

Original Article

Cytology and high-risk human papillomavirus testing to reduce colposcopies in women with postcoital bleeding

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ABSTRACT

INTRODUCTION. Women with postcoital bleeding (PCB) are traditionally referred for colposcopy. A 2022 regional strategy recommends colposcopy only if abnormalities are found in cytology, human papillomavirus testing or by gynaecological examination (GE). This study explores the number of reduced colposcopies using this triad to detect precancerous or cancer lesions and examines the potential for implementing this strategy with general practitioners (GPs).

METHODS. Between 1 January 2022 and 15 October 2024, a retrospective cohort study was conducted on women referred with PCB identified using the International Classification of Diseases, tenth version (ICD-10) diagnosis codes "DN930 Bleeding after coitus" and "DN930B Contact bleeding". Data were collected from the hospital's electronic patient files and the Danish Pathology Database.

RESULTS. Data from a total of 392 patients were analysed. Among 199 low-risk patients with normal tests, 161 (81%) avoided colposcopy. The combined sensitivity of cytology and hrHPV tests was 97%, detecting 33 of 34 CIN2+ cases. One CIN2+ case was missed by both tests but identified by GE. GP agreed with gynaecologists in 53% of abnormal GE findings, whereas 47% were misclassified as normal GE findings.

CONCLUSIONS. Combining cytology, hrHPV testing and GE reduces unnecessary colposcopies in low-risk women with PCB by 81%. The triadic approach successfully identified all CIN2+ cases. Implementing the new strategy with GPs is challenging due to GPs' limited accuracy in identifying abnormal GE findings.

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Human papillomavirus (HPV) is a common sexually transmitted virus associated with cervical cancer [1]. In Denmark, women aged 23 to 65 years are offered free cervical cancer screening every 3-5 years, with the latest data showing a national screening coverage of 74% [2, 3]. Additionally, HPV vaccination has been provided free of charge to young people up to the age of 18 years. It has also been included in the national vaccination programme, with recent data indicating an 87% coverage rate [4, 5]. Despite these measures, symptoms like postcoital bleeding (PCB), defined as bleeding during or right after intercourse, remain a diagnostic challenge, as they can indicate benign conditions, such as cervicitis or polyps, or more severe conditions like cervical cancer [6]. Traditionally, guidelines recommend that women with PCB should be referred for colposcopy to exclude

malignancy [7, 8]. However, studies show that only a small percentage of PCB cases involve cervical intraepithelial neoplasia (CIN) requiring treatment, defined as CIN2+ (CIN II, CIN III, adenocarcinoma in situ and cervical cancer), raising concerns about the overuse of colposcopy [9]. In July 2022, hospitals in the Region of Southern Denmark introduced a new diagnostic strategy to reduce unnecessary colposcopies in such cases. Following the new strategy, women with PCB initially undergo cytology and high-risk human papillomaviruses (hrHPV) testing, with referral to colposcopy only if results show either abnormal cytology, positive hrHPV or an abnormal gynaecological examination (GE). This new approach is referred to as the three-test strategy. Research shows that hrHPV testing has a higher sensitivity and negative predictive value than cytology for detecting CIN II and III, supporting its use in targeted diagnostics [10]. Postmenopausal women with negative results from all three tests (cytology, hrHPV and GE) are referred for endometrial diagnostics to exclude other causes. As an additional safety measure, patients are instructed to return if PCB symptoms persist for more than three months, ensuring that no cases are missed.

The primary objective of this study was to estimate the reduction in the number of colposcopies in women with PBC when using the hrHPV and cytology tests before referral to colposcopy. The secondary objective was to assess the diagnostic ability of cytology and hrHPV testing in the referred women by identifying CIN2+ cases, ensuring that no significant lesions, particularly cervical cancer, were missed during the transition to the new strategy. As a third objective, the study also sought to determine if the new strategy could be implemented by general practitioners (GPs).

METHODS

The project was approved by the hospital administration and registered with the Region of Southern Denmark (Acadre 23/42232). In accordance with the General Data Protection Regulation, approval for data storage and access to patient records was granted on 5 September 2024. A retrospective cohort study was conducted at the Department of Gynaecology and Obstetrics at the University Hospital of Southern Denmark.

The study included women who had PCB and underwent a cytology and hrHPV test between 1 January 2022 and 15 October 2024. The study population was identified using the International Classification of Diseases, tenth version (ICD-10) diagnosis codes "DN930 Bleeding after coitus" and "DN930B Contact bleeding." Data were extracted from the Electronic Patient Journal, which provided HPV vaccination and cancer staging. Furthermore, the Danish Pathology Database provided data on cytology, hrHPV, histology results and previous CIN cases.

Colposcopy reduction

To estimate the reduction in colposcopy referrals, patients were categorised into low- and high-risk groups using the following criteria: Low-risk was defined as women with normal cytology, negative hrHPV and normal GE findings (the cervix was described as normal, plump or with an erythroplakia). High-risk women had either abnormal cytology, positive hrHPV or abnormal GE findings, including visible tumours, polyps or vulnerable mucosa, defined clinically as areas that bleed upon contact or gentle brushing during speculum examination.

Cytology and high-risk human papillomavirus tests for cervical intraepithelial neoplasia 2+ detection

CIN2+ cases were identified in the Danish Pathology Database, and their cytology and hrHPV test results were analysed to evaluate their sensitivity. As part of the follow-up, the database was also reviewed to assess whether patients returned with symptoms or had new samples taken after the initial evaluation to ensure that no CIN2+ cases were missed.

Implementation of the new strategy with general practitioners

This analysis used gynaecologists' clinical findings during GE as the "gold standard" for identifying cervical abnormalities. These examinations were performed by either gynaecology specialists or specially trained nurses at outpatient gynaecology clinics. Patients who had normal cytology and negative hrHPV results were divided into two groups based only on the gynaecologists' GE findings, using the same definitions of normal and abnormal findings as described in the *Colposcopy reduction* section.

Lastly, the GPs' GE findings, as described in the referral letter, were compared with those of the gynaecologists.

Statistical analysis

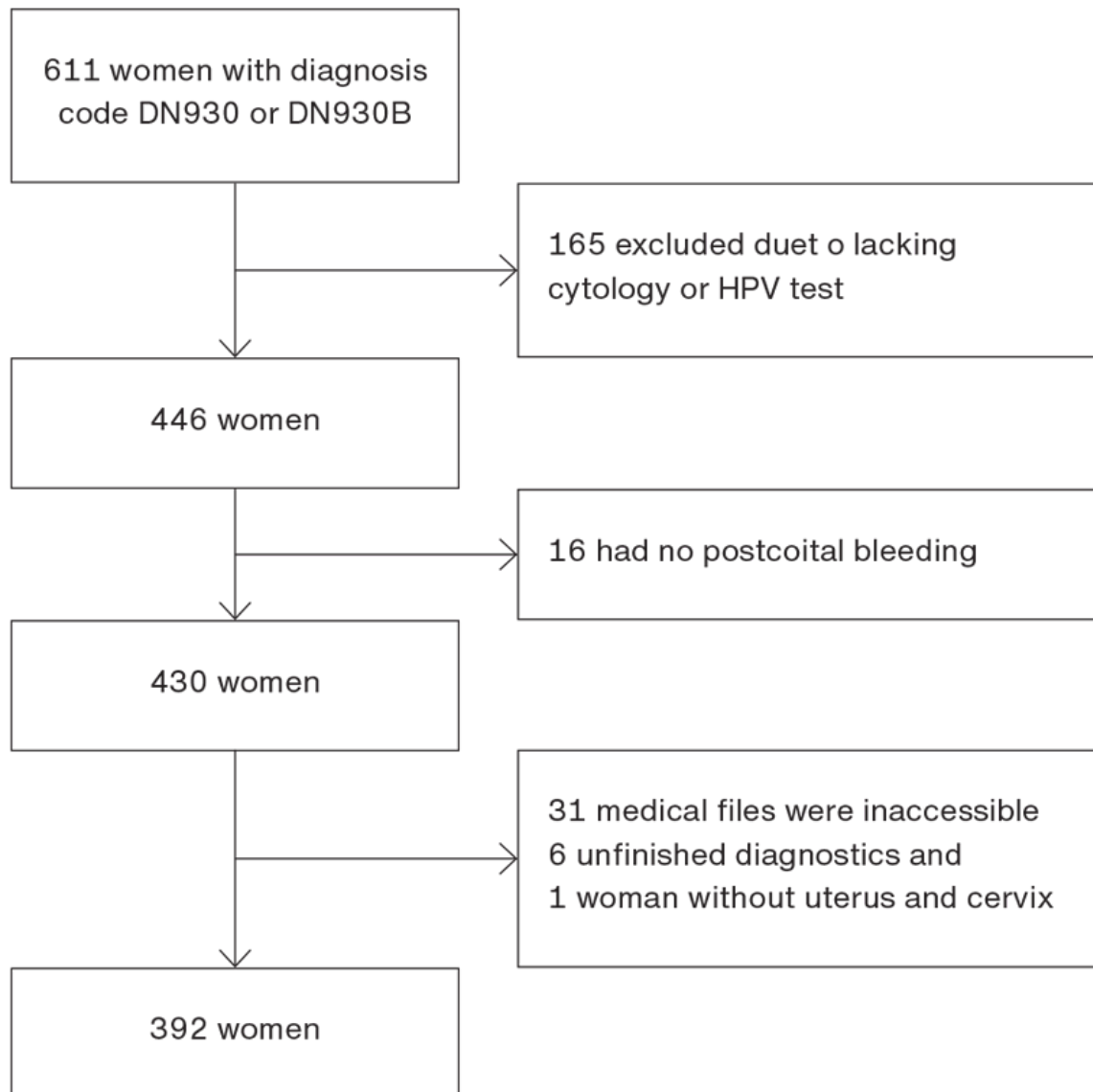
Data were analysed using STATA18.5 (StataCorp, College Station, TX) and stored on a secure SharePoint platform. Missing data were categorised as missing. The chi-squared test was used for categorical data, and ANOVA was used for continuous data. A p-value below 0.05 was considered significant.

Trial registration: not relevant.

RESULTS

In this study, 611 women were identified with the ICD-10 diagnosis codes "DN930 postcoital and contact bleeding" and "DN930B for Contact bleeding". The study population of 392 women was included in the final analysis, as illustrated in **Figure 1**.

FIGURE 1 Flow chart of the inclusion and exclusion criteria of women with postcoital bleeding.



HPV = human papillomavirus.

The study population had a mean age of 35 (range:17-81) years and a median follow-up time of 15 months (interquartile range: 8-21 months). A total of 34 CIN2+ cases were identified; among these, five were cases of carcinoma. Most CIN2+ cases were found among patients aged 30-49 years. Specifically, 58% of CIN II cases were observed in patients aged 17-29 years. No carcinoma cases were found among this patient group. Furthermore, 71% of CIN III cases and 60% of carcinoma cases were detected in patients aged 30-49 years. The remaining 40% of carcinoma cases were found in patients aged 50 years or older, as detailed in **Table 1**.

TABLE 1 Histological results in cervical biopsies.

	Not neoplasia		Cervical neoplasia				Cancer			
	normal	inflammation	CIN I	CIN II	CIN III	adenoCIS	carcinoma	Not taken	Total	p value
n (%)	101 (25.7)	64 (16.3)	19 (4.8)	12 (3.0)	17 (4.3)	0	5 (1.2)	174 (44.3)	392 (100)	
<i>Age</i>										
Mean ± SD, yrs	41.9 ± 11.9	39 ± 11.3	38 ± 13.3	30.3 ± 11.4	38.5 ± 9	0	45 ± 6	40.4 ± 12.5	40.18 ± 12.1	0.0008
Age groups, n (%):										
17-29 yrs	17 (16.83)	11 (17.1)	8 (42.1)	7 (58.3)	4 (23.5)	0	0	33 (18.9)	80 (20.4)	
30-49 yrs	54 (53.46)	44 (68.7)	5 (26.3)	3 (25)	12 (70.5)	0	3 (60)	103 (59.1)	224 (57.1)	
≥ 50 yrs	30 (29.7)	9 (914.0)	6 (31.5)	2 (16.6)	1 (5.8)	0	2 (40)	38 (21.8)	88 (22.4)	
<i>Post-menopausal?, n (%)</i>										0.367
Yes	13 (12.8)	8 (12.5)	2 (10.5)	0	1 (5.8)	0	1 (20)	25 (14.3)	50 (12.7)	
No	87 (86.1)	56 (87.5)	17 (89.4)	12 (100)	16 (94.1)	0	4 (80)	148 (85.0)	340 (86.7)	
Missing data	1 (1)	0	0	0	0	0	0	1 (0.5)	2 (0.5)	
<i>HPV vaccination?, n (%)</i>										0.031
Yes	30 (29.7)	20 (31.2)	11 (57.9)	8 (66.6)	7 (41.1)	0	0	56 (32.1)	132 (33.6)	
No	22 (21.7)	17 (26.5)	4 (21)	3 (25)	6 (35.2)	0	4 (80)	51 (29.3)	107 (27.2)	
Missing data	49 (48.5)	27 (42.1)	4 (21.0)	1 (8.3)	4 (23.5)	0	1 (20)	67 (38.5)	153 (39)	
<i>Previous CIN?, n (%)</i>										0.176
Yes	21 (20.8)	14 (21.9)	4 (21.0)	3 (25)	3 (17.6)	0	0	26 (14.9)	71 (18.1)	
No	77 (76.2)	47 (73.4)	12 (63.1)	7 (58.3)	13 (76.5)	0	4 (80)	136 (78.1)	296 (75.4)	
Missing data	3 (2.9)	3 (4.7)	3 (15.8)	2 (16.6)	1 (5.9)	0	1 (20)	12 (6.9)	25 (6.3)	
<i>Cytology results, n (%)</i>										0.000
Normal	86 (85.1)	55 (85.9)	13 (68.4)	4 (33.3)	5 (29.4)	0	0	163 (93.6)	326 (83.21)	
Inflammation	1 (1)	0	0	0	0	0	0	8 (4.6)	9 (2.30)	
ASCUS or LSIL	13 (12.8)	6 (9.4)	5 (26.3)	6 (50)	6 (35.3)	0	0	3 (1.7)	39 (9.9)	
ASCH or HSIL	1 (1)	2 (3.1)	1 (5.2)	2 (16.6)	5 (29.4)	0	3 (60)	0	14 (3.6)	
AGC or adenoCIS	0	1 (1.5)	0	0	1 (5.9)	0	1 (20)	0	3 (0.7)	
Carcinoma	0	0	0	0	0	0	1 (20)	0	1 (0.2)	
<i>hrHPV in cytology sample, n (%)</i>										0.000
Type 16	3 (2.9)	1 (1.5)	1 (5.2)	1 (8.3)	2 (11.7)	0	3 (0.6)	0	11 (2.8)	
Type 18	0	0	1 (5.2)	0	1 (5.8)	0	1 (20)	0	3 (0.7)	
Other types	17 (16.8)	21 (32.8)	13 (68.4)	10 (83.3)	13 (76.5)	0	0	5 (2.8)	79 (20.1)	
Negative	81 (80.2)	42 (65.6)	4 (21.0)	1 (8.3)	1 (5.9)	0	1 (20)	169 (97.1)	299 (76.1)	
<i>Risk stratification with colposcopy/biopsy, n (%)</i>										
Low-risk ^a	24 (63.1)	14 (36.8)	0	0	0	0	0	0	38 (100)	0.061
High-risk ^c	53 (37.3)	40 (28.1)	19 (13.3)	10 (7)	15 (10.5)	0	5 (3.5)	0	142 (100)	0.544

AdenoCIS = adenocarcinoma in situ; AGC = atypical glandular cells; ASCH = atypical squamous cells cannot rule out HSIL; ASCUS = atypical squamous cells of undetermined significance; CIN = cervical intraepithelial neoplasia; GE = gynaecological examination; HPV = human papillomavirus; hrHPV = high-risk HPV; HSIL = high-grade squamous intraepithelial lesion; LSIL = low-grade squamous intraepithelial lesion; SD = standard deviation.

a) Risk stratification includes 350 women from the total study population (N = 392).

b) Normal cytology, hrHPV-negative, normal GE.

c) Normal cytology, hrHPV-negative, abnormal GE, see Methods for details.

Colposcopy reduction

For colposcopy reduction analysis, the study focused on 350 patients, excluding 42 women. The excluded women underwent both hrHPV testing and cytology. However, they were still referred to colposcopy as they were assessed during the transition to the new guidelines and thus followed the previous protocol. However, we included them in the overall population to strengthen our sensitivity and specificity analyses. Among the 199 low-risk patients, 161 (81%) avoided colposcopy, whereas 38 (19%) underwent colposcopy despite being low-risk, which was a deviation from the guidelines. Among the 151 high-risk patients, 142 (94%) underwent colposcopy as recommended; however, nine (6%) were not referred despite being high-risk.

No CIN2+ cases were found in either group that did not follow the guidelines during the follow-up and control period.

Cytology and high-risk human papillomavirus tests for cervical intraepithelial neoplasia 2+ detection

The entire study population of 392 women was included to evaluate the effectiveness of cytology and hrHPV tests in detecting CIN2+ lesions. Among the 221 women who underwent colposcopy, the overall prevalence of CIN2+ was 34 (15%). As shown in Table 2, the combined use of cytology and hrHPV had the highest sensitivity (97%), detecting 33 out of 34 CIN2+ cases. The last remaining case was identified through a GE, which revealed a high-risk GE finding. All of the five cancer cases were detected by cytology, but hrHPV failed to detect one

adenocarcinoma. GE identified two of the three cancers with higher International Federation of Gynaecology and Obstetrics (FIGO) stages (IB-IIB) but missed the IIB case and the last two early-stage cancers (FIGO IA and IA1), for which normal GE findings were consistent with their microscopic nature.

TABLE 2 Cytology, high-risk human papillomavirus test and gynaecological examination in women with postcoital bleeding and cervical intraepithelial neoplasia 2+.

Detection method	Sensitivity, %	Specificity, %	PPV, %	NPV, %	CIN2+-detected, n/N	Missed CIN2+ cases, n	Cancer detection
Cytology, alone	73.5	88.5	37.9	97.2	25/34	9	All 5 cases detected
hrHPV, alone	91.2	82.7	33.3	99.0	31/34	3	4/5: missed 1 adenocarcinoma
Cytology + hrHPV, combined	97.1	76.3	28.0	99.6	33/34	1	5/5
GE, alone					1/34	33	2/3 advanced cases: missed a stage CIN IIB

CIN = cervical intraepithelial neoplasia; GE = gynaecological examination; hrHPV = high-risk human papillomavirus; NPV = negative predictive value; PPV = positive predictive value.

Implementation of the new strategy with general practitioners

To evaluate how well GPs' GE evaluations align with those performed by gynaecologists, a total of 283 patients were included in the analysis as described in the Methods section. The results are presented in **Table 3**. Gynaecologists classified 223 patients as having normal GE findings, considered the "gold standard". GPs agreed with this classification in 192 (86%) cases but misclassified 31 (14%) patients as having abnormal GE findings. None of these 31 patients were later found to have CIN2+ during follow-up. Among the 60 patients with abnormal GE findings identified by gynaecologists as the "gold standard", GPs correctly identified 31 (52%) cases, whereas 29 (48%) were misclassified as normal. Among these, one patient was later found to have a polypoid change, with a biopsy confirming a CIN III lesion.

TABLE 3 Gynaecological examination by general practitioner versus gynaecologist in women with postcoital bleeding.

	GP assessment, n (%)		
	normal	abnormal	total
Normal	192 (86)	31 (14)	223 (100)
Abnormal	29 (48) ^a	31 (52)	60 (100)
Total	221 (78)	62 (22)	283 (100)

CIN = cervical intraepithelial neoplasia; GP = general practitioner.

a) 1 case of CIN III was detected during follow-up in this group (false negative).

DISCUSSION

This study evaluated a new diagnostic strategy for women with PCB to estimate the reduction in colposcopies, assessed a combined three-test approach for CIN2+ detection, and examined its implementation with GPs.

Colposcopy reduction

The results show that cytology and hrHPV testing reduce unnecessary colposcopies performed in low-risk women with PCB by 81%. However, all unnecessary colposcopies were not avoided, indicating a need for further measures. As shown in Table 1, the three-test combination exhibits a high sensitivity and negative predictive value, suggesting that colposcopy is unnecessary in low-risk women.

Among low-risk patients who were still referred for colposcopy outside the guidelines, 35 out of 38 patients (92%) had no documented indication. In these cases, colposcopy was performed by gynaecologists, often due to a family history of cervical cancer or because the new guideline was not yet fully implemented in routine practice.

Cytology and high-risk human papillomavirus tests for cervical intraepithelial neoplasia 2+ detection

This study highlights the importance of the three-test strategy for effective risk assessment. Even though hrHPV testing has a high sensitivity, it may miss some CIN2+ cases, as also noted in a study highlighting the limitations of the test [11]. Combined testing with the addition of cytology improves accuracy and reduces the likelihood of missing CIN2+ cases. Even so, cytology requires additional resources [12]. However, if both tests fail, GE may still detect these cases, which makes it a valuable addition to the diagnostic approach.

Cytology detected abnormalities in all five carcinoma cases, whereas hrHPV testing missed one case despite the presence of FIGO stage IIb adenocarcinoma, showing its limitations as a stand-alone method. Notably, this case also had a normal GE, which is unusual for a tumour at this stage, highlighting the limitations of relying solely on GE findings. HPV types 16 or 18 were identified in four cases, which confirms their strong link to cervical cancer [13]. Among women under 29 years, there were few cases of CIN2+ and no carcinomas, likely owing to high vaccination coverage, a finding supported by other studies [14].

Implementation of the new strategy at the general practitioner level

In the three-test approach, GPs can perform cytology and hrHPV tests, but GE interpretation remains challenging [15]. Significant discrepancies between GP and gynaecologist interpretations raise concerns about the feasibility of introducing the strategy with GPs. While GPs often identified normal findings, some were misclassified as abnormal, potentially leading to unnecessary referrals. More concerning, abnormal findings were occasionally missed or incorrectly assessed as normal, including one case of stage IIb carcinoma. This highlights a need for additional training or support, especially given the varying experience levels among GPs, which may impact diagnostic accuracy. Targeted training or the use of specialised staff could improve consistency, and real-time image sharing with gynaecologists may further enhance accuracy, as shown by Champin et al. [16].

Strengths and limitations

A strength of this study is its 33-month inclusion period, with most patients (368 out of 392) having at least three months of follow-up. This allows reassessment in cases of persistent PCB and re-evaluation if the initial diagnosis was incorrect. The pathology database also ensures accurate documentation, with all cytology and hrHPV tests being registered [17].

However, this study also has some limitations. First, the retrospective design may have led to incomplete or missing data. Second, using gynaecologists' evaluations as the "gold standard" may potentially introduce bias due to interobserver variation, as their interpretations may differ. Third, verification bias remains a limitation, as biopsies were not performed in all patients. However, our analysis focused on the subgroup with positive hrHPV or cytology results, for whom biopsy was consistently performed. Finally, follow-up time was not uniform across all patients, which could introduce bias if early- and late-included women differ systematically. Future research

should address these limitations through large-scale prospective testing to enhance the diagnostic strategy and its application with GPs.

CONCLUSIONS

This study demonstrates that using a combination of cytology, hrHPV testing and GE can reduce colposcopy referrals in low-risk women with PCB by 81%, while maintaining an excellent 100% accuracy for detecting CIN2+ cases. However, implementing this approach with GPs is challenging due to their lower accuracy in identifying abnormal GE findings compared to gynaecologists. Despite this, the strategy shows great potential for improving diagnostic precision, minimising unnecessary procedures and enhancing patient care in PCB management. To achieve these benefits, further training and the development of standardised guidelines for GPs are necessary.

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