not inducing malignant changes. HPV infects basal cells of the squamous epithelium, and induction of malignant transformation occurs when the viral gene products, E5, E6 and E7, are expressed. E5 enhances cell growth by stabilizing growth factor receptors. E6 and E7 interact with and inhibit tumor suppressor genes. This inhibition makes the host cell unable to control apoptosis and combined with increased sensitivity to growth signals, it leads to uncontrolled cell-growth and the risk of multiple mutations.[8]

Other factors

For the malignant transformation to occur the HPV infection has to be persistent. Immunosuppression, genetic predisposition, host factors and environmental factors may influent on the risk of persistent infection and add to the risk of cervical cancer.[5]

Screening tests

The Papanicolaou smear

The Papanicolaou (Pap) smear has been used in cervical cancer screening for more than 60 years.[6] The test is based on cell samples from the outer opening of the cervix, which is hotspot for cervical intraepithelial neoplasia to occur. For the conventional smear the cells are smeared on slides and fixed. A newer technology is liquid-based cytology (LBC), where the sampled cells are fixed immediately in a preservative liquid and then subsequent distributed uniformly in a thin layer on a slide. An additional advantage of LBC is that only a portion of the sample is used to prepare the slide, the remaining can be used for additional tests, e.g. HPV-tests. Preparing the slides include Papanicolaou staining. This method stains the squamous cells with a transparent cytoplasmic color that varies with cell maturity.[6]

One review, which investigated the accuracy of cytology, found that sensitivity ranged from 30% to 87%; specificity from 86% to 100%.[9] These values are the result of several potential sources of error. Depending on the expertise of the physician the sample might not be collected correctly, and depending on the laboratory and the qualifications of the technologist reading error might occur. Interpretation is subjective resulting in interobserver variability.[5]

The HPV-test

Screening for hrHPV DNA takes a step back in the malignant progress compared to cytology, where the changes already has happened.
In contrast to cytology, the HPV-tests have high negative predictive value, but lack specificity, meaning that the test in most cases correctly will identify women with precursor lesions, however HPV-infected women with no cellular dysplasia are tested positive too.[5]
DNA, RNA and different markers associated with malignant changes can be used to identify HPV infection in cell samples. Polymerase Chain Reaction (PCR) and hybridization are the two main methods.[5]

Methods

Sources and selection criteria

Articles referred to in this thesis are found in PubMed using the MeSH Database. Different MeSH terms were used to find the references for the introduction part. The articles were selected based on their relevance and publication date, so that the most recent available were chosen first. Reviews were preferred and from citations in these original literature was found. General information about HPV and cervical cancer was found in educational books.

To identify the three main articles following MeSH-terms were used: “Papillomaviridae”, “Uterine Cervical Neoplasms”, “Early Detection of Cancer”, “Randomized Controlled Trial (Publication Type)” and “Mass Screening”. The search was restricted to articles published in 2011 to 2016 by a 5-years publication criteria. In total nine articles matched this search, but only three of them were relevant for this thesis (figure 1). Additional publications were identified with details about each trial design.

Data analysis

An analysis of the three main articles will lead to an estimate of the efficacy of hrHPV testing as a primary screening method. This will be based on the credibility of the articles, measured by an analysis of potential bias.

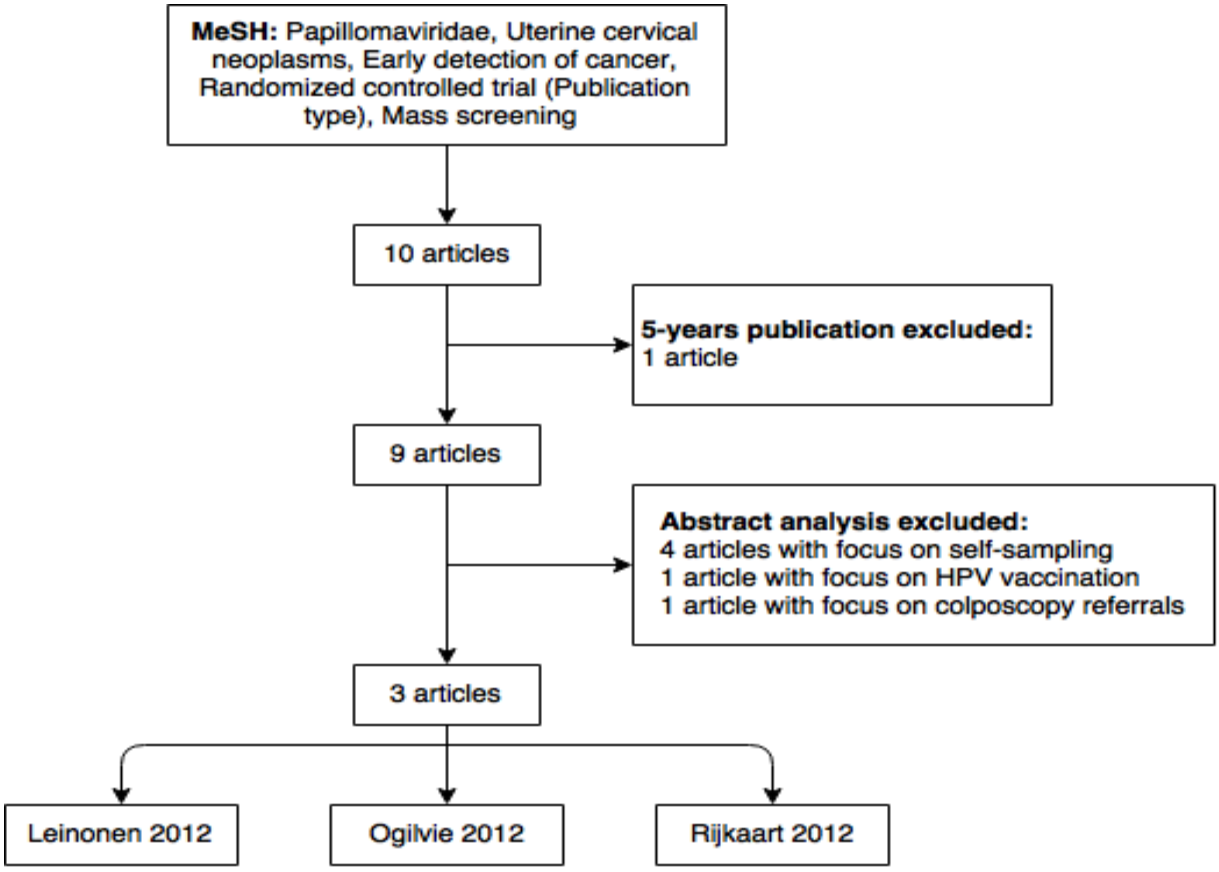

Figure 1: Flow diagram showing article identification process with exclusion criteria. Leinonen 2012[1], Ogilvie 2012[2], Rijkaart 2012[3]

Results

In the following section the three main studies will be presented and an estimate of the efficacy of hrHPV tests compared to cytology calculated.

In the introduction of both Leinonen 2012[1], Ogilvie 2012[2] and Rijkaart 2012[3] current knowledge and literature is presented. In the light of the importance of an effective screening program, the need for additional investigations concerning the efficacy of hrHPV testing is clarified. However, specifying incidence or prevalence of cervical cancer would put the investigations into perspective and further emphasize the severity.

The main objective of all three studies is to investigate the efficacy of hrHPV testing as primary screening method, and it will be the focus of this presentation.

To address the hypothesis, a population of women participating in a cervical cancer screening program have to be identified. To investigate the effect one group of women (the control arm) has to follow the usual screening procedure, while the other group (the intervention arm) will be tested for hrHPV (table 1). Randomization will avoid confounding.

Table 1: Summary of methods used in the the intervention arm and control arm sorted by study

	Study design	
Article	**Intervention arm**	**Control arm**
Leinonen 2012	hrHPV testing and cytology triage of HPV-positive women.	Cytology
	Test: Hybrid Capture 2 assay	**Test:** Conventional cytology
	Classification: HPV-positive or HPV-negative	**Classification:** Papanicolaou classification (2003-2005), Bethesda classification hereafter
Ogilvie 2012	hrHPV testing and cytology triage of HPV-positive	Cytology
	Test: Hybrid Capture 2 assay	**Test:** Liquid-based cytology
	Classification: HPV-positive or HPV-negative	**Classification:** Bethesda classification
Rijkaart 2012	hrHPV testing combined with cytology	Cytology combined with hrHPV testing (blinded)
	Test: GP5+/6+ PCR-enzyme immunoassay and conventional cytology	**Test:** Conventional cytology and GP5+/6+ PCR-enzyme immunoassay
	Classification: HPV-positive or HPV-negative and according to CISOE-A classification	**Classification:** CISOE-A classification system

Leinonen 2012

"Detection rates of precancerous and cancerous cervical lesions within one screening round of primary human papillomavirus DNA testing: prospective randomized trial in Finland"

The target population was women aged 25 to 65, participating in the cervical cancer screening program from 2003 to 2007 in Southern Finland. Through a database eligible women were selected, and an invitation letter with information of the new test sent out. The women became randomized, and upon screening visit the assigned test was revealed.

Study size estimates have been calculated to identify the minimum population size to test for differences in screening effects.[10]

Of the 203.788 women eligible for randomization, 66.410 in the HPV arm and 65.785 in the control arm attended screening. There is no analysis of background characteristics of the participating and non-participating women.

There was no blinding in this trial, since personnel involved were aware of all test results. All necessary data and results were gathered from databases, where the women's identifiers were linked individually.

Leinonen 2012 recognize some weaknesses and limitations in their study due to possible opportunistic Pap testing and potential defects in randomization.

Ogilvie 2012

"Primary cervical cancer screening with HPV testing compared with liquid-based cytology: results of round I of a randomized controlled trial – the HPV FOCAL Study"

The study population is women aged 25 to 65, who receive care from a study participating family physician in British Columbia, Canada. Some exclusion criteria are applied, most of which exclude women with a higher risk of HPV infection.
The women were recruited either directly attending cervical screening or by letter.

Power calculations have been made measuring the adequate sample size.[11] The planned sample size was 33.000 in total; however, only 18.648 women of the 44.099 invited were eligible for randomization. There is no data reporting the final desired sample size. 5.271 declined participation, 475 did not meet inclusion criteria and 24.086 did not respond to invitation letter. No analysis of non-responders has been made.

In the HPV FOCAL Study the eligible women are randomized into three arms: control arm, intervention arm (screening interval: 4 years) and safety check arm (screening interval: 2 years). Ogilvie 2012 presents results from round I combining the safety and intervention arm as a HPV arm, because of identical management at baseline and initial follow-up.

Samples are collected at baseline screen and can be used both as cytologic sample and for hrHPV test. Randomization occurs through a database upon laboratory receipt; independent of the physician and the participant. All the relevant stages of the process are blinded.

Trial arms are balanced on different parameters (Ogilvie 2012: Table 1), and both known and unknown confounders are presumed to be randomly distributed.

Ogilvie 2012 presents results from the baseline screen and subsequent screening round for women recommended for a second test. Missing data from the subsequent screening round is treated as being missing at random; an assumption that already observed data does not depend on the missing data. There is no reason to think that the missing test results will influence on the results already analyzed.
Data from the second screening round is not available as the trial is still proceeding, and this might explain the fact that discussion and conclusion are not clearly separated.

Rijkaart 2012

"Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomized controlled trial"

The study population is women participating in the nationwide screening program in the Netherlands from 1999 to 2002. Women are invited for cervical cancer screening when they reach 30 and until 60 with a 5-year interval.

Together with the invitation for the regular screening, the women were informed about POBASCAM and invited to participate.
Some exclusion criteria were applied, excluding women with increased risk of cervical cancer or women who because of older age would not routinely receive screening in connection with the second round.

Power calculations have been performed to ensure that the study would be large enough to detect significant difference in outcome. A study size of 44.000 participants were estimated to be sufficient.

After sampling of cervical cells, the women were randomized using computer-generated random numbers. The cytologic samples are classified according to the CISOE-A classification system used in the Netherlands, which can be compared to the Bethesda system.[12]

Of 44.938 women randomized, 19.999 in the intervention arm and 20.106 in the control arm attended first screen. At second screen some were lost to follow-up and others excluded. However, the main analyses were done by intention-to-screen; all women randomized were included.

Both molecular and cytotechnicians were blinded and unaware of the sample's assignment. In both groups the pathologists were aware of cytology, but not HPV result. Two experienced pathologists made reviews of all CIN biopsies, and they were blinded for both cytology and HPV results. The original diagnoses were used in the analyses.

Rijkaart 2012 recognizes some weaknesses in their study design owed to possible variation in histological classification of cervical lesions between different centers.

Study results are summarized in table 2.

Table 2: Summary of results presented sorted by study

	Results
Leinonen 2012	hrHPV detects more cervical cancer and CIN lesions than primary cytology. Detection of CIN3 was strongly decreased among women who were HPV-negative at baseline compared to women with normal cytology. The follow-up is too short to make statement about potential changes in cervical cancer incidence.
Ogilvie 2012	Increased detection rates of CIN2 or worse in the hrHPV arm compared to the control arm.
Rijkaart 2012	Women randomized to the HPV arm had fewer CIN3 or worse including cervical cancer detected in the second screening round compared to the control arm. Women who tested HPV-negative had significantly fewer CIN3 or worse than women who had normal cytology at baseline screen.

Overall the three studies agree that hrHPV testing has some benefits compared to cytology, however they estimate the efficacy in different ways. To compare the data, a common estimate can be calculated and, if reasonable, a weighted average too.

Weighted average

To assess the efficacy of hrHPV testing compared to cytology a relative risk of CIN2 detection in the first screening round can be estimated for each study (table 3). Using hrHPV tests instead of cytology may lead to earlier detection of cell changes, why CIN2 is used as outcome.

The weighted average can be estimated to 1,54 (95%CI:1,36;1,74); meaning that the risk of CIN2 detection is 1,54 times higher with hrHPV screening compared to cytology screening. The difference is significant. Calculations can be found in appendix 1 (table 4 and 5), and a forest plot is made to visualize the comparison (appendix 2, figure 2).

Table 3: No. of CIN2 detected and relative risk (RR) sorted by study and a weighted average

Article	Intervention arm		Control arm		RR (95%CI)	Weighted average (95%CI)
	No. CIN2	Total	No. CIN2	Total		
Leinonen 2012	345	66.410	201	65.784	1,70 (1,43;2,02)	1,54 (1,36;1,74)
Ogilvie 2012	201	12.472	67	6.115	1,46 (1,11;1,93)	
Rijkaart 2012	168	19.999	127	20.106	1,33 (1,06;1,67)	

Discussion

Results from the previous section suggest that hrHPV screening may be more efficient than cytology. However, stated findings depend on the validity of the studies.

Prospective randomized controlled trials with long term follow-up provide a high level of evidence. The essence of matter is a successful randomization, which distribute all known and unknown confounders equally. Problem is that a complete randomization rarely exists throughout the entire follow-up.

Randomization

To keep avoiding confounder problems and selection bias, data has to be analyzed by intention-to-screen. In this way the effect of potential crossover and dropouts can be eliminated. However, this is only stated in Rijkaart 2012.
Neither Leinonen 2012 nor Ogilvie 2012 mention this type of analysis why both studies are prone to confounding and selection bias.
Both studies have dropouts after randomization; even though the dropouts might be randomly as well, it is impossible to know if unknown confounders are still equally distributed. An analysis of the non-participating women will expose distribution of known confounders, however neither side have made such an extensive analysis. However, in Leinonen 2012 women who did not attend screening in the HPV arm had significantly fewer cases of cervical cancer than women in the control arm. This suggests that the randomization has failed.

A background analysis of the participating women would make it possible for the reader to evaluate the randomization, however only Ogilvie 2012 presents such data. Number of sexual partners and smoking status are some of the factors that might influence on the risk of cervical cancer and they should be equally distributed in the two groups.

In Leinonen 2012 the women have the possibility to refuse the hrHPV test after randomization, and this might introduce potential confounders. The benefits of randomization are lost, because the characteristics of the women may no longer be equally divided.
These women represent crossovers, and the effect of their choice can be eliminated by intention-to-screen or prevented by blinding of study participants.

Blinding and information bias

In a randomized controlled trial blinding will ensure that outcome cannot be influenced by individuality, but rather reflects the true correlation with the intervention.
Interpreting cytology and histological samples will always be subjective tasks why information bias might happen. Depending on study design (blinded or not) it can be either non-differential or differential misclassification.

In Leinonen 2012 the study is not blinded which makes differential misclassification possible. Histologists might interpret the biopsies differently knowing the status of hrHPV. If hrHPV positive samples are more likely to be classified as CIN1 or worse, then it will cause information bias. Cervical lesions will be overrepresented in the HPV arm, resulting in overestimation of the benefits of hrHPV testing at first screen.

In Ogilvie 2012 all relevant aspects of the study are blinded. A misclassification would be based on outcome and independent of to which group the slides belong. By gathering all cytologic samples in

one center, all biopsies in two centers, and by following standard practice if there is discordance between the cytologic and histologic evaluation, it is tried to reduce the risk of information bias.

Similarly, in the study presented in Rijkaart 2012 relevant stages of the process are blinded. To avoid the risk of non-differential misclassification two experienced pathologists made reviews of all the CIN biopsies, both were blinded for HPV and cytology results. The original diagnosis agreed with the second in 97% of cases.[13]

However, in both cases, non-differential misclassification will lead to bias towards the null hypothesis, which cannot explain an observed difference.

Selection bias

In a randomized controlled trial selection bias might happen after randomization if the dropouts are dependent of both exposure and outcome. If the dropouts are distributed randomly between the two groups, then the consequence will be a smaller study.
In total 4.304 women refuse the hrHPV test after randomization in the study of Leinonen 2012, however their choice is made independent of outcome, simply because it has not yet occurred. Their choice will not cause selection bias.
With no analysis of the non-participating women in Ogilvie 2012 it is not possible to decide whether dropouts are associated to both exposure and outcome. Only the study of Rijkaart 2012 will be free of selection bias because of the intention-to-screen principle.

External validity

Generally, dropouts before randomization have no effect on internal validity, but might affect external validity; it is no longer given that the study population reflects the target population and can be applied directly.
In Leinonen 2012 the target population is all women eligible for screening, and they are all randomized.
In Rijkaart 2012 some women are not enrolled because the general practitioners did not have time to explain the study objectives. If these women represent a particular group with increased risk of cervical cancer, then the risk in the study population no longer reflects the overall risk the women participating in the screening program.

In British Columbia, where the study population of Ogilvie 2012 is sampled from, family practices are not listed and women may visit different physicians.
Furthermore, participants can only be recruited in family practices willing to participate. Given these circumstances an estimation of the proportion of women, who actually received and read their invitation letter cannot be made. Consequently, external validity might be compromised.

All three studies have limitations and strengths. Leinonen 2012 presents a large study with more than 200.000 participants eligible for randomization compared to 44.938 in Rijkaart 2012 and 18.648 in Ogilvie 2012. However, with no blinding of participants and technologists, information bias might be present and crossovers cannot be avoided, putting randomization at risk. Ogilvie 2012

presents a smaller study, however all relevant parts of the process are blinded and randomization seems succeeded.
The results presented by Rijkaart 2012 are estimated by intention-to-screen analyses, which ensure the intern validity concerning confounding and selection bias. Furthermore, the study is blinded; making sure that differential misclassification becomes unlikely.

Weighted average

An estimate for the detection of CIN2 was calculated in the result section, however, for a weighted average to make sense there must be some equality between the studies according to intervention and outcome.

Intervention

The study presented by Ogilvie 2012 is the only one to use LBC, while both Leinonen 2012 and Rijkaart 2012 use conventional cytology. Different studies have shown either no difference or improved performance for liquid-based cytology compared to conventional cytology.[6]

Furthermore, Ogilvie 2012 and Leinonen 2012 use Hybrid Capture 2 assay as hrHPV test, while Rijkaart 2012 uses a GP5+/6+ PCR method. The two methods, however, have shown to perform much the same according to sensitivity and specificity.[3]

Based on this it is assumed that the difference in the choice of methods will not affect the estimation of a weighted average to a greater extent. However, if LBC performs better than convention cytology, the benefits of hrHPV testing might be overestimated if you compare hrHPV to LBC.

Outcome

In Leinonen 2012 numbers of CIN2 detected are presented in table 2. Numbers of the women who attended screening are used and the two age groups are combined. This comparison relies on the effect of screening method, why women not attending screening are not included. The numbers represent the quantity of women who are diagnosed with CIN2 in a 5-year follow-up collected from different databases.
In Rijkaart 2012 numbers are found in table 1 presenting detection of CIN2 in baseline and subsequent screen. Numbers from the two screens combined are used.
In Ogilvie 2012 no concrete numbers of CIN2 detection are found; instead detection rates per 1.000 are presented in table 2. Results from baseline and subsequent screen are used (appendix 1, table 4).

The weighted average is based on weights depended on standard error; the largest study will enter with greatest influence. Consequently, our result must be read with caution due to potential bias in Leinonen 2012.

To further investigate the efficacy of hrHPV testing, it would have been of interest to evaluate the effect gained in a prolonged follow-up permitting results from a second screening round. If hrHPV screening detects precancerous lesions earlier than cytology, subsequent screens will show a decrease in precancerous and cancerous lesions, corresponding to the data in Rijkaart 2012.

Additionally, results presented in this thesis would have been supported further by including more studies. A more broadly-based search in PubMed might have revealed other results.
A complete investigation of hrHPV tests as primary screening test is beyond the scope of this thesis, and studies investigating the potential benefits from different points of view are needed. Both the financial and individual costs have to compare with the benefits of the method. Other aspects are the consideration of a prolonged screening interval and starting age.

Conclusion

The presented literature supports the use of hrHPV screening as a primary screening method with cytology triage. Both Leinonen 2012 and Ogilvie 2012 found an increased detection of precancerous lesions in the HPV arm compared to the control arm. The study presented by Rijkaart 2012 is the only one to demonstrate results from a second screening round, and it is shown that hrHPV screening decreases the risk of precancerous and cancerous lesions prospectively.
As a comparison between the HPV and control arm in the three studies, a relative risk of CIN2 detection was found and a weighted average estimated. Comparing hrHPV screening to cytology screening the risk of CIN2 detection is 1,54 times higher. Based on data from the first screening round, this too supports the use of hrHPV screening. However, these findings must be read in the light of potential bias, and further prolonged investigations are needed to address a potential beneficial effect prospectively.

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Table 4: Calculation of relative risk (RR) with 95% confidence interval (CI)

	No. CIN2	Others	Total	Risk per 100	RR	SE(ln(RR))	95%CI
Leinonen 2012							
Intervention	345	$66410 - 345 = 66065$	66410	$\frac{345}{66410} = 0{,}52$	$\frac{0{,}52}{0{,}31} = 1{,}70$	$\sqrt{\frac{1}{345} - \frac{1}{66410} + \frac{1}{201} - \frac{1}{65784}} = 0{,}0886$	$\exp(\ln(1{,}70) \pm 1.96 \cdot 0{,}0886) = [1{,}43;2{,}02]$
Control	201	$65784 - 201 = 65583$	65784	$\frac{201}{65784} = 0{,}31$			
Ogilvie 2012							
Intervention	201*	$12472 - 201 = 12271$	12472	$\frac{201}{12472} = 1{,}61$	$\frac{1{,}61}{1{,}10} = 1{,}46$	$\sqrt{\frac{1}{201} - \frac{1}{12472} + \frac{1}{67} - \frac{1}{6115}} = 0{,}1400$	$\exp(\ln(1{,}46) \pm 1.96 \cdot 0{,}1400) = [1{,}11; 1{,}93]$
Control	67**	$6115 - 67 = 6048$	6115	$\frac{67}{6115} = 1{,}10$			
Rijkaart 2012							
Intervention	168	$19999 - 168 = 19831$	19999	$\frac{168}{19999} = 0{,}84$	$\frac{0{,}84}{0{,}63} = 1{,}33$	$\sqrt{\frac{1}{168} - \frac{1}{19999} + \frac{1}{127} - \frac{1}{20106}} = 0{,}1172$	$\exp(\ln(1{,}33) \pm 1.96 \cdot 0{,}1172) = [1{,}06; 1{,}67]$
Control	127	$20106 - 127 = 19979$	20106	$\frac{127}{20106} = 0{,}63$			

***Intervention**	$\frac{16{,}1}{1000} \cdot 12472 = 201$	****Control**	$\frac{11{,}0}{1000} \cdot 6115 = 67$

Table 5: Calculation of weighted average with 95% confidence interval (CI)

Stratum	Estimat	SE	Weight (w)	Estimate x weight
Leinonen 2012	$\ln(1{,}70) = 0{,}5308$	0,0886	$\frac{1}{0{,}0886^2} = 127{,}39$	$127{,}39 \cdot 0{,}5308 = 67{,}62$
Ogilvie 2012	$\ln(1{,}46) = 0{,}3809$	0,1400	$\frac{1}{0{,}1400^2} = 51{,}02$	$51{,}02 \cdot 0{,}3809 = 19{,}43$
Rijkaart 2012	$\ln(1{,}33) = 0{,}2851$	0,1172	$\frac{1}{0{,}1172^2} = 72{,}80$	$72{,}80 \cdot 0{,}2851 = 20{,}76$
Sum	–	–	$\sum \mathrm{w} = 251{,}212$	$\sum \mathrm{Estimate} \cdot \mathrm{w} = 107{,}81$
	Estimate		SE	95% CI
Weighted average	$\frac{107{,}81}{251{,}212} = 0{,}4292$		$\frac{1}{\sqrt{251{,}212}} = 0{,}0631$	$0{,}4292 \pm 1.96 \cdot 0{,}0631 = [0{,}3055; 0{,}5528]$
Exponentialized	1,54		–	[1,36;1,74]

Appendix 2

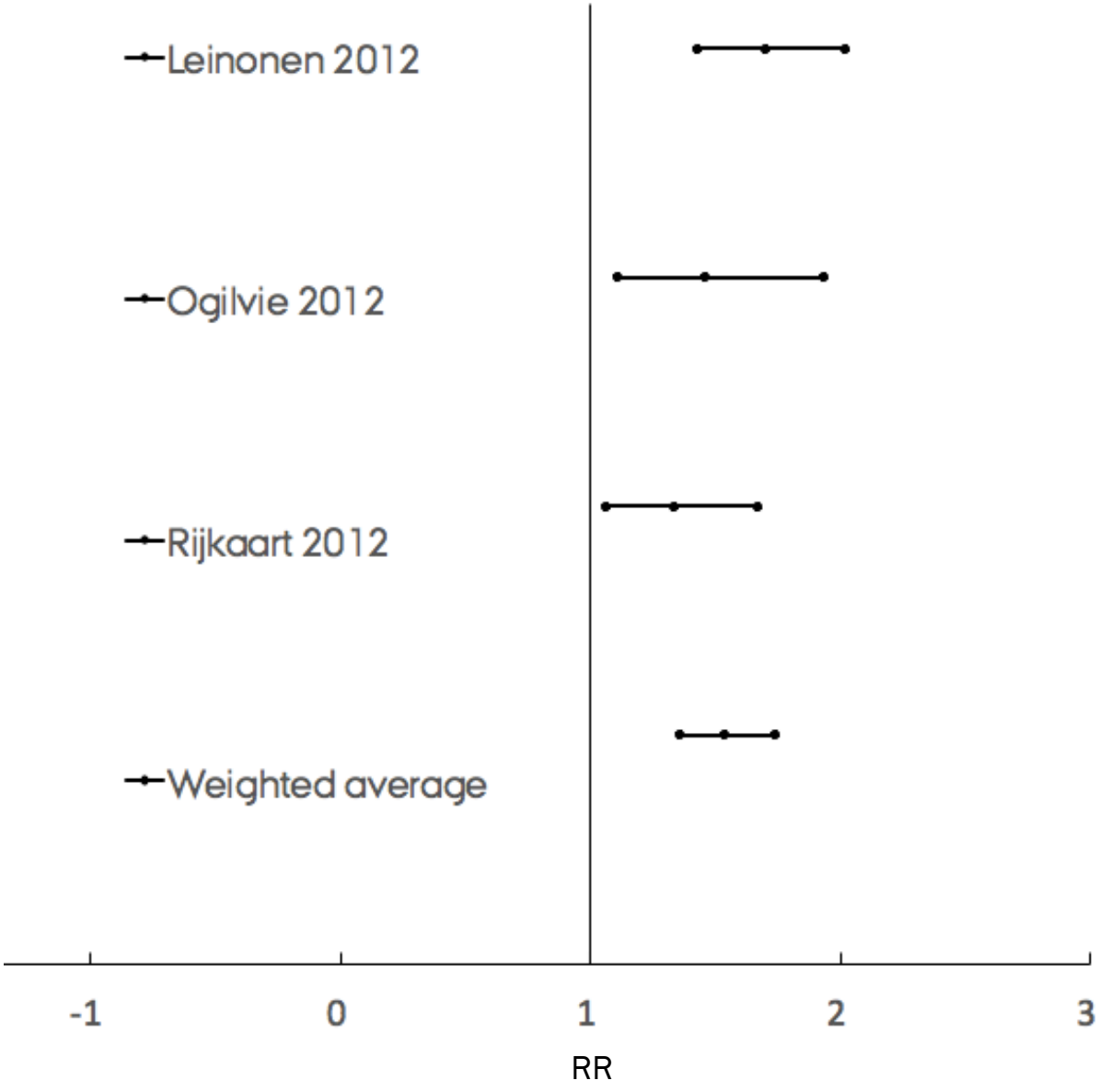

Figure 2: Relative risk (RR) for detection of CIN2 and 95% CI for Leinonen 2012, Ogilvie 2012, Rijkaart 2012 and a weighted average.