Department of Public Health, Community Medicine, Faculty of Health Sciences

Triage of women with ASC-US/LSIL cytology: the added value of implementing an HPV-test

Mbachi Ruth Msomphora
Master thesis in [Public Health/Folkehelsevitenskap], December 2014

Main supervisor: Finn Egil Skjeldestad, Professor, Department of Clinical Medicine, UIT The Arctic University of Norway.

Co-supervisor: Sveinung Wergeland Sørbye, Senior consultant at Department of Clinical Pathology, University Hospital of North Norway
Acknowledgements

The background for my Master thesis in Public Health bows down to the opportunity I had as a Heath Care Subjects Librarian at UiT The Arctic University of Norway, to participate in facilitating Evidence-based Medicine by giving courses, especially to odontology and health care students at the Faculty of Heath Sciences, including my participation in working with national clinical procedures in cooperation with the Norwegian Knowledge Centre for the Health Services at the University Hospital of North Norway. Thank you to Professor Hans-Peter Müller for the support and encouragement in the whole process for my pursuing a Master of Science degree in Public Health, which gave me the opportunity to writing this thesis.

I acknowledge the University Hospital of North Norway (UNN) for the provision of data used in this thesis. I also extend my acknowledgement to my co-supervisor, Sveinung Wergeland Sørbye (MD, PhD), the senior consultant at Department of Clinical Pathology at UNN, for his assistance in making this data acquisition from UNN possible.

I am very grateful for the advice and assistance, especially in the analysis of the data I got from my main supervisor Professor Finn Egil Skjeldestad (MD, PhD), Department of Clinical Medicine, UiT The Arctic University of Norway.

I sincerely thank both of my supervisors, Finn Egil Skjeldestad and Sveinung Wergeland Sørbye for their great support and guidance in writing this thesis. Through the process of writing this thesis, I have learnt a lot from them. Their constructive comments and clarifications about the processes used in the triage algorithms involved in this study have significantly strengthened my thesis.
Finally my thanks also go to the Department of Community Medicine, Faculty of Health Sciences, UiT The Arctic University of Norway, for giving me the opportunity of pursuing this very interesting master’s degree in Public Health (MPH), which have expanded my professional development and horizon to a wider and higher level.

December, 2014 Mbachi Ruth Msomphora
**Abstract**

**Background:** Within the Norwegian cervical cancer screening program women were recommended biopsy after three consecutive smears of minor lesions (ASC-US/LSIL) 6-12 months apart until 2005. In 2005 the recommendations for biopsy changed to the 2nd ASC-US/LSIL, if the HPV-test was positive. In this study we determine the outcomes of secondary cervical cancer screening in two time-periods before (1996-1998) and after (2006-2008) implementation of an HPV test.

**Methods:** This study has a historical prospective design by including in retrospect all screening- and follow-up tests for women aged 25 to 69 years old kept by the Department of Clinical Pathology, University Hospital of North Norway. All women with a first ASC-US/LSIL smear in 1996-98 and 2006-08 were identified and compared on outcomes such as proportion of cases resolved within 42 months of index smear and incidence of CIN2+. The Department of Clinical pathology has used an HPV mRNA test (NorChip PreTect HPV-Proofer) since the autumn of 2005 in secondary cervical cancer screening (triage).

**Results:** Over the years January 1st 1991 and December 31st 2010 UNN laboratory processed 635 287 smears. After exclusion criteria, the study population comprised of 1405 women during 1996-1998 (study group A) and 738 women with a valid HPV-test in triage during 2006-2008 (study group B). In these subsets 16.2% (227/1405) in 1996-98 and 18.8% (139/738) in 2006-08 of the women were eligible for colposcopy/biopsy according to national screening recommendations. In 2006-08, when the HPV mRNA test was applied, the mean time to resolve an ASC-US/LSIL was 9.1 months (range 3-41) relative 23.1 months (range 4-42) in 1996-98 (p<0.001). Significantly more cases were solved in study group B, including HPV-testing at 1st follow-up (82.4%) compared to study group A (53.6%) within 42 months of follow-up. The positive predictive value of CIN2+ 52.0% (64/124) in study group B was
significantly higher compared to that of study group A 41.7% (68/163), when there was indication for biopsy according to the screening algorithm over the time-periods.

**Conclusion:** In triage of women with ASC-US/LSIL, the HPV mRNA test significantly reduced the time from the first abnormal cytology until biopsy and had higher predictive values compared with repeat cytology.

**Key words**

Triage; ASC-US / LSIL; Women; Algorithm; HPV mRNA; Cytology; Index; Smear; Biopsy
### List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACIS</td>
<td>Adenocarcinoma in situ</td>
</tr>
<tr>
<td>ASC-H</td>
<td>Atypical Squamous Cells of High-grade</td>
</tr>
<tr>
<td>ASC-US</td>
<td>Atypical Squamous Cells of Undetermined Significance</td>
</tr>
<tr>
<td>CC</td>
<td>Cervical Cancer</td>
</tr>
<tr>
<td>CIN</td>
<td>Cervical Intraepithelial Neoplasia</td>
</tr>
<tr>
<td>E6/E7</td>
<td>The two primary oncoproteins of high risk HPV types. The “E” designation indicates that these two proteins are expressed early in the HPV life cycle.</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>HPV</td>
<td>Human Papillomavirus</td>
</tr>
<tr>
<td>HSIL</td>
<td>High-grade Squamous Intraepithelial Lesion</td>
</tr>
<tr>
<td>LSIL</td>
<td>Low-grade Squamous Intraepithelial Lesion</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger Ribo Nucleic Acid</td>
</tr>
<tr>
<td>N</td>
<td>Number</td>
</tr>
<tr>
<td>P</td>
<td>Probability</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive Predictive Value</td>
</tr>
<tr>
<td>REK</td>
<td>Regional Ethics Committee</td>
</tr>
<tr>
<td>UiT</td>
<td>University of Tromsø</td>
</tr>
<tr>
<td>UNN</td>
<td>University Hospital of North Norway</td>
</tr>
</tbody>
</table>
Table of Contents

Acknowledgements...........................................................................................................2

Abstract.............................................................................................................................4

List of Abbreviations.........................................................................................................6

Table of Contents...............................................................................................................7

List of tables and figures...................................................................................................9

1 Introduction ......................................................................................................................10
  1.1a Triage with cytology..................................................................................................11
  1.1b Triage with HPV mRNA test....................................................................................12

2 Main objectives ...............................................................................................................15
  2.1 Study contribution.....................................................................................................15

3 Outcomes of efficacy ......................................................................................................16

4 Material and Methods ...................................................................................................17
  4.1 Design .......................................................................................................................17
  4.2 Data analysis...............................................................................................................18

5 Ethical approval ...............................................................................................................18

6 Results .............................................................................................................................18
  6.1 Study group ...............................................................................................................18
  6.2 Most recent smears to index smear ...........................................................................19
  6.3 Follow-up after index ASC-US/LSIL .........................................................................19
  6.4 Histology....................................................................................................................20

7 Discussion.......................................................................................................................21
  7.1 Time interval taken from most recent smear to index smear.................................21
  7.2 Proportion of biopsy indication within 42 months of follow-up after index ASC- US/LSIL.................................................................22
7.3 Proportion of resolved cases within 42 months of follow-up after index ASC-US/LSIL

7.4 Patient compliance (Acceptability)

7.5 Proportion of women diagnosed with CIN2+

7.6 Representativeness and validity of the study

7.7 Strength and weaknesses of the study

8 Conclusion remarks

8.1 Recommendation

9 References
List of tables

**Table 1:** Age by study group 18

**Table 2:** Case solution/status follow-up within 42 months after index ASC-US/LSIL by study group 19

**Table 3:** Histology by biopsy indication and study group 20

List of figures

**Figure 1.** Flow-chart indicating the guidelines for follow-ups during first study period 1996–98 12

**Figure 2.** Flow-chart indicating the guidelines for follow-ups during second study period 2006–08 13
1. Introduction

Cervical cancer is the third most common cancer affecting women worldwide. About 300 women in Norway get cervical cancer each year and 80 of these die from the disease \(^1\). Research suggests that cervical cancer is caused by infection with Human Papillomavirus (HPV) \(^2\). A persistent infection with HPV can develop serious abnormalities (precancer) in the cervix. If not treated they may develop into cancer \(^3\), \(^4\). Hence detecting and treating precancerous lesions before cancer development can reduce the risk of cancer in the population \(^5\), \(^8\).

In Norway a National Cervical Cancer screening programme was established in 1995. As a part of the programme, women aged 25–69 years are invited to cytological screening every 3 years \(^1\). Of the 400,000 smears evaluated/tested every year, estimates of 17,000 women are retested (triaged) due to minor cytological lesions (atypical squamous cells of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesion (LSIL)) \(^1\). The existing general consensus is that women with high-grade cytologic lesions (ASC-H/HSIL) should be referred immediately for further exploration \(^6\), \(^38\). The Norwegian guidelines recommend treatment with conisation of the cervix after histological confirmed high-grade dysplasia (CIN2+) \(^1\). Different countries have different guidelines for management of women with low-grade squamous intraepithelial lesion (LSIL) or equivocal cytological abnormalities (ASC-US) \(^11\). Hence the management of women with minor cytologic lesions (ASC-US/LSIL) remains controversial \(^39\). The option in Norway for the women detected at primary screening with minor cytological lesions (ASC-US/LSIL) is triage (secondary screening) by repeat cytology and/or HPV-testing. Depending on the secondary screening results, referral decisions are made. For instance, the woman can be referred to gynecologist for colposcopy/biopsy, followed-up with cytology after 12 months or returned to normal screening after 3 years \(^5\).
According to Solomon et al., the role of triage is:

“... to identify which women with ASC-US / LSIL are at risk and require colposcopy and which women can be spared the anxiety and costs associated with intensified follow-up”.

In Norway before 2005 secondary screening (repeat cytology) was done after 6 month intervals up to 18 months (Fig. 1, i.e. Triage with cytology) from primary cytology, but eventually from 2005, repeat cytology and HPV-test is done after 6-12 months (Fig. 2, i.e. Triage with HPV mRNA test).

1.1a. Triage with cytology

In the time before 2006, triage at UNN was conducted by using repeat cytology (Fig. 1). The screening result corresponding to ASC-US and LSIL was recommended to take new cytology after 6 months. If the result from the repeated cytology was high grade cytology (ASC-H/HSIL), then immediate colposcopy and biopsy was recommended, and if it was normal return to screening was recommended (women returned to normal screening). For other results, e.g. ASC-US and LSIL, new cytology was recommended after 12 months and if this was normal, women were returned to regular screening. But if the result from this was again ASC-US / LSIL for 3 consecutive-smears within 18 months, then the women were sent for colposcopy and biopsy. The colposcopy and biopsy were done for treatment confirmation. If the results from colposcopy and biopsy were CIN2+, then the women were treated, but if it is benign / CIN1, then a new cytology was recommended.
13 consecutive smear within 18 months showing ASC-US/LSIL

Figure 1. Flow-chart indicating the guidelines for follow-ups during first study period 1996–1998.

1.1b. Triage with HPV mRNA test

From the year 2006 HPV mRNA test was introduced in triage at UNN. As indicated in Fig. 2 below, if the result of the index cytological screening test (primary screening) was ASC-US or LSIL, a new cytology samples an HPV mRNA test (triage test) was recommended after 6 months (maximum 12 months) from index cytology. Depending on the secondary testing results the following five options were recommended: if the result was HPV mRNA negative and normal cytology, women were recommended to return to normal screening. If the results were HPV mRNA negative and ASC-US/LSIL a new screening test (new cytology) was recommended within 12 months from triage (18 months from primary screening). For the next two options where the alternatives were HPV mRNA positive and ASC-US/LSIL or ASC-H/HSIL (regardless of HPV result), women were immediate referred for colposcopy and biopsy. For the fifth option where HPV mRNA test is positive and the simultaneous cytology is normal, it was recommended that the women should go for a new HPV test in about 6 months from triage (12 months from primary screening). Depending on the positive or negative result of this follow-up test the woman should be referred to colposcopy and biopsy or returned to normal screening.
The natural history of minor cytologic lesions is difficult to predict since these lesions (ASC-US/LSIL) often regress spontaneously, and only 10-15% require treatment during follow-up. Hence referring all women with ASC-US/LSIL lesions for colposcopy/biopsy would mean an increase in overdiagnosis and risk of overtreatment. Referring all women with first time ASC-US / LSIL is not considered as good medical practice in Norway, especially due to lack of availability of colposcopic services. Nevertheless, although most women with an ASC-US/LSIL smear result do not have clinically significant disease, a substantial proportion of such cases do have confirmed high-grade cervical intraepithelial neoplasia or worse (CIN2+) after histopathologic examination. For instance, from a population of screened women in USA, it is reported that approximately 1/3 of CIN2+ lesions were discovered on follow-ups of a previous smear with ASC-US. In Norway, approximately 10,000 women with high grade cytology (ASC-H/HSIL) or positive triage are referred to colposcopy, and more than 3000 women are treated by conisation because of high-grade changes confirmed by biopsy. However out of the 3000 women treated, only about
10% would develop cancer without treatment, i.e. estimates 2700 cases out of the 3000 women are over treated, but we do not know which women would develop cancer without treatment\textsuperscript{1,7}. Ferris et al. point out that approximately 25% of the women detected with minor cytological lesions (e.g. ASC-US/LSIL) will have cervical intraepithelial neoplasia (CIN), documented by colposcopically detected biopsy \textsuperscript{10}. Hence given such information, including the reported evidence concerning the etiologic role of oncogenic human papillomavirus (HPV) infections in the development of cervical cancer and CIN \textsuperscript{13, 14, 17, 33-37}, careful triage for detecting the CIN2+ is crucial. To achieve this, identification of the most effective method for distinguishing CIN2+, i.e. high-grade cervical dysplasia with risk of progression to invasive cancer, is required.

The assay PreTect HPV-Proofer (NorChip AS, Norway) detects the E6/E7 oncogene expression which is necessary for malignant transformation \textsuperscript{9, 18}. Thus the PreTect HPV-Proofer may be a possible candidate to identify underlying cervical cancer precursors with high probability of progression to cancer among women with minor cytological abnormalities. The assay, i.e. the HPV E6/E7 mRNA test, was included in the cervical cancer screening program of University Hospital of North Norway in 2006 \textsuperscript{1,7}. Hence the efficacy of CIN2+ diagnosis of screening programme in Norway before and after introduction of HPV E6/E7 mRNA test i.e. between two time period 1996-1998 and 2006-2008 respectively, is an interesting case to evaluate.
2. Main objective

To study outcomes of secondary screening in the Norwegian cervical cancer programme within two time periods, 1996-1998 (ref. Fig. 1) and 2006-2008 (ref. Fig. 2) i.e. before and after introducing the HPV mRNA test, respectively.

H₀: There is no difference in efficacy of the secondary screening before and after introducing the HPV mRNA test.

2.1. Study contribution

In this thesis, our interest is to evaluate and compare the efficacy of the routine diagnostic practice at the University Hospital of North Norway before and after including the use of the HPV E6/E7 mRNA test PreTect HPV-Proofer in triage of women with minor cytological cervical lesions (ASC-US and LSIL) for the detection of CIN2+. Effectiveness in terms of proportion of women and time taken from first abnormal PAP-smear (ASC-US / LSIL) to diagnosis for CIN2+ and biopsy is considered. Besides, the consideration also based on percent difference in referrals with time-taken to 1) repeat cytology or HPV mRNA test at triage, 2) colposcopy/biopsy and 3) return to normal screening programme, including percent difference in cases lost to follow-up and unresolved cases. Therefore basing on the outcomes of secondary screening of women within the periods 1996-1998 and 2006-2008 as described in this paragraph above, this thesis tries to identify a better algorithm for retesting, i.e. between repeat cytology alone and cytology in combination with HPV mRNA testing. The identified algorithm will then be recommended as the better method to follow at the University Hospital of North Norway. This study will therefore contribute to the insights for quality assurance in handling triage women with ASC-US / LSIL at the University Hospital of North Norway.
Biopsy-confirmed CIN2+ was used as clinical end point for the study. CIN2+ as a wording for inclusion of all high grade cervical lesions, CIN2, CIN3, cervical carcinoma in situ (CIS), adenocarcinoma in situ (ACIS) and cervical cancer (CC).

3. Outcomes of efficacy

• Proportion of women followed-up by age
  - in 1996-98 within 42 months after index ASC-US/LSIL
  - in 2006-08 within 42 months after index ASC-US/LSIL

• Proportion of women having a diagnostic decision (resolved) by screening period within 42 months after index ASC-US / LSIL, that is:
  - proportion of women having biopsy
  - proportion of women returning to screening programme after a conclusive follow-up in either normal cytology or successful treatment

• Proportion of women unresolved by screening period within 42 months after index ASC-US/LSIL, that is:
  - proportion of women with incomplete follow-up
  - proportion of women with biopsy indication, but not taken
  - proportion of women with no biopsy indication, but still need follow-up

• Proportion of women detected CIN1, CIN2, CIN3, ACIS or CC
  - By biopsy indication
  - By screening period
4. Materials and methods

Over the years 1st January 1991 and 31st December 2010 the Cytology laboratory, Department of Clinical Pathology, University Hospital of North Norway, processed 635,287 smears. After exclusion of smears from women not residents of Troms and Finnmark counties, double entries of smears, cytology codes not consistent with cervical pathology, and less severe diagnoses when more than one diagnosis in the same smear, 94,841 women with 452,796 smears were eligible for analysis. From this sample we identified 2,727 women, aged 25-69 years, who had 1st ASC-US/LSIL (index smear) during 1996-1998 (study group A) and 2006-2008 (study group B), no previous history of HSIL (cytology) and CIN (histology) before index smear. We refined the study population to women who had 1st follow-up after 3 and 18 months after index smear. After these exclusions the study population comprised 1,405 women during 1996-1998 and 738 women with a valid HPV-test in triage during 2006-08.

As the screening interval is 3 years, we assessed follow-up after 1st ASC-US/LSIL within 42 months after index smear. We defined cases as returning to screening or having a biopsy collected within completed 42 months after index cytology as solved cases, whereas women not met for follow-up, incomplete follow-up, having a biopsy taken without relevant indications, not met for biopsy when indicated, or completed follow-up after 42 months of index smear, as unresolved cases.

4.1. Design

As illustrated above, this study has a historical prospective design by including in retrospect all screening- and follow-up tests for women aged 25 to 69 years old kept by the Department of Clinical Pathology, University Hospital of North Norway.
4.2. Data analysis

All analyses were done in SPSS, version 19.0, with Chi-square test (categorical variables), Mann-Whitney test (continuous variables) and survival analyses with p-value < 0.05 as significance level.

5. Ethical approval

After application for approval to the Regional Committee for medical and health research ethics, North Norway (REC North), the REC North decided that this type of the project, which is categorised as quality assurance project, is not required to be submitted before the committee.

6. Results

6.1. Study group

There was no difference in mean age (40.8/40.4 years, range 25-69 years) between the study groups A and B. However, a significant higher proportion of women were either younger (25-34 yrs.) or older (45-69 yrs.) in the 1996-1998 respective the 2006-2008 time-period (Table 1)

<table>
<thead>
<tr>
<th>Study group</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=1405</td>
<td>N=738</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>25-34</td>
<td>36.6</td>
<td>32.8</td>
</tr>
<tr>
<td>35-44</td>
<td>26.4</td>
<td>32.9</td>
</tr>
<tr>
<td>45-69</td>
<td>37.0</td>
<td>34.3</td>
</tr>
</tbody>
</table>
6.2. Most recent smear to index smear

Index smear represented first smear ever for 11-12% among respondents regardless of study group. Most recent smear were normal (99%) or inconclusive (1%). The interval from most recent smear to index smear was significantly shorter for study group A (mean 14.9 months, range 1-96 months) compared with study group B (mean 28.4 months, range 1-190 months).

6.3. Follow-up after index ASC-US/LSIL

All cases had at least one follow-up after index ASC-US / LSIL. Significantly more cases were solved in study group B, including HPV-testing at 1st follow-up (82.4%), compared to study group A (53.6%) within 42 months of follow-up (Table 2). A total of 6.4% in study group A and 5.8% in study group B had indications for biopsy without having any biopsies collected. Women without biopsy represents more than 1/3 of all women referred to biopsy in the first study-period and 25% of all women referred to biopsy in the last study-period.

Table 2: Case solution/status follow-up within 42 months after index ASC-US/LSIL by study group.

<table>
<thead>
<tr>
<th>Case solution</th>
<th>Study group</th>
<th>Study period</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=1405</td>
<td>N=738</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case solution</td>
<td>Status</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Unresolved cases</td>
<td>Incomplete follow-up</td>
<td>35.4</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biopsy done, not indicated</td>
<td>4.6</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biopsy indicated, not done</td>
<td>6.4</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>Resolved cases</td>
<td>Back to screening</td>
<td>42.0</td>
<td>65.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biopsy indicated, done</td>
<td>11.6</td>
<td>16.8</td>
<td></td>
</tr>
</tbody>
</table>

The cumulative proportion of cases resolved by 12, 24 and 36 months of follow-up were 11.9%, 38.2% and 57.2% in study group A relative 75.3%, 88.2% and 91.7% in study group B (p<0.001, survival analysis). The mean time to resolve a case were 9.1 months in study group B (range 3-41) and 23.1 months (range 4-42) in study group A (p<0.001).
6.4. Histology

Among women who had no indication for biopsy the histology results were normal in 2/3 of the cases in the last study period relative 3 out for 4 in the first study period (Table 3). For CIN2+ the relative proportions were 20.0% and 12.6% in study group B versus study group A.

<table>
<thead>
<tr>
<th>Study group</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study period</strong></td>
<td>1996-1998</td>
<td>2006-2008</td>
</tr>
<tr>
<td>N=1405</td>
<td>N=738</td>
<td></td>
</tr>
<tr>
<td><strong>Biopsy</strong></td>
<td>Not indicated</td>
<td>Indicated</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td><strong>Inconclusive</strong></td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td><strong>Normal</strong></td>
<td>76.6</td>
<td>42.9</td>
</tr>
<tr>
<td>CIN1</td>
<td>9.4</td>
<td>15.3</td>
</tr>
<tr>
<td>CIN2</td>
<td>6.3</td>
<td>19.0</td>
</tr>
<tr>
<td>CIN3</td>
<td>6.3</td>
<td>20.9</td>
</tr>
<tr>
<td>ACIS*</td>
<td>0</td>
<td>1.2</td>
</tr>
<tr>
<td>CC**</td>
<td>0</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*ACIS - adenocarcinoma in situ  
**CC - cervical cancer

In study group A and study group B 18.0% (253/1405) and 22.6% (167/738) were recommended biopsy. We received biopsy from 64.4% (163/253) in study group A and 74.3% (124/167) in study group B. Significantly more cases CIN2+ were diagnosed in the study group B relative study group A, 52.0% (64/124) versus 41.7% (68/163), when there was indication for biopsy according to the screening algorithms (higher PPV in study group B). The proportion CIN1 cases were evenly distributed between study group A and B when indications were present.
7. Discussion

Only women between the ages 25-69 years old are included in our study, because this is the recommended age group within the Norwegian cervical cancer programme 1.

The overall study analysis indicates that the algorithm that was used to triage women with ASC-US / LSIL cytology in 2006 – 2008 is more effective than that used in 1996 – 1998 time period. In secondary screening of women with ASC-US / LSIL cytology, repeat cytology combined with HPV mRNA test is better than repeat cytology alone. This is due to the following findings:

7.1. Time interval taken from index smear to decision making

The results indicate that the algorithm used to triage women in 2006 - 2008 took shorter time interval from index smear to decision making compared to that used in 1996 – 1998. This means that in triage, it is more time-consuming using repeat cytology alone compared to triage with both repeat cytology and HPV cotesting, i.e. ≈ 50% shorter. Thus the combination of cytology and HPV mRNA test in triage of women with ASC-US / LSIL will help in reducing the time before a decision is made. Delay in decision making is a disadvantage for women 10, 20, 22. Using the 2006-2008 algorithm to triage women would save further expenses for clinicians’ time, patient inconvenience, tracking and recall efforts and other costs like transportation and all other resources spent on screening or test measures 10, 20, 21. It would facilitate immediately referrals for colposcopy or biopsy examination. Therefore algorithm used to triage ASC-US / LSIL women in the time period 2006-2008 is more effective compared to that used in 1996-1998.
7.2. Proportion of biopsy indication within 42 months of follow-up after index

**ASC-US/LSIL**

Biopsies were taken from more women with no biopsy indication (4.6%) in group A compared to group B (2%). The proportions of women with biopsy indication was higher (22.6%) in group B compared to group A (18%). This is most likely due to increased sensitivity that HPV mRNA test brought into the triage algorithm of 2006-2008. The results confirm the findings of Sørbye et al. 7, i.e. meaning that the HPV mRNA test in combination with the cytology has a higher sensitivity but also specificity than cytology alone. Thus the addition of the HPV mRNA test to triage ASC-US / LSIL women adds effectiveness to the algorithm. The PPV for CIN2+ were higher in group B than in group A. Using HPV mRNA test we increasingly find the right women than it was before in 1996-1998. Higher sensitivity of the screening algorithm using HPV mRNA test gives a higher degree of assurance that the remaining women (normal cytology and negative HPV mRNA test), considered to be at low risk for harbouring a significant cervical cancer is correct/true, and could then be sent back to “normal screening” after three years, where they will be monitored by less expensive cervical cytology test 5, 10. Higher specificity and higher PPV using HPV mRNA test implies that the problem of false positive results can be avoided, thereby preventing unnecessary follow-up, unnecessary colposcopies, biopsies and risk of over-treatment 9, 10, 23, 31.
7.3. Proportion of resolved cases within 42 months of follow-up after index ASC-US/LSIL

Our findings show that the proportion of resolved cases in 2006 – 2008 was higher than that in 1996-1998 (Table 2) within 42 months of follow-up after index ASC-US/LSIL. On average, less than half the time (9.1 months) was taken to resolve cases in 2006-2008 (group B) compared to 1996-1998 (group A), i.e. 23.1 months. Besides, group B’s cumulative proportion of resolved cases 75%, 88.2% and 91.7% within 12, 24 and 36 months of follow-up respectively is illustrated to be significantly much higher relative to 11.9%, 38.2% and 57.2% of group A (p<0.001). This implies that in triage, it takes less time to resolve cases using HPV mRNA test in combination with cytology than using cytology alone. The combination of HPV mRNA test with cytology helps in identifying more women of those women at high risk for cancer within a shorter time than just using repeated cytology test. This will therefore ensure the prevention of more cancer within a shorter period of time. Since the concept of triage is to resolve, and the more the resolved is better, then the test method used in 2006-2008 period, i.e. HPV mRNA test in combination with cytology, is considered better compared to that used in the period 1996-1998, i.e. cytology test alone.

7.4. Patient compliance (Acceptability)

The results illustrate a significantly high number of women with incomplete follow-up (35.4%) in group A compared to only 9.8% in group B. This shows that patient’s compliance was higher in the time period 2006-2008 than in 1996-1998, implying that the algorithm with a combination of HPV mRNA test and cytology is better compared to that with cytology alone. This is most likely because the guidelines for HPV-testing were more explicit and the recommendations of follow-up were clearer than before. HPV-testing is new, and the doctors had to explain the guidelines for their patients. Maybe some HPV positive women were scared when the “cancer virus” were detected, leading to a higher compliance. The
Department of Clinical pathology also sends reminders if the recommended follow-up test was not performed.

7.5. Proportion of women diagnosed with CIN2+

In agreement with what is pointed out above, the proportion of women recommended colposcopy/biopsy was higher in 2006-2008 than in 1996-1998, i.e. 18.8% and 16.2% respectively. If we assume that the proportion of CIN2+ among women with ASC-US / LSIL is the same in 1996-1998 as in 2006-2008, it appears that the CIN2+ proportion detected by HPV mRNA test in combination with the cytology was higher, compared to CIN2+ detected by the cytology alone, despite the presence or absence of biopsy indication (Table 3). This is expected because HPV mRNA test in combination with cytology is reported to be more sensitive and specific than cytology alone. Sensitivity will increase with addition of HPV mRNA test than cytology alone. The histology results indicated no development of cervical cancer (CC) in the colposcopy/biopsy referrals in 2006-2008 which is good. There were a few cases of CC in referrals from 1996-1998. High specificity and high PPV of the HPV mRNA test reduce the number of colposcopies and biopsies compared to cytology alone. This means the 2006-2008 algorithm can help in avoidance of unnecessary referrals to treatment, which is good. For every case referred, there is a cost involved, i.e. to the woman and the community/society. Thus the HPV mRNA test in combination with the cytology test method in triage should help in reducing the number of referral women with low risk of high grade dysplasia to treatment. It can reduce unnecessary follow-up cost and the patients’ psychological stress. The question is whether we have dealt with the right women, because it is very rare to optimally achieve that everyone within a certain time diagnosed with disease (precancerous and cancer) should be placed in referral group and all the other placed in the normal screening group. However with the illustrated results of this study, the
algorithm used in 2006-2008 shows to be good enough to at least achieve this compared to the 1996-1998 test algorithm.

The positive predicted value (PPV) for CIN2+ using the HPV mRNA test in combination with cytology was higher compared to cytology test alone. For example, in the biopsy indicated cases, PPV for CIN2+ was 52.0% compared to 41.7% in the time period 2006-2008 and 1996-1998 respectively (Table 3). This may imply that triage testing with HPV mRNA perform well to detect CIN2+ in women with cytology test of both ASCU-US and LSIL, which is unlike the HPV DNA test as it is reported to not perform well for the women detected with LSIL ¹⁰. As such even if the earlier findings report that the immediate triage testing with HPV DNA test (HC II) is potentially robust strategy for the women detected with ASC-US ¹⁰, ³¹, our current study results does not agree with it. As such the HPV mRNA test in combination with cytology method appears to be a potentially robust strategy for triaged women with previously cytology test reports of ASC-US / LSIL.

7.6. Representativeness and validity of the study
This study is very representative and valid for the Norwegian society, since all age- groups in accordance to the National cervical cancer programme are included ¹. However it may not be generalizable to other nations with different screening programs and different algorithms for triage.

7.7. Strength and weaknesses of the study
This study has no problems of selection bias or analytical bias since it is based on historical design. All eligible women screened in Troms and Finnmark County were included. But the results may underestimate the true potentials of HPV mRNA test in combination with the cytology method for several reasons.
First not all women with indication of HPV mRNA test actually got it. Since the HPV mRNA test requires liquid based cytology (LBC), besides the guidelines i.e. age and time from index cytology, fewer women with ASC-US / LSIL for the period 2006-2008 were included in our material compared to women with ASC-US / LSIL in the period of 1996-1998. Thus fewer cases available for HPV mRNA test method than for cytology method alone. It is therefore most likely that the test method for the period 2006-2008 might have detected more cases of CIN2+ and prevented more cancer if more women were tested by HPV mRNA.

Second, some cells might have been lost during the process of preparing Pap smear of the cytological collection into liquid cytology transport fluids. Hence fewer cervical cells might have been available for liquid-based HPV testing. As such it would not be wrong to assume that the liquid-based HPV test performance might have detected more cases of CIN2+ if more cervical cells had been collected into vials.

Third, sensitivity and specificity for the test methods was not determined in this study. This could have made our results even more conclusive. The sensitivity and specificity was not calculated because the study analysis started with ASC-US / LSIL, such that nothing about cases of women who had ASC-H / HSIL or normal cytology was known. We did not have biopsy from all the women in the study, to enable us calculate the true sensitivity and specificity of the tests. However despite that the current study was unable to calculate the sensitivity and specificity, the performance of both test methods in 1996-1998 and 2006-2008 can still be assessed using the positive predictive value (PPV) of CIN2+ detection calculated (Table 3).

In addition, a complete evaluation of the test performance of the algorithm also requires an assessment of the resources and services spent on screening or test measures (cost-effective
analysis)\textsuperscript{10,20}. But this is not set as one of the elements to be considered in this thesis. Hence, this is another limitation of this study. The cost-effective analysis is useful for the determination of the overall utility of the use of HPV mRNA test in combination with cytology and subsequent HPV triage testing as a practical cervical neoplasia screening system. However, since most of the costs in the screening programme are due to consultations\textsuperscript{12}, our study still provides a necessary evaluation of the involved test performance. According to the senior consultant S.W. Sørbye at the Department of Clinical Pathology at the University Hospital of North Norway (UNN), approximately 500.000 consultations cost 1.000 million NOK including the women’s travel costs and time for not being at work. Hence a single consultation will cost about NOK 2.000. The HPV mRNA test in combination with cytology costs NOK 325 more than the cytology alone, but using repeat cytology we need one more consultations than the HPV-test for decision making, i.e. resolved or not resolved case. As such the extra cost of a HPV-test is a small fee to pay if we can reduce the number of consultations using cytology. Thus the HPV mRNA test in combination with the cytology is proven significantly less expensive compared to cytology test alone. The thesis therefore provides part of the necessary foundation for a complete evaluation of the secondary test methods used in the two periods, i.e. 1996 – 1998 and 2006 - 2008.

In general, the discussion above shows that following 2006-2008 algorithm, unlike that used in 1996-1998, will increase the degree of assurance that only women with cervical lesions that confer an increased risk of cervical cancer are identified. Ensuring that only women requiring further follow-up and/or treatment to prevent progress are subjected to colposcopy and biopsy examination, i.e. that they should be referred immediately for further exploration\textsuperscript{38,40-42} and at the same time ensuring that the referrals should not result in substantial costs for the health care system\textsuperscript{28} and avoid creating unnecessary feelings of anxiety and discomfort for women\textsuperscript{29}. 

\textsuperscript{28}
8. Concluding remarks

The screening algorithm for follow-up of women with ASC-US / LSIL using HPV mRNA test in combination with cytology takes significantly shorter time in resolving cases compared to the cytology test alone. The positive predictive value for mRNA HPV test in combination with cytology is higher compared to those of repeat cytology. Substituting a strategy of HPV mRNA test in combination with cytology in triage women seems promising but probably needs evaluation in long-term trials of large volume. This would be an interesting topic in a future study with inclusion of a detailed cost-effectiveness analysis, such that all the required calculations can conclusively be done in order to make hard recommendations on which method to follow for quality assurance in handling triage women with ASC-US / LSIL at the University Hospital of North Norway.

8.1. Recommendation

Between the two triage algorithms examined in this study, we can recommend the University Hospital of North Norway to follow the algorithm used in the time period 2006-2008. This is because our current study results illustrates that the HPV mRNA test in combination with the cytology is the better and more effective algorithm to triage women with ASC-US / LSIL cytology compared to cytology test alone, which was used in the time period 1996-1998.
9. References


12. Sørbye SW, the senior consultant at Department of Clinical Pathology at the University Hospital of North Norway (UNN). Personal communication 2012.


