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RESEARCH ARTICLE

Clinical utility of p16/Ki67 dual-stain cytology for detection of cervical intraepithelial neoplasia grade two or worse in women with a transformation zone type 3: A cross-sectional study

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Abstract

Objective: To evaluate the clinical utility of p16/Ki67 dual-stain (DS) compared with cytology for detecting cervical intraepithelial lesion grade two or worse (CIN2+) in women with a transformation zone type 3 (TZ3).

Design: Cross-sectional study.

Setting: Colposcopy clinics in Central Denmark Region.

Population: Women aged 45 years or older referred for colposcopy because of an abnormal screening test.

Methods: All women had a cervical sample collected for cytology and DS testing and underwent large-loop excision of the transformation zone (LLETZ).

Main outcome measure: Sensitivity, specificity and negative (NPV) and positive (PPV) predictive values of DS for CIN2+ detection were compared to those of cytology.

Results: Of 166 women eligible, 93 (56.0%) were included in the final analysis. Median age was 68 years (interquartile range [IQR] 63.4–70.5 years). Most women were postmenopausal (95.7%) and referred based on a positive human papillomavirus screening test (86.0%). Fifty-two women (55.9%) were DS-positive, 29 (55.8%) of whom had CIN2+ detected. Twenty-seven (29.0%) women had atypical squamous cells of undetermined significance or worse (ASC-US+), and CIN2+ was detected in 21 women (77.8%). DS had a higher sensitivity (96.7% versus 70.0% $p = 0.021$) and NPV (97.6% versus 86.4%, $p = 0.018$) compared with cytology for CIN2+ detection. In contrast, the specificity (63.5% versus 90.5% $p < 0.001$) and PPV (55.8% versus 77.8%, $p = 0.001$) were lower for DS compared with cytology.

Conclusions: Dual stain may be a valuable risk marker to guide clinical management of women with a TZ3. The superior NPV of DS suggests that a diagnostic excision may safely be avoided in DS-negative women.

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kits for this study and had the opportunity to comment on the article. Roche Diagnostics had no influence on the scientific process and no editorial rights pertaining to this manuscript. The authors retained the right to submit the manuscript.

KEY WORDS

cervical intraepithelial neoplasia, colposcopy, human papillomavirus, large-loop excision of the transformation zone, liquid-based cytology, p16/Ki67 dual-stain cytology, postmenopausal, transformation zone type 3

Tweetable abstract: DS-negative postmenopausal women with a TZ3 have a low risk of CIN2+ and may undergo follow up instead of LLETZ.

1 | INTRODUCTION

Cervical cancer screening aims to detect and treat cervical precancers, thereby reducing cervical cancer incidence and mortality.¹ Screening with cytology and/or high-risk human papillomavirus (HPV) testing may identify women who are at increased risk of disease, whereas colposcopy and biopsies are important parts of the diagnostic workup.² In postmenopausal women, colposcopy is challenging because of atrophy, retraction and limited visualisation of the transformation zone (TZ).³ This may challenge the collection of biopsies, potentially resulting in increased risk of missing cervical intraepithelial neoplasia (CIN). In a recent publication we describe how biopsies missed more than half (54.5%) of the CIN grade two or worse (CIN2+) cases detected in specimens from large-loop excision of the transformation zone (LLETZ).⁴ Scandinavian and Australian guidelines suggest that a diagnostic LLETZ may be considered in women with an abnormal screening result when visualisation of the TZ is incomplete (i.e. TZ3).⁵⁻⁷ The main disadvantages of LLETZ are the increased risk of overtreatment and complications such as stenosis, which may compromise follow up. In our recent study,⁴ 61% of women aged 45 years or older with a TZ3 had no disease detected in the LLETZ specimen, suggesting a significant risk of overtreatment if LLETZ is to be performed in all screen-positive women with a TZ3. Therefore, a biomarker for correct risk stratification is urgently needed because this would enable a discrimination of women with a TZ3 at increased risk of CIN2+ who need excisional treatment from those who can safely undergo follow up. One such marker could be p16/Ki67 dual-stain cytology (DS), which has been shown to provide better risk stratification of HPV-positive women compared with cytology alone when used in primary screening.⁸⁻¹¹ However, it remains unclear if DS can be used as a tool to guide clinical management of women with a TZ3 at colposcopy.

This study aimed to evaluate the clinical utility of DS versus cytology for CIN2+ detection in a referral population of women with a TZ3.

2 | METHODS

2.1 | Setting and study design

This cross-sectional study was conducted from March 2019 through June 2021 at the Departments of Obstetrics and Gynaecology in Central Denmark Region in collaboration

with the Department of Pathology, Randers Regional Hospital, Denmark.

Denmark has an organised cervical cancer screening programme, with screening, clinical follow up and treatment offered free of charge. Women aged 23–59 years are screened with liquid-based cytology (hereafter ‘cytology’), whereas women aged 60–64 years undergo HPV-based screening. In addition, from April 2019 women aged 65–69 were invited for one additional HPV-based screening test as part of an intervention study in Central Denmark Region.¹² Women with abnormal screening results were referred for colposcopy and managed clinically according to national guidelines (Table S1).^{7,13,14}

2.2 | Participants and samples

Samples used in the present study were collected as part of another clinical study.⁴ In brief, women were eligible for enrolment if they were aged 45 years or above, referred for colposcopy because of an abnormal screening result (Table S1), and had a TZ3 at colposcopy according to the 2011 International Federation of Cervical Pathology and Colposcopy nomenclature.^{15,16} Women were excluded if they wanted to become pregnant, if excision was not technically possible (e.g. pain, narrow vagina, atrophy or pelvic organ prolapse), had previous history of excisional treatment or hysterectomy, received anti-coagulant medical treatment, or if they underwent repeated colposcopy for cervical dysplasia. Before enrolment, women received written and oral information about the study, and all included women signed an informed consent form. Before colposcopy, a cytology sample was collected in SurePath (BD Diagnostics, Burlington, NC, USA) for morphological assessment, and HPV and DS testing. All women had multiple biopsies taken for the purpose of another study and underwent a diagnostic LLETZ immediately after colposcopy using local anaesthesia (Citanest Dental Octapressin, Dentsply).

Women answered questions on basic characteristics (e.g. smoking, number of lifetime sexual partners, parity). Further, information on previous screening history was retrieved from the National Danish Pathology Databank¹⁷ using the woman's personal identification number, a unique code assigned to all Danish residents at birth or upon immigration.¹⁸ The National Danish Pathology Databank holds data on all cytopathological and histopathological examinations at an individual level for all Danish residents since 1997, and for some examinations back to 1970.¹⁷

2.3 | Cytology, HPV and p16/Ki67 dual stain

Cytology slides were reviewed by experienced cyto-technicians using computer-assisted microscopy (BD FocalPoint GS Imaging System) and categorised using the Bethesda 2014 grading system.¹⁹ Cytology results were deemed invalid if too few squamous cells were present (i.e. ≤ 5000 cells per slide). Cytology results were grouped as normal cytology and atypical squamous cells of undetermined significance (ASC-US+); low-grade squamous intraepithelial lesions (LSIL); atypical squamous cells, cannot rule out high-grade squamous intraepithelial lesion (ASC-H) or high-grade squamous intraepithelial lesion (HSIL). HPV DNA testing was performed using the clinically validated Cobas 4800 HPV DNA test (Roche Diagnostic) according to the manufacturer's instructions. This assay enables individual detection of HPV 16 and HPV 18, and pooled detection of 12 other high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).²⁰ DS was performed using the residual SurePath cell pellet in all included women regardless of HPV result. Details of the dual staining have been described elsewhere.²¹ In brief, DS was performed using the commercial US Food and Drug Administration approved CINtec PLUS assay (Roche Diagnostics).²² Slides were stained using the automated BenchMark ULTRA immunostainer (VENTANA, Roche Diagnostics) according to the manufacturer's instructions. Each staining run had one external cytology-positive control (HSIL), and cells stained with either p16 or Ki67 were used as internal positive controls.

Dual-stain slides were reviewed independently by two cyto-technicians who were blinded to all study data (i.e. HPV genotype, cytology and histology results) except for the age of the woman. Both cyto-technicians received training in interpretation of DS slides according to the manufacturer's recommendations.²¹ In case of discrepancy between the cyto-technicians' results, a consensus-based decision was performed based on a revision in a multi-headed microscope. Slides were considered positive if one or more DS-positive cells were detected, without consideration of morphology and cellularity criteria. For each slide the number of DS-positive cells was recorded. Slides were deemed negative if no DS-positive cell(s) were detected and the Bethesda 2014 criterion of squamous cellularity (i.e. ≥ 5000 cells per slide) was fulfilled. Slides were considered invalid if the cellularity criteria were not fulfilled or if one or both proteins (i.e. p16 or Ki67) were not stained. In case of an invalid DS slide, a second slide preparation and staining from the residual cell pellet was performed.

Cytology, HPV testing and DS testing were performed at the Department of Pathology, Randers Regional Hospital, Denmark, which is responsible for analysing all cervical cytology samples in Central Denmark Region (approximately 90 000 samples annually).

Clinical management was not based on DS results.

Histopathological examination of cervical biopsies and LLETZ specimens was performed in routine laboratories at the Departments of Pathology, Randers and Viborg Regional Hospital and graded according to the CIN classification²³ as

follows; <CIN2 (normal and CIN1) and CIN2+ (CIN2, CIN3, unclassifiable CIN [i.e. the full height of the epithelium is not discernible], adenocarcinoma in situ and cancer). The histopathological result of the LLETZ specimen served as the reference standard.⁴

No patients or patient organisations were involved in the development, design or implementation of this study.

2.4 | Statistical analysis

For analysis, only women with valid results on cytology, HPV and DS were included. CIN2+ in the LLETZ specimen was used as the primary outcome instead of CIN grade 3 or worse because CIN2 is the threshold for excisional treatment in older women in most countries, including Denmark and the UK.¹⁴ Age and body mass index were calculated and presented as median with interquartile ranges (IQR).

For cytology and DS, we calculated specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) for CIN2+ detection with corresponding 95% confidence intervals (CI). Specificity and sensitivity for cytology and DS, respectively, were compared using the McNemar's chi-square test. To test the robustness of our findings we conducted a sensitivity analysis excluding women with unclassifiable CIN. Further, we have restricted the analyses of DS positivity by cytology and histology to women who have undergone HPV-based screening.

Finally, we calculated sensitivity, specificity, PPV and NPV using different thresholds of DS-positive cells (1, ≥ 2 , ≥ 6 and > 50) for CIN2+ detection and calculated the Youden's index ($YI = \text{sensitivity} + \text{specificity} - 1$). Cases with one DS-positive cell served as reference for the comparison between different thresholds.

Data were entered and stored in REDCap.^{24,25} All statistical analyses were conducted using STATA version 17 (StataCorp LP, College Station, TX, USA), and for comparison of PPV and NPV we used the method developed by Leisenring et al. using the DTComPair package in R.²⁶ Values of p less than 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Study population

One-hundred and sixty-six women were assessed for eligibility, 99 (59.6%) of whom were enrolled (Figure 1). Six women (3.6%) were subsequently excluded because of invalid test results, leaving a total of 93 women (56.0%) for final analyses.

The median age of the included women was 68.0 years (IQR 63.4–70.5 years) with 89 (95.7%) women being postmenopausal (Table 1). Most women were non-smokers (72.0%), parous (90.4%) and had no previous history of abnormal cytology (73.1%). The majority of women reported no new sexual partner within the past 2 years (83.9%), and 55 (59.1%) reported having five or more lifetime sexual

partners. The majority had undergone HPV-based screening with reflex cytology (82.8%) (Table 1).

3.2 | p16/Ki67 dual stain by cytology and histology

Of the 93 included women, 66 (71.0%) had normal cytology, 8 (8.6%) had low-grade cytology (ASC-US and LSIL) and 19 (20.4%) had high-grade cytology (ASC-H and HSIL) (Table S2). DS positivity increased with the severity of cytology from normal to high-grade (39.4% versus 94.7%, $p < 0.001$), respectively (Table S2).

Restricting the analyses to women who have undergone HPV-based screening showed similar results to those stated above (Table S2).

3.3 | Performance of p16/Ki67 dual-stain cytology and cytology for CIN2+ detection

A total of 52 women (55.9%) were DS positive, of whom 29 (55.8%) had CIN2+ detected (Table 2). With respect to cytology, 27 (29.0%) women had ASC-US+, of whom 21 (77.8%) had CIN2+ detected (Table 2). Compared with cytology, DS was more sensitive in detecting CIN2+ (70% versus 96.7%, respectively, $p = 0.021$), but less specific (90.5% versus 63.5%, $p < 0.001$) (Table 2). The NPV of DS was significantly higher compared with cytology (97.6% versus 86.4%, $p = 0.018$), whereas the PPV of DS was significantly lower compared with the PPV for cytology (55.8% versus 77.8%, $p = 0.001$) (Table 2). When restricting to women referred as the result of HPV-based screening ($n = 80$), we found no major differences in sensitivity, specificity, PPV and NPV compared with the overall results (Table 2).

Excluding unclassifiable CIN from analysis resulted in a significant decrease in PPV for DS (55.8% versus 34.1%,

$p = 0.004$) and cytology (77.8 versus 52.6%, $p = 0.004$), respectively. The sensitivity, specificity and NPV for DS and cytology did not change (data not shown).

3.4 | Performance of p16/Ki67 dual stain at different thresholds

We evaluated the performance of DS for CIN2+ detection at thresholds of one, two or more, six or more, and more than 50 DS-positive cells (Table 3). Increasing the threshold from one DS-positive cell to two or more DS-positive cells lead to a slight reduction in DS positivity from 55.9% to 51.6% ($p = 0.56$) as well as increased specificity (63.5–69.8%, $p = 0.13$) and PPV (55.8–60.4%, $p = 0.04$). Sensitivity and NPV were almost identical compared with one-cell cutoff (Table 3). Cutoff values at six or more and more than 50 DS-positive cells resulted in higher specificity and PPV but at the cost of lower sensitivity and NPV compared with a cutoff of one cell (Table 3).

4 | DISCUSSION

4.1 | Main findings

Compared with cytology, DS had superior sensitivity and NPV for CIN2+ detection in women aged 45 years or above with a TZ3 at colposcopy. In contrast, specificity and PPV were significantly lower for DS compared with cytology. Using two or more DS positive cells as threshold increased the specificity and PPV without lowering sensitivity and NPV for CIN2+ detection. Taken together, these results suggest that DS-negative women with a TZ3 can undergo follow up with repeated cervical sampling instead of diagnostic LLETZ.

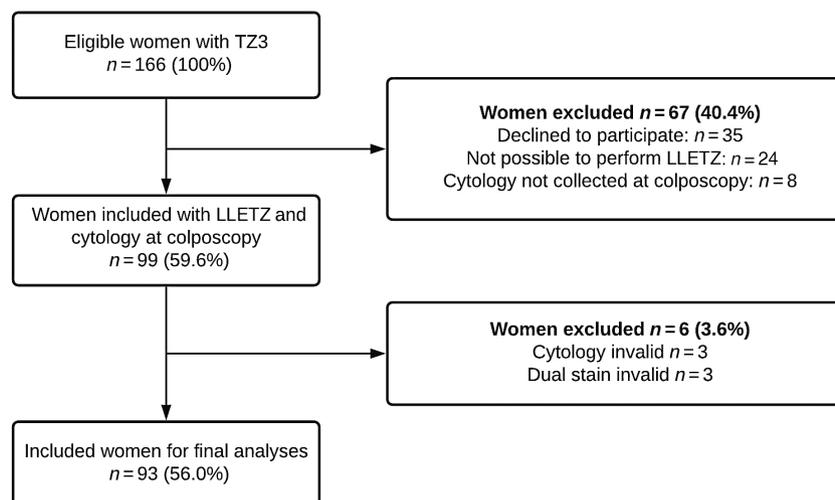


FIGURE 1 Flow chart for study population. LLETZ, large-loop excision of the transformation zone; TZ3, transformation zone type 3

TABLE 1 Basic characteristics for the included women ($n = 93$)

| | Women |
|---|------------------|
| Age (years), median (IQR) | 68.0 (63.4–70.5) |
| Body mass index (kg/m^2), median (IQR) | 24.4 (21.8–27.7) |
| Smoking, n (%) | |
| No | 67 (72.0) |
| Yes | 20 (21.5) |
| Missing | 6 (6.5) |
| Menopausal status, n (%) | |
| Postmenopausal | 89 (95.7) |
| Premenopausal | <3 |
| Missing | <3 |
| Parity, n (%) | |
| Nulliparous | 5 (5.4) |
| Parous | 85 (90.4) |
| Missing | 3 (3.2) |
| Referral test, n (%) | |
| Primary HPV with reflex cytology | 77 (82.8) |
| Primary cytology with reflex HPV test | 4 (4.3) |
| Cytology only | 9 (9.7) |
| HPV test only | 3 (3.2) |
| Previous history of abnormal cytology (\geq ASC-US), n (%) | |
| No | 68 (73.1) |
| Yes | 25 (26.9) |
| Lifetime sexual partners, n (%) | |
| <5 | 27 (29.0) |
| 5–10 | 36 (38.7) |
| >10 | 19 (20.45) |
| Missing | 11 (11.8) |
| New sexual partners within the past 2 years, n (%) | |
| No | 78 (83.9) |
| Yes | 7 (7.5) |
| Missing | 8 (8.6) |
| HPV vaccination, n (%) | |
| No | 80 (86.0) |
| Yes | 7 (7.5) |
| Missing | 6 (6.5) |

Abbreviations: \geq ASC-US, atypical squamous cells of undetermined significance; ASC-US; HPV, human papillomavirus; IQR, interquartile range.

4.2 | Strengths and limitations

A key strength of this study is that all the included women had a LLETZ performed, which minimised the risk of underestimating CIN2+, as we in a recent study found that biopsies missed 54% of the CIN2+ cases detected in the LLETZ specimens.⁴ Moreover, all samples (i.e. cervical cytology, biopsies and LLETZ) were collected on the same day and analysed in the same laboratory, limiting temporal and inter-laboratory variation, respectively. To reduce interpretation bias, cytotechnicians were blinded to all study data except the age of

TABLE 2 Sensitivity, specificity, PPV, and NPV for p16/Ki67 dual stain and cytology for CIN2+ detection in women referred for colposcopy without a visible TZ

| Statistical measures | DS positive (I+) % (95% CI) | Cytology (ASC-US+) % (95% CI) | p^a value |
|--|-----------------------------|-------------------------------|-------------|
| All women ($n = 93$) | | | |
| Positivity | 55.9 (45.6–65.8) | 29.0 (20.6–39.2) | <0.001 |
| Sensitivity | 96.7 (82.8–99.9) | 70.0 (50.6–85.3) | 0.021 |
| Specificity | 63.5 (50.4–75.3) | 90.5 (80.4–96.4) | <0.001 |
| PPV | 55.8 (41.3–69.5) | 77.8 (57.7–91.4) | 0.001 |
| NPV | 97.6 (87.1–99.9) | 86.4 (75.7–93.6) | 0.018 |
| Women referred based on primary HPV screening ($n = 80$) | | | |
| Positivity | 52.5 (41.4–63.3) | 23.8 (15.6–34.4) | <0.001 |
| Sensitivity | 95.7 (78.1–99.9) | 60.9 (38.5–80.3) | 0.021 |
| Specificity | 64.9 (51.1–77.1) | 91.2 (80.7–97.1) | <0.001 |
| PPV | 52.4 (36.4–68.0) | 73.7 (48.8–90.9) | 0.014 |
| NPV | 97.4 (86.2–99.9) | 85.2 (73.8–93.0) | 0.017 |

Note: HPV any type: HPV 16/18/other high-risk HPV types. p16/Ki67 dual-stain cytology threshold is one positive cell.

Abbreviations: ASC-US+, atypical squamous cells of undetermined significance; CI, confidence interval; DS, dual stain; CIN2+, cervical intraepithelial lesion grade two or worse; NPV, negative predictive value; PPV, positive predictive value; TZ, transformation zone.

^aValue of p between cytology and DS.

the women. Limitations include the relatively small sample size making the results less robust. Moreover, we cannot rule out that excluded women differed from those included with respect to basic characteristics. However, we do not believe that this has caused selection problems, because women were excluded before the results of cytology, DS and LLETZ.

4.3 | Interpretation

Until now, studies have mainly investigated the performance of DS for triage of HPV-positive women in younger screening populations^{8,9,27} and in screen-positive women with ASC-US or LSIL.²⁸ Fewer studies have investigated the use of DS in a referral population like ours (i.e. a high-risk population).^{29,30} In the present study, we found a significantly higher sensitivity (96.7%) and NPV (97.6%) of DS for CIN2+ detection compared with cytology, which is comparable to the above-mentioned studies among women referred for colposcopy.^{29,30} Packet et al. reported a sensitivity and NPV for DS of 95% and 94%, respectively, in a subgroup of women with inconclusive colposcopy (due to bleeding, inflammation or incomplete visualisation of the TZ).²⁹ A recent study investigated the use of DS in HPV-positive women with low-grade cytology and TZ3 undergoing LLETZ and reported a sensitivity of 100% and NPV of 100% of DS for CIN2+ detection, whereas the specificity and PPV were 73.8% and 76.1%, respectively.³¹ These findings are in line with our results although there are important differences in the median age

TABLE 3 Performance of p16/Ki67 dual stain for CIN2+ detection at different thresholds of p16/Ki67 dual stain positive cells (*n* = 93)

| Characteristic | Positivity % (95% CI) | <i>p</i> ^a | <i>p</i> ^b | Sensitivity % (95% CI) | <i>p</i> ^a | <i>p</i> ^b | Specificity % (95% CI) | <i>p</i> ^a | <i>p</i> ^b | PPV % (95% CI) | <i>p</i> ^a | <i>p</i> ^b | NPV % (95% CI) | <i>p</i> ^a | <i>p</i> ^b | Youden's index |
|--|--------------------------|-----------------------|-----------------------|---------------------------|-----------------------|-----------------------|---------------------------|-----------------------|-----------------------|-------------------|-----------------------|-----------------------|-------------------|-----------------------|-----------------------|-------------------|
| Cytology, ASC-US+ | 29.0 (20.6–39.2) | Ref | NA | 70.0 (50.6–85.3) | Ref | NA | 90.5 (80.4–96.4) | Ref | NA | 77.8 (57.7–91.4) | Ref | NA | 86.4 (75.7–93.6) | Ref | NA | 0.605 |
| DS ≥ 1 positive cells (<i>n</i> = 52) | 55.9 (45.6–65.8) | <0.001 | Ref | 96.7 (82.8–99.9) | 0.021 | Ref | 63.5 (50.4–75.3) | <0.001 | Ref | 55.8 (41.3–69.5) | <0.001 | Ref | 97.6 (87.1–99.9) | 0.018 | Ref | 0.602 |
| DS ≥ 2 positive cells (<i>n</i> = 48) | 51.6 (41.0–62.1) | <0.002 | 0.557 | 96.7 (90.2–100.0) | 0.021 | 1.000 | 69.8 (58.5–81.2) | <0.001 | 0.125 | 60.4 (46.6–74.3) | 0.009 | 0.043 | 97.8 (93.5–100.0) | 0.014 | 0.362 | 0.665 |
| DS ≥ 6 positive cells (<i>n</i> = 38) | 40.9 (30.8–51.5) | 0.089 | 0.041 | 90.0 (79.3–100.0) | 0.109 | 0.500 | 82.5 (73.2–91.9) | 0.063 | <0.001 | 71.1 (56.6–85.5) | 0.218 | 0.001 | 94.5 (88.5–100.0) | 0.071 | 0.238 | 0.725 |
| DS > 50 positive cells (<i>n</i> = 27) | 29.0 (20.1–39.4) | 1.000 | <0.001 | 76.7 (61.5–91.8) | 0.774 | 0.031 | 93.7 (87.6–99.7) | 0.688 | <0.001 | 85.2 (71.8–98.6) | 0.340 | 0.001 | 89.4 (82.0–96.0) | 0.513 | 0.023 | 0.703 |

Note: Cytology positivity = ASC-US+ (atypical squamous cells of undetermined significance) is defined as: ASC-US, Youden's index = (Sensitivity + Specificity – 1).

Abbreviations: ASC-US+, atypical squamous cells of undetermined significance; CIN2+, cervical intraepithelial neoplasia (grade 2 or higher); DS, dual stain p16/Ki67; NA, not applicable.

^a*p* value: comparison with cytology as reference.

^b*p* value: comparison with DS ≥ 1 as reference.

(68 years in our study versus 39 years in the study by Manley et al.).³¹ To our knowledge, only one study has reported the performance of DS in postmenopausal women.³² In that study 'histologic' outcome was based on biopsies from the ectocervix or cervical cytology in the case of a lack of referral for colposcopy, which may have affected the CIN2+ detection rate. They reported sensitivity, specificity, NPV and PPV of 57.1%, 94.3%, 96.3% and 46.2%, respectively, with the sensitivity being somewhat lower than in our study.³² This discrepancy may be explained by differences in the study design and characteristics of the study population across studies. In a recent study,⁴ we found that biopsies missed more than half of the CIN2+ cases detected in the LLETZ specimens, suggesting that the histological outcome of biopsies may be suboptimal for use as reference in women with a TZ3.

Several countries have either switched to or are currently transitioning to HPV-based screening, with triage being performed to improve specificity and reduce the risk of unnecessary colposcopy. The most used triage strategies include cytology and partial, or in a few settings, extended HPV genotyping. However, the sensitivity of cytology decreases with increasing age and may not be the optimal triage choice for older HPV-screen-positive women.³³ Other settings may use genotyping with HPV 16- and/or HPV 18-positive women being referred directly for colposcopy. Given that previous studies^{34,35} have reported a significant decline in the prevalence of HPV 16 and HPV 18 in cervical cancer cases with increasing age, this triage strategy may be suboptimal as well. More studies are needed to determine the best method for triage of older HPV-screen-positive women.

Across countries, the diagnostic workup in postmenopausal women with a TZ3 poses a major clinical challenge. There are different ways to obtain histological material from the cervix, for example, by endocervical curettage (ECC).^{6,36} However, ECC has been reported to be painful and the diagnostic value of ECC to detect CIN2+ in women who have a TZ3 is not well addressed.^{37,38} A recent study³¹ found DS of cervical cytology samples from HPV-positive women with low-grade cytology to have superior sensitivity, NPV and PPV for CIN2+ detection compared with ECC. However, as the mean age was 39 years, the value of ECC may not be fully comparable to an older postmenopausal population.³¹ Another practice in the diagnostic work-up in postmenopausal women referred because of abnormal screening results and who have a TZ3 is a diagnostic LLETZ.^{5–7} However, a diagnostic LLETZ in all women referred for colposcopy will probably lead to a significant risk of overtreatment. For example, in a previous study we found that 68% of women referred for colposcopy had <CIN2 (normal and CIN1) detected in the LLETZ specimens.⁴

In the present study, our results suggest that DS is of great value in clinical management because of a high sensitivity and because a negative DS test provides greater reassurance against CIN2+ than a negative cytology. Hence, DS may enable a risk stratification of women into those in need of immediate excisional treatment while allowing those with a negative DS test to undergo follow up. However, future

studies are needed to determine the appropriate follow-up interval and to replicate our findings focusing on women with a TZ3.

5 | CONCLUSION

The high NPV of DS suggests that postmenopausal women with a negative DS can safely undergo follow up with repeated cervical sampling instead of diagnostic LLETZ. Therefore, DS may be a valuable risk marker to guide clinical management of postmenopausal women with a TZ3 at colposcopy.

AUTHOR CONTRIBUTIONS

The idea and the overall design of this study were conceived by LWG, MT, BA, PB, LKP, NW, MC and AH. Recruitment, data collection, data management and analysis of samples were by RB, PNC, PB, MT and LWG. Data analyses were conducted by MT, NW, MC, AH and LWG. LWG prepared the first draft with guidance/supervision from MT, NW, MC, PB, BA, LKP, PB and AH. Critical revision of the manuscript for important intellectual content was provided by NW, MC, MT, LWG, BA, RB, PNC, PB, LKP and AH. All authors approved the final manuscript before publication.

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CONFLICT OF INTEREST

LWG, MT and LKP have received speaker's fees from Astra Zeneca, outside the submitted work. LWG, MT and BA are participating in other studies with HPV test kits and CINtec plus kits sponsored by Roche, outside the submitted work. MT has received honoraria from Roche Diagnostics for lectures on CINtec plus, outside the submitted work. AH has received reagents from Roche, Denmark for free, outside the submitted work. MC: Enduring Guidelines for Cervical Cancer Screening and Management (U.S. National Cancer Institute; Steering Committee). International Neoplasia Society's Task Force for the development of anal cancer screening guidelines (Working Group Co-Chair). LKP: Speakers fee from Merck on subjects outside this study. PNC, RB, NW and PB report no conflict of interest. Completed disclosure of interest forms are available to view online as supporting information.

DATA AVAILABILITY STATEMENT

The data set generated and analysed in this study is not available for the public due to Danish legislation. Data can be made available on request from researchers who meet the criteria for access to patient's confidential data and upon approval from the Danish Data Protection Agency.

ETHICS STATEMENT

The project was listed at the record of processing activities for research projects in the Central Denmark Region (j.no. 1-16-02-528-18) and was deemed exempt from the need for ethics approval (j.no:1-10-72-4-17).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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