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# Management of low-grade cervical cytology in young women. Cohort study from Denmark

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**ABSTRACT**

**Objective:** Cytology findings of atypical squamous cells of unknown significance (ASCUS) or low-grade squamous intraepithelial lesion (LSIL) are common among women under 30, but evidence on best management strategy is insufficient. We therefore investigated how different management strategies used in Denmark influenced biopsy rates and detection of cervical intraepithelial neoplasia (CIN).

**Methods:** Register-based cohort study including Danish women aged less than 30 years and born 1980–95, with ASCUS/LSIL as their first abnormal cervical cytology in 2008–16. Rates and relative risks (RR) of biopsy and detection of CIN3+, CIN2 and < CIN2 during two years follow-up were compared between women referred directly to colposcopy after ASCUS/LSIL or undergoing additional testing, including mRNA or DNA test for high risk HPV or repeat cytology.

**Results:** 19,946 women with ASCUS and 19,825 with LSIL were included in the study of whom 92% had adequate information about follow-up. Among women referred directly to biopsy, CIN3+ was detected among 21%, CIN2 in 17%, while 62% had < CIN2. Repeating cytology after 6 months reduced the biopsy rate to 44% of which 53% had < CIN2. Biopsy rates with HPV test were 67% for DNA test, 77% with 14-type mRNA test and 58% with 5-type mRNA test. The detection of CIN3+ was somewhat higher, between 13% and 14% for the three HPV tests vs. 11% with repeat cytology. However, the detection of < CIN2 (not indicating treatment) also increased with RR 2.11 (95% CI 2.01–2.21) for 14-type mRNA test, 1.35 (95% CI 1.29–1.41) for 5-type mRNA test, and 1.86 (95% CI 1.76–1.97) with HPV DNA test.

**Conclusions:** The choice of management strategy influences both the detection rate for severe lesions (CIN3+) and the proportion of women followed up for potentially insignificant findings.

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**KEYWORDS**
Cervical cancer screening; ASCUS; LSIL

## Introduction

Cervical screening with cytology has helped reduce cervical cancer incidence and mortality in high-income countries [1]. In countries with organized screening programs, women with normal cytology will be recalled for screening after a time period depending on age and national/local recommendations, while women with high-grade intraepithelial lesion (HSIL) are recommended further assessment, including colposcopy and supplementary testing, including biopsy or endocervical abrasio, to verify if cervical intraepithelial neoplasia (CIN) is present [2].

An organized cervical screening program aims to obtain the best possible balance between reducing the risk of cervical cancer and limiting overmanagement. Although many countries are adopting HPV test as a primary screening test, this is usually not recommended for women under the age of 30 years who will still be screened with cytology, also in Denmark [3]. However, the positivity rate for cytology is quite high in this age group and therefore management strategy becomes essential. The optimal strategy is not fully established and probably depends on the woman's age [2]. Women with atypical cells of unknown significance (ASCUS) or low-grade squamous intraepithelial lesions (LSIL) are at much lower risk of having CIN2+ than women with HSIL. Referral of all women with ASCUS or LSIL directly to colposcopy would result in overmanagement and overtreatment,

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and constitute a burden for women in terms of inconvenience and worry and in risks related to biopsy taking. In addition, it may be an inappropriate use of healthcare resources. Additional testing or triage of women with ASCUS or LSIL is therefore recommended prior to referral for colposcopy. In this light, the objective of the present study was to examine the outcome of different strategies used in Denmark for women below 30 years of age with ASCUS or LSIL.

## Material and methods

### Data and definitions

The study was a register-based, retrospective cohort study set in Denmark. All residents in Denmark are assigned a unique personal identifier which allows for cross linking of official healthcare and other registers. Women born in 1980 to 1995 and resident in Denmark through to study end in 2018 were identified through the Civil Registration System [4]. For these women, cervical pathology results were retrieved in the Danish Pathology Register (DPR) [5]. This register contains all cytology and histology diagnoses with SNOMED codes from all pathology departments in Denmark, and was established in 1997. Cervical cytology is classified according to the Bethesda system and histology from the cervical region according to the CIN classification. Women were included in the study if they had a cervical cytology sample diagnosed with ASCUS or LSIL between 2008 and 2016, were below age 30, with no previous abnormal cervical pathology result and no histology sample taken on the same date as the index cytology sample. This was the index sample. We included the index sample and all cervical pathology and HPV results for the subsequent 2 years.

National screening guidelines were published in 2007 [3]. Consequently, we included index samples from 1st January 2008 to 31st December 2016 allowing for two years of follow-up (see Lexis diagram in Suppl. fig. 1). If more than one histology diagnosis was recorded for a woman during the two years, the most severe was used.

In Denmark, national recommendations for cervical cancer screening are published by the Danish Health Authority, but the screening program is implemented by the five regions of the country, and the recommendations are not legally binding. All regions invite women according to the guidelines, i.e., cytology screening every three years from age 23–49 years and every five years until age 59 years, followed by a primary HPV-test ("exit test") between age 60 and 64 years, five years after the last cytology test. For women aged less than 30 years with ASCUS or LSIL, the Danish guidelines recommend either mRNA HPV test of the ASCUS/LSIL sample or repeat cytology after 6 months. For women 30 years or older, HPV DNA test is an additional option for ASCUS [3,6]. In addition to the recommended methods, some pathology departments have periodically used HPV-DNA test also for women under 30 years, and/or for LSIL.

Test methods were introduced and/or changed gradually and more than one strategy may have been in use simultaneously in the same department. We therefore grouped women based on the actual tests performed as per the pathology register. For HPV tests, the register reports that HPV test was performed, and in the majority of cases, whether DNA or mRNA test was used. However, which DNA or mRNA test was used, is not coded in the register. For mRNA tests, we were able to classify into 5-type and 14-type mRNA test groups based on information from the departments on assay used at that point in time. In total, we identified 5 main strategies: repeat cytology, 14-type mRNA test, 5-type mRNA test, DNA test (any assay), or direct referral to colposcopy (see table in supplement for an overview of the laboratories and their main recommended strategies). Women who could not be classified into any of the five groups were labeled unclassified. Furthermore, we subdivided each group based on whether or not the woman had been adequately followed up, and women lacking follow-up were excluded from the analysis of outcomes. Table 1 shows the criteria for each group.

### Statistics

We calculated the proportion of women followed up with each strategy by year and index diagnosis, and the proportion of HPV tests that were positive by test method (5-type mRNA/14-type mRNA/DNA HPV test) and index diagnosis.

The main analysis was the comparison of outcomes between the different follow-up strategies. The outcomes included were:

- Biopsy: women with a cervical biopsy histology result in the DPR
- CIN3+: women with a histology result of CIN3 or cervical cancer in DPR
- CIN2: women with histology result of CIN2 in DPR
- <CIN2: women with histology result of CIN1, normal, or no intraepithelial lesions in DPR

These groups reflect the different follow-up options. Conization is recommended for CIN3+, while not recommended for normal and CIN1 biopsy finding, which should be followed up after 6–12 months. For CIN2 the recommendation changed in 2012 from conization to follow-up.

We calculated the number and proportion of women with each outcome during two years following initial ASCUS or LSIL test for each follow-up strategy. For women undergoing HPV test or direct referral, risk ratios with 95% confidence intervals (CI) were calculated for each outcome relative to those who underwent repeat cytology. Results were adjusted for year of birth to account for variation between the birth cohorts in HPV vaccination rates and age at vaccination [7].

A consequence of a follow-up period of two years may be that some samples not resulting from the initial screening episode (the index sample) are included. Although we considered this less likely, given the recommended screening interval of 3 years, we repeated the analysis considering 9 months of follow-up only. Women under 23 years are not invited for screening through the Danish program and may differ from women screened on invitation, so we repeated the analyses only for women aged 23 and above. SAS 9.4 and Stata IC 15.1 were used for the analysis.

**Table 1.** definition of triage strategies and whether women had adequate follow-up.

| Triage strategy | Definition | Adequate follow-up (included in analysis) | Inadequate follow-up (excluded from analysis) |
|---|---|---|---|
| Repeat cytology | • No HPV test performed on the index sample<br>AND<br>• Next sample in pathology register was cytology<br>AND<br>• No histology sample was taken at the time of repeat cytology | Either:<br>• Abnormal cytology on repeat cytology<br>AND<br>• Subsequent histology sample<br><br>Or:<br>• Normal cytology on repeat cytology | • Abnormal cytology on repeat cytology<br>AND<br>• No additional samples in register during 2 years |
| 14-type mRNA test | • hr-HPV mRNA test was performed on the index cytology sample<br>AND<br>• Index date and laboratory where 14-type test was used | At least one more sample registered after index | No tests beyond the index (according to guideline, women with negative mRNA-test should be retested with cytology after 12 months, so regardless of mRNA result we would expect additional test within 2 years) |
| 5-type mRNA test | • hr-HPV mRNA test was performed on the index cytology sample<br>AND<br>• Index date and laboratory where 5-type test was used | At least one more sample registered after index | No tests beyond the index (women with negative mRNA-test should be retested with cytology after 12 months, so regardless of mRNA result we would expect additional test within 2 years) |
| DNA | • hr-HPV DNA test was performed on the index cytology sample | Either:<br>• HPV DNA test negative<br><br>Or:<br>• HPV DNA test positive<br>AND<br>• at least one sample registered after index | • HPV DNA test positive<br>AND<br>• no further tests beyond index sample |
| Direct referral | • Next cervical sample in the pathology register was histology (with or without cytology)<br>AND<br>• No HPV test was performed on index sample | Women in this group were identified based on the presence of a histology result and so by definition have all been followed up | |
| Unclassifiable | Either:<br>• no HPV test on the index sample<br>AND<br>• no further tests beyond the index sample<br>Or:<br>• HPV test on index sample but no information on type (mRNA or DNA) | | All excluded from the analysis |

## Results

A total of 39,771 women were included in the study, 19,946 with ASCUS and 19,825 with LSIL. The median age was 24.1 years (range 14–29 years), and 31,918 (80%) were 23–29 years when the index sample was collected. The proportion of women belonging to birth cohorts offered HPV-vaccination through the childhood immunization program (birth years 1993–95) was 6.7%.

Overall, about half of the women had repeat cytology, 46% of ASCUS and 57% of LSIL (Table 2). HPV DNA testing was used more often for ASCUS than LSIL; and 2,158 women (5.4%) were unclassifiable, including 1,875 without repeat cytology and no HPV-test of index sample, and 283 with an unspecified HPV test. The proportion with repeat cytology declined over time from 79% in 2008 to 34% in 2015, reflecting a gradual introduction of HPV testing. In 2016, the use of DNA testing for ASCUS declined and the 5-type mRNA test went out of use in Denmark. Consequently the proportion with repeat cytology increased to 52% (data not shown). The proportion of samples testing positive for HPV depended on the index diagnosis and test method, being higher for LSIL and DNA test and lower for ASCUS and 5-type mRNA test (Table 3).

In total 36,453 (92%) could be classified according to the 5 strategies and had adequate follow-up within 2 years (Table 4). The proportion of women undergoing biopsy was higher with HPV test than with repeat cytology and highest

**Table 2.** Women under 30 years with ASCUS or LSIL in Denmark 2008–2016 by index diagnosis and follow-up.

| Follow-up | ASCUS | | LSIL | | Total | |
|---|---|---|---|---|---|---|
| Repeat cytology | 9,604 | 48% | 11,366 | 57% | 20,970 | 53% |
| 14-type mRNA test | 1,647 | 8% | 1,542 | 7.8% | 3,189 | 8% |
| 5-type mRNA test | 3,306 | 17% | 4,047 | 20% | 7,353 | 19% |
| DNA | 2,916 | 15% | 839 | 4.2% | 3,755 | 9% |
| Direct referral for colposcopy | 1,439 | 7% | 907 | 4.5% | 2,346 | 6% |
| Not classified | 1,034 | 5% | 1,124 | 5.7% | 2,158 | 5% |
| Total | 19,946 | 100% | 19,825 | 100% | 39,771 | 100% |

**Table 3.** HPV test results by index diagnosis and HPV test method, excluding samples with unknown HPV test and/or unknown result.

| | ASCUS | | | | | LSIL | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Positive | | Negative | | Total[a] | Positive | | Negative | | Total[a] |
| 14-type mRNA test | 1,131 | 69% | 515 | 31% | 1,646 | 1,209 | 78% | 333 | 22% | 1,542 |
| 5-type mRNA test | 1,106 | 36% | 1,964 | 64% | 3,070 | 1,655 | 44% | 2,136 | 56% | 3,791 |
| DNA test | 2,165 | 75% | 736 | 25% | 2,901 | 745 | 90% | 84 | 10% | 829 |
| Total | 4,402 | 58% | 3,215 | 42% | 7,617 | 3,609 | 59% | 2,553 | 41% | 6,162 |

[a]Totals are lower than in Table 2 because results were missing for some samples.

**Table 4.** Biopsy rates and proportion with CIN3+, CIN2, or < CIN2 in Danish women under 30 years with ASCUS or LSIL by management strategy and whether follow-up was adequate, 2008–2016.

| | Repeat cytology, with follow-up | 14-type mRNA, with follow-up[a] | 5-type mRNA, with follow-up | DNA test, with follow-up[a] | Direct referral, with follow-up[a] | Total with follow-up[a] | Unclass or no follow-up | Total |
|---|---|---|---|---|---|---|---|---|
| Number with ASCUS/LSIL | 20.634 | 3.021 | 6.789 | 3.663 | 2.346 | 36.453 | 3.318 | 39.771 |
| Number with biopsy[b] | 9.022 | 2.314 | 3.929 | 2.454 | 2.346 | 20.065 | | |
| % biopsy/(ASCUS/LSIL) | 44% | 77% | 58% | 67% | 100% | 55% | | |
| Number with CIN3+ | 2.225 | 435 | 862 | 475 | 492 | 4.489 | | |
| % CIN3+/(ASCUS/LSIL) | 11% | 14% | 13% | 13% | 21% | 12% | | |
| % CIN3+/biopsy | 25% | 19% | 22% | 19% | 21% | 22% | | |
| Number with CIN2 | 1.975 | 381 | 899 | 453 | 388 | 4.096 | | |
| % CIN2/(ASCUS/LSIL) | 10% | 13% | 13% | 12% | 17% | 11% | | |
| % CIN2/biopsy | 22% | 16% | 23% | 18% | 17% | 20% | | |
| Number with < CIN2 | 4.797 | 1.496 | 2.147 | 1.521 | 1.445 | 11.406 | | |
| % <CIN2/(ASCUS/LSIL) | 23% | 50% | 32% | 42% | 62% | 31% | | |
| % <CIN2/biopsy | 53% | 65% | 55% | 62% | 62% | 57% | | |
| Number with ASCUS | 9.463 | 1.557 | 3.034 | 2.857 | 1.439 | 18.350 | 1.596 | 19.946 |
| Number with biopsy[b] | 3.657 | 1.138 | 1.587 | 1.918 | 1.439 | 9.739 | | |
| % biopsy/ASCUS | 39% | 73% | 52% | 67% | 100% | 53% | | |
| Number with CIN3+ | 1.070 | 257 | 367 | 347 | 384 | 2.425 | | |
| % CIN3+/ASCUS | 11% | 17% | 12% | 12% | 27% | 13% | | |
| % CIN3+/biopsy | 29% | 23% | 23% | 18% | 27% | 25% | | |
| Number with CIN2 | 789 | 194 | 352 | 329 | 216 | 1880 | | |
| % CIN2/ASCUS | 8% | 12% | 12% | 12% | 15% | 10% | | |
| % CIN2/biopsy | 22% | 17% | 22% | 17% | 15% | 19% | | |
| Number with < CIN2 | 1.786 | 686 | 860 | 1.238 | 833 | 5.403 | | |
| % <CIN2/ASCUS | 19% | 44% | 28% | 43% | 58% | 29% | | |
| % <CIN2/biopsy | 49% | 60% | 54% | 65% | 58% | 55% | | |
| Number with LSIL | 11.171 | 1.464 | 3.755 | 806 | 907 | 18.103 | 1.722 | 19.825 |
| Number with biopsy[b] | 5.365 | 1.176 | 2.342 | 536 | 907 | 10.326 | | |
| % biopsy/LSIL | 48% | 80% | 62% | 67% | 100% | 57% | | |
| Number with CIN3+ | 1.155 | 178 | 495 | 128 | 108 | 2.064 | | |
| % CIN3+/LSIL | 10% | 12% | 13% | 16% | 12% | 11% | | |
| % CIN3+/biopsy | 22% | 15% | 21% | 24% | 12% | 20% | | |
| Number with CIN2 | 1.186 | 187 | 547 | 124 | 172 | 2.216 | | |
| % CIN2/LSIL | 11% | 13% | 15% | 15% | 19% | 12% | | |
| % CIN2/biopsy | 22% | 16% | 23% | 23% | 19% | 21% | | |
| Number with < CIN2 | 3.011 | 810 | 1.287 | 283 | 612 | 6.003 | | |
| % <CIN2/LSIL | 27% | 55% | 34% | 35% | 67% | 33% | | |
| % <CIN2/biopsy | 56% | 69% | 55% | 53% | 67% | 58% | | |

ASCUS: Atypical cells of unknown significance; LSIL: low-grade squamous intraepithelial lesion; CIN: Cervical intraepithelial neoplasia.
[a]These numbers only include women with adequate follow-up as per definition in Table 1 and are therefore lower than numbers in Table 2 which include all women.
[b]Number with biopsy may be higher than the sum of histology diagnoses because some samples had unknown diagnosis.

for 14-type mRNA test at 77%, followed by DNA (67%), and 5-type mRNA test (58%). The detection of all histology results increased with HPV triage, including non-severe lesions (<CIN2) and normal biopsies. The same pattern was seen when considering ASCUS or LSIL separately.

Correspondingly, the relative risks were above one for all outcomes when HPV triage and direct referral were compared with repeat cytology (Table 5). For 14-type mRNA test, DNA test, and direct referral, the relative risks were higher for < CIN2 than for CIN2 and CIN3+. Adjustment for year of birth and index diagnosis did not change these results. Looking at ASCUS separately, the results followed the same pattern, but for LSIL, the RR for < CIN2 when using HPV triage was not elevated to the same extent. Figure 1 visualizes the relative risks for the adjusted analysis presented in Table 5.

Repeating the analyses using only 9 months of follow-up resulted in a higher proportion of women lacking follow-up (24%, mostly from the repeat cytology group) but did not change the results of the analysis (data not shown). Restricting the analysis to women aged 23 and above at index did not change the results either (data not shown).

## Discussion

### *Main findings*

During the past decade, several strategies have been used in Denmark for women below 30 years with ASCUS or LSIL. The strategy used had an impact on the benefits and the harms of cervical screening of these young women. The detection

**Table 5.** Relative risk of CIN3+, CIN2, <CIN2 for women with ASCUS or LSIL triaged with HPV-tests or referred directly for colposcopy vs. women with repeat cytology.

| | 14-type mRNA test vs repeat cytology | | 5-type mRNA test vs. repeat cytology | | DNA test vs repeat cytology | | Direct referral vs repeat cytology | |
|---|---|---|---|---|---|---|---|---|
| | RR | 95% CI | RR | 95% CI | RR | 95% CI | RR | 95% CI |
| All women – unadjusted/crude | | | | | | | | |
| CIN3+ | 1.34 | 1.21–1.47 | 1.18 | 1.09–1.27 | 1.20 | 1.10–1.32 | 1.94 | 1.78–2.12 |
| CIN2 | 1.32 | 1.19–1.46 | 1.38 | 1.28–1.49 | 1.29 | 1.17–1.42 | 1.73 | 1.56–1.91 |
| <CIN2 | 2.13 | 2.04–2.23 | 1.36 | 1.30–1.42 | 1.79 | 1.71–1.87 | 2.65 | 2.54–2.76 |
| All women – adjusted for birth year | | | | | | | | |
| CIN3+ | 1.48 | 1.34–1.63 | 1.19 | 1.10–1.28 | 1.37 | 1.24–1.51 | 1.94 | 1.78–2.12 |
| CIN2 | 1.31 | 1.18–1.46 | 1.37 | 1.27–1.48 | 1.30 | 1.18–1.44 | 1.73 | 1.56–1.91 |
| <CIN2 | 2.07 | 1.97–2.17 | 1.35 | 1.29–1.41 | 1.72 | 1.63–1.80 | 2.66 | 2.55–2.77 |
| All women – adjusted for index diagnosis | | | | | | | | |
| CIN3+ | 1.33 | 1.21 – 1.46 | 1.18 | 1.09 – 1.27 | 1.18 | 1.08 – 1.30 | 1.9 | 1.74 – 2.08 |
| CIN2 | 1.33 | 1.20 – 1.48 | 1.38 | 1.28 – 1.49 | 1.4 | 1.27 – 1.55 | 1.79 | 1.62 – 1.98 |
| <CIN2 | 2.17 | 2.08 – 2.27 | 1.36 | 1.30 – 1.41 | 1.97 | 1.87 – 2.07 | 2.79 | 2.68 – 2.91 |
| All women – adjusted for index diagnosis and birth year | | | | | | | | |
| CIN3+ | 1.47 | 1.33–1.62 | 1.19 | 1.10–1.28 | 1.35 | 1.22–1.50 | 1.91 | 1.74–2.08 |
| CIN2 | 1.34 | 1.20–1.49 | 1.37 | 1.27–1.48 | 1.43 | 1.29–1.59 | 1.79 | 1.62–1.98 |
| <CIN2 | 2.11 | 2.01–2.21 | 1.35 | 1.29–1.41 | 1.86 | 1.76–1.97 | 2.80 | 2.68–2.92 |
| ASCUS – unadjusted/crude | | | | | | | | |
| CIN3+ | 1.46 | 1.29–1.65 | 1.07 | 0.96–1.20 | 1.07 | 0.96–1.20 | 2.36 | 2.13–2.61 |
| CIN2 | 1.49 | 1.29–1.73 | 1.39 | 1.24–1.57 | 1.38 | 1.22–1.56 | 1.80 | 1.57–2.07 |
| <CIN2 | 2.33 | 2.18–2.50 | 1.50 | 1.40–1.61 | 2.30 | 2.16–2.44 | 3.07 | 2.89–3.26 |
| ASCUS – adjusted for birth year | | | | | | | | |
| CIN3+ | 1.62 | 1.42–1.84 | 1.07 | 0.96–1.20 | 1.20 | 1.06–1.35 | 2.34 | 2.11–2.60 |
| CIN2 | 1.52 | 1.30–1.78 | 1.38 | 1.23–1.57 | 1.43 | 1.25–1.63 | 1.80 | 1.57–2.08 |
| <CIN2 | 2.21 | 2.04–2.38 | 1.49 | 1.39–1.60 | 2.15 | 2.01–2.29 | 3.07 | 2.89–3.27 |
| LSIL – unadjusted/crude | | | | | | | | |
| CIN3+ | 1.18 | 1.01–1.36 | 1.27 | 1.16–1.41 | 1.54 | 1.30–1.82 | 1.15 | 0.96–1.39 |
| CIN2 | 1.20 | 1.04–1.39 | 1.37 | 1.25–1.51 | 1.45 | 1.22–1.72 | 1.79 | 1.55–2.06 |
| <CIN2 | 2.05 | 1.94–2.17 | 1.27 | 1.20–1.34 | 1.30 | 1.18–1.44 | 2.50 | 2.37–2.64 |
| LSIL – adjusted for birth year | | | | | | | | |
| CIN3+ | 1.30 | 1.01–1.36 | 1.29 | 1.17–1.43 | 1.89 | 1.57–2.26 | 1.18 | 0.98–1.42 |
| CIN2 | 1.19 | 1.03–1.38 | 1.36 | 1.24–1.50 | 1.43 | 1.19–1.71 | 1.78 | 1.54–2.06 |
| <CIN2 | 2.04 | 1.92–2.16 | 1.27 | 1.20–1.34 | 1.30 | 1.17–1.44 | 2.51 | 2.37–2.65 |

CIN: cervical intraepithelial neoplasia; RR: risk ratio. CI: confidence interval.

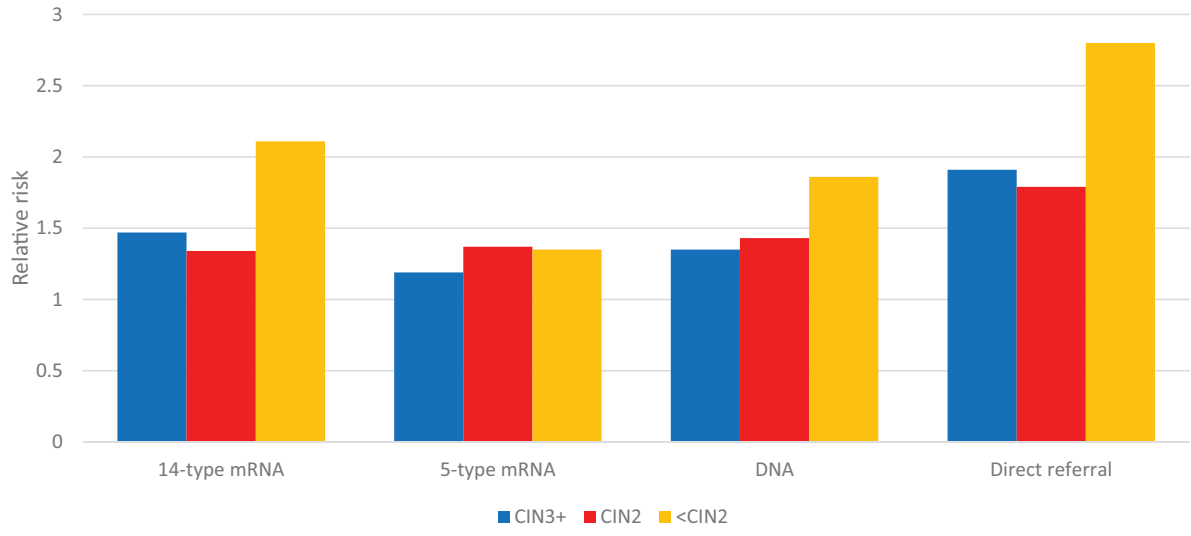


**Figure 1.** Relative risks for CIN3+, CIN2 and < CIN2 with HPV test or direct referral to colposcopy compared to repeat cytology. Results adjusted for index diagnosis and birth year.

of CIN3+ was somewhat higher, 13% to 14% with HPV testing vs. 11% with repeat cytology. Compared with repeat cytology, HPV testing led to more women undergoing biopsy, and more women had biopsies that turned out normal or with CIN1-2 which does not indicate treatment. Direct referral to colposcopy led to as many as 62% of women undergoing biopsies with < CIN2.

Except for 5-type mRNA test, the relative risks for biopsies with < CIN2 were higher than the relative risks for high grade lesions, indicating overmanagement. The risk of having biopsies not indicating treatment was approximately doubled with 14-type mRNA test (2.11) or HPV DNA test (1.86) and almost tripled with direct referral to colposcopy (2.80).

### Other studies

Several studies have shown higher rates of either underlying CIN2+ or higher rates of progression to CIN2+ or HSIL in women with ASCUS or LSIL who are HPV positive than women who are HPV negative [8,9]. A negative HPV test thus serves to identify women at low risk who can safely return to the screening program. However, studies also confirm that HPV-tests are often positive in ASCUS/LSIL, especially among younger women [10–13] and absolute rates of CIN2+ are low, appr. 5–10% over 5 years in women under 30 years [13].

Several studies [13–15], a Cochrane review from 2013 [16] and two recent metaanalyses [17,18] have reported increased sensitivity for CIN2+ with HPV test, whether mRNA or DNA compared to repeat cytology. In the Cochrane review, both methods had similar specificity in ASCUS cases, but the specificity of DNA test in LSIL was significantly lower than that of repeat cytology. In addition, the absolute specificity of both methods was low, potentially leading to many unnecessary referrals and biopsies, especially in younger women. The Cochrane review only included studies using the HC2 assay, whereas assays used in Denmark may be more sensitive and increase overmanagement [19].

Another Cochrane review compared immediate colposcopy to repeat cytology and found that immediate colposcopy led to increased detection of potentially significant as well as insignificant lesions, as in our study, but also concluded that over two years of cytological surveillance the detection of severe lesions was similar to immediate colposcopy [20]. A Swedish study found that repeat cytology within 6 months maintained a reduced risk for invasive cancer while reducing the colposcopies for the youngest women in this age group [21]

A meta-analysis on studies of 5-type mRNA tests concluded that these tests reduce referrals to colposcopy and overtreatment but due to their lower sensitivity compared to other HPV tests cannot reliably exclude CIN2+ and therefore women with ASCUS or LSIL and negative 5-type mRNA test still need cytological follow-up [22]. This is in line with findings from other settings [23–25]. Comparison of 14-type mRNA test to DNA test has shown either comparable sensitivity for CIN2 + [10] or slightly lower sensitivity of 14-type mRNA test [26] and higher specificity of the 14-type mRNA test. However, in a Norwegian study the detection rate of CIN3+ were the same for the 5-type HPV mRNA test and a 14-type HPV DNA test [27].

Other options besides the ones in use in Denmark are available and may offer advantages. One option is dual staining cytology which appears to lower the number of false positives and increase the proportion of correct referrals for colposcopy [11], although a model-based economic evaluation of triage of ASC-US/LSIL in women 25–33 years, showed HPV mRNA triage to be more cost-efficient than dual staining [28]. Another option is so-called delayed triage, with HPV testing performed at the time of the repeat cytology and not on the initial ASCUS/LSIL sample. This was the approach taken in Norway, when triage of ASCUS and LSIL was introduced [12,29]. Comparing the previous routine (repeat cytology only) to the new routine (repeat cytology and HPV test), the new routine led to increase in referrals to colposcopy and increased detection rate of CIN2+ while rates fell in women referred back to the screening program, indicating better risk stratification but at the expense of more women undergoing colposcopy and biopsy. Specificity was lowest for women under 34 years, at 47% [12,29].

### Clinical implications

Although most women with ASCUS, including those with positive hr-HPV test, will never develop CIN3 or cancer, a considerable proportion of all CIN3 diagnoses are made in women whose initial screening result was ASCUS [16], indicating the need for follow-up of these women. Repeated cytological examination constitutes a burden to women and the healthcare sector. On the other hand, referring all women with ASCUS directly to colposcopy and possibly biopsies result in overmanagement, as also indicated by our study. A specific triage test with a low positivity rate translates into a low referral rate for colposcopy, which is very appealing for triage situations. In our material, the 5-type HPV mRNA test was the most specific triage test.

In current Danish guidelines, HPV positive cases are referred for colposcopy. This may lead to overmanagement, however, because many HPV infections and minor lesions clear spontaneously. As in our study, the Cochrane review reported high HPV positivity rates in LSIL. LSIL is a common manifestation of HPV infection even in cases where the HPV infection never progresses to more severe lesions or cancer, and the ability of the test to distinguish between cases with or without severe underlying lesions or risk for development of such lesions is limited [16]. Correspondingly, our study has shown that HPV test used this way, especially DNA test and 14-type mRNA test, leads to many unnecessary biopsies.

Additionally, not all CIN3 and only a minority of CIN2 progress to cancer so a substantial proportion of women will still undergo biopsy and potentially conization unnecessarily, even when referral to colposcopy is restricted to HPV-positive cases [30]. An alternative might be to use DNA test or 14-type mRNA test to identify which women with ASCUS/LSIL need cytological follow-up after 6 months rather than to select women for colposcopy at their first occurrence of ASCUS/LSIL, while using genotyping or 5-type mRNA test to select women for immediate referral to colposcopy. In Norway, in women with ASC-US/LSIL, only HPV type 16/18 positive are referred for colposcopy while other HPV-types are followed-up with repeated HPV-testing after 12 months [31].

The Danish guidelines recommend only HPV mRNA test and not DNA test for women under age 30. However, our results show similar outcome for the 14-type mRNA test and DNA test and therefore do not support the general recommendation to prefer an mRNA test to a DNA test in this age group. The 5-type mRNA test is more specific according to our results, but not currently in use in Denmark.

### *Strengths and limitations*

The availability of nationwide registers allowed us to have population-based, complete data on women with ASCUS/LSIL cervical samples and to base our observations on how the triage strategies perform in practice and not in an experimental study setting. In addition, this minimizes loss of follow-up.

Conization would have been a relevant outcome but was not included because the policy for conization changed over the years from conization of CIN2+ to only CIN3+, especially for premenopausal women. This change was formalized in 2012 in new guidelines from the Danish Society for Obstetrics and Gynecology, but implemented gradually across the five Danish regions. The risk for conization therefore depends not only on histology result (and hence triage method), but also on whether the more restrictive policy had been adopted in a woman's region of residence at the time she was screened. Indeed, in our data, for women with an index date before 1st January 2012, 42% of those with CIN2 as worst histology were conizised in contrast with 20% in those with index date after 1st January 2012; and 17% after January 2016.

Liquid based cytology (LBC) was introduced gradually over the years, and so was the use of HPV triage. LBC imay lead to detection of more intraepithelial lesions than conventional cytology, especially ASCUS and LSIL [29,30] so the differences in outcomes may therefore depend not only on the triage method but also, at least in part, on whether conventional or liquid based cytology was used. The cytology method is documented only for a minority of samples, however, so we were unable to account for this in the analysis.

The pathology register does not hold information on whether cytology sampling was due to invitation from the screening program, opportunistic screening, or symptoms. Conceivably, women referred directly to colposcopy (which is not in line with official recommendations) may have consulted their GP for symptoms or they may have been referred based on observations made by the GP at the routine screening. Consequently, they may not be completely comparable to women in the other groups which may, at least in part, explain the higher rates of CIN seen with direct referral. This may in particular be the case for women under 23 years not invited to screening. We therefore repeated the analysis only for women aged 23 and above, which did not change the results (data not shown).

It is possible that some women had not completed follow-up within the two years, especially women in the repeat cytology group. When the repeat cytology is normal, the recommendation is for a third cytology after 12 months (i.e., 18 months after the initial cytology) and referral to colposcopy within 3 months (21 months after the initial sample) if ASCUS+. Biopsy rates and proportion of women diagnosed with any degree of CIN may therefore be underestimated if delays in obtaining pathology results and booking new appointments led the 21 months period to extend beyond the 2 years of follow-up. However, we expect that most women would be retested within the recommended time frame and in addition, extending the follow-up to 3 years would result in inclusion of samples taken as a result of reaching the next screening invitation and not as follow-up of the index sample.

Finally, this study covered a historical period where most women had not been vaccinated against HPV prior to reaching screening age. From 2016, birth cohorts vaccinated through the childhood immunization program started entering the screening program, and rates of HPV infection, cytological lesions and CIN are likely to change in the future with implications for screening and triage strategies.

## Conclusion

In conclusion, the optimal strategy for assessment of ASCUS and LSIL is still not clear, and the choice will depend on the health authorities' and women's preferred balance between benefits and harms. Based on our study, repeat cytology or triage with 5-type mRNA test seems to offer advantages compared to HPV testing in terms of fewer unnecessary biopsies. Alternatively, using HPV testing to select women with ASCUS/LSIL for repeat cytology (rather than for immediate referral to colpocopy) might be a suitable option. More research, including on women's preferences and possibly triage strategies not currently in use in the Danish program, is needed to establish the optimal management strategy for young women with ASCUS or LSIL.

## Ethical approval

This was a register-based study with no contact to patients, their relatives and/or health care providers. Access to data approved by the Danish Data Protection Agency under the University of Copenhagen (SUND-2016-22).

## Disclosure statement

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