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
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

Triage; Cytology

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.

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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 invited 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

- 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

<table>
<thead>
<tr>
<th>Programmes</th>
<th>Screened women</th>
<th>% HPV+ among screened women</th>
<th>% of HPV+ women referred immediately to colposcopy</th>
<th>% of compliance to 1-year HPV testing</th>
<th>% HPV+ at 1-year retesting</th>
<th>% of CIN2+ detected at immediate referral</th>
<th>Overall % of screened women referred to colposcopy</th>
<th>Overall detection of CIN2+ per 1000 screened women (n)</th>
<th>Overall % of HPV+ women referred to colposcopy</th>
<th>Overall detection of CIN2+ per 100 HPV+ women</th>
<th>Overall % PPV for CIN2+ of colposcopy referral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adria</td>
<td>3448</td>
<td>5.1</td>
<td>38.9</td>
<td>95.0</td>
<td>49.7</td>
<td>58.3</td>
<td>3.5</td>
<td>1.4 (5)</td>
<td>68.5</td>
<td>2.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Alta padovana</td>
<td>7985</td>
<td>4.4</td>
<td>42.0</td>
<td>86.7</td>
<td>48.3</td>
<td>79.6</td>
<td>2.9</td>
<td>3.7 (32)</td>
<td>66.3</td>
<td>8.1</td>
<td>12.3</td>
</tr>
<tr>
<td>Este</td>
<td>5596</td>
<td>5.5</td>
<td>20.0</td>
<td>75.0</td>
<td>66.0</td>
<td>70.8</td>
<td>3.1</td>
<td>2.8 (16)</td>
<td>59.6</td>
<td>4.8</td>
<td>8.3</td>
</tr>
<tr>
<td>Lanciano</td>
<td>11,692</td>
<td>9.2</td>
<td>23.3</td>
<td>65.7</td>
<td>35.1</td>
<td>94.3</td>
<td>3.9</td>
<td>5.1 (57)</td>
<td>40.7</td>
<td>4.6</td>
<td>10.6</td>
</tr>
<tr>
<td>Padova</td>
<td>10,095</td>
<td>4.9</td>
<td>56.9</td>
<td>77.4</td>
<td>51.7</td>
<td>88.7</td>
<td>3.7</td>
<td>6.3 (60)</td>
<td>74.8</td>
<td>13.0</td>
<td>17.5</td>
</tr>
<tr>
<td>Reggio Emilia</td>
<td>3271</td>
<td>6.5</td>
<td>40.6</td>
<td>95.5</td>
<td>42.1</td>
<td>70.6</td>
<td>4.3</td>
<td>5.4 (17)</td>
<td>66.9</td>
<td>8.0</td>
<td>11.3</td>
</tr>
<tr>
<td>Rovigo</td>
<td>6727</td>
<td>5.1</td>
<td>32.4</td>
<td>92.9</td>
<td>61.9</td>
<td>49.0</td>
<td>3.6</td>
<td>4.4 (25)</td>
<td>70.8</td>
<td>7.4</td>
<td>10.5</td>
</tr>
<tr>
<td>Teramo</td>
<td>7498</td>
<td>7.8</td>
<td>40.7</td>
<td>36.5</td>
<td>26.8</td>
<td>89.6</td>
<td>3.6</td>
<td>3.2 (25)</td>
<td>46.4</td>
<td>3.8</td>
<td>8.1</td>
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<tr>
<td>Torino</td>
<td>12,332</td>
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<td>23.8</td>
<td>91.6</td>
<td>59.1</td>
<td>71.4</td>
<td>3.9</td>
<td>7.0 (84)</td>
<td>65.3</td>
<td>11.4</td>
<td>17.5</td>
</tr>
<tr>
<td>Val Camonica</td>
<td>4235</td>
<td>5.0</td>
<td>46.2</td>
<td>79.8</td>
<td>44.6</td>
<td>93.1</td>
<td>3.4</td>
<td>2.9 (13)</td>
<td>65.9</td>
<td>4.7</td>
<td>6.8</td>
</tr>
<tr>
<td>Total age standardised</td>
<td>72,869</td>
<td>5.9</td>
<td>36.5</td>
<td>79.6</td>
<td>48.5</td>
<td>76.6</td>
<td>3.6</td>
<td>4.2 (334)</td>
<td>62.5</td>
<td>6.8</td>
<td>10.7</td>
</tr>
<tr>
<td>Total not standardised</td>
<td>6,1</td>
<td>6.1</td>
<td>34.7</td>
<td>74.5</td>
<td>48.8</td>
<td>80.6</td>
<td>3.6</td>
<td>4.6 (334)</td>
<td>58.9</td>
<td>7.5</td>
<td>12.6</td>
</tr>
<tr>
<td>Standard deviation between statistical units (programme/age group combinations)</td>
<td>574 (mean 1214)</td>
<td>2.4</td>
<td>13.9</td>
<td>20.6</td>
<td>17.0</td>
<td>14.6</td>
<td>1.4</td>
<td>3.6</td>
<td>12.8</td>
<td>5.0</td>
<td>7.2</td>
</tr>
</tbody>
</table>

HPV = human papillomavirus; PPV = positive predictive value.

Except otherwise specified values are age standardised assuming equal number of women in all age groups.
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) 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
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% to 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 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-Salto 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 twofold 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 POBAS-CAM 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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