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Original Research

Impact of variations in triage cytology interpretation on human papillomavirus–based cervical screening and implications for screening algorithms

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Abstract ***Background:*** Women positive to human papillomavirus (HPV+) testing at cervical screening need triage, typically cytology and immediate colposcopy in case of atypical squamous cells of undetermined significance (ASCUS) or worse (ASCUS+) or, in cytology-normal HPV+ women, HPV test repeat after 1 year and colposcopy referral if still HPV+. Our hypothesis was that substantial variations in triage positivity and sensitivity may produce

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Triage;
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little variation in overall referral to colposcopy and on sensitivity of the entire screening process.

Methods: Centre- and age-aggregated data from 72,869 women aged 35–64 years were derived from 10 organised screening programmes which have piloted HPV screening in Italy since 2012. Overall colposcopy referral was evaluated as a function of immediate colposcopy referral and overall CIN2+ detection as a function of the proportion of all CIN2+ detected by immediate referral (a proxy of cytology's sensitivity). We fitted additive regression models, adjusted for centre, age, compliance to HPV retesting and to colposcopy, by generalised estimation equations.

Results: The proportion of HPV+ women directly referred to colposcopy varied across programmes (20–57%; average 37%) and so did CIN2+ detection (49–94%; average 77%). Overall, 63% (range 41–75%) of HPV+ were referred to colposcopy either immediately or at HPV repeat. An absolute 10% increase in immediate colposcopy referral resulted in 4.2% (95% CI: 3.3–5.1%) increase in overall referral. An absolute 10% increase in cytology's sensitivity resulted in a 1.1% (95% CI: 0.1–2.0%) increase in overall CIN2+ detection.

Conclusions: Repeat HPV testing limits the effect of subjectivity of cytology interpretation on overall referral and sensitivity. These will change only slightly when replacing cytology with another test if the interval to HPV repeat remains unchanged.

1. Introduction

Human papillomavirus (HPV) DNA testing is more sensitive than cytology for high-grade cervical intraepithelial neoplasia (CIN) but less specific [1], entailing the need for triaging HPV-positive (HPV+) women before referral to colposcopy [2].

HPV DNA testing with cytology triage has been applied in randomised controlled trials (RCTs). All these RCTs, despite differences in details, included the referral of HPV+ women with abnormal cytology (immediate, reflex, triage) to colposcopy and the invitation of HPV + women with normal cytology to repeat HPV DNA testing (second triage). RCTs consistently showed earlier detection of high-grade CIN [3–5] and reduced cancer incidence [2] in the HPV arm compared to the cytology arm, demonstrating the efficacy of this approach. Also importantly, long-period biopsy rates were similar in the two arms while the biopsy rate was doubled with direct referral to colposcopy of all HPV+ women [2]. Immediate triage by cytology and genotyping for HPV16/18 with retesting after 1 year for HPV of women negative to such tests were recommended in 2013 in the United States of America [6].

Despite these achievements, there is still room for improvements in HPV-based screening and intensive research is ongoing. The accuracy of cytology interpreted with knowledge of HPV status [7,8] and of biomarkers like genotyping [9], p16 overexpression (alone [10,11] or combined with Ki67 [12–14]), methylation of human [15–17] and viral [18–20] genes, and expression of the E6 viral oncoprotein [21] among HPV+ women have been studied.

It must be noted that, when retesting is applied, the overall triage process is done of two phases, one immediate (e.g. reflex cytology) for all HPV+ women and a second one delayed (HPV retesting) for those negative to immediate triage. HPV+ women can be referred to colposcopy and have a high-grade CIN detected in either of the two phases. In order to improve HPV-based screening protocols, studying the effect of the overall process is of obvious interest [22]. Indeed, programme's cost and women's discomfort depend on overall referral to colposcopy, not just on the referral due to immediate triage. The sensitivity of the overall process represents the probability that a precancerous lesion prevalent at baseline is detected either by the immediate triage test or by delayed HPV retesting. These lesions, if missed, would not be detected until the subsequent screening round and could progress to invasion in the meanwhile. Overall sensitivity therefore is essential to decide the length of the interval between completion of the triage process and a new screening round. Conversely, the sensitivity of the immediate triage test affects the risk of invasive cancer before HPV repeat and informs about the safe length of the interval before such repeat (see Discussion).

If more women are positive to immediate triage (and referred to immediate colposcopy), then fewer will be retested (and referred to delayed colposcopy). In addition, given the high sensitivity of HPV DNA testing, lesions not detected by immediate triage are expected to be detected as a result of retesting, except those which regress in the interval or occurring in women who missed or had a falsely negative retest. Therefore, we expect the two phases to be closely interdependent and that (a) referral to colposcopy due to the immediate triage test has a limited impact on overall referral and (b) the sensitivity of the immediate triage test has very limited impact, if any, on the sensitivity of the overall process.

To test this hypothesis we used a first extensive survey of routine HPV-based screening with cytology triage

started in Italy in 2013 [23]. As wide variability in the interpretation of triage cytology emerged across centres, we evaluated on over 73,000 women the impact of such variability on overall referral to colposcopy and overall detection of high-grade CIN or cancer by the end of the screening round (i.e. phase 1 + phase 2).

2. Patients and methods

2.1. Organisation of cervical screening and introduction of HPV-based screening in Italy

The Italian Ministry of Health (MOH) issues rules of health management, including the list of 'Essential Assistance Levels' (EALs), and funding. However, health services are under the responsibility of 20 regional health administrations that must guarantee EALs in exchange of funds. Organised screening programmes for cervical cancer have been active in Italy mainly since the late 1990s and are now part of EALs. Until recently, programmes regularly invited women aged 25–64 years for cytology every 3 years. Individual screening data are systematically registered at a regional/local level. Regular national surveys are conducted and published yearly by the national centre for screening monitoring on behalf of the national MOH [24,25]. For this purpose standardised tables of aggregated data are collected from each screening programme. A 'Programme' is hereafter defined as the entity from which aggregated data were obtained. In general this corresponds to an organisational unit that manages and coordinates the different steps of screening, from invitation to diagnostic assessment and treatment. These units are similarly organised but their size is variable (Table 1).

During the last 5 years a number of pilot programmes using the HPV test as primary screening test started in different areas of Italy. In 2012 an Italian Health Technology Assessment report [26] based on a systematic review of the literature endorsed the superiority of HPV-based versus cytology-based screening. It also estimated that the recommended HPV-based screening protocol would decrease from 422 to 290 euro the overall cost of screening women from 35 to 64 years of age, due to the shift from 3- to 5-year intervals (despite the increase of the cost per screening round, especially the first with HPV). In 2013, the national MOH recommended a progressive transition to HPV-based cervical screening with HPV testing every 5 years in women aged 30–35 to 64 years. Surveys of HPV-based screening started collecting data in 2013.

2.2. Screening protocol

Italian guidelines recommend stand-alone HPV testing with cytological triage. Women have samples taken for both HPV and cytology (either a single sample in liquid medium or, less commonly, a conventional smear and another sample for HPV) and are first tested for HPV. If such test is negative they are returned to a new screening round. Cytology is prepared and interpreted only for HPV + women. If cytology is atypical squamous cells of undetermined significance or worse women are referred to colposcopy. If cytology is normal women are reinvited for another HPV test after 1 year. If such test is still positive women are referred for colposcopy. Otherwise they are returned to a new screening round (Fig. 1).

2.3. Data collection

Nineteen organised screening programmes (out of 119 performing cervical screening) conducted HPV-based primary screening in 2012.

For each local programme we collected tables including the number of

a) HPV+ and HPV− women among those screened;
b) HPV+ women referred immediately to colposcopy, having actually had it and having had CIN or cancer detected on immediate triage;
c) women invited to HPV retesting after 1 year, who complied, were still HPV+, were referred to colposcopy, actually had it and had a CIN or cancer detected on such occasion.

Tables show women by 5-year age groups and according to whether they had been previously screened by HPV within organised screening. The use of HPV-based screening in opportunistic activity up to 2012 was plausibly minimal.

As the protocol entails 1-year repeats of the HPV test, data on the women invited for a primary HPV test in 1 year were collected across two years, using a cohort approach. For women invited to HPV-based screening during 2012 and screened up to April 2013 tables (a) and (b) were collected in June/July 2013 and tables (c) in September 2014.

2.4. Data analysis

We included data from 10 programmes that provided complete data. Nine programmes were excluded because of lack of sufficient data (mainly age stratification, n = 6), substantial protocol differences (n = 2), or extremely small size (n = 1). Combinations of screening programme and 5-year age groups were the statistical units (n = 60). The few women aged 35–64 years who had been previously screened by HPV were excluded.

For each centre and 5-year age group we estimated:

- The overall proportion of HPV+ women referred to colposcopy as a function of the proportion of HPV+ women immediately referred to colposcopy because of abnormal

Table 1
Selected features of screening programmes, Italy, 2012–2014.

Programmes	Screened women	% HPV+ among screened women	% of HPV+ women referred immediately to colposcopy	% of compliance to 1-year HPV retesting	% HPV+ at 1-year retesting	% of CIN2+ detected at immediate referral	Overall % of screened women referred to colposcopy	Overall detection of CIN2+ per 1000 screened women (n)	Overall % of HPV+ women referred to colposcopy	Overall detection of CIN2+ per 100 HPV+ women	Overall % PPV for CIN2+ of colposcopy referral
Adria	3448	5.1	38.9	95.0	49.7	58.3	3.5	1.4 (5)	68.5	2.4	3.6
Alta padovana	7985	4.4	42.0	86.7	48.3	79.6	2.9	3.7 (32)	66.3	8.1	12.3
Este	5596	5.5	20.0	75.0	66.0	70.8	3.1	2.8 (16)	59.6	4.8	8.3
Lanciano	11,692	9.2	23.3	65.7	35.1	94.3	3.9	5.1 (57)	40.7	4.6	10.6
Padova	10,095	4.9	56.9	77.4	51.7	88.7	3.7	6.3 (60)	74.8	13.0	17.5
Reggio Emilia	3271	6.5	40.6	95.5	42.1	70.6	4.3	5.4 (17)	66.9	8.0	11.3
Rovigo	6727	5.1	32.4	92.9	61.9	49.0	3.6	4.4 (25)	70.8	7.4	10.5
Teramo	7498	7.8	40.7	36.5	26.8	89.6	3.6	3.2 (25)	46.4	3.8	8.1
Torino	12,332	5.9	23.8	91.6	59.1	71.4	3.9	7.0 (84)	65.3	11.4	17.5
Val Camonica	4235	5.0	46.2	79.8	44.6	93.1	3.4	2.9 (13)	65.9	4.7	6.8
Total age standardised	72,869	5.9	36.5	79.6	48.5	76.6	3.6	4.2 (334)	62.5	6.8	10.7
Total not standardised		6.1	34.7	74.5	48.8	80.6	3.6	4.6 (334)	58.9	7.5	12.6
Standard deviation between statistical units (programme/age group combinations)	574 (mean 1214)	2.4	13.9	20.6	17.0	14.6	1.4	3.6	12.8	5.0	7.2

HPV = human papillomavirus; PPV = positive predictive value.
Except otherwise specified values are age standardised assuming equal number of women in all age groups.

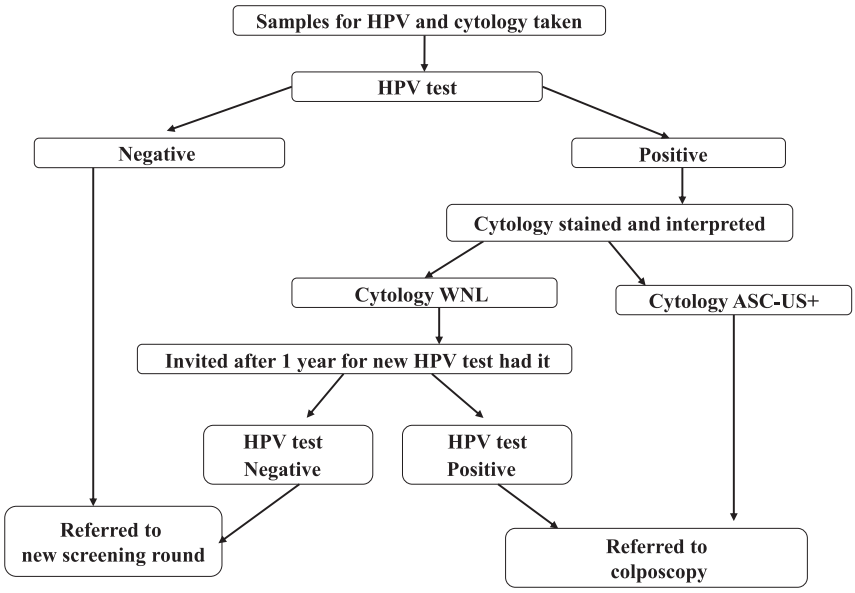

Fig. 1. Italian protocol for HPV-based screening. HPV = human papillomavirus.

cytology (i.e. of the positivity rate of the immediate triage test).

- The overall detection of CIN2+ among HPV+ women as a function of the proportion of CIN2+ detected by immediate colposcopy referral in that programme. This latter proportion comes near to the sensitivity of cytology for CIN2+, except for lesions missed on both occasions. The prevalence of CIN2+ among HPV-infected women in strata of age and screening history is stable [27] and, therefore, overall age-adjusted detection of CIN2+ among HPV+ women can be considered a proxy of the sensitivity of the entire process and it shows whether it is a function of the sensitivity of immediate triage. We also studied the overall CIN2+ detection as a function of immediate referral to colposcopy.
- The overall positive predictive value (PPV) for CIN2+ of colposcopy referral (either immediate or after 1 year) both as a function of the positivity rate and of sensitivity of cytology.

Adjusted risk differences were obtained from additive regression models assuming binomial distribution of errors. Additive (linear) models were used as they are more easily interpretable and more a priori plausible than multiplicative (logarithmic) models. The direction and statistical significance of associations and goodness of fit did not vary in any case by model (data not shown). Clustering of women was taken into account by using generalised estimation equations [28]. All models were adjusted by 5-year age group and compliance to immediate colposcopy (done because cytology was abnormal); 1-year HPV retesting; and delayed colposcopy in persistently HPV+ women. Compliances to colposcopy were not available by age group and therefore we used the centre-specific raw value for all age groups of the same centre. Statistical analyses were performed using SAS version 9.2 and STATA version 14.

3. Results

We included 72,869 women. Table 1 shows age-standardised (assuming equal size of each age group) values of selected screening features of the 10 programmes included in the analysis. On average 5.9% of screened women were HPV+ and among them 36.5% were directly referred to immediate triage. Such proportion varied between programmes from 20.0% to 56.9%. The remaining 63.5% of HPV+ women were referred to delayed triage. Eighty percent of them complied and 48.5% of compliers were still HPV+. As a result of the entire screening programme (phases 1 + 2) 3.6% of screened women were referred to colposcopy and 4.2 per 1000 had CIN2+ detected. Referral and detection of CIN2+ among HPV+ women were 62.5% and 6.8% respectively. Some 76.6% of CIN2+ was detected at immediate colposcopy (range across programmes: 49.0%–94.3%). Overall PPV for CIN2+ of colposcopy referral was 10.7%. Both compliance to immediate colposcopy and to delayed colposcopy was >92% in all programmes except in Lanciano (83.2% for immediate and 59.6% for delayed colposcopy) and Teramo (53.7% and 45.3%, respectively).

Table 2 shows adjusted regression-estimated risk differences for selected end-points and explanatory variables. An absolute increase in direct referral to colposcopy of 10 absolute percent points (e.g. from 25% to 35%) increased the overall referral by 4.2% (95% CI: 3.3%–5.1%). The proportion of CIN2+ detected by immediate colposcopy was weakly positively associated with overall detection of CIN2+ among HPV+ women but the proportion of HPV+ women immediately referred to colposcopy was not. An increase of 10 absolute percent points in the proportion of CIN2+ detected by immediate colposcopy was estimated to

Table 2
Regression-estimated risk difference and corresponding 95% confidence intervals (CIs) by selected end-points and explanatory variables, Italy, 2012–2014.

End-point and explanatory variable	Difference (95% CI)
Overall referral to colposcopy for a 10% absolute increase in the proportion of HPV+ women immediately referred to colposcopy	4.2% (3.3% to 5.1%)
Overall detection of CIN2+ for a 10% absolute increase of the proportion of CIN2+ detected by immediate referral	1.1% (0.1% to 2.0%)
Overall detection of CIN2+ for a 10% absolute increase of the proportion of HPV+ women immediately referred to colposcopy	0.4% (−0.5% to 1.2%)
Overall PPV for CIN2+ for a 10% absolute increase of the proportion of HPV+ women immediately referred to colposcopy	−0.2% (−1.4% to 1.2%)
Overall PPV for CIN2+ for a 10% absolute increase of the proportion of CIN2+ detected by immediate referral	1.4% (−0.1% to 2.9%)
Probability of being HPV+ at 1-year HPV re-testing for a 10% absolute increase of the proportion of HPV+ women immediately referred to colposcopy	−3.2% (−5.0% to −1.4%)

HPV = human papillomavirus; PPV = positive predictive value.
Differences in end-points are for an increase of 10 absolute percent points (e.g. from 25% to 35%) of the explanatory variable.
Adjusted by 5-year age group, compliance to 1-year HPV retesting, to immediate colposcopy (because of abnormal cytology) and to colposcopy at 1 year (because of persistent HPV infection).

result in an absolute 1.1% (0.1–2.0%) increase in the overall detection of CIN2+ among HPV+ women. An increase of 10 absolute percent points in immediate referral was not associated with overall detection of CIN2+ among HPV+ women (0.4%; −0.5% to 1.2%). Overall PPV for CIN2+ of colposcopy referral was not associated with either immediate triage sensitivity or immediate colposcopy referral. Finally, the probability of being HPV+ at 1-year HPV retesting slightly but significantly decreased (−3.2%; CI: −5.0% to −1.4%) for a 10 absolute percent points increase in immediate colposcopy referral.

4. Discussion

4.1. Effect of referral rate and sensitivity of the triage test on those of the entire triage process

The proportion of HPV+ women judged to have abnormal cytology and thus referred for immediate colposcopy varied substantially among Italian screening programmes because of different thresholds applied in interpretation. Indeed, also the proportion of CIN2+ detected by immediate colposcopy varied among programmes suggesting variability in the sensitivity of cytology. Beyond cytology, our present study is therefore representative of any scenario in which any type of triage test with different threshold and sensitivity were used. There is therefore the possibility to assess the effect of this variability on overall referral to colposcopy, sensitivity for CIN2+ and PPV resulting from the entire process.

Our results show that, in a protocol that includes 1-year repeat HPV testing in women initially triage-negative, moving from an immediate triage test that classifies only a small proportion of women as positive to another triage test that classifies a high proportion of women as positive has a limited effect on the final referral of the overall triage process (therefore on resource consumption and on efficiency). An increase in 10 absolute percent points in immediate triage results in only 4.2 absolute percent points in the latter. In the Vrije Universiteit Medical Centre-Saltro laboratory population-based cervical screening (VUSA)-screen cohort study [29], stand-alone cytology and cytology plus 16/18 genotyping cotesting as immediate triage tests entailed 21.6% and 43.4% immediate referral respectively (a two-fold increase) but the overall referral with 1-year HPV repeat, was 65.7% and 72.5% respectively (just a 11% increase). Variation in overall referral could be larger if triage test negativity is associated with HPV infection clearance because in such a case fewer triage-negative women would be HPV+ at repeat. In our study the probability of being positive at 1-year HPV retesting decreased only marginally with the increase of immediate referral, suggesting that this possibility is small with cytology. Clearance, however, would be higher in women positive for non-16 genotypes [30] and, possibly, in p16 negative women. Nevertheless, the mentioned association should be very strong in order to have a substantial effect on the overall screening process.

We also found a very weak relationship between the proportion of CIN2+ detected by immediate colposcopy and overall age-adjusted detection of CIN2+ among HPV+ women suggesting that the overall sensitivity of the entire screening process was little affected by the sensitivity of immediate triage. This observation is consistent with the hypothesis that most lesions missed by cytology at baseline are detected at HPV retesting. Regression of CIN2+ in the 1-year interval could explain their small increase when cytology's sensitivity is higher. In the VUSA-screen study [29] stand-alone cytology had 62.7% sensitivity for CIN2+ among HPV+ women and cotesting by cytology plus 16/18 genotyping 81.5% sensitivity. However, after 1-year repeat of HPV testing the estimated overall sensitivity was very similar:

98.6% and 100% respectively. We found no significant association between overall CIN2+ detection and the proportion of HPV+ women immediately referred to colposcopy.

4.2. Strengths and limitations

Our results are based on a survey of routine implementation of HPV-based screening in nearly 73,000 women. They combine, therefore, high real-life representativeness with strong statistical power.

In principle, local conditions associated to higher or lower CIN2+ detection (e.g. previous screening history of the population, quality of colposcopy or broader criteria of interpretation of histology) could have acted as confounders if also associated with the sensitivity of cytology or with the proportion of HPV+ women directly referred to colposcopy. Such variables were not measurable in our study. However, to obscure a large positive effect of the immediate triage test's sensitivity on the overall CIN2+ detection they should have acted as negative confounders, that is, higher sensitivity of histology interpretation should have been strongly associated with lower sensitivity of cytology, which is rather implausible. It must also be kept in mind that we included only women at their first screen with HPV. In POBASCAM and NTCC the proportion of HPV+ women was about halved at the second round with HPV [31]. Therefore the overall referral to colposcopy is expected to drop from the second round with HPV.

4.3. Implications for the Italian screening programme

A high variability in the interpretation of triage cytology was observed. High variability in referral to colposcopy by cytology was observed in the past among Italian regions [24,25] and even more among EU countries [32]. In Italy cytology variability was progressively reduced by intensive educational activity of standardisation [24,25] that will now focus on triage cytology in HPV-based screening.

It is, however, reassuring that subjectivity in cytology interpretation has little impact: despite large variation in immediate referral, overall referral was stably around 3.6%. This value happens to coincide with the overall referral (3.5%) we assumed for colposcopy referral at the first round with HPV when we estimated the costs of the future HPV-based screening in Italy and the corresponding savings compared to cytology [26]. Also CIN2+ detection was relatively stable showing that repeating the HPV test acts as a safety net for variations in cytology interpretation.

4.4. Implications for HPV-based screening algorithms

Our results indirectly suggest that replacing cytology with another triage test without changing the remaining of the algorithm (HPV retesting at 1 year in triage-negative women) is likely to have little effect on resource consumption and overall screening sensitivity.

Positivity at HPV repeat largely depends on the interval before HPV retesting. Overall referral can therefore be reduced by prolonging the interval between HPV tests. Longer intervals obviously entail the risk that a CIN2+ missed by the immediate triage test may progress into invasive carcinoma before HPV retesting. This risk is clearly a function of the cross-sectional sensitivity of the triage test(s) without HPV repeat and, therefore, the use of an extremely sensitive triage test (or combination of tests) could be efficient even if it leads to high immediate referral provided that the extra sensitivity is accompanied by a prolongation of the interval before HPV repeat.

This study shows that routine data collected for screening monitoring can be used for research purposes and can provide relevant information in order to optimise screening methods.

Conflict of interest statement

GR, MZa, SF, ST, AC, MC, AD, FC, NS and MZo have no conflicts of interest to declare.

PGR, as former principal investigator of an independent study, funded by the Italian Ministry of Health, data owner, made agreements with Roche diagnostics, Hologic Genprobe, Abbott, and Qiagen to obtain reagents at reduced price or for free.

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References

[1] Arbyn M, Ronco G, Anttila A, Meijer CJLM, Poljak M, Ogilvie G, et al. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine 2012 Nov 20; 30(Suppl. 5):F88–99.

[2] Ronco G, Dillner J, Elfström KM, Tunesi S, Snijders PJF, Arbyn M, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet 2014;383(9916):524–32.

[3] Naucler P, Ryd W, Törnberg S, Strand A, Wadell G, Elfgren K, et al. Human papillomavirus and Papanicolaou tests to screen for cervical cancer. N Engl J Med 2007 Oct 18;357(16):1589–97.

[4] Kitchener HC, Almonte M, Thomson C, Wheeler P, Sargent A, Stoykova B, et al. HPV testing in combination with liquid-based

cytology in primary cervical screening (ARTISTIC): a randomised controlled trial. Lancet Oncol 2009;10:672–82.
[5] Rijkaart DC, Berkhof J, Rozendaal L, van Kemenade FJ, Bulkmans NWJ, Heideman DAM, et al. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial. Lancet Oncol 2012;13:78–88.
[6] Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, et al. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obst Gynecol 2013;121:829–46.
[7] Bergeron C, Giorgi-Rossi P, Cas F, Schiboni ML, Ghiringhello B, Dalla Palma P, et al. Informed cytology for triaging HPV-positive women: substudy nested in the NTCC randomized controlled trial. J Natl Cancer Inst 2015;107(12). http://dx.doi.org/10.1093/jnci/dju423. dju423.
[8] Leinonen M, Nieminen P, Kotaniemi-Talonen L, Malila N, Tarkkanen J, Laurila P, et al. Age-specific evaluation of primary human papillomavirus screening vs conventional cytology in a randomized setting. J Natl Cancer Inst 2009;101:1612–23.
[9] Castle PE, Stoler MH, Wright TC, Sharma A, Wright TL, Behrens CM. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. Lancet Oncol 2011;12:880–90.
[10] Carozzi F, Confortini M, Dalla Palma P, Del Mistro A, Gillio-Tos A, De Marco L, et al. Use of p16-INK4A overexpression to increase the specificity of human papillomavirus testing: a nested substudy of the NTCC randomised controlled trial. Lancet Oncol 2008;9:937–45.
[11] Carozzi F, Gillio-Tos A, Confortini M, Del Mistro A, Sani C, De Marco L, et al. Risk of high-grade cervical intraepithelial neoplasia during follow-up in HPV-positive women according to baseline p16-INK4A results: a prospective analysis of a nested substudy of the NTCC randomised controlled trial. Lancet Oncol 2013;14:168–76.
[12] Petry KU, Schmidt D, Scherbring S, Luyten A, Reinecke-Lüthge A, Bergeron C, et al. Triaging Pap cytology negative, HPV positive cervical cancer screening results with p16/Ki-67 Dual-stained cytology. Gynecol Oncol 2011;121:505–9.
[13] Wentzensen N, Fetterman B, Castle PE, Schiffman M, Wood SN, Stiemerling E, et al. p16/Ki-67 dual stain cytology for detection of cervical precancer in HPV-positive women. J Natl Cancer Inst 2015 Sept 15;107(12). http://dx.doi.org/10.1093/jnci/djv257. pii: djv257. Print 2015 Dec.
[14] Uijterwaal MH, Polman NJ, Witte BI, van Kemenade FJ, Rijkaart D, Berkhof J, et al. Triaging HPV-positive women with normal cytology by p16/Ki-67 dual-stained cytology testing: baseline and longitudinal data. Int J Cancer 2015 May 15;136(10):2361–8. http://dx.doi.org/10.1002/ijc.29290 [Epub 2014 Nov 6].
[15] De Strooper LMA, Meijer CJLM, Berkhof J, Hesselink AT, Snijders PJF, Steenbergen RDM, et al. Methylation analysis of the FAM19A4 gene in cervical scrapes is highly efficient in detecting cervical carcinomas and advanced CIN2/3 lesions. Cancer Prev Res (Phila) 2014 Dec;7:1251–7. http://dx.doi.org/10.1158/1940-6207.CAPR-14-0237 [Epub 2014 Oct 3].
[16] De Strooper LMA, Hesselink AT, Berkhof J, Meijer CJLM, Snijders PJF, Steenbergen RDM, et al. Combined CADM1/MAL methylation and cytology testing for colposcopy triage of high-risk HPV-positive women. Cancer Epidemiol Biomarkers Prev 2014 Sep;23(9):1933–7. http://dx.doi.org/10.1158/1055-9965.EPI-14-0347 [Epub 2014 Jun 24].
[17] Verhoef VMJ, Bosgraaf RP, van Kemenade FJ, Rozendaal L, Heideman DAM, Hesselink AT, et al. Triage by methylation-marker testing versus cytology in women who test HPV-positive on self-collected cervicovaginal specimens (PROHTECT-3): a randomised controlled non-inferiority trial. Lancet Oncol 2014 Mar;15(3):315–22. http://dx.doi.org/10.1016/S1470-2045(14)70019-1 [Epub 2014 Feb 13].
[18] Mirabello L, Sun C, Ghosh A, Rodriguez AC, Schiffman M, Wentzensen N, et al. Methylation of human papillomavirus type 16 genome and risk of cervical precancer in a Costa Rican population. J Natl Cancer Inst 2012 Apr 4;104(7):556–65. http://dx.doi.org/10.1093/jnci/djs135 [Epub 2012 Mar 23].
[19] Wentzensen N, Sun C, Ghosh A, Kinney W, Mirabello L, Wacholder S, et al. Methylation of HPV18, HPV31, and HPV45 genomes and cervical intraepithelial neoplasia grade 3. J Natl Cancer Inst 2012 Nov 21;104(22):1738–49. http://dx.doi.org/10.1093/jnci/djs425 [Epub 2012 Oct 23].
[20] Vasiljević N, Scibior-Bentkowska D, Brentnall A, Cuzick J, Lorincz A. A comparison of methylation levels in HPV18, HPV31 and HPV33 genomes reveals similar associations with cervical precancers. J Clin Virol 2014 Mar;59(3):161–6. http://dx.doi.org/10.1016/j.jcv.2013.12.014 [Epub 2014 Jan 8].
[21] Valdez M, Jeronimo J, Bansil P, Qiao Y-L, Zhao F-H, Chen W, et al. Effectiveness of novel, lower cost molecular human papillomavirus-based tests for cervical cancer screening in rural China. Int J Cancer 2016 Mar 15;138:1453–61. http://dx.doi.org/10.1002/ijc.29877 [Epub ahead of print].
[22] Wentzensen N, Schiffman M, Palmer T, Arbyn M. Triage of HPV positive women in cervical cancer screening. J Clin Virol 2016;76(Suppl. 1):S49–55.
[23] Ronco G, Giorgi Rossi P, Giubilato P, Del Mistro A, Zappa M, Carozzi F, et al. A first survey of HPV-based screening in routine cervical cancer screening in Italy. Epidemiol Prev 2015 May–Jun;39(3 Suppl. 1):77–83.
[24] Ronco G, Iossa A, Naldoni C, Pilutti S, Anghinoni E, Zappa M, et al. A first survey of organized cervical cancer screening programs in Italy. Tumori 1998;84:624–30.
[25] Ronco G, Giubilato P, Carozzi F, Maia G, Giorgi Rossi P, Zappa M, et al. Extension of organized cervical cancer screening programmes in Italy and their process indicators, 2011–2012 activity. Epidemiol Prev 2015 May–Jun;39(3 Suppl. 1):61–76.
[26] Ronco G, Biggeri A, Confortini M, Naldoni C, Segnan N, Sideri M, et al. Health technology assessment report: HPV DNA based primary screening for cervical cancer precursors. Epidemio Prev 2012;36(3–4 Suppl. 1):1–72 [Italian].
[27] Giorgi-Rossi P, Franceschi S, Ronco G. HPV prevalence and accuracy of HPV testing to detect high-grade cervical intraepithelial neoplasia. Int J Cancer 2012 Mar 2105;130(6):1387–94.
[28] Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika 1986;73:13–22.
[29] Rijkaart DC, Berkhof J, van Kemenade FJ, Coupe VMH, Hesselink AT, Rozendaal L, et al. Evaluation of 14 triage strategies for HPV DNA-positive women in population-based cervical screening. Int J Cancer 2012;130:602–10.
[30] Richardson H, Kelsall G, Tellier P, Voyer H, Abrahamowicz M, Ferenczy A, et al. The natural history of type-specific human papillomavirus infections in female university students. Cancer Epidemiol Biomarkers Prev 2003 Jun;12(6):485–90.
[31] Veldhuijzen NJ, Berkhof J, Gillio-Tos A, De Marco L, Carozzi F, Del Mistro A, et al. The age distribution of type-specific high-risk human papillomavirus incidence in two population-based screening trials. Cancer Epidemiol Biomarkers Prev 2015;24:111–8.
[32] Ronco G, van Ballegooijen M, Becker N, Chil A, Fender M, Giubilato P, et al. Process performance of cervical screening programmes in Europe. Eur J Cancer 2009;45:2659–70.