J Med Screen
0(0) 1–4

DOI: 10.1177/0969141319864991
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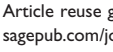

The impact of knowledge of HPV positivity on cytology triage in primary high-risk HPV screening

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Abstract

Objective: Several studies have shown that there is an upward shift in the classification of cervical cytology when high-risk human papillomavirus (hrHPV) status is known to be positive. The Netherlands implemented primary hrHPV screening with reflex cytology as the primary screening test in 2017. Prior to implementation of the new programme, we investigated whether knowledge of hrHPV status influences cytology rating.

Methods: Using a set of 200 cytology slides that had been previously tested, two pairs of cytotechnicians rated 100 slides per pair twice: first without knowledge of hrHPV status and then, after a wash-out period of two months, with knowledge of hrHPV status.

Results: We found that hrHPV positive slides were more likely to be rated up over the referral threshold (i.e. from negative for intraepithelial lesion or malignancy to atypical squamous cells of undetermined significance+) than hrHPV negative slides at the second review when hrHPV status was known (relative risk = 3.2; 95% confidence interval: 1.3–7.9).

Conclusions: If the same upward shift in ratings were to be observed in the national programme, it may have implications for referrals of women with low-grade lesions.

Keywords

hrHPV screening, cervical cytology, cervical cancer screening

Date received: 11 December 2018; accepted: 1 July 2019

Introduction

In 2017, the Netherlands replaced primary cytology with primary high-risk Human Papillomavirus (hrHPV) screening in the national cervical cancer screening programme. All eligible women (aged 30–60) are offered hrHPV screening every five years, with reflex cytology when hrHPV is found. Several studies have found that knowledge of positive HPV status can result in upward rating of cervical cytology.[1–6] In the renewed Dutch programme, all cytology slides reviewed will be hrHPV positive. An upward shift in cytology rating may result in more referrals. We aimed to investigate whether cytotechnicians would classify cytology slides higher when positive hrHPV status is known.

Methods

A set of 200 unmarked glass slides (~50% hrHPV positive), taken between August 2013 and July 2014 and adjusted for age and expected proportion of cytological abnormalities was selected from the Dutch screening comparison study (DuSC).[7] This set was divided into two sets of 100, each allocated to a pair of cytotechnicians for review.

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Four experienced cytotechnicians volunteered for this study and were grouped into two pairs. Prior to the implementation of the hrHPV screening programme, each pair reviewed 100 slides twice: once without hrHPV status, and after a two-month wash-out, with hrHPV status and reordered slides. Analysts were asked to rate slides in one of the following categories (equivalent Bethesda classification shown in brackets):

- Pap 0 (Inadequate quality)
- Pap 1 (Negative for intraepithelial lesion or malignancy (NILM))
- Pap 2 (Atypical squamous cells of undetermined significance (ASC-US))
- Pap 3a1 (Low-grade squamous intraepithelial lesion (LSIL))
- Pap 3a2 (High-grade squamous intraepithelial lesion (HSIL))
- Pap 3b (HSIL)
- Pap 4 (Carcinoma in situ or worse)

There were 800 individual observations from the entire dataset; 400 observations from each review (100 paired observations per cytotechnician). Twenty slides (10% of sample; 9 hrHPV positive, 11 hrHPV negative) were excluded due to the incorrect hrHPV status being accidentally provided at the second review, resulting in 360 paired observations from 180 slides. Switches in rating between review 1 and 2 were classified as upgrades (e.g. NILM to ASC-US), downgrades (e.g. LSIL to NILM), no change (e.g. NILM at both ratings) or to/from inadequate. Ratings from NILM to ASC-US+ or vice versa were classified as switches over or below the referral threshold. The net increase/decrease in referrals was calculated by subtracting the number of upgrades over the referral threshold at the second review from the number of downgrades below the referral threshold at the second review.

Data analysis was performed using SAS Base 9.4 and IBM SPSS Statistics v25. The highest classification was selected for three records categorized in multiple categories (e.g. 'Pap 0/1'). Risk estimates were calculated. Proportional risk difference was calculated using Wald asymptotic test of equality. Wald asymptotic confidence limits were calculated for proportions.

Results

HrHPV positive slides were more likely to be upgraded over the referral threshold at the second review than hrHPV negative slides (relative risk (RR) = 3.2; 95% confidence interval (CI): 1.3%–7.9%). There was a net increase in ratings that would result in referral between the first and second review of 12 for hrHPV positive slides and a net decrease of 18 for hrHPV negative slides. Overall, hrHPV negative slides were downgraded 29 times (15.9%; 95% CI: 10.6%–21.3%), compared with 15 times (8.4%; 95% CI: 4.3%–12.5%) for hrHPV positive slides ($p = 0.03$). Conversely, hrHPV positive slides were upgraded 22 times (12.4%; 95% CI: 7.8%–17.2%), compared with seven times (3.8%; 95% CI: 1.0%–6.7%) for hrHPV negative slides ($p = 0.003$). Results by Bethesda classification are shown in Figure 1.

Discussion

This study suggests that there may be an upward shift in the rating of cervical cytology slides when positive hrHPV status is known. Our results show that hrHPV positive slides were rated upwards more often than hrHPV negative slides, and more often over the referral threshold. This is consistent with previous literature.[3,4] Upgrading cytology when hrHPV status is positive was previously observed in Dutch observational data. Between 2007 and 2016, hrHPV testing was used in some laboratories, as an additional test at six months for women with ASC-US/LSIL at primary screening. Under this policy, significantly fewer slides were rated NILM, and significantly more slides were rated ASC-US/LSIL at six-month follow-up when hrHPV status was known.[8] Similar results were also seen in the regular monitoring of the Dutch national screening programme, with more slides rated at ASC-US or higher when hrHPV testing was performed at six-month follow-up.[9]

Two studies[1,2] found that prior knowledge of hrHPV status resulted in an increased sensitivity for CIN 2+ lesions, which points to an increase in true positive referrals. However, first results of the new hrHPV screening programme show both increased CIN 2+ detection and more unnecessary referrals (<CIN 2) compared with the cytology-based programme,[10] suggesting that there may be an influence of upward cytology ratings on the number of referrals of women with low-grade lesions. As all women with hrHPV positive, ASC-US+ primary screens are directly referred in the new hrHPV-based programme, an increase in slides rated as ASC-US may lead to more women with low-grade lesions being referred unnecessarily. This is concerning, as overtreatment of low-grade lesions also presents risks of harm. To mitigate the impact of potential cytology upgrading within the Dutch programme, training was provided at all five screening programme laboratories on morphological differences between Pap classifications (Personal Communication, 28 February 2018), and professional education continues to be provided.

This study has several strengths. Because this study was conducted prior to implementation of the new hrHPV screening programme, cytotechnicians were still reviewing both hrHPV positive and negative slides. The cytotechnicians in this study were experienced in evaluating cervical cytology. The distribution of age and abnormalities reflects the screened population, as slides were drawn

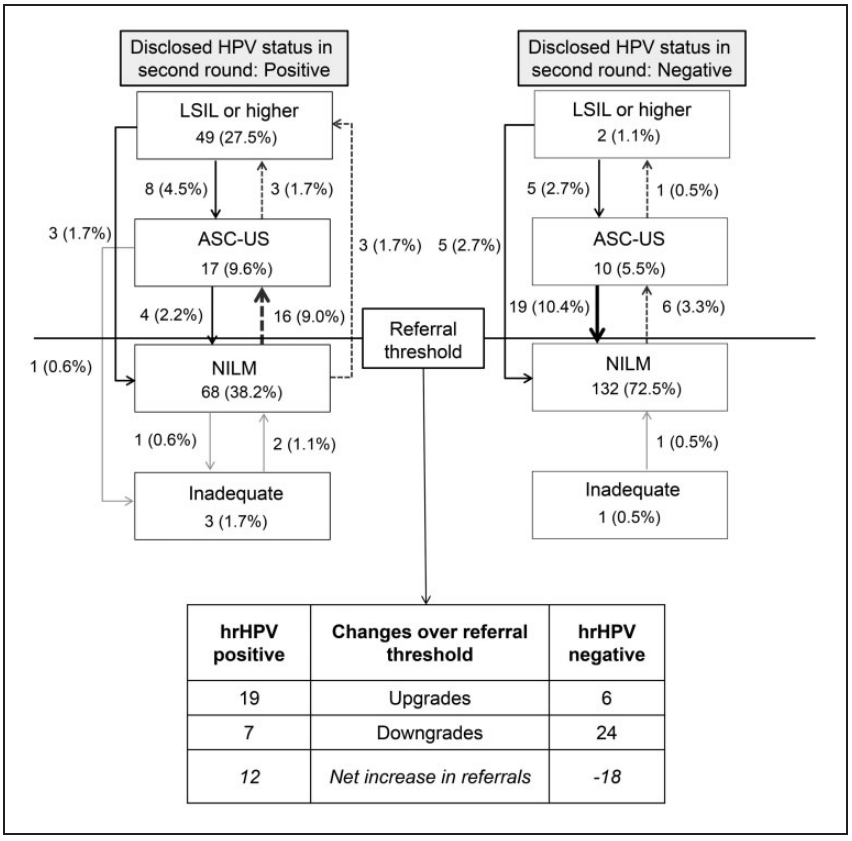

Figure 1. Flowchart of switches in cytology ratings between slide review 1 and slide review 2 by hrHPV status, rounded percentages. Percentages are rounded to one decimal place. As such, totals may not sum to 100%. Dashed black arrows represent upgrades and solid black arrows represent downgrades. Solid grey arrows represent changes to or from 'inadequate'. Numbers in the figure represent pairs of observations for one analyst, not the total count of slides; 360 pairs of observations are included in this figure.
NILM: negative for intraepithelial lesion or malignancy; ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion.

from the screening programme. The study also has some limitations. The small sample size has an impact on statistical power. Additionally, 10% of slides were excluded, due to incorrect HPV status provided at the second review.

Conclusion

Our study suggests that knowledge of hrHPV status may result in an upward shift in cytology ratings. While appropriate training is being provided to cytotechnicians, continued monitoring of unnecessary referrals will be essential, to mitigate risks of overtreatment following referrals of women with low-grade lesions.

Acknowledgements

The authors wish to thank D Sie-Go, J van Dam, E van den Heuvel, P Balk, A Hamminga, L Motie and E Hazenberg for their assistance in organising and conducting this study.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: CA Aitken and IMCM de Kok work on projects for the National Evaluation of the Dutch Cervical Cancer Screening Programme funded by the Dutch National Institute for Public Health and the Environment (RIVM).

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by the Dutch National Institute for Public Health and the Environment (RIVM).

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