Tampere University

JOHANNA PULKKINEN

Atypical Endocervical Cells in Pap Smears

Diagnostic Challenges and the Role of HPV Primary Screening

Tampere University Dissertations 785

Tampere University Dissertations 785

JOHANNA PULKKINEN

Atypical Endocervical Cells in Pap Smears

Diagnostic Challenges and the Role of HPV Primary Screening

ACADEMIC DISSERTATION
To be presented, with the permission of
the Faculty of Medicine and Health Technology
of Tampere University,
for public discussion in the auditorium F114
of the Arvo building, Arvo Ylpön katu 34, Tampere,
on 2 June 2023, at 12 o'clock.

ACADEMIC DISSERTATION
Tampere University, Faculty of Medicine and Health Technology
Fimlab Laboratories Oy
Finland

Responsible supervisor and Custos	Docent Ivana Kholová Tampere University Finland	
Pre-examiners	Docent Anne Ahtikoski University of Turku Finland	Docent Kirsi Hämäläinen University of Eastern Finland Finland
Opponent	Professor Olli Carpén University of Helsinki Finland	

The originality of this thesis has been checked using the Turnitin OriginalityCheck service.

Cover design: Roihu Inc.

ISBN 978-952-03-2861-0 (print)
ISBN 978-952-03-2862-7 (pdf)
ISSN 2489-9860 (print)
ISSN 2490-0028 (pdf)
http://urn.fi/URN:ISBN:978-952-03-2862-7

Carbon dioxide emissions from printing Tampere University dissertations have been compensated.

PunaMusta Oy – Yliopistopaino
Joensuu 2023

To my family

ACKOWLEDGEMENTS

The research for this thesis was performed at the Department of Pathology of Fimlab Laboratories Oy and at the Faculty of Medicine and Health Technology of Tampere University. This work was supported by grants from VTR Funding, the Eemil Aaltonen Foundation, and the Ida Montin Foundation.

First and foremost, I want to express my deepest gratitude to my supervisor, Docent Ivana Kholová, M.D., Ph.D., who, despite her very busy schedule, found the time to guide me through this project. With endless patience and an encouraging positive attitude, she was always there to help me overcome my obstacles. With her expertise in the field of cytopathology, research and academic writing, she has provided me with a great deal of excellent practical advice. During this project, she has supported me professionally and personally in many hardships of life, for which I am sincerely grateful.

I also highly appreciate the background support Professor Timo Paavonen, M.D., Ph.D., has provided for this thesis.

I would like to acknowledge all my co-authors for their contributions and collaboration. For Heini Huhtala, M.Sc., I am deeply grateful for her patience in repeatedly reviewing the basics of our statistical methods and helping me interpret our results. Her professional skills and participation were essential for the success of this thesis. I want to express my appreciation to Sinikka Hollmén, M.D., Leena Krogerus, M.D., Ph.D. and Marita Laurila M.D. for finding the time to participate. I am especially grateful to Leena Krogerus, M.D., Ph.D., for all the valuable ideas, advice and comments she provided during the study design and while proofreading the manuscripts. I want to thank Saara Kares, M.Sc., for all the help she gave me with data collection.

I wish to thank my official reviewers, Docent Anne Ahtikoski, M.D., Ph.D., and Docent Kirsi Hämäläinen, M.D., Ph.D., for all the insightful comments and valuable advice in preparing the final version of this thesis.

I thank the thesis supervision committee members, Docent Jyrki Parkkinen, M.D., Ph.D. and Virva Pohjolainen, M.D., Ph.D., for their true interest in the project and for all the encouragement and companionship during the dark moments of this journey.

I am grateful to my chief physician, Mika Tirkkonen, M.D., for providing me with the organisational opportunity of leave of absence, which enabled me to complete this thesis. I wish to thank my colleagues at Fimlab Laboratories for their friendly and supportive working environment. I appreciate the prevailing educational approach in the atmosphere and the senior pathologists generously sharing their knowledge and skills.

I cannot thank my friends enough for all their support. I especially want to express my gratitude to my oldest friend, Sanna Valkama, who has been there for me in all the highest and lowest moments of my life and is always willing to listen and help, however possible. I also want to thank Maina Korteniemi and Maija Ylivuori for their solid presence during this project and for providing me with much-needed peer support.

I want to thank all my family members for their support. In particular, I wish to express my appreciation for my parents, who have always believed in me. For them, I am forever deeply grateful for all the unconditional love and encouragement I have received. I wish to thank my siblings, their spouses and my in-laws for their stable and unfailing presence in my life and for expanding my views in so many ways.

Finally, I want to thank my dear husband, Mika Ala-Lipasti, for always being there for me. He is my true partner in everything. His sense of humour and sometimes brutal honesty help me keep things in perspective. For my greatest achievements, Juuli, Eemeli and Elle, I am endlessly grateful for all the joy and fulfilment they bring to my life. I am thankful that they make sure I keep my priorities straight and challenge me every day to grow as a person.

Tampere, March 2023
Johanna Pulkkinen

ABSTRACT

Cervical cancer is the fourth most common malignancy among women worldwide and the third most common cause of cancer mortality. Cervical cancer comprises two distinct histological types: squamous cell carcinoma (SCC) and endocervical adenocarcinoma (EAC), of which SCC is the most common. In recent decades, both the relative and absolute incidences of EAC have increased, particularly in high-income countries.

Most existing cervical cancer research is based on SCC and its precursor lesions. In this thesis, we focused on the diagnostics of EAC and its precursor lesion, adenocarcinoma in situ (AIS). We investigated the cytomorphological features associated with EAC and AIS, along with the features obscuring their diagnoses and the features leading to their false-positive diagnoses. Additionally, we evaluated the diagnostic reproducibility in cytology, as well as the performance of HPV primary screening in detecting EAC and AIS.

We found that most histological lesions behind cytological endocervical cell atypia are purely squamous. A combination of cytomorphological features, including palisading cell borders, nuclear pleomorphism and the lack of single atypical cells in conventional Pap smears, predict histological EAC and AIS. The reproducibility of the more severe cytopathological diagnoses is of a moderate level and better than that of the milder cytological changes and glandular and squamous features. In EAC and AIS Pap smears, marked nuclear enlargement and nuclear pleomorphism are the most common features in Pap smears, with good consensus of the neoplastic nature and of the endocervical cell origin of the lesion. In turn, degenerative changes and a lack of nuclear enlargement are the most frequent features encountered in samples with low consensus.

Squamous metaplasia, significant mixed inflammation, tubal metaplasia and microglandular hyperplasia are the most common benign causes of the misdiagnosis of endocervical cell atypia in cytology. Lack of nuclear crowding and lack of degenerative changes are the best cytomorphological features in separating benign endocervical cell atypias from those harbouring malignancy.

In our study, no neoplastic lesions were found among hrHPV-negative patients presenting with endocervical cell atypia in cytology. One hrHPV-negative gastric-

type EAC was missed by the HPV primary screening during the investigated 4-year screening period.

TIIVISTELMÄ

Maailmanlaajuisesti kohdunkaulasyöpä on naisten neljänneksi yleisin syöpä. Naisten syöpäkuolemien aiheuttajana kohdunkaulasyöpä on kolmanneksi yleisin.

Mikroskooppisesti kohdunkaulasyöpä koostuu kahdesta eri syöpätyypistä, levyepiteelisolusyövästä ja lieriöepiteelisolusyövästä. Näistä levyepiteelisolusyöpä on selvästi yleisempi. Viime vuosikymmenten aikana lieriöepiteelisolusyöpien kokonaismäärä, sekä määrä suhteessa levyepiteelisolusyöpien määrään, on kasvanut etenkin korkean elintason maissa.

Enemmistö aiemmista kohdunkaulasyöpätutkimuksista käsittelee levyepiteelisolusyöpää ja sen esiastemuutoksia. Tässä väitöskirjatutkimuksessa keskityimme lieriöepiteelisolusyöpään ja sen esiastemuutokseen, in situ- tasoiseen lieriöepiteelisolusyöpään. Tutkimme irtosolunäytteistä lieriöepiteelisolusyöpää ja sen esiastemuutosta ennustavia solupiirteitä sekä solupiirteitä, jotka johtavat diagnostisiin virhetulkintoihin. Lisäksi tutkimme irtosolunäytteisiin perustuvan diagnostiikan toistettavuutta sekä HPV-perusteista kohdunkaulasyöpäseulontaa lieriöepiteelisolumuutosten näkökulmasta.

Totesimme, että enemmistö lieriöepiteelisoluperäisiksi tulkituista irtosolunäytteiden muutoksista on puhtaasti levyepiteelisolusyövän esiastemuutoksista aiheutuneita. Irtosolunäytteissä samanaikaisesti esiintyneet paaluaitamaisesti järjestäytyneet solujen tumat solukasojen reunoilla, kooltaan ja muodoltaan huomattavan vaihtelevat solujen tumat sekä yksittäisten poikkeavien solujen puuttuminen, ennustavat lieriöepiteelisolusyövän ja sen esiastemuutoksen löytymistä kudosnäytteestä.

Vaikea-asteisimpiin irtosolumuutoksiin liittyvien diagnoosien toistettavuus on kohtalaista tasoa. Lieviin solumuutoksiin liittyvien diagnoosien toistettavuus on matala-asteisempaa. Samoin arvioitaessa lieriösoluperäisten solupoikkeavuuksien erottamista levyepiteelisoluperäisistä solupoikkeavuuksista, irtosolunäytteiden diagnoosien toistettavuus on matala-asteisesta.

Niissä lieriöepiteelisolusyöpää tai sen esiastemuutosta edustavissa irtosolunäytteissä, joissa yhteisymmärrys solujen pahanlaatuisuudesta ja lieriöepiteliaalisesta alkuperästä on hyvä, tavallisimpia solupiirteitä ovat huomattava tumakoon kasvu sekä huomattava tuman koon ja tuman muodon vaihtelu.

Vastaavasti näytteissä, jossa solujen luonteesta ja alkuperästä ei synny yhteisymmärrystä, tavallisimpia solupiirteitä ovat hajoamiseen liittyvät solumuutokset sekä tumakoon kasvun puuttuminen.

Levyepiteelimetaplasia, merkittävä sekasoluinen tulehdus, tubaalinen metaplasia ja mikroglandulaarinen hyperplasia ovat yleisimpiä hyvänlaatuisia muutoksia, jotka irtosolunäytteissä johtavat virheelliseen lieriöepiteelisolupoikkeavuuden tulkintaan. Solupiirteitä, jotka parhaiten erottelevat hyvänlaatuisiin muutoksiin liittyvät lieriöepiteelisolupoikkeavuudet pahanlaatuisiin muutoksiin liittyvistä lieriöepiteelisolupoikkeavuuksista ovat tumien ruuhkautumisen puuttuminen sekä solun hajoamiseen liittyvien muutosten puuttuminen.

Ainuttakaan pahanlaatuista muutosta ei löytynyt aineistomme potilailta, joilla oli negatiivinen HPV- testitulos sekä irtosolunäytteessä lieriöepiteelisolupoikkeavuus. HPV- seulonnassa yksi HPV- negatiivinen, gastrista alatyyppia oleva lieriöepiteelisolusyöpä jäi löytymättä neljän peräkkäisen vuoden seulontaikäisten naisten aineistossa.

CONTENTS

List of Tables

ABBREVIATIONS

AIS	adenocarcinoma in situ
AEC-FN	atypical endocervical cells, favour neoplastic
AEC-NOS	atypical endocervical cells, not otherwise specified
AEM-NOS	atypical endometrial cells, not otherwise specified
AGC-NOS	atypical glandular cells, not otherwise specified
AGUS	atypical glandular cells
AKT1	oncogene AKT Serine/Threonine Kinase 1
ARID1A	protein coding gene AT-Rich Interaction Domain 1A
ARID1B	protein coding gene AT-Rich Interaction Domain 1B
ASC-H	atypical squamous cells, cannot exclude HSIL
ASC-US	atypical squamous cells, undetermined significance
ASDR	age-standardised death rate
ASIR	age-standardised incidence rate
ASMR	age-standardised mortality rate
BIRST	Bethesda Interobserver Reproducibility Study
BRAF	B-Raf Proto-Oncogene, Serine/Threonine Kinase
CDKN2A	protein coding gene Cyclin Dependent Kinase Inhibitor 2A
CI	confidence interval
CIN	cervical intraepithelial lesion
CIN2+	cervical intraepithelial lesion 2 or worse
CIN3+	cervical intraepithelial lesion 3 or worse
DNA	deoxyribonucleic acid
DS	dual stain
EAC	endocervical adenocarcinoma

ERBB2	protein coding gene Erb-B2 Receptor Tyrosine Kinase 2
ERBB3	protein coding gene Erb-B2 Receptor Tyrosine Kinase 3
FOXL2	transcription factor Forkhead Box L2
gAIS	gastric-type adenocarcinoma in situ
GCO	Global Cancer Observatory
GNAS	protein coding gene GNAS Complex Locus
HE	hematoxylin and eosin
HPV	human papillomavirus
hrHPV	high-risk human papillomavirus
HSIL	high-grade squamous intraepithelial lesion
IARC	International Agency for Research on Cancer
IECC	International Endocervical Adenocarcinoma Criteria and Classification
K	kappa
Ki-67	marker of proliferation Ki-67
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
LBC	liquid-based cytology
LIS	laboratory information system
LSIL	low-grade squamous intraepithelial lesion
NILM	negative for intraepithelial lesion or malignancy
NORDCAN	Association of the Nordic Cancer Registries
NRAS	oncogene N-ras
OR	odds ratio
p16	tumour suppressor protein 16
PAP	Papanicolaou
PCR	polymerase chain reaction
PI3K/Akt/mTOR	intracellular signalling pathway controlling the cell cycle
PIK3CA	oncogene Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha

PTEN	tumour suppressor gene Phosphatase and Tensin Homolog
SCC	squamous cell carcinoma
SD	standard deviation
SMAD4	transcription factor and tumour suppressor gene SMAD Family Member 4
SMARCA4	SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily A, Member 4
STK11	tumour suppressor gene Serine/Threonine Kinase 11
TBSRCC	The Bethesda System for Reporting Cervical Cytology
TP53	tumour suppressor gene Tumour Protein P53
WHO	World Health Organization

ORIGINAL PUBLICATIONS

Publication I	Pulkkinen, J., Huhtala, H., Kholová, I., 2021. The role of Pap smear in the diagnostics of endocervical adenocarcinoma. APMIS. 2021; 129(4): 195–203. https://doi.org/10.1111/apm.13115.
Publication II	Pulkkinen, J., Huhtala, H., Krogerus, L.A., Hollmén, S., Laurila, M., Kholová, I., 2022. Endocervical cytology: Inter- and intra-observer variability in conventional pap smears. Acta Cytol. 2022;66(3):206–215. https://doi.org/10.1159/000522212.
Publication III	Pulkkinen, J., Kares, S., Huhtala, H., Kholová, I., 2021. Detection and outcome of endocervical atypia in cytology in primary HPV screening programme. Diagnostics (Basel). 2021;11(12):2402. https://doi.org/10.3390/diagnostics11122402.
Manuscript IV	Pulkkinen, J, Huhtala H., Kholova I. False-positive atypical endocervical cells in conventional Pap smears: Cyto-histological correlation and analysis. Submitted 28.11.2022.

AUTHORS CONTRIBUTION

Studies I–IV: The author was responsible for project administration, conducting the literature searches and collection of the laboratory and clinical data. The author participated in the analysis and interpretation of the data together with the supervisor and the co-author, who performed the statistical analyses. The author wrote the original drafts, generated the associated tables and figures and edited the final papers with the help of the supervisor and co-authors. The author submitted the manuscripts to the journals with the help of the supervisor.

Studies III and IV: The author participated in the study design together with the supervisor.

1 INTRODUCTION

Most new cervical cancer cases occur in low- and middle-income countries, where cervical cancer mortality rates are generally the highest. High-income countries have implemented cervical cancer screening programmes, which have been successful in reducing both the incidence and mortality rates of cervical cancer. (Bray et al., 2012; GCO, 2022; IARC, 2022, Zhang et al., 2021).

The reductions seen in cervical cancer incidence rates in high-income countries are due to the reduced numbers of squamous cell carcinoma (SCC). Simultaneously, both the relative and absolute number of endocervical adenocarcinoma (EAC) cases has increased; currently, the incidence of EAC in high-income countries is around 15% (Holl et al., 2015; Pimenta et al., 2013; Smith et al., 2000).

Traditionally, the screening and diagnosis of cervical cancer has been based on cytology. Since the causal relationship of high-risk human papillomavirus (hrHPV) infection in nearly all SCC and most EAC cases has become evident, HPV testing along with or instead of cervical cytological sampling has become more common both in clinical practice and in cervical cancer screening (Bosch et al., 2002; Moljin et al., 2016; de Sanjose et al., 2010; Pirog et al., 2014; Wallboomers et al., 1999).

The hrHPV test has a high negative predictive value for cervical cancer (Katki et al., 2011; Ogilvie et al., 2018). In the risk stratification of hrHPV-positive patients, a cervical cytological sample is still the main method used.

In cervical cytology, the reproducibility of diagnostic categories is generally quite low. In particular, milder atypias and endocervical cell changes have been shown to be problematic. (Confortini et al., 2006; Lee et al., 2002; Lepe et al., 2018; Simsir et al., 2003).

The aim of this study was to find tools to improve the diagnostics of EAC and its precursor lesion adenocarcinoma in situ (AIS). We focused on cytomorphology in an attempt to define the earliest features predicting EAC and AIS, which would enable earlier recognition of patients harbouring a malignant lesion and requiring immediate intervention. Additionally, we focused on the features leading to false-positive and false-negative cytological interpretations and thus to unnecessary and costly follow-ups and procedures, as well as missed malignancies.

2 REVIEW OF THE LITERATURE

2.1 Cervical cancer incidence, mortality, and trends

In 2020, cervical cancer was the fourth most common cancer among women worldwide following breast, colorectal and lung cancer, with an estimated 604,000 new cases diagnosed yearly. As also estimated, cervical cancer was responsible for around 342,000 cancer deaths, making it the third most common cause of cancer mortality after breast and lung malignancies. Although both the age-standardised incidence rate (ASIR) and age-standardised death rate (ASDR) show a decreasing trend of cervical cancer globally, most new cervical cancer cases occur in low- and middle-income countries, where the mortality rates are generally the highest (Bray et al., 2012; GCO, 2022; IARC, 2022; Zhang et al., 2021). Countries with a very low incidence of cervical cancer (ASIR less than 5 per 100,000) are mostly located in Western Asia or the western part of Central–South Asia (Arbyn et al., 2020).

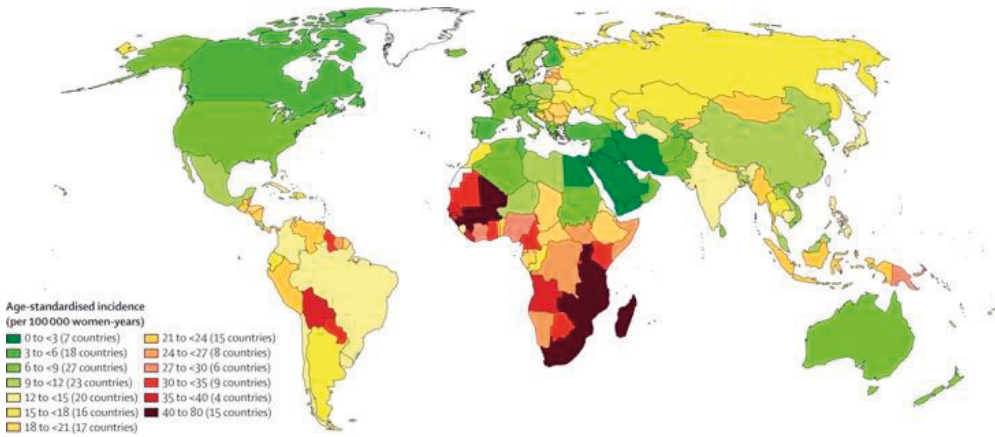

Figure 1. Age- standardised incidence of cervical cancer by country. Reprinted from Arbyn (Arbyn et al., 2020).

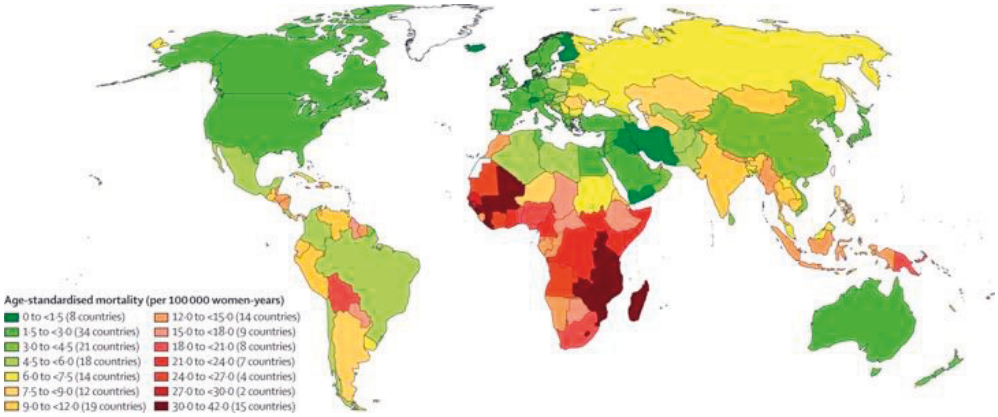

Figure 2. Age- standardised mortality of cervical cancer by country. Reprinted from Arbyn (Arbyn et al., 2020).

In Europe, cervical cancer is the eighth most common cancer among women (Ferlay et al., 2018). Its burden varies regionally, with Northern, Western and Southern Europe presenting generally with only a modest incidence rate (ASIR < 10 per 100,000) and Eastern Europe with a high incidence rate (ASIR > 15 per 100,000). The lowest ASIR of 3.5 per 100,000 has been reported in Malta and the highest ASIR of 25.0 per 100,000 in Latvia. In addition, the mortality rates among European countries are variable, with the highest age-standardised mortality rates (ASMR) reported in Bulgaria (ASMR 8.9) and the lowest in Finland (ASMR 0.9) (Arbyn et al., 2021).

In Finland, the cervical cancer ASIR of 4.5 per 100,000 is one of the lowest in the world and lower than the ASIR range of 8.2–10.4 per 100,000 seen in other Nordic countries. This corresponds to 168 new cervical cancer cases detected in Finland annually, according to statistics from 2012–2016 (NORDCAN, 2022). Both in Finland and globally, there is a peak in incidence rate in the age group of 30–40-year-old women (Arbyn et al., 2020; NORDCAN, 2022). In Finland, the ASIR of cervical cancer in this age group is about 12 per 100,000 (NORDCAN, 2022).

In recent decades, the introduction of cervical cancer screening programmes has led to an overall decrease in cervical cancer incidence and mortality, both in Europe in general and in Nordic countries in particular (Arbyn et al., 2009; Bray et al., 2005; Laara et al., 1987; Vaccarella et al., 2014). In Europe, though, the reduction rate has been variable in different countries; in some European countries, mainly in Eastern Europe, the latest reports have continued to show an increasing trend (Arbyn et al., 2009; Zhang et al., 2021).

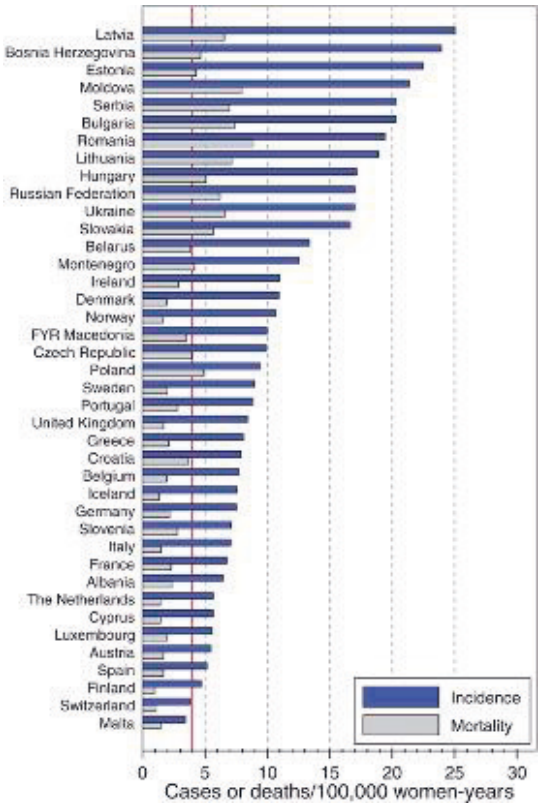

Figure 3. World age-standardised incidence and mortality rates for cervical cancer in Europe; estimates for 2018. The red line represents the World Health Organization (WHO) elimination target (4/100,000 per year). Reprinted from Arbyn et al. (2021). Original source of data: IARC GLOBOCAN.

In Finland, the incidence rate of cervical cancer began to decrease in the 1960s, when the national cervical cancer screening programme was introduced. The incidence rate continued to drop until the 1990s, after which it remained at the same level. According to the latest national report, the incidence of cervical cancer differs among different socioeconomic classes today. Women with only basic education have nearly twice the incidence rate of cervical cancer as women with higher education (Syöpärekisteri, 2020).

Currently, cervical cancer represents 1.1% of all cancers in Finland and is responsible for 1.0% of cancer deaths (NORDCAN, 2022). At present, the survival

rate in cervical cancer is around 70%. Among women with only a basic level of education, the mortality is 2.5 times higher than among women with higher education (Syöpärekisteri, 2022).

The reduction seen in cervical cancer incidence rates is due to the reduced rates of squamous cell carcinoma (SCC). According to numerous reports, the incidence of adenocarcinoma (EAC) had risen until the early 21st century, with an increase of up to 29.1% in the reported age-adjusted incidence rates (Bray et al., 2005; Gunnell et al.; Reimers et al., 2009; Sasieni et al., 2004; Smith et al., 2000; Wang et al., 2004). Additionally, a rise in the incidence of EAC, specifically in the younger age groups of women under 45 years, has been observed (Bulk et al., 2005). The most recent studies have reported stable levels of EAC, which, in association with declining SCC rates, still result in increased relative incidence rates (van der Horst et al., 2017; Mancini et al., 2017).

Globally, EAC is estimated to represent 9.4% of all cervical cancers (Pimenta et al., 2013). However, its relative percentage varies considerably by country and region. In developed countries, EAC comprises 14.2% to 18.7% of cervical cancers (Holl et al., 2015; Pimenta et al., 2013; Smith et al., 2000).

At the same time, the increase in the incidence of AIS has been even more pronounced (Gunnell et al., 2007; van der Horst et al., 2017; Orumaa et al., 2019; Wang et al., 2004), especially in females aged 25–39 (van der Horst et al., 2017). In a recent study covering the years 2008–2015, a declining trend of AIS was observed among women aged 21–24 years, which was speculated to represent the effect of the HPV vaccination (Cleveland et al., 2020). In the older age groups, the AIS incidence remained stable.

2.2 Histological classification

Cervical carcinoma comprises two histological types: SCC and EAC. According to the 5th edition (the latest) of the WHO Classification of Female Genital Tumors, both subtypes are further divided into HPV-associated and HPV-independent categories, described below (WHO Classification of Tumors Editorial Board, 2020).

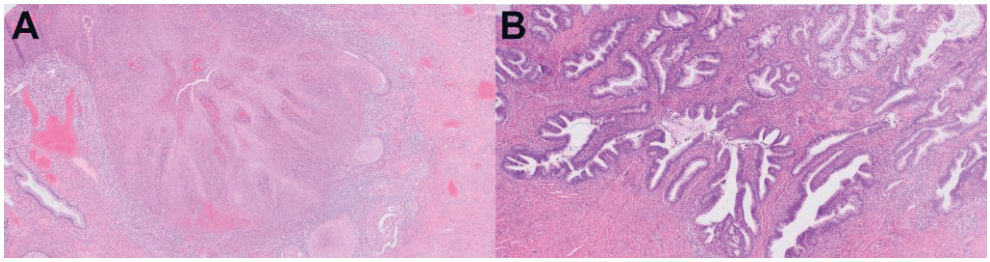

Figure 4. Cervical carcinoma comprises two distinct histological types: squamous cell carcinoma (A) and adenocarcinoma (B). Hematoxylin and eosin stain, magnification 5x (A) and 5x (B).

The histological subtypes in the HPV-associated adenocarcinoma category are the usual type EAC and the mucinous EAC. Mucinous cytoplasm is found in 10–50% of tumour cells of the usual type and more than 50% of tumour cells of the mucinous type. Characteristics of both types include apical mitoses and apoptotic bodies, which are recognisable at low magnification.

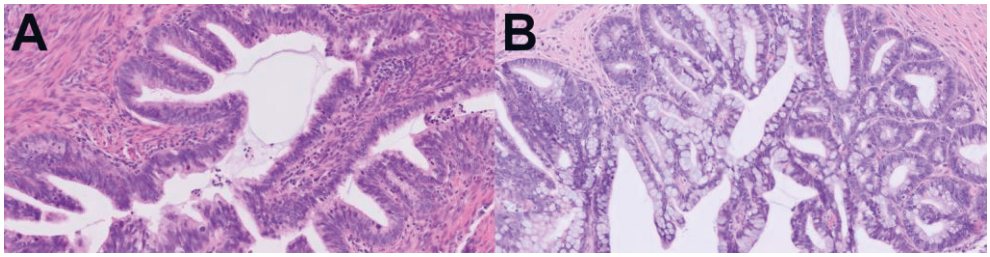

Figure 5. Characteristic for HPV-associated adenocarcinomas are apical mitoses and apoptotic bodies, which are recognisable at low magnification in light microscopic examination. Endocervical adenocarcinoma, usual type (A) and intestinal endocervical adenocarcinoma with goblet cells (B). Hematoxylin and eosin stain, magnification 20x (A) and 20x (B).

The usual subtype includes the villoglandular variant, which is an exophytic papillary tumour with mild atypia and absent or minimal stromal invasion. The mucinous type has four variants: 1) mucinous, EAC-NOS with normal endocervix-resembling mucinous tumour cells; 2) intestinal EAC with goblet cells and/or enteric differentiation seen in ≥ 50% of the tumour cells; 3) signet-ring cell EAC with ≥ 50% of the tumour consisting of loose, round cells with an intracytoplasmic mucin vacuole displacing the nucleus; and 4) stratified mucin-producing carcinoma with invasive nests of stratified epithelium with intracytoplasmic mucin.

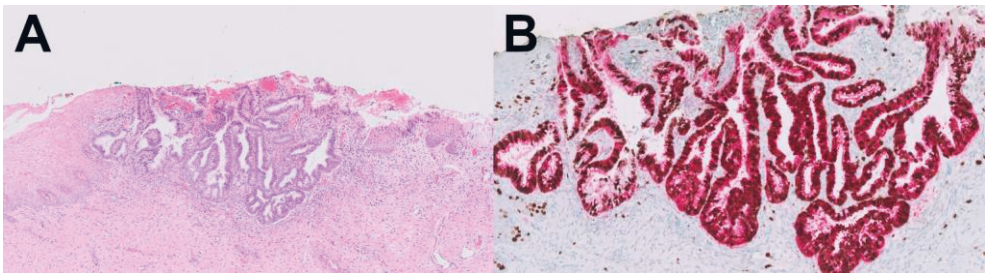

Figure 6. Adenocarcinoma in situ at squamocolumnar junction (A). The lesion shows block-type p16 positivity and high proliferation in the KI-67 stain (B). Hematoxylin and eosin stain, magnification 10x (A) and dual stain p16/Ki-67, magnification 20x (B).

HPV-associated EAC has a precursor lesion AIS, in which the neoplastic changes are confined to the pre-existing glandular structures without stromal invasion. In AIS, the nuclei are typically pseudostratified and hyperchromatic, and apical mitoses and basal karyorrhexis usually are easily identified. The morphological spectrum of AIS includes mucin depletion, obvious mucinous cells, goblet cells, ciliated cells and monolayered changes. A specific subtype is the stratified mucin-producing intraepithelial lesion, in which intracytoplasmic mucin is seen in all layers of stratified epithelium, often peripherally cuffed by basaloid or reserve cells.

The histological types included in the HPV-independent adenocarcinoma category are the gastric type EAC, the clear cell type EAC, the mesonephric type EAC and the endometrioid type EAC.

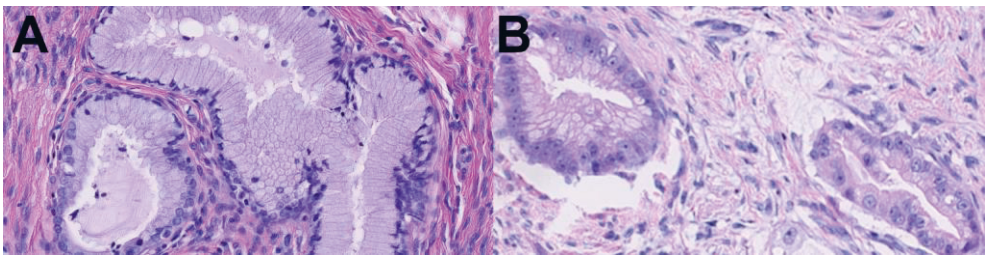

Figure 7. The gastric type endocervical adenocarcinoma is the most common subtype in the HPV-independent adenocarcinoma category. No apical mitosis or apoptotic bodies can be appreciated. An extremely well-differentiated example (A) and a case with more pronounced atypia (B). Hematoxylin and eosin stain, magnification 20x (A) and 20x (B).

The tumour cells in gastric type EAC typically have abundant clear or pale eosinophilic cytoplasm and distinct cell borders. Apical mitoses and apoptotic bodies are inconspicuous. Morphology can vary from extremely well-differentiated to poor.

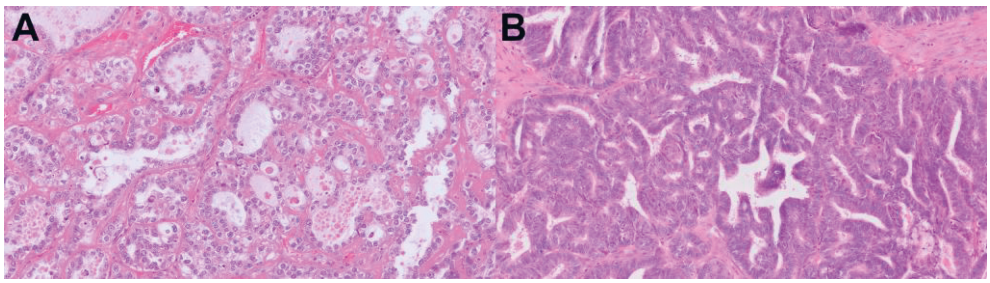

Figure 8. Tumour cells with prominent cell boundaries, clear cytoplasm and minimal cell stratification are typical for clear cell type EAC. There are tubular and solid structures in the partly hyalinised stroma (A). Endometrial type EAC is morphologically identical to endometrioid carcinoma of the uterine corpus (B). Hematoxylin and eosin stain, magnification 20x (A) and 20x (B).

In clear cell type EAC, tumour cells with prominent cell boundaries and clear, eosinophilic or granular cytoplasm show only minimal stratification. Mitoses are rare and stromal hyalinisation is common. Architecture is tubulocystic, papillary, solid or a variable mixture of growth patterns. Although endometrioid type EAC lacks apical mitoses and apoptotic bodies at scanning magnification, it can resemble mucin-poor usual type EAC. Before diagnosing endometrial type EAC, an endometrial primary tumour and HPV infection should be excluded.

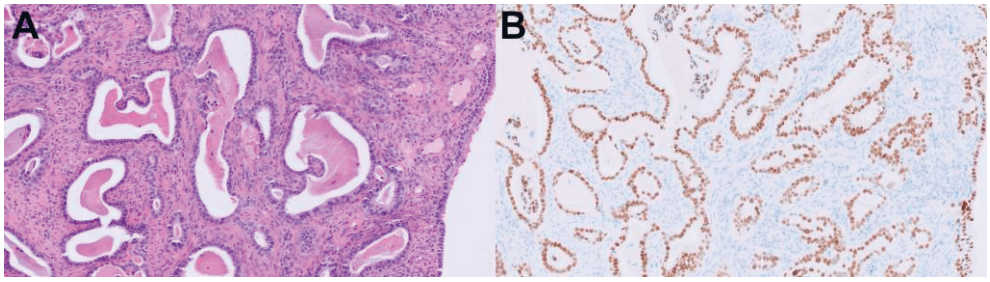

Figure 9. In mesonephric type EAC, the best recognised growth pattern includes tubular glands lined with cuboidal cells and filled with dense eosinophilic secretions (A). The mesonephric type EAC is typically positive with PAX8 and GATA3 and negative with hormone receptors (B). Hematoxylin and eosin stain, magnification 20x (A) and PAX8 immunohistochemical stain, magnification 20x (B).

Classically, in mesonephric-type EAC, there are tubular glands lined with cuboidal cells with uniform nuclei, but several other growth patterns can occur. The glandular lumina are typically filled with dense eosinophilic secretions. The mitotic activity is variable. The endometrial type EAC is identical to its endometrial counterpart.

Of the HPV-independent adenocarcinomas, only the gastric type EAC has an established precursor lesion, gastric type adenocarcinoma in situ (gAIS). In gAIS, cells morphologically similar to cells seen in gastric-type EAC are confined to pre-existing endocervical glands. Currently, lobular endocervical glandular hyperplasia and its atypical variant are also considered most likely to represent the spectrum of gAIS (WHO Classification of Tumours Editorial Board, 2020).

While in SCC, hrHPV prevalence rates approaching 100% have been published (Wallboomers et al., 1999, Muñoz et al., 2003), in EAC, hrHPV is less common, with a reported range of 62% to 90% of the cancers positive (An et al., 2005; Chen et al., 2016; Hodgson et al., 2019; Holl et al., 2015; Moljin et al., 2016; de Sanjose et al., 2010; Smith et al., 2007; Pirog et al., 2014). Both in SCC and EAC, the hrHPV-negative cancers present at an older age and at a more advanced clinical stage than the hrHPV-positive tumours (Chen et al., 2016; Moljin et al., 2016; Pirog et al., 2014; Radomska et al., 2021; Stolnicu et al., 2018; Tjalma et al., 2013).

As described above, the EAC is a heterogenic group of tumours, including histological subtypes originating from hrHPV-independent pathways, as is accepted by the current WHO 2020 classification system (WHO Classification of Tumours Editorial Board, 2020). The most common histological subtype is the usual type, which comprises 59%–89.3% of the EAC, according to previous studies (An et al., 2005; Chen et al., 2016; Holl et al., 2015; Moljin et al., 2016; Park et al., 2013; Pirog et al., 2014; Radomska et al., 2021; Stolnicu et al., 2018). In the same studies, the proportion of the rarer EAC subtypes ranged from 1.6% to 20.8% for tumours now classified as the gastric type, 0% to 6.3% for the clear cell type, 0% to 6.7% for the endometrioid type and 0% to 8% for the not otherwise specified type. Most of the studies have also included the serous type (which has been withdrawn from the current WHO classification), with prevalence ranging from < 1% to 5.0% (An et al., 2005; Chen et al., 2016; Holl et al., 2015; Moljin et al., 2016; Park et al., 2013; Pirog et al., 2014; Stolnicu et al., 2018).

The different classification systems used in different studies are probably responsible, in part, for some of the variation seen in the relative proportions of the histological subtypes. This variation also likely reflects the fairly low histological diagnostic reproducibility of these subtypes (Chen et al., 2016; Hodgson et al., 2019;

Holl et al., 2015). However, there seems to be some true regional variation in the EAC subtype distribution observed between both countries and continents (Holl et al., 2015; Pirog et al., 2014).

2.3 Etiology

The causal relationship of hrHPV infection with nearly all cervical SCC and with the majority of EAC is well documented (Bosch et al., 2002; Moljin et al., 2016; de Sanjose et al., 2010; Pirog et al., 2014; Wallboomers et al., 1999). Thus, hrHPV infection is currently generally accepted as the most important etiological risk factor for cervical cancer. However, HPV infection alone is not sufficient to cause invasive cancer. Additional modifications in intra- and intercellular signalling cascades and in cell-mediated immune responses are needed (zur Hausen, 2000; McBride, 2022; Olusola et al., 2019). Additionally, sexual behaviour-related variables, other gynaecological infections, changes in vaginal microbiome and immune suppression, including HIV and smoking, have been associated with an increased risk of developing cervical cancer (Doulgeraki et al., 2022; Castellagué et al., 2006).

The usual EAC subtype presents with the strongest association with hrHPV infection, with a reported range of 60% to 95% of the tumours being positive (Chen et al., 2016; Holl et al., 2015; Jenkins et al., 2020; Moljin et al., 2016; Park et al., 2013; Pirog et al., 2014). In the same studies, the presence of hrHPV in the rarer subtypes also varied greatly, but when detected in microdissected tumour tissue only, hrHPV was present in 0% of the gastric and serous types of EAC and in 0% to 13% of the clear cell and endometrioid types (Jenkins et al., 2020; Moljin et al., 2016). Likewise, when diagnosed according to the International Endocervical Adenocarcinoma Criteria and Classification (IECC), on which the current WHO 2020 classification is based, only 3% of the tumours in the HPV-independent category showed hrHPV positivity (Stolnicu et al., 2018).

Currently, the genomes of nearly 450 different HPV types have been isolated and sequenced. Of these, 220 are listed as reference types by the HPV Reference Centre (McBride, 2022). Around 40 HPV genotypes cause infections in the anogenital area and of them, genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are classified as carcinogenic and generally referred to as high-risk types (Bouvard et al., 2009; Halec et al., 2013; zur Hausen, 2000). Genotypes 26, 30, 34, 53, 66, 67, 68, 69, 70, 73, 82, 85 and 97 are included in the categories of probably or possibly carcinogenic HPV types and they are generally referred to as probable/possible high-risk types (Bouvard et al., 2009; Halec et al., 2013).

Table 1. The HPV genotypes currently defined as carcinogenic and probably or possibly carcinogenic by the IARC*; correspondingly referred to as the high-risk and the probable/possible high-risk genotypes.

High-risk HPV genotypes	Probable/possible high-risk genotypes
16	25
18	30
31	34
33	53
35	66
39	67
45	68
51	69
52	70
56	73
58	82
59	85
	89

* IARC (International Agency for Research on Cancer)

Worldwide, the two most common HPV genotypes encountered in cervical cancer are HPV16 and HPV18, which together constitute 70% to 98.3% of HPV-positive EAC cases (An et al., 2005; Chen et al., 2016; Holl et al., 2015; Moljin et al., 2016; Park et al., 2013; Pirog et al., 2014; de Sanjose et al., 2010; Smith et al., 2007; Tjalma et al., 2013). HPV18 is more common in EAC than in SCC, and, for example, in Denmark, Greece, Germany and Korea, HPV18 is the major HPV genotype detected in EAC (Holl et al., 2015; Park et al., 2013; Smith et al., 2007). Most often, HPV45 is the third most common HPV genotype encountered in EAC, although in some countries the third type has been reported to be HPV31, HPV33, HPV52 or HPV not otherwise specified (An et al., 2005; Chen et al., 2016; Holl et al., 2015; Park et al., 2013; Pirog et al., 2014; de Sanjose et al., 2010; Tjalma et al., 2013). Other HPV genotypes described in association with EAC are genotypes 6, 30, 35, 39, 45, 51, 53, 56, 58, 59, 66, 68 and 73, which all show a regionally variable but generally low distribution of around 2% and under (Chen et al., 2016; Holl et al., 2015; Moljin et al., 2016; Park et al., 2013; Pirog et al.; de Sanjose et al., 2010; Smith et al., 2007).

Infection with multiple hrHPV genotypes occurs in 7%–13% of EAC, most frequently in subtypes other than the usual EAC (An et al., 2005; Chen et al., 2016; Holl et al., 2015; Pirog et al., 2014; Tjalma et al., 2013). In most multiple infections, HPV16 and/or HPV18 are present (An et al., 2005; Chen et al., 2016; Holl et al., 2015; Pirog et al., 2014; Tjalma et al., 2013). Based on studies on squamous lesions, HPV genotypes 16, 18 and 45 seem to lead to development of neoplasia faster than the other hrHPV genotypes (Tjalma et al., 2013). HPV16 and the much rarer genotype HPV33 have been shown to have the highest progression rate and the highest cumulative risk of invasive cancer (Demarco et al., 2020).

AIS is accepted as the precursor lesion of invasive EAC, but there are no well-established earlier precursors for AIS, as there is low-grade squamous intraepithelial lesion (LSIL) for high-grade intraepithelial lesion (HSIL) and SCC (Zaino, 2002; WHO Classification of Tumours Editorial Board, 2020). In general, the pathogenesis of AIS and EAC is less well defined than the progression from LSIL to HSIL and subsequently to SCC. There is evidence that at least most of the AIS and EAC originate in the region of the squamocolumnar junction or transformation zone (Lee et al., 2000; Zaino, 2002). It has been suggested that reserve cells committed to glandular differentiation in the area become infected by oncogenic HPV and start to proliferate, which eventually leads to the development of AIS (Stoler, 2000).

AIS is diagnosed in women 5 to 20 years younger than in those with invasive EAC (Holt et al., 2015; Lee et al., 2000; Plaxe et al., 1999; Zaino 2002). The mean time of development of AIS has been reported to be shorter (21.0 months) among women who have been HPV-positive at the study baseline compared with HPV-negative women (28.7 months; Ault et al., 2011). No data addressing the possible spontaneous regression rate of AIS were found.

Molecularly, the different histological subtypes of EAC present with different mutational patterns. The most common abnormalities encountered in the HPV-positive usual type EAC include mutations in the PIK3CA, KRAS, NRAS, GNAS, FOXL2, AKT1, PTEN and TP53 genes and abnormalities in segments of the PI3K/Akt/mTOR signalling cascade (Jenkins et al., 2020; Lou et al., 2015; Ojesina et al., 2014; Stolnicu et al., 2021; Tornesello et al., 2014; Wright et al., 2013).

Some of the gastric type EAC are associated with Peutz-Jeghers syndrome, but somatic mutations of STK11 have also been identified in addition to mutations in TP53, CDKN2A, ERBB2/ERBB3, KRAS, AKT1, GNAS, SMAD4, PIK3CA, BRAF and several other genes (Garg et al., 2019; Jenkins et al., 2020; Park et al., 2021; Selenica et al., 2021; Stolnicu et al., 2021). Molecularly, the gastric type EAC is a heterogenic group of tumours in which mutations of the TP53, STK11, CDKN2A,

ATM and NTRK genes are more common than in HPV-associated tumours (Hodgson et al., 2020).

The data on the rarer EAC subtypes are sparse. In clear cell type EAC, microsatellite instability and mutations of the TP53 and PIK3CA genes have been described (Boyd et al., 1996; Jenkins et al., 2020). In mesonephric type EAC, mutations in KRAS or NRAS are common, and they often present with mutations in chromatin remodelling genes ARID1A/B or SMARC4 and with chromosomal abnormalities, including copy number gains of 1q, loss of 1p, and gain of chromosomes 10 and 12 (Mirkovic et al., 2015; Mirkovic et al., 2017). No data on the mutational profile of the endometrioid-type EAC were found.

2.4 Cytological diagnostics

2.4.1 Classification

Cytological endocervical cell abnormalities are currently classified according to The Bethesda System for Reporting Cervical Cytology 2014 (TBSRCC). The Bethesda System was originally introduced in 1988, with updates in 1991, 2001 and 2014 (Nayar & Wilbur, 2015). Prior to the implementation of The Bethesda System, Pap smears were diagnosed according to the Papanicolaou classification system, which is a method introduced by Dr George Papanicolaou first in a conference paper in 1928 and later as a more defined version in a publication in 1941 (Classes in Oncology, 1973; Papanicolaou et al., 1941). In the cervical Papanicolaou classification system, the cytological samples are divided into five diagnostic categories as follows: 0 represents inadequate samples, 1 represents normal cells, 2 represents reactive cellular changes, 3 represents slightly worrisome cellular changes of uncertain nature, 4 represents cellular changes severely suspicious for malignancy and 5 represents cytologically malignant cells. According to the current TBSRCC 2014, endocervical cell abnormalities are classified into four diagnostic categories, as described below (Nayar & Wilbur, 2015).

The category of atypical endocervical cells, not otherwise specified (AEC-NOS), includes samples with nuclear features exceeding the changes normally encountered in reactive and reparative processes, which at the same time are not severe enough to confidently designate them as malignant. The features include some cell crowding, nuclear overlapping or pseudostratification, nuclear enlargement up to 3 to 5 times the normal, some variation in nuclear size and shape and mild hyperchromasia and

chromatin irregularities with increased nuclear to cytoplasmic ratio. Cell borders are usually preserved and distinct (Nayar & Wilbur, 2015).

The category of atypical endocervical cells, favour neoplastic (AEC-FN), includes samples with clearly worrisome cytomorphological features, which at the same time are not quantitatively or qualitatively sufficient for a malignant interpretation. The features include sheets and strips of cells with increased nuclear-to-cytoplasmic ratio, nuclear crowding, overlapping or pseudostratification, sometimes with ill-defined cell borders. There is nuclear enlargement with hyperchromasia, coarseness of chromatin and chromatin irregularities. Additionally, occasional rosettes, feathering, mitoses or apoptotic bodies can be observed (Nayar & Wilbur, 2015).

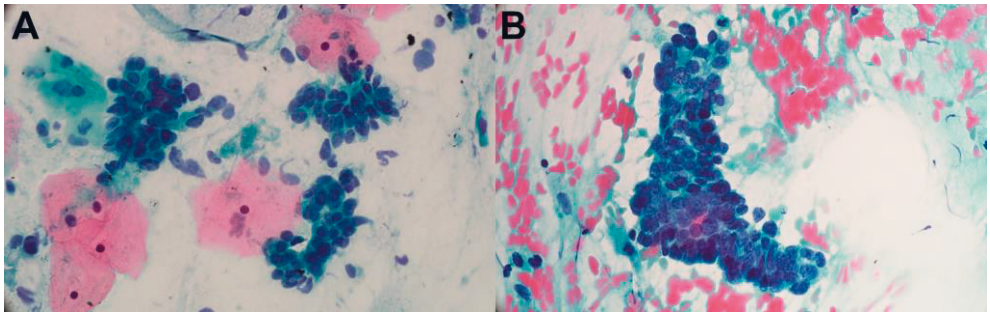

Figure 10. Atypical endocervical cell fragments with mild nuclear enlargement, nuclear crowding and some nuclear overlapping classified as AEC-NOS (A). A papillary-like endocervical cell fragment classified as AEC-FN shows a palisading border, nuclear enlargement with chromatin abnormalities, nuclear crowding and nuclear overlapping (B). Papanicolaou stain, magnification 40x (A) and 60x (B).

In the categories of AIS and EAC, the cytomorphological features are mostly similar as described above for AEC-FN, but they are more abundant. Rosettes, feathering, mitoses and apoptotic bodies are more frequently encountered; in addition, loss of honeycomb pattern and palisading nuclear arrangement can be observed. The variation in nuclear size can be more pronounced, especially in EAC. In EAC, macronucleoli and necrotic tumour diathesis are also encountered, the latter indicating stromal invasion.

2.4.2 Performance and mimics of AEC, AIS and EAC

The reported incidence of endocervical cell atypia varies from 0.1% to 2.1%, with an average of 0.29%, according to a large meta-analysis of 24 studies (Schnatz et al.,

2006). The Bethesda categories AEC-NOS, AEC-FN and AIS have all been proven to have a progressively better association with neoplasia (Burja et al., 1999; Lai et al., 2009; Westin et al., 2008; Zardo et al., 2009) and also specifically with endocervical glandular malignancies (Selvaggi, 2016). However, in many cases diagnosed as endocervical cell atypia or malignancy in cytology, the histological sample has only shown evidence of non-neoplastic changes, squamous or endometrial lesions or other mainly gynaecological malignancies (Burja et al., 1999; Geldenhuys et al., 2007; Kim et al., 1999; Kim et al., 2017; Lai et al., 2009; Pradhan et al., 2016; Rabelo-Santos et al., 2008; Schnatz et al., 2006; Zardo et al., 2009; Zhao et al., 2009).

In detecting neoplasia, the sensitivity of a single Pap smear has varied widely, from 15.3% to 100%, which can be explained at least partly by the heterogeneity seen in the studied sample selection (Geldenhuys et al., 2007; Kim et al., 1991; Kim et al., 2017; Krane et al., 2001; Schoolland et al., 2002; Zardo et al., 2009; Zhao et al., 2009). In general, in studies also including Pap smears diagnosed as AEC-NOS, AEM-NOS, AGC-NOS, negative for intraepithelial lesion or malignancy (NILM) and insufficient instead of only smears with possible or definite cytological high-grade features, the sensitivity has been lower. When samples representing only the Bethesda categories AIS and EAC were examined, histological proof of endocervical malignancy was found in 26% to 82% of the cases (Geldenhuys et al., 2007; Lee et al., 1995; Nasu et al., 1999; Westin et al., 2008; Zardo et al., 2009). However, among these cases, other clinically significant lesions were detected in nearly all (Geldenhuys et al., 2007; Lee et al., 1995; Nasu et al., 1999; Westin et al., 2008; Zardo et al., 2009).

Squamous lesions are the most common neoplastic lesions after cytological glandular cell atypia, accounting for up to 77% of the premalignant or malignant cases detected (Burja et al., 1999; Pradhan et al., 2016; Rabelo-Santos et al., 2008; Westin et al., 2008). When only the TBSRCC categories of AIS and EAC have been examined, the relative portion of squamous lesions generally decreases, but they have still been reported to account for 6.3% to 53% of the neoplastic lesions (Geldenhuys et al., 2007; Lee et al., 1995; Nasu et al., 1999; Zardo et al., 2009; Zhao et al., 2009).

When all categories of cytological glandular cell atypia were examined as one group, 41.8% to 79% of the cases showed no histological premalignant or malignant changes (Chen et al., 2008; Kim et al., 1999; Kim et al., 2017; Lai et al., 2009; Lee et al., 1995; Nasu et al., 1993; Pradhan et al., 2016; Rabelo-Santos et al., 2008; Westin et al., 2008). Out of the benign conditions detected, inflammatory changes with or without squamous metaplasia, tubal metaplasia, microglandular hyperplasia and endocervical or endometrial polyps are the most frequently encountered entities in

follow-up histologies (Chen et al., 2008; Nasu et al., 1993; Selvaggi et al., 2016; Zhao et al., 2009).

Of the individual cytomorphological features, feathering is the strongest predictor of endocervical glandular neoplasia in terms of separating them from the squamous lesions and from the benign and reactive conditions of the endocervix (Burja et al., 1999; Rabelo-Santos et al., 2008). Loss of polarity, papillary groups and palisading borders have also been detected more frequently in malignant endocervical lesions than among the benign endocervical changes (Burja et al., 1999; Rabelo-Santos et al., 2008). Likewise, general neoplasia-associated features, including enlarged nuclei, increased nuclei to cytoplasmic ratio and coarsely granular chromatin, have been more common in endocervical neoplastic lesions than among the benign conditions of the endocervix (Burja et al., 1999; Rabelo-Santos et al., 2008).

In addition to feathering, papillary groups, pseudostratified strips, enlarged nuclei and coarsely granular chromatin have been among the cytomorphological features performing the best in differentiating the neoplastic endocervical lesions from the neoplastic squamous lesions (Burja et al., 1999; Rabelo-Santos et al., 2008).

2.4.3 Reproducibility

As discussed above, although the diagnostic criteria for cytological endocervical cell changes are defined by TBSRCC, in practice, classifying glandular lesions according to their severity and separating them from squamous lesions and from the benign and reactive conditions of the cervix has proven to be challenging. In previous studies, the interobserver reproducibility of the TBSRCC endocervical cell categories has mostly been poor, with kappa value (K) variation from 0.002 to 0.36, with the best agreement generally achieved in the more severe categories (Confortini et al., 2006; Lee et al., 2002; Simsir et al., 2003). In differentiating glandular atypias from squamous atypias, the K range, with values from 0.015 to 0.61, has been reported (Lepe et al., 2018; Niu et al., 2019; Moreira et al., 2008). At the level of benign vs any atypia and low-grade atypia vs high-grade atypia, a slightly better consensus has been reached with respective K values ranging from 0.37 to 0.46 and from 0.21 to 0.74 (Confortini et al., 2006; Joste et al., 1999; Lee et al., 2002; Niu et al., 2019; Simsir et al., 2003). No significant differences in reproducibility in recognising glandular and neoplastic features between conventional Pap smears and liquid-based cytology (LBC) samples have been documented (Lee et al., 2002; Moreira et al., 2008).

2.4.4 Cervical cancer screening

Currently, in Finland, women aged 30–60 years are invited to participate in cervical cancer screening every fifth year. In some municipalities, 25- and 65-year-olds are also included in the programme. The primary screening method has traditionally been the conventional Pap smear, but in recent years, primary hrHPV screening has been implemented in some regions for women aged 30 years and over (Anttila et al., 2010; Kares et al., 2019; Kotaniemi-Talonen et al., 2005; Veijalainen et al., 2016; Veijalainen et al., 2019; Veijalainen et al., 2021).

Briefly, according to the national guidelines, if HPV primary screening is applied, all HPV-positive women with NILM or ASC-US and HPV-negative women with ASC-US are referred to a screening control after 12 months. The control included both HPV tests and cytological samples. If the repeated hrHPV test is positive and/or if there is any cytological atypia, the woman is referred for colposcopy. If the hrHPV test is negative and there is no cellular atypia, the patient receives an invitation to the next screening round five years after the original invitation.

In cytology-based screening, women with a cytological diagnosis of ASC-US and those under age 30 with a cytological diagnosis of LSIL are referred to a screening control with a cytological sample after 12 months. If there is any cytological atypia detected in the screening control, the woman is referred to colposcopy. If there is no cellular atypia, the woman receives an invitation to the next screening round five years from the original invitation.

Both in HPV primary screening and in cytology-based screening, all the diagnoses in the TBSRCC endocervical cell category always lead straight to colposcopy, despite the HPV status of the patient. Similarly, LSIL in women over 30 and squamous atypias ASC-H or worse are always referred to colposcopy.

After the colposcopic examination, which usually includes tissue samples, patients are treated according to national guidelines. Despite the colposcopic findings or procedures they lead to, all patients receive an invitation for the next screening round (Käypähoito, 2021).

Many developed high-income countries have adopted similar cytology-based and nowadays even HPV-based screening programmes, although there are differences in screening practices, targeted age groups, screening intervals and follow-up algorithms among countries (Chrysostomou et al., 2018; Elfström et al., 2015; Eun et al., 2020; Farnsworth, 2016; Maver et al., 2020). Most European Union countries are implementing, planning or piloting cervical cancer screening, but fully established population-based programmes have been reported to exist only in a minority of countries (Chrysostomou et al., 2018). Both the invitational coverage and the

participation rates of screening vary between the European countries with the highest coverage achieved in the Nordic countries, the U.K., Netherlands and Italy (Elfström et al., 2015; Salciccioli et al., 2021).

In middle-income countries, the utilisation of screening programmes has generally been suboptimal; in many low-income countries, cervical cancer screening is practically non-existent. This is due to multiple issues, including the lack of public health policies and health care resources and the lack of infrastructure, which are all needed for the implementation of screening and treatment strategies. Even if the necessary health care facilities exist, low public awareness of cervical cancer and other cultural issues are inhibiting females from participating in screening in many low-income countries (Vu et al., 2018).

In earlier studies, hrHPV testing has been more efficient in detecting women with cancer or precancerous lesions than cytology alone (Katki et al., 2011; Naucler et al., 2007; Ogilvie et al., 2018; Ronco et al., 2010; Schiffman et al., 2018; Wright et al., 2015). Particularly with endocervical glandular lesions, the HPV test performed better. According to previous publications, up to 79.0% of EAC and up to 82.2% of AIS were detected by positive hrHPV test in comparison to respective detection rates of 15% to 45.4% and 40% to 53.2% for EAC and AIS seen with cytology (Katki et al., 2011; Schiffman et al., 2018). Additionally, a negative hrHPV test has been shown to predict a benign end result better than a negative cytological sample (Katki et al., 2011; Ogilvie et al., 2018). However, 22% of EAC and about 15% of AIS have been reported to remain negative with both detection methods (Katki et al., 2011; Schiffman et al., 2018).

Many of the earlier publications on the Finnish population similarly report increased detection rates of cancer and precancerous lesions in HPV primary screening triaged with cytology compared with cytology-based screening only (Anttila et al., 2010; Leinonen et al., 2012; Malila et al., 2013). Among women aged 35 years and older, HPV primary screening with cytology triage has also been reported to be more specific in detecting cancer and precursor lesions than cytology (Leinonen et al., 2009).

According to a recent study, nearly one-tenth of HSIL and AIS can remain simultaneously negative with two different commercial hrHPV tests, although HPV DNA is detectable with PCR in lesional tissue (Reich et al., 2020). However, testing hrHPV-positive has been shown to substantially increase the risk of developing EAC (Castellagué et al., 2006). Even up to 14 years before the malignant diagnosis, HPV16 and HPV18 infections detected in cytologically normal specimens have been reported to be associated with significantly increased risks of later developing AIS

and EAC (Dahlström et al., 2010). In addition, among women diagnosed with AGC in screening, the incidence of EAC was markedly higher than among cytologically normal controls until up to 15.5 years (Wang et al., 2016). If there is only mild cytological glandular atypia, the presence of hrHPV infection significantly increases the likelihood of neoplasia (Zeferino et al., 2011). Nevertheless, among younger women, screening with HPV has also been shown to lead to the overdiagnosis of regressive CIN2 lesions (Murphy et al., 2012; Ronco et al., 2010; Wright et al., 2015). Similar data on glandular atypia were not issued.

3 AIMS OF THE STUDY

The aim of the thesis was to find tools to improve the cytological diagnostics of endocervical glandular lesions to improve the early detection of patients harbouring a malignant lesion and requiring immediate intervention and differentiating them from those whose glandular cell atypia is a result of a non-neoplastic process. The specific aims were as follows:

1. To define the first cytomorphological features or combinations of features predicting histological EAC or AIS and to document their development over time (I).

2. To evaluate inter- and intraobserver variability in cervical cytology with a focus on the cytomorphological features of the diagnostic Pap smears of EAC and AIS cases with the best and worst interobserver consensus (II).

3. To assess the outcomes of two consecutive HPV primary cervical cancer screening rounds among patients presenting with cytological endocervical glandular cell atypia in the first screening round (III).

4. To define the most common benign histological lesions behind mild cytological endocervical glandular cell atypia and to find specific cytomorphological features to predict them (IV).

4 STUDY POPULATION, MATERIALS AND METHODS

4.1 Study population and study design

4.1.1 Study I

All available Pap smear samples from 60 patients treated for EAC or AIS at Tampere University Hospital between 2008 and 2014 were blindly analysed in search of 38 cytomorphological features, categorised into background, architectural, cellular and nuclear features (Table 2). The time from cytological sampling to histological confirmation of the EAC or AIS diagnosis was calculated for each smear. HPV status of the patient when available and Pap smear type (screening vs. clinical) were collected.

Table 2. Cytomorphological features analysed in Pap smears.

Background features	Architectural features	Cellular features	Nuclear features
Clean	Scant cellularity	Columnar cell shape	Enlarged nuclei
Bloody	High cellularity	Cuboidal cell shape	Nuclear hyperchromasia
Inflammatory	Single atypical cells	Irregular cell borders	Nuclear membrane irregularity
Inflammatory debris	Loss of honeycomb pattern	Increased nucler/cytoplasmic ratio	Nuclear crowding
Necrotic	Loss of polarity	Degeneration	Oval nuclei
Apoptotic debris	Pseudostratified strips	Regeneration	Elongated nuclei
	Palisading borders	Atrophy	Nuclear pleomorphism
	Rosettes		Nucleoli
	Feathering		Macronucleoli
	Papillary groups		Finely granular chromatin
			Coarsely granular chromatin
			Chromatin clearing
			Nuclear vacuole
			Mitotic figures
			Apoptotic bodies

The Pap smear samples of 30 patients with histologically proven HSIL were used as a control group and investigated for the same 38 cytomorphological features. The

average age of the patients in the EAC/AIS group was 43.0 (SD ± 14.6, range 22–83) and 36.7 (SD ± 10.0, range 21–54) in the control HSIL group.

The EAC/AIS cases with combined squamous lesions were excluded from the statistical analyses. Altogether, the cytomorphological features of 256 Pap smears were analysed in an attempt to define cytomorphological features or combinations of features that would predict histological AIS and EAC. The aim was to define the earliest cytological changes and their development over time.

4.1.2 Study II

Out of the Pap smear samples analysed in Study I, a subgroup of 167 Pap smears was randomly selected. These Pap smears represented samples from 27 patients with EAC, 23 patients with AIS and 28 patients with HSIL. The selected Pap smears included samples in all TBSRCC 2014 categories (Nayar & Wilbur, 2015) from NILM to HSIL, AIS and endometrial adenocarcinoma according to the diagnoses given in routine practice. The average age of the patients in the EAC/AIS group was 43.1 (SD ± 14.4, range 22–83) and 36.3 (SD ± 10.1, range 21–54) in the HSIL group.

Four cytopathologists with at least 20 years of experience in gynaecological cytopathology blindly diagnosed the 167 Pap smears according to the TBSRCC 2014 (Nayar & Wilbur, 2015) to assess interobserver agreement. A couple of months later, a subgroup of 20 Pap smears was re-evaluated by the same cytopathologists for intraobserver agreement. These 20 samples comprised 12 Pap smears with diagnostic agreement of ≥3 observers on the first round and 8 Pap smears with diagnostic disagreement of all observers on the first round. The smears represented 8 cases of EAC or AIS, 6 cases of EAC or AIS in combination with HSIL and 6 cases of HSIL.

Inter- and intraobserver variability was evaluated in four categories: 1) squamous vs glandular, 2) NILM, 3) atypical and 4) preneoplastic/neoplastic. The atypical category included TBSRCC diagnostic categories ASC-US, AEC-NOS and AGC-NOS, which all resulted in a control Pap smear during the time of the study design. The preneoplastic/neoplastic category included TBSRCC diagnostic categories LSIL, HSIL, ASC-H, AEC-FN, AIS and EAC. The original, in the routine practice given cytological diagnoses, were included in the interobserver part of the study and named Observer 0 in the analyses.

In addition, the diagnostic Pap smears of the EAC/AIS group with diagnostic agreement of ≥3 observers (n = 8) and diagnostic disagreement of ≥3 observers (n = 14) were cytomorphologically analysed for typical features.

4.1.3 Study III

In the Pirkanmaa region during the years 2012–2015, a total of 93,439 women aged 35 to 60 were invited to HPV primary screening for cervical cancer. Of these, 66,147 (70.8%) participated. The HPV primary screening sample included both the hrHPV test and the conventional Pap smear. All the Pap smears of the hrHPV-positive women were analysed microscopically. During the 2012–2016 period, 10% of HPV-negative cases were assessed cytologically as part of the laboratory quality assurance programme.

Of the screening participants, 87 (0.13%) presented with AEC-NOS or AEC-FN with or without squamous atypia in their Pap smears. The cohort of this study consisted of 87 women and included their findings in two consecutive cervical cancer screening rounds.

The HPV status of each patient, their Pap smear diagnoses and the findings on the consequent histological samples at each screening round or follow-up visit were retrieved from the LIS of Fimlab Laboratories. An additional LIS search encompassing the study period from 2012–2020 was performed to find the EAC and AIS cases possibly missed by the HPV primary screening. In the analyses, the patients were divided into four groups based on their Pap smear diagnoses during the first screening round. The first group included cases with AEC-NOS only, the second group included cases with AEC-NOS and a simultaneous squamous atypia, the third group included cases with AEC-FN only and the fourth group included cases with AEC-FN and a simultaneous squamous atypia.

4.1.4 Study IV

The study was based on 45 Pap smear samples taken in 2013–2019 and diagnosed with AEC-NOS without simultaneous cytological squamous atypia. Altogether, 30 of the Pap smears represented histologically proven benign changes with a negative follow-up history until the end of 2021, and 15 of the Pap smears represented biopsy-proven EAC or AIS. Of the benign cases, 29 had a follow-up time of five or more years, and one case had a follow-up of four years. Only cases with hrHPV test results were included in the study cohort.

All Pap smears were blindly analysed in search of 38 cytomorphological features consisting of background, architectural, cellular and nuclear features (Table 2). The biopsies originally diagnosed as benign were re-evaluated and assessed for any inflammatory, reactive, metaplastic, hyperplastic or neoplastic histological changes

that could explain the endocervical cell atypia seen in the Pap smears. The association of cytomorphological features with specific histological entities was statistically evaluated.

4.2 Methods

4.2.1 Cytological samples

In Studies I–IV, the cytological samples were conventional Pap smears collected in clinical settings or as part of a cervical cancer screening programme. In Studies I and II, some of the older samples were classified according to the Papanicolaou Classification System. Diagnoses of these samples were converted to corresponding diagnoses according to the TBSRCC 2014 (Nayar & Wilbur, 2015), which was the classification system applied in all four studies.

4.2.2 Histological samples

The histological samples evaluated in Study IV were pre-existing biopsies from routine clinical practice or from follow-up visits to the cervical cancer screening programme. The biopsies were stained with hematoxylin and eosin stains. P16 immunostaining or p16/Ki-67 dual stain was available in seven of the 30 (23.3%) cases in the histologically benign group and in 12 of the 15 (80%) cases in the EAC/AIS group.

4.2.3 HPV test

The HPV samples in Studies I, III and IV were collected simultaneously with Pap smears in clinical settings or as a part of a cervical cancer screening programme. For the hrHPV DNA detection, the Abbot RealTime hrHPV PCR assay (RealTime, Abbot, Wiesbaden, Germany) was used. The test recognises 14 different hrHPV genotypes. Genotypes 16 and 18 were reported separately, and genotypes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 were grouped together and reported as 'hrHPV other than 16 or 18'.

4.2.4 Statistical analyses

In Studies I and II, statistical analysis was performed with the SPSS program, version 25 (IBM SPSS Statistics for Windows, Version 25.0, IBM Corp., Armonk, NY, USA) and in Study IV with SPSS version 28 (IBM SPSS Statistics for Windows, Version 28.0, IBM Corp., Armonk, NY). In Studies I and IV, univariate associations were examined using chi-square or Fisher's exact tests and any P-value < 0.05 was considered significant. To investigate the combination of cytomorphological features predicting EAC and AIS in Study I and benign histotypes in Study IV, a forward stepwise multivariable logistic regression analysis was performed using probability values of < 0.05 for the entry of features. In Study II, Kappa (κ) values were calculated and the strength of association was defined as proposed by Landis and Koch: 1 for perfect agreement, 0.81–0.99 for almost perfect agreement, 0.61–0.80 for substantial agreement, 0.41–0.60 for moderate agreement, 0.21–0.40 for fair agreement, 0–0.20 for slight agreement and < 0 for poor agreement (Landis & Koch, 1997). All statistical analyses were performed by a professional statistician.

4.3 Ethical considerations

Since Studies I–IV were based on pre-existing samples produced by routine clinical practice or by the national cervical cancer screening programme, the studies were conducted without the informed consent of each individual. The studies were approved by the Regional Ethics Committee of the Expert Responsibility Area of Tampere University Hospital (R13094, R16022). The Declaration of Helsinki was followed in the design and performance of the studies.

5 SUMMARY OF THE RESULTS

5.1 Study I

Clinical samples represented 72.6% (146/201) of the Pap smears in the EAC/AIS group while 27.4% (55/201) of the smears were cervical cancer screening samples. Of the screening sample cases, 10.9% (6/55) were hrHPV-positive, representing genotypes HPV16 in two cases, HPV18 in two cases and hrHPV other than HPV16 or HPV18 in two cases. There were no hrHPV-negative cases among patients with EAC or AIS.

The clinical causes leading to clinical Pap smear sampling are summarised in Figure 8. Notably, only 11.0% (16/146) of the clinical Pap smears were taken because of patient-reported symptoms, and 70.0% of the patients in the EAC/AIS group did not report any symptoms during their cervical cytological sampling history. Infection-related issues were the most frequent symptoms reported by the patients (62.5%, 10/16), followed by abnormal uterine bleeding (37.5%, 6/16). Abnormal uterine bleeding was the symptom that best predicted EAC and AIS, since it led straight to a cancer diagnosis in 83.0% of the cases when reported.

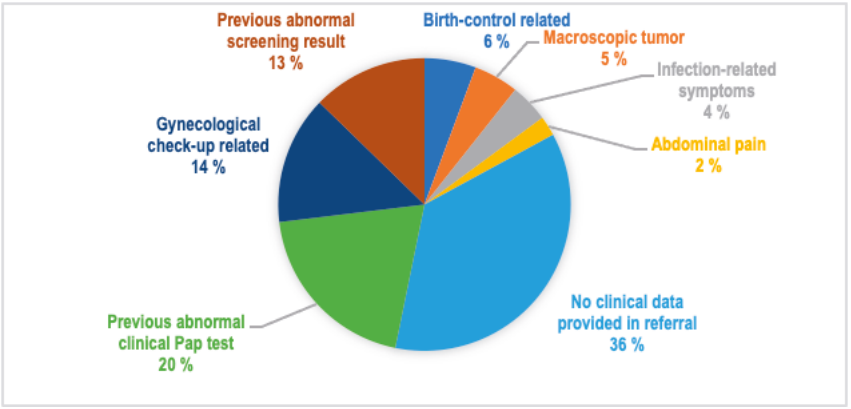

Figure 11. Indications of clinical Pap testing.

In the statistical analysis of the cytomorphological features, none of the single features showed an association with EAC or AIS. Nevertheless, 70% of the neoplastic Pap smears in this group were correctly diagnosed as glandular. In further investigation of cytomorphological features, a combination of palisading cell borders, nuclear pleomorphism and the lack of single atypical cells emerged and showed an association with EAC and AIS, with corresponding odd ratios (OR) of 5.89 (95% confidence interval (CI) 1.96–17.70), 3.71 (95% CI 1.14–12.02) and 10.76 (95% CI 1.20–59.50) and P-values of 0.002, 0.034 and 0.005, respectively. The same trend could be seen in Pap smears even up to five years before the histological diagnosis, with respective ORs of 4.98 (95% CI 1.78–13.88), 3.24 (95% CI 1.09–9.62) and 10.70 (95% CI 2.01–56.89) for palisading cell borders, nuclear pleomorphism and the lack of single atypical cells and corresponding P-values of 0.02, 0.34 and 0.05.

In summary, in Pap smears, the combination of cytomorphological features, including palisading cell borders, nuclear pleomorphism and the lack of single atypical cells, was the strongest predictor of histological EAC and AIS.

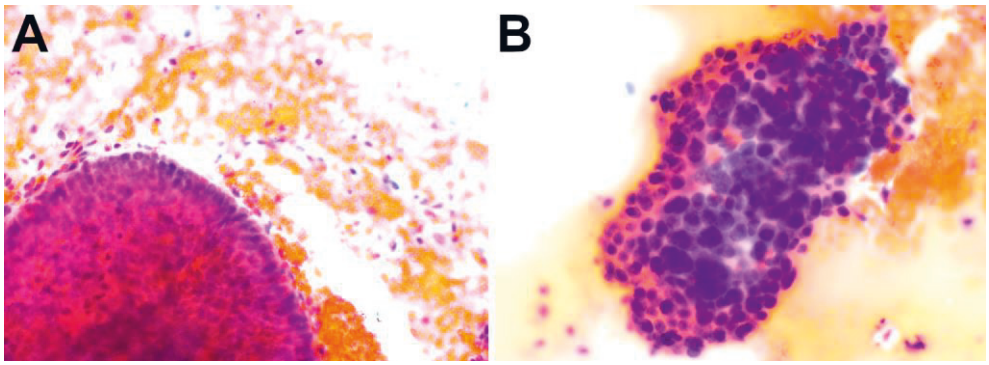

Figure 12. Combination of palisading cell borders (A), nuclear pleomorphism (B) and lack of single atypical cells in Pap smears predicts EAC and AIS. Papanicolaou stain, magnification 40x (A) and 40x (B).

5.2 Study II

Agreement among three observers was reached in 46.5% (82/167) of the evaluated Pap smears of which nearly half (39/82, 47.5%) represented samples categorised as NILM originally. Combinations of squamous and glandular diagnoses represented almost 10% of all diagnoses given by the cytopathologists. When only one of the diagnoses was taken into account in these cases, a consensus of three observers was reached in 62.3% (104/167) of the cases. Among the diagnostic Pap smears representing histological EAC or AIS without combined squamous lesions, a consensus diagnosis of ≥ 3 observers was reached in 87.5% (35/40) of the cases. All the diagnoses given by the observers were AEC-FN, HSIL or worse. In addition, 92.5% (37/40) of the diagnoses were glandular.

In the interobserver part of the study, a huge variation in agreement between the different observer pairs was seen, with κ-value variation from poor to substantial (Table 3). The highest overall κ-value, 0.412, was reached in recognising preneoplastic/neoplastic cytological features. In the category of squamous vs glandular, substantial agreement was reached in five out of six interobserver analyses among observer pairs 0, 2, 3 and 4. However, Observer 1 showed only poor to slight agreement with the other observers, which led to an overall κ-value of 0.314.

In addition, the categories NILM and atypical proved to be problematic. The overall κ-value was 0.272 in the NILM category and only 0.082 in the atypical category.

In the intraobserver part of the study, a stronger consensus was reached in all categories. The only substantial overall κ-value in the study ($\kappa = 0.616$) was reached in this part of the study, in the squamous vs glandular category. In the preneoplastic/neoplastic category, the overall κ-value was 0.491 and in the NILM and atypical categories, it was 0.345 and 0.241, respectively.

Table 3. The interobserver kappa values in the categories of recognising negative, atypical, preneoplastic/neoplastic and squamous versus glandular features in Pap smears. Modified from Pulkkinen et al., 2022.

Kappa values between observer pairs in recognizing NILM samples					
	Observer 1	Observer 2	Observer 3	Observer 4	Overall κ
Observer 0	-0.018	0.633	0.357	0.444	
Observer 1		0.036	0.035	-0.047	
Observer 2			0.474	0.469	
Observer 3				0.159	
					0.272
Kappa values between observer pairs in recognizing atypia*					
	Observer 1	Observer 2	Observer 3	Observer 4	Overall κ
Observer 0	-0.050	0.203	-0.088	0.169	
Observer 1		0.072	0.200	0.080	
Observer 2			0.092	0.259	
Observer 3				-0.037	
					0.082
Kappa values between observer pairs in recognizing prenoplasia/neoplasia**					
	Observer 1	Observer 2	Observer 3	Observer 4	Overall κ
Observer 0	0.229	0.456	0.400	0.379	
Observer 1		0.376	0.356	0.351	
Observer 2			0.483	0.536	
Observer 3				0.496	
					0.412
Kappa values between observer pairs in differentiating atypical or preneoplastic/neoplastic squamous and glandular features					
	Observer 1	Observer 2	Observer 3	Observer 4	Overall κ
Observer 0	-0.073	0.503	0.620	0.613	
Observer 1		-0.062	-0.027	0.052	
Observer 2			0.689	0.631	
Observer 3				0.664	
					0.314

* Includes diagnoses ASC-US, AEC-NOS and AGC-NOS
** Includes diagnoses LSIL, HSIL, ASC-H, AEC-FN, AIS and EAC

In the descriptive analysis of the cytomorphological features of the diagnostic EAC/AIS Pap smears with agreement of ≥ 3 observers, nuclear enlargement was seen in all (8/8) samples. Nuclear enlargement > 2 x normal size was encountered in 87.5% (7/8) of the smears, nuclear pleomorphism in 75% (6/8) of the smears and crowded fragments with scant cellular cytoplasm and nuclear stratification in 62.5% (5/8) of the smears. In addition, at least one of the architectural features previously described as being associated with EAC or AIS (rosettes, feathering, palisading cell borders or papillary groups; Burja et al., 1999; Rabelo-Santos et al., 2008) was present in all (8/8) samples.

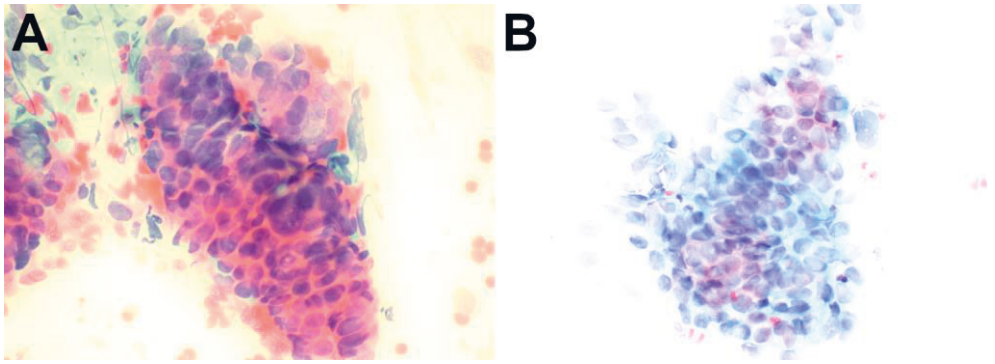

Figure 13. In diagnostic EAC and AIS Pap smears, nuclear enlargement, nuclear pleomorphism and crowded fragments with scant cellular cytoplasm and nuclear stratification were the most common features in smears, with good interobserver agreement of the neoplastic nature and of the endocervical cell origin of the cellular changes (A). Lack of nuclear enlargement and degenerative changes were the most common features explaining the diagnostic discrepancy (B). Papanicolaou stain, magnification 40x (A) and 40x (B).

Among the diagnostic EAC/AIS Pap smears with diagnostic disagreement among ≥ 3 observers, the lack of nuclear enlargement > 2x normal size, which was encountered in 92.8% (13/14) of the smears, was the most common feature explaining the diagnostic discrepancy. Among these smears, degenerative changes occurred more frequently (62.5% vs 25%).

In summary, the reproducibility of the preneoplastic/neoplastic cytological diagnoses was better than the reproducibility of the diagnoses in the atypical and NILM categories in both the inter- and intraobserver parts of the study. The intraobserver reproducibility was higher in all evaluated categories compared to the respective interobserver values. Nuclear enlargement, nuclear pleomorphism and

crowded fragments with scant cellular cytoplasm and nuclear stratification were the most common cytomorphological features encountered in Pap smears with good interobserver agreement of the neoplastic nature and of the endocervical cell origin of the cellular changes. Lack of nuclear enlargement and degeneration were the most common features causing interobserver disagreement.

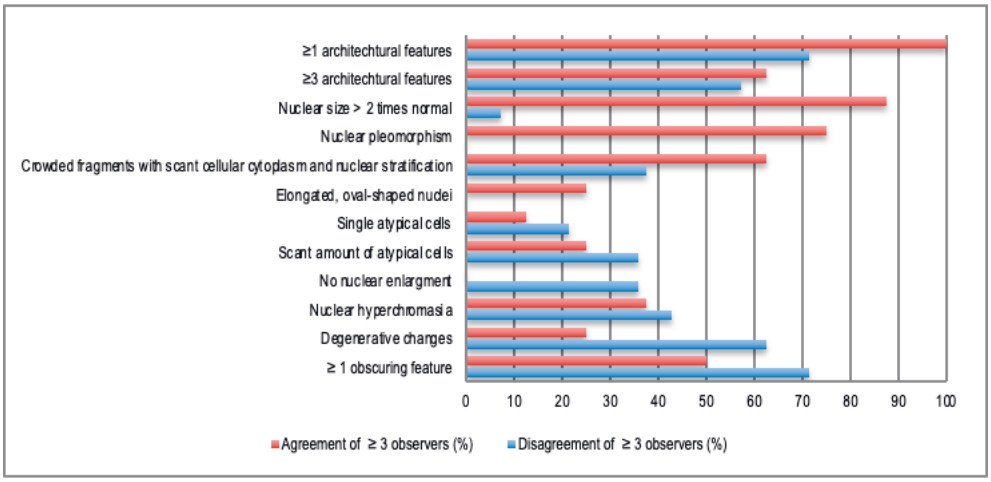

Figure 14. The most common cytomorphological features observed in diagnostic Pap smears of histologically verified EAC and AIS cases with diagnostic agreement of ≥ 3 observers and diagnostic disagreement of ≥ 3 observers. Reprinted from Pulkkinen et al., 2022.

5.3 Study III

In the first screening round, 60.7% (37/61) of the AEC-NOS-only cases were hrHPV-positive and resulted in one AIS and six HSIL in the histological follow-ups. At the second screening round, 5 years and 9 months after the initial diagnosis of glandular atypia, one of the HSIL cases was diagnosed with an additional AIS. There were no histological high-grade lesions among the hrHPV-negative cases.

Among the cases with AEC-NOS and squamous atypia, only one hrHPV-negative case was diagnosed as AEC-NOS + ASC-US. No neoplastic lesions were found in the follow-ups of the case. The remaining 94.1% (16/17) of the cases presented with hrHPV positivity. In the follow-ups of hrHPV-positive AEC-NOS + ASC-US cases (n = 5), two histological HSILs were diagnosed, and in the follow-ups of AEC-NOS + HSIL/ASC-H cases (n = 9), four histological HSIL and two combinations of histological EAC and LSIL were found. Among the cases with

AEC-NOS + LSIL (n = 2), no histological high-grade lesions were encountered. During the second screening round, no additional high-grade histological lesions were diagnosed.

HrHPV was positive in all cases of AEC-FN only (n = 7) and AEC-FN + LSIL (n = 2). There were no cases diagnosed with AEC-FN + ASC-US or AEC-FN + HSIL/ASC-H. In the AEC-FN-only group, two HSIL and one EAC were histologically diagnosed during the first screening round and an additional AIS was found during the second screening round. The diagnosis of the AIS case was reached 6 years and 11 months after the initial cytological diagnosis of AEC-FN. Among the AEC-FN + LSIL cases, no high-grade histological lesions were found during the two screening rounds.

Table 4. The high-grade histological lesions detected on two consecutive screening rounds in 2012–2015 and 2017–2020, including the initial cytological diagnoses of the lesions and the hrHPV genotypes. Modified from Pulkkinen et al., 2021.

Histological lesion	Cytological diagnosis	HPV genotype*
HSIL	AEC-NOS	other
HSIL	AEC-NOS	other
HSIL	AEC-NOS	other
HSIL	AEC-NOS	other
HSIL	AEC-NOS	16
HSIL	AEC-NOS + ASC-US	other
HSIL	AEC-NOS + ASC, US	16, other
HSIL	AEC-NOS + ASC-H	16
HSIL	AEC-NOS + ASC-H	16, 18
HSIL	AEC-NOS +HSIL	16, other
HSIL	AEC-NOS + HSIL	16
HSIL	AEC-FN	16
HSIL	AEC-FN	other
HSIL, later AIS	AEC-NOS	16, 18, other
AIS	AEC-NOS	16
AIS	AEC-FN	18
EAC + LSIL	AEC-NOS +HSIL	16
EAC+ LSIL	AEC-NOS + HSIL	18
EAC	AEC-FN	16

* In the screening, hrHPV genotypes 16 and 18 were reported separately and the remaining 14 genotypes recognised by The Abbot RealTime hrHPV PCR assay were reported as 'other hrHPV than HPV16 or HPV18'.

In summary, the high-grade lesions detected in histology during the two consecutive screening rounds were three EAC, two AIS, one AIS + HSIL and 13 HSIL, concluding that 68.4 % (13/19) of the lesions were purely of squamous origin. In 53.8% (7/13) of the histological HSIL-only cases, the primary cytological diagnosis in screening was glandular only, without a squamous component. Of the EAC and AIS cases, 33.3% (2/6) were HPV18 positive and 66.7% (4/6) HPV 16 positive. Of the histological HSIL cases, 42.9% were positive with an hrHPV genotype other than HPV16 or HPV18. Statistically, HPV genotypes (16, 18 or other) or combinations of them showed no prediction specifically for endocervical malignancies or squamous lesions, which might have been due to the small number of positive cases in the series. There were no histological high-grade lesions among the hrHPV-negative cases. No endometrial malignancies were found in the study cohort.

The database search revealed one hrHPV-negative gastric-type mucinous EAC missed by the HPV primary screening.

The study revealed a relatively high drop-out rate from the screening protocol, since during the first screening round, 17.5% (11/63) of the hrHPV-positive patients and 29.2% (7/24) of the hrHPV-negative patients did not attend their follow-ups. Of the originally hrHPV-positive women, 38.1% (24/63) did not attend the next screening round or follow-up. In all, 25.4% (16/63) of the originally hrHPV-positive patients dropped out of the screening protocol, with a persistent hrHPV infection at their last visit.

5.4 Study IV

In the study group comprising AEC-NOS Pap smears with only histologically proven benign changes and a negative follow-up history, 66.7% (20/30) of the patients were hrHPV-negative at the study baseline. The hrHPV-positive cases represented infections with HPV16 (n = 1), HPV18 (n = 2), and hrHPV other than 16 or 18 (n = 7). All hrHPV-positive cases at baseline turned negative during the study period. The AEC-NOS patients in the EAC/AIS group were all hrHPV-positive, with genotypes HPV16 and 18 accounting for 73.3% (11/15) of the cases.

The most common histological findings among the benign group were squamous metaplasia (22/30, 73.3%), significant mixed inflammation (16/30, 53.3%), tubal metaplasia (10/30, 33.3%) and microglandular hyperplasia (6/30, 20.0%). Significant mixed inflammation and squamous metaplastic changes were often encountered in the same specimen and were sometimes associated with mucosal surface ulceration.

Reserve cell hyperplasia, eosinophilic change, granulation tissue and follicular inflammation were seen in only a few cases. There were no polyps, premalignant or malignant lesions in any of the histological follow-up samples.

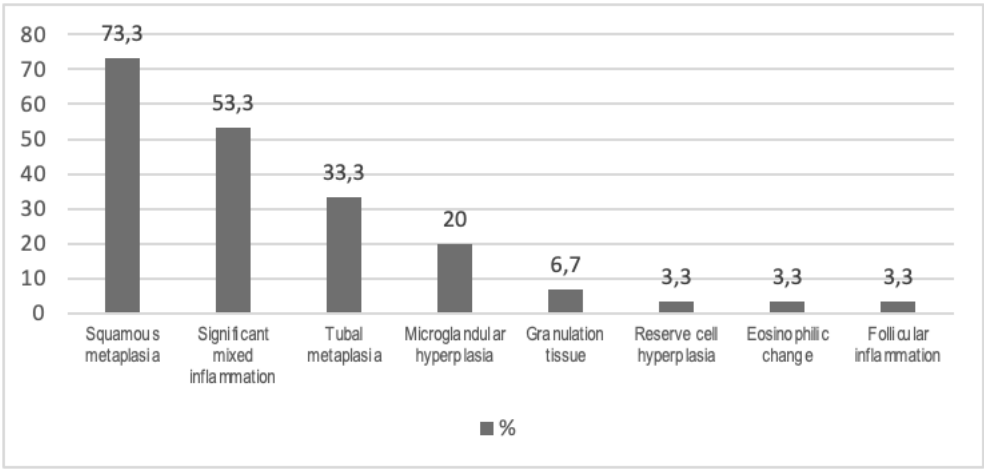

Figure 15. The histological lesions among benign-proven AEC-NOS cases. Squamous metaplasia and significant mixed inflammation were often encountered in the same histological specimen.

In the statistical analysis of cytomorphological features, inflammatory background in Pap smears was associated with significant mixed inflammation in histology (48.4% vs. 14.3%, P = 0.029). Nuclear crowding and nuclear elongation were more frequent in Pap smears of patients who had no significant inflammation in their follow-up biopsies (75.9% vs 37.5%, P = 0.01 and 42.9% vs 9.7%, P = 0.017). Nuclear elongation was also associated with microglandular hyperplasia (66.7% vs 12.8%, P = 0.010). A finely granular chromatin pattern was more commonly seen in immature squamous metaplasia than in other histological findings (20.0% vs 0%, P = 0.032). In contrast, degeneration was more frequently recognised in Pap smears without squamous metaplasia or immature squamous metaplasia in the follow-up histology (52.2% vs 19.0%, P = 0.015 and 50.0% vs 13.3%, P = 0.017). Overall, scant cellularity in Pap smears was associated with histological findings of granulation tissue formation (50.0% vs 0%, P = 0.044). In addition, granulation tissue in histology was associated with palisading cell borders seen in cytology (100% vs 16.3%, P = 0.036).

In summary, none of the manifested cytomorphological features were specific enough to be diagnostic or strong enough to confidently leave the cytological atypia without histological confirmation. Further statistical analysis of the features did not show any combinations of features suggestive of a specific benign entity. In separating the benign AEC-NOS Pap smears from those representing EAC or AIS, degeneration and nuclear crowding were the best cytomorphological features (26.7% vs 60.0%, P = 0.030 and 50.0% vs 86.7%, P = 0.017) to distinguish them.

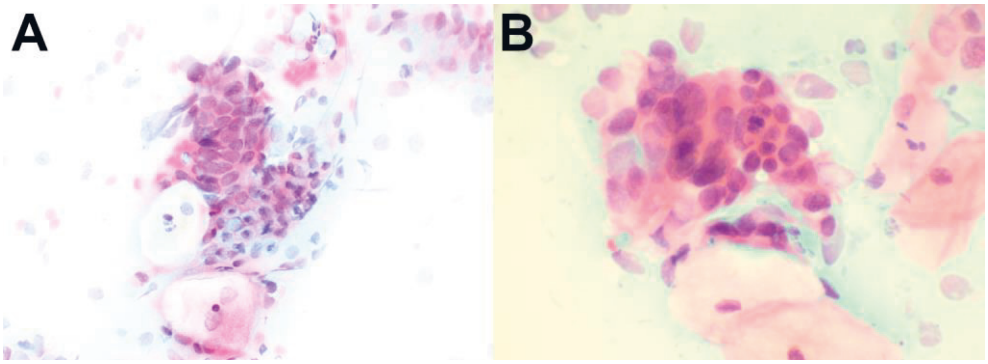

Figure 16. Nuclear crowding (A) and degenerative changes (B) were more common among AEC-NOS Pap smears representing histological EAC or AIS than among the benign-verified cases. Papanicolaou stain, magnification 40x (A) and 60x (B).

6 DISCUSSION

6.1 Study I

In our study, there was a simultaneous squamous lesion in histology in 25.5% of the EAC/AIS cases, which is not surprising since most cervical glandular and squamous cell neoplasias share the same hrHPV-related etiology (Pirog et al., 2014; de Sanjose et al., 2010; Walboomers et al., 1999). None of the 38 cytomorphological features investigated in our study showed an association specifically with EAC or AIS when evaluated alone. Even so, pathologists were often able to differentiate the neoplastic squamous lesions from the neoplastic glandular ones in Pap smears. In the diagnostic Pap smears of the histologically confirmed HSIL-only cases, the cytological diagnoses given were of squamous origin in all cases. Among the histologically proven EAC- or AIS-only cases, the given neoplastic cytological diagnoses were glandular in 70% of the cases.

In the further analysis of the cytomorphological features, a combination of palisading cell borders, nuclear pleomorphism and the lack of single atypical cells showed an association with EAC and AIS that could be seen in Pap smears up to five years before the histological diagnosis.

Similar findings regarding the combination of several cytological features associated with endocervical malignancies instead of isolated features alone have also been published previously (Conrad et al., 2018; Mariani et al., 2014). In agreement with our findings, nuclear pleomorphism was among the combination of these features in a study by Mariani et al. (2014). In addition, nuclear enlargement, increased nuclear-to-cytoplasmic ratio and cells occurring in sheets and strips with cell crowding and nuclear overlap were described (Mariani et al., 2014). In the study by Conrad et al. (2018), a combination of abundant tumour cellularity, nuclear size from 3 to 6 times the normal, abundant 3-dimensional tumour cell groups, round cell shape and cytoplasmic neutrophils were reported to predict a correct diagnosis of EAC.

In earlier descriptive analyses of the features associated with EAC or AIS, both nuclear pleomorphism and the rarity of single atypical cells were mentioned (Nasu et al., 1993; Lee et al., 1997). Palisading cell borders have also been found to be

among the features that predict glandular neoplasia (Burja et al., 1999; Nasu et al., 1993).

In summary, the cytological features reported to predict EAC and AIS in the earlier literature are plentiful and, in some areas, even controversial. The most frequently recurring findings seem to be the general neoplasia-associated features, including marked nuclear enlargement or nuclear pleomorphism, changes in the nuclear-to-cytoplasmic ratio, cell crowding and nuclear piling or stratification, which are most often encountered in association with at least one of the architectural features, including rosettes, feathering, palisading cell borders or papillary groups (Burja et al., 1999; Conrad et al., 2018; Lee et al., 1995; Lee et al., 1997; Mariani et al., 2014; Nasu, et al., 1993; Raab et al., 1995; Rabelo-Santos et al., 2008; Torres et al., 2005). In separating the glandular lesions from the squamous lesions, papillary groups, feathering and palisading borders have been among the best features, as have decreased cytoplasm and irregular nuclear membranes (Burja et al., 1999; Rabelo-Santos et al., 2008; Torres et al., 2005).

During the collection of the study samples, Pap smears of patients diagnosed with AEC-NOS were followed by only a control Pap smear instead of the current policy of colposcopy, which practically always includes histological sampling (Käypähoito, 2021). In our study, AEC-NOS was reported in 17 EAC/AIS-only cases with a wide time range of 0–121 months before the histological confirmation of the neoplastic diagnosis. When considering the evolution of the cytomorphological features, if there are no follow-up biopsies, it can only be speculated that the prediagnostic Pap smears already harboured a significant clinical lesion.

Because of the location of the endocervical cell malignancies, reaching the correct diagnosis can be challenging, even if a colposcopy is performed. According to previous findings, at worst, the sensitivity of a colposcopy in detecting endocervical lesions has been as low as 9.8%, and the probability of a significant lesion after a normal colposcopy finding is as high as 87.5% (Costa et al., 2007; Ullal et al., 2009).

Reflex hrHPV testing in AEC-NOS cases has been shown to be useful in selecting the right patients for colposcopy and histological sampling (Chen et al., 2008). Most importantly, hrHPV negativity has been reported to have a high negative predictive value in AEC-NOS cases as well as in the follow-up of conservatively treated AIS cases (Chen et al., 2008; Costa et al., 2007). On the other hand, among older women, a large proportion of malignancies behind cytological glandular atypias are of endometrial origin, and in their diagnostics, hrHPV testing is not helpful (Kim et al., 2017; Pradhan et al., 2016; Zhao et al., 2009).

Finland has a national cervical cancer screening programme available for women aged 30 to 60. Additionally, in some municipalities, women aged 25 and/or 65 years are tested. Participation in even a single screening at the age of 30 or later has been shown to be associated with a reduced cervical cancer risk, with the highest protective effects in the older age groups (Lönnberg et al., 2012). In addition to the cervical cancer screening programme, opportunistic screening is performed; according to a previous study, this comprises 60% of the samples taken for screening purposes and is responsible for 71% of the total screening costs annually (Salo et al., 2014).

In addition, in our study, 72.6% of the Pap smears of the EAC/AIS cases represented clinical samples and only 27.4% were screening programme samples. However, in 45% of the EAC/AIS cases, the screening Pap smear led to biopsies or follow-ups, which eventually led to the diagnosis of glandular neoplasia. Notably, in our study, only 11% of the clinical Pap smears were taken because of a symptom reported by the patient.

6.1.1 Strengths and limitations

The investigation of the cytological features was blinded, which may be considered a strength of the study. The association of cytomorphological features with EAC and AIS histology was evaluated statistically instead of only descriptively. Furthermore, the statistical analyses were performed by a professional statistician. To the best of our knowledge, this is the first study in which the development of cytomorphological features predicting EAC and AIS has been investigated in relation to time.

Since no liquid-based specimens were available, the cytomorphological analysis included only conventional Pap smears, which can be seen as a limitation. In addition, some of the evaluated Pap smears were old, with suboptimal preservation possibly interfering with the interpretation of the cytomorphological features; this may have influenced the results.

6.2 Study II

In the current TBSRCC 2014, there are five diagnostic categories for squamous cell lesions and a total of 10 diagnostic categories for glandular cell lesions in addition to the diagnoses “Insufficient” and “NILM” (Nayar & Wilbur, 2015). In practice, the

combination of squamous and glandular diagnoses is common; this was also seen in our study, since our observers used 26 different diagnoses or combinations of diagnoses to describe the findings in 167 Pap smears. For statistical purposes, all the components of the diagnoses had to match exactly for the diagnoses to be considered equal. In our study analyses, the large number of allowed diagnostic options probably accounted for some of the diagnostic diversity seen in comparison to studies with fewer diagnostic categories to choose from (Confortini et al., 2016; Joste et al., 1996; Lepe et al., 2018; Simsir et al., 2003). This likely contributed to the fact that exact agreement was reached in only 47.5% of the Pap smears, which is lower than the 55.1% and 62.8% reported earlier in the Bethesda Interobserver Reproducibility study (BIRST) and in the BIRST-2 (Kurtycz et al., 2017; Sherman et al., 2007). In BIRST, the agreement was seen to improve from 55.1% to 82.3% when evaluation was performed only at the level of negative vs non-negative (Sherman et al., 2007).

In general, the reproducibility of diagnoses in the squamous cell and NILM categories has been fairly good and much better than the 33% agreement achieved in the glandular cell category in BIRST-1 (Kurtycz et al., 2017). In our study, compared to some previous studies, cytological squamous and glandular features were well recognised, with half of the observer pairs and half of the observers in the intraobserver part of the study reaching substantial $\varkappa$- values (Lepe et al., 2018; Moreira et al., 2008). In our study, the variation between the observer pairs was much wider than in the other studies, which led to only fair overall agreement in comparison to the ranges reported earlier (Lepe et al., 2018; Moreira et al., 2008; Niu et al., 2019).

In the preneoplastic/neoplastic category, our results were also in line with some of the previously published studies with moderate overall interobserver and intraobserver reproducibility (Lee et al., 2002; Lepe et al., 2018). The highest interobserver $\varkappa$- value of 0.67 in this category was encountered in a setting of histological AIS cases and their Pap smears from the year preceding the histological diagnosis (Niu et al., 2019). In turn, the best intraobserver values, from 0.63 to 0.74 for high-grade lesions, were described in a setting of LSIL vs HSIL (Joste et al., 1996).

In our study, the Pap smears diagnosed as ASC-US or AEC-NOS were grouped together since, during the study design according to the Finnish national guidelines, these diagnoses led only to a control Pap smear instead of colposcopy and histological sampling. The interobserver agreement in this category was simply poor and even slightly worse than that described by others (Lee et al., 2002). Also, in terms

of intraobserver agreement, only a fair level of reproducibility was reached in this category.

Likewise, in the category of NILM samples in our study, there was a large variation between the observer pairs ranging from negative to substantial $\varkappa$- values and leading to a fair overall agreement that was lower than the fair or moderate level of reproducibility achieved in earlier studies (Confortini et al., 2006; Simsir et al., 2003). In our study, reproducibility was only fair also in terms of intraobserver agreement.

Similarly, only a fair level of interobserver agreement has been described on cervical histological samples when the evaluation of the severity of the lesion is based on haematoxylin eosin-stained slides only (Ismail et al., 1989; Hodgson et al., 2019; McGluggage et al., 1998). As seen with Pap smears, the agreement on more severe lesions has been better than the reproducibility of less severe diagnoses (Grenko et al., 2000; McGluggage et al., 1998). In addition, when the macroscopic characteristics of atypical transformation zones have been investigated, the agreement between colposcopists has shown variation from poor to substantial, depending on the feature evaluated (Sellors et al., 1990; Vallikad et al., 2017). Thus, at all levels in the diagnostics of cervical lesions, interobserver variability must be addressed.

The generally only fair-to-moderate level of interobserver agreement achieved is not unique for cervical samples. Similar findings, including cytological diagnoses, have been reported for other organs, such as the salivary gland and breast (Layfield et al., 2020; Viswanathan et al., 2020).

However, when dealing with cervical cytological samples, the most important issue is to identify the cases requiring immediate intervention or follow-up from those that do not. In this sense, it is not crucial whether an atypia or a preneoplastic/neoplastic finding in cytology is deemed squamous or glandular if the follow-up is the same. Recognising glandular atypia, however, can guide the clinician to pay special attention to the endocervical canal and its sampling.

In the same vein, it is reassuring that the preneoplastic/neoplastic diagnoses are the ones for which cytopathologists have the best agreement. The NILM and the diagnoses in the atypical group are more challenging. The low reproducibility of these diagnoses presumably leads to some unnecessary follow-ups and diagnostic procedures and probably also to some missed malignancies.

In our study, the intraobserver reproducibility was higher in all evaluated categories in comparison to the values reached in the interobserver part of the study. Since the number of the evaluated Pap smears in the first round was quite high and there was a time interval of couple of months between the two rounds, we find it

unlikely that observers would have been able to remember the cases they had reviewed or the diagnoses they had given.

In the morphological analysis of the diagnostic Pap smears of EAC and AIS patients, nuclear size > 2 times the normal and nuclear pleomorphism were the most common features observed in smears correctly diagnosed as a glandular neoplasia, with good agreement on the specific diagnosis among observers. Marked nuclear enlargement or nuclear pleomorphism have also been among the combination of features associated with EAC and AIS in previous studies and as a single feature described in association with glandular neoplasia (Conrad et al., 2018; Lee et al., 1995; Lee et al., 1997; Mariani et al., 2014; Pulkkinen et al., 2021; Rabelo-Santos et al., 2008; Torres et al., 2005). In our study, crowded fragments with scant cellular cytoplasm and at least one architectural feature (rosettes, feathering, palisading cell borders or papillary groups) were also more commonly encountered among samples with good diagnostic agreement. Nuclear crowding, with or without changes in the nuclear-to-cytoplasmic ratio, has also been described in association with glandular malignancies by others (Lee et al., 1995; Lee et al., 1997; Mariani et al., 2014). In addition, in all previous descriptive analyses, at least one of the architectural features was encountered in association with AIS or EAC (Nasu et al., 1993; Lee et al., 1995; Lee et al., 1997). Of these features, the best predictors of glandular neoplasia have been shown to be papillary groups, feathering and palisading borders (Burja et al., 1999; Rabelo-Santos et al., 2008).

In our study, degeneration was among the two features obscuring the diagnosis of glandular neoplasia, a finding published earlier by others (Lee et al., 1995). The lack of enlarged nuclei was also more commonly encountered in smears with poor interobserver agreement in our study.

In summary, in cervical cytology, the diagnostic reproducibility is variable throughout the diagnostic categories and substantial levels of agreement are only occasionally reached. Agreement on severe cytological changes is generally better than the reproducibility of milder atypias and negative samples. In our study, the general neoplasia-associated features of marked nuclear enlargement and nuclear pleomorphism were the most frequent features in the EAC and AIS Pap smears, with good consensus of the neoplastic nature and of endocervical glandular origin of the lesion. In addition, at least one architectural feature (rosettes, feathering, palisading cell borders or papillary groups) was present in all these samples. In turn, degeneration and the lack of enlarged nuclei were the most common features obscuring the diagnosis of endocervical glandular neoplasia.

6.2.1 Strengths and limitations

The sample size was relatively large, with 167 Pap smears analysed independently by four observers in the interobserver part of the study. Both the sample size and the number of observers can be seen as strengths of the study. Additionally, a subgroup of 20 samples was later re-evaluated by the same observers, which allowed the intraoberver analysis to be performed with several observers. In the morphological analysis of the diagnostic Pap smears of the EAC and AIS cases with good and poor interobserver consensus, the cytological features described were retrieved from the materials of Study I. Thus, the Pap smears were investigated blinded, which is an additional strength in evaluating the significance of the findings. The sample size was small, though; therefore, only a descriptive analysis of features was performed.

The study comprised only conventional Pap smears without any LBC preparations. Some of the smears were old, with suboptimal preservation possibly interfering with the interpretation of the cytological features and the classification of the samples. This might have influenced the end results. For the analyses, we did not have a cytological reference diagnosis, but instead used the majority of agreed diagnoses of three observers.

There was a change in the national guidelines during the study period, as a result of which the AEC-NOS cases were also sent for colposcopy instead of taking only a control Pap smear (Käypähoito, 2021). Therefore, some of the investigated categories no longer correspond to clinical management, which can be seen as a flaw.

In the statistical analysis, we found that one of the observers consistently agreed less frequently with the others. The observer in question participated in the study design and knew that the histological AIS and EAC cases were overrepresented in comparison to HSIL cases and that there were no samples with a benign final histology included. This can be seen as a limitation, since the knowledge of the number of final diagnoses might have influenced the observer's interpretation of the cytological features in favour of glandular diagnoses and malignancies.

6.3 Study III

In this study, no endocervical malignancies were found among patients with cytological endocervical cell atypia and a negative hrHPV result, which mirrors the fact that most endocervical adenocarcinomas are associated with hrHPV infection (Moljin et al., 2016; Pirog et al., 2014; de Sanjose et al., 2010). There were no cases with HSIL or SCC, either supporting the earlier finding that most of the mild

cytological glandular atypias, in fact, represent benign histological changes or only histological LSIL-level changes (Burja et al., 1999; Kawano et al., 2020; Kim et al., 2017; Lee et al., 1995; Polat et al., 2021; Pradhan et al., 2016; Torres et al., 2005).

Endocervical glandular atypia was reported in 0.13% of Pap smears, which is in the same range as the 0.1–1.84% described by others (Ajit et al., 2013; Burja et al., 1999; Chen et al., 2008; Lai et al., 2008; Lee et al., 1995; Kim et al., 2017; Nasu et al., 1993; Pradhan et al., 2016; Selvaggi et al., 2016; Zhao et al., 2009). Of the AEC-NOS Pap smears, 37.3% were hrHPV-negative, which is significantly less than the 79.8% reported in an earlier study (Chen et al., 2008).

Of the high-grade histological lesions detected among the AEC cases with or without combined squamous atypia, 68.4% were purely of squamous origin; this is in the same range as the 37% to 77% reported earlier (Hare et al., 2003; Nasu et al., 1993; Rabelo-Santos et al., 2008).

The high proportion of squamous lesions might be explained by the extension of HSIL to endocervical glands, which is known to be a common cause of false positive cytological glandular diagnoses and is reported in up to 63% of HSIL specimens originally diagnosed as atypical glandular cells (AGUS) in cytology (Kumar et al., 2009; Selvaggi, 1994; Selvaggi, 2002). The difficulty in correctly categorising mild cytological changes in particular is also a well-recognised problem, and it is probably partly responsible for our findings (Confortini et al., 2006; Lee et al., 2002; Lepe et al., 2018; Moreira et al., 2008; Pulkkinen et al., 2022; Simsir et al., 2003).

The hrHPV test has been proven to be positive for cancer and precancerous lesions more often than the simultaneously taken cytology is abnormal; the difference in performance has been more pronounced among cases subsequently diagnosed with AIS or EAC than among CIN3 or SCC cases (Katki et al., 2011; Schiffman et al., 2018; Wright et al., 2015). As reported by most previous studies, in addition to detecting more SCC precursors, the implementation of hrHPV testing has also led to the earlier detection of SCC precursors, as well as to reduced numbers of CIN3 lesions and invasive carcinomas later at follow-up in comparison to cytology alone (Horn et al., 2019; Kitchener et al., 2009; Murphy et al., 2012; Naucler et al., 2007; Ogilvie et al., 2018; Rijkaart et al., 2012; Ronco et al., 2010; Ronco et al., 2014). Among cases screened with cytology only, a high percentage of the invasive cancers detected later were adenocarcinomas (Ronco et al., 2010). In addition, normal findings in cytological screening have been shown to be associated with a significantly lower risk reduction rate for EAC than for SCC in comparison to unscreened women (Wang et al., 2020). On the other hand, a negative hrHPV test result in screening has been shown to predict a negative outcome better than

cytology (Gauge et al., 2014; Horn et al., 2019; Katki et al., 2011; Kitchener et al., 2011; Ogilvie et al., 2018); this was also conversely seen in our study since there were no high-grade histological lesions among the hrHPV-negative cases.

In our study, only two neoplastic lesions were diagnosed in the second screening round. Both lesions were glandular and represented AIS. The time lag from the initial hrHPV positivity and the cytological glandular atypia seen at the study baseline was 5 years and 9 months for the first case and 6 years and 11 months for the second case. Similar reports of cytological AGUS diagnosed with AIS or EAC after a long follow-up period of up to 8 years suggest either a long evolution of the clinically detectable lesions or mirroring the difficulties in their diagnosis, both in cytology and in clinical practice (Boddington et al., 1976; Kim et al., 2017; Soofer et al., 2000).

A database search covering the study period of 2014–2021 revealed one hrHPV-negative mucinous gastric-type adenocarcinoma missed by the primary HPV screening. The patient was hrHPV-negative at screening and therefore the Pap smear was not investigated, and no follow-ups were scheduled. The carcinoma was diagnosed two years later at a gynaecological check-up scheduled for other reasons. It can only be speculated that including a Pap smear in the screening protocol would have led to an earlier diagnosis in this case since up to 22% of EAC and about 15% of AIS have been proven to be negative both in cytology and in the hrHPV test (Austin et al., 2018; Katki et al., 2011; Schiffman et al., 2018). Adding a cytological sample to hrHPV screening would increase the total costs and, based on previous calculations, would lead to an earlier detection of at most five cases per million women in one year (Schiffman et al., 2018). In any case, mucinous gastric-type adenocarcinomas are known for their blunt cytomorphology, making their cytological diagnostics challenging. Characteristic cytological features and features separating them from the usual HPV-associated adenocarcinomas have been described. The features include monolayered and honeycomb sheets, distinct cell borders, vacuolated and/or foamy cytoplasm, intracytoplasmic neutrophil entrapment, sometimes vesicular nuclei with prominent nucleoli, nuclear overlapping and mostly only focal nuclear enlargement (Kawakami et al., 2015; Negri et al., 2021; Ryu et al., 2021).

In LBC samples, adding a p16/Ki-67 dual stain to the diagnostics has been reported to increase the detection of at least histological HSIL cases among hrHPV-positive and cytology-negative patients (Trzeszcz et al., 2021). On the other hand, a positive Hepika test on a cytological sample seems to be a good predictor of invasive carcinoma, both squamous and glandular, compared to precursor lesions only (Gustinucci et al., 2021). Adding p16 stains and Hepika tests to the screening

protocol in selected cases would probably increase or at least promote the detection of some AIS and EAC cases. Since p16 and Hepika are both surrogate markers for hrHPV infection, they are unlikely to be of any benefit in the diagnosis of the practically always hrHPV-negative gastric-type endocervical adenocarcinomas (Holl et al., 2015; Jenkins et al., 2020; Moljin et al., 2016; Nicolás et al., 2019; Pirog et al., 2014; Roiguez-Carunchio et al., 2015).

6.3.1 Stregths and limitations

In the study, the participation rate in screening (70.8%) was in the same range as reported earlier in Finland (Elfström et al., 2015), but the high drop-out rate observed in follow-up was unexpected. This can be considered a limitation of the study, since of the originally hrHPV-positive patients, 25.8% did not attend their follow-ups with a persistent hrHPV infection as their last finding. This might have affected the end results and left some cancers and premalignant lesions undiagnosed or unreported. During the study period, there was one death from other reasons revealed by the LIS. Since in Finland the different regional and private sector practitioners' databases do not communicate, we do not know whether the patients dropping out of the screening protocol moved, were diagnosed and treated elsewhere or simply chose not to participate.

We performed a database search that covered the study period to find cases missed by the HPV primary screening. As discussed earlier, for some AIS and EAC cases, reaching the diagnosis can take years. Thus, some malignancies can still emerge later.

6.4 Study IV

In our study, squamous metaplasia, inflammation or a combination of these were the most frequent findings in the follow-up histologies of AEC-NOS Pap smears. This is in line with many of the earlier publications (Ajit et al., 2013; Kawano et al., 2020; Nasu et al., 1993; Polat et al., 2021; Schindler et al., 1998; Selvaggi, 2016; Torres et al., 2005; Zhao et al., 2009). Inflammation is known to induce reactive nuclear atypia and metaplastic squamous changes as a protective response. Thus, encountering them in the same specimen is not unexpected (Ajit et al., 2013; Nasu et al., 1993; Polat et al., 2021; Zhao et al., 2009). Inflammatory background in the AEC-NOS Pap smears in our study correlated with significant mixed inflammation

in the follow-up histological samples. If the inflammatory infiltrate in Pap smear is pleomorphic and also includes tingle-body macrophages, the associated mild endocervical cell atypia has been postulated to most likely represent a benign, reactive change (Wood et al., 2007).

Besides infection and inflammation, reactive and reparative cytological atypia can be induced by several other benign or even malignant conditions affecting the uterine cervix (Levine et al., 2005; Ng et al., 2003; Rimm et al., 1999). However, in many of the earlier studies, the etiology of the reactive/reparative atypia has not been defined (Ghorab et al., 2000; Lee et al., 1995; Nasu et al., 1993; Ronnet et al., 1999).

According to earlier literature, the nuclei of the reactive/reparative endocervical cells can be normal-sized, uniformly enlarged or sometimes prominently anisonuclear. Prominent nucleoli can also be encountered. The chromatin pattern is usually even and fine, although even nuclear hyperchromasia has been described (Ghorab et al., 2000; Lee et al., 1995; Nasu et al., 1993; Ronnet et al., 1999; Torous et al., 2021; Wood et al., 2007). Additionally, crowded cell groups and sheets with nuclear stratification have been reported, but without feathering, rosettes or palisading cell borders (Ghorab et al., 2000; Lee et al., 1995; Ronnet et al., 1999; Torous et al., 2021).

In this study, nuclear crowding in cytology did not correlate with significant inflammation in the follow-up histological specimens. Instead, in the absence of significant histological inflammation, the nuclei of the endocervical cells were more frequently interpreted as elongated in cytology. A finely granular chromatin pattern showed an association with immature squamous metaplasia but not with significant histological inflammation.

The third most common benign condition encountered in our study was tubal metaplasia. According to previous publications, tubal metaplasia often presents as strips, sheets or groups of cells showing variable-sized nuclei with crowding and piling in addition to occasional hyperchromasia and mitotic figures (Selvaggi et al., 1997; Torous et al., 2021; Wilbur, 2016; Wood et al., 2007). Significant chromatin abnormalities, apoptotic bodies and tumour diathesis are absent. If encountered, cilia and terminal bars are the most characteristic, although not pathognomonic, of metaplasia only (Torous et al., 2021; Wilbur, 2016; Wood et al., 2007). In our study, none of the investigated cytological features correlated with tubal metaplasia in histology.

Microglandular hyperplasia was present in one-fifth of the benign histological follow-up specimens in our study. In cytology, microglandular hyperplasia was associated with nuclear elongation. According to TBSRCC 2014, nuclear elongation

is a feature of AEC-FN and cytological AIS (Nayar & Wilbur, 2015). Nuclear elongation was not mentioned in any of the earlier studies in association with microglandular hyperplasia (Alvarez-Santíne et al., 1999; Selvaggi et al., 1997; Wood et al., 2007; Yahr et al., 1991). Therefore, the power of our findings awaits a larger series. The cytomorphological features that have been previously described in association with microglandular hyperplasia include three-dimensional cell groups, small gland-like spaces, variation in cell size, either cubical, cylindrical or rounded nuclei and, in rare cases, hyperchromasia and prominent mitotic activity (Abi-Raad et al., 2014; Alvarez-Santíne et al., 1999; Selvaggi et al., 1997; Wood et al., 2007).

The scant cellularity in Pap smears was associated with granulation tissue formation in the histological samples. This could be expected since the granulation tissue most often lacks the surface epithelium. In addition, palisading cell borders correlated with the histological findings of granulation tissue. No other reports of the cytological features of granulation tissue in Pap smears have been found in the literature.

Some histological changes were encountered in all our benign follow-up histological samples of AEC-NOS Pap smears, which is in line with many of the previous studies (Ajit et al., 2013; Chen et al., 2008; Kawano et al., 2020; Selvaggi, 2016). However, in other reports, in 10% to 71% of cases, the histological samples harboured no abnormalities, possibly explaining the cytological glandular atypia diagnosed (Burja et al., 1999; Hare et al., 2003; Kim et al., 2017; Nasu et al., 1993; Schindler et al., 1998; Schnatz et al., 2006).

It has been proven that HPV infections have a high spontaneous clearance rate and that most of the mild squamous lesions spontaneously regress (Moscicki et al., 1998; Moscicki et al., 2004; Petry et al., 2018; Loopik et al., 2021) In comparison to squamous lesions, the evolution of EAC and its precursor lesions is less well established. There are no similar data published that address the possible spontaneous clearance rate of the endocervical lesions. In theory, HPV infection could induce later regressing changes in the glandular epithelium as well, thereby providing an explanation for why sometimes no histological changes explaining the cytological glandular atypia can be found. Since endocervical lesions are diagnostically challenging for both clinicians and pathologists, the discrepancy between cytological and histological diagnoses can also naturally be due to a sampling or diagnostic error (Confortini et al., 2006; Lee et al., 2002; Lepe et al., 2018; Pulkkinen et al., 2022; Simsir et al., 2003).

In AEC-NOS Pap smears, degeneration and nuclear crowding were more common among cases histologically confirmed as malignant. Thus, degeneration and

nuclear crowding were the strongest cytomorphological features separating the AEC-NOS Pap smears harbouring malignancy from the histologically benign proven cases. Degeneration has also been one of the cytological features obscuring the neoplastic nature of atypical cells in Pap smears in previous studies (Lee et al., 1995; Pulkkinen et al., 2022). Similarly, in agreement with our findings, crowded fragments have also been among the combination of cytological features in cases correctly diagnosed as endocervical cell malignancy in previous studies (Lee et al., 1995; Mariani et al., 2014; Pulkkinen et al., 2022).

In conclusion, additional tools besides cytology are needed for the diagnosis of endocervical malignancies to reliably distinguish mild glandular atypias related to benign pathologies from those harbouring a neoplastic lesion. All of the histologically benign verified AEC-NOS cases in our study were either hrHPV-negative at the study baseline or turned hrHPV-negative during the study period. The majority of AIS and EAC are hrHPV-associated (Moljin et al., 2016; Pirog et al., 2014; de Sanjose et al., 2010). Until more specific biomarkers become available, a negative hrHPV test seems to be the best predictor of a benign outcome among AEC-NOS cases. Meanwhile, some overdiagnostics and overtreatment most likely occurs among the problematic hrHPV-positive AEC-NOS cases.

6.4.1 Strengths and limitations

The fact that this was a blinded study instead of only a retrospective descriptive analysis of the cytomorphological features behind benign lesions can be counted as a strength. In addition, we had histologically malignant proven AEC-NOS Pap smears as a control group. All our benign cases had a relatively long follow-up period, and every case had an HPV status history available. These both support the interpretation that the cases in the benign group were truly non-neoplastic.

The relatively small sample size of 30 benign and 15 malignant AEC-NOS Pap smears can be seen as a limitation of the study and as a possible explanation as to why the investigated cytomorphological features did not show more specific correlations to any of the histological lesions. Our strict inclusion criteria limited the possibility of a larger series.

7 FUTURE DIRECTIONS

7.1 Prognostic biomarkers of cervical cancer

As hrHPV testing has become widely applied as a diagnostic tool and screening method, it has become evident that additional methods are needed in the risk stratification of hrHPV-positive patients. While LBC-based cytological sampling has simultaneously replaced the conventional Pap smear in many high-income countries as the main cytological method, the dual-stain (DS) with p16 and Ki-67 has already been quite broadly studied with regard to squamous lesions and as a possible future screening tool. While the role of DS in the diagnosis of EAC and mild cytological endocervical cell atypias has not yet been widely studied, DS has proven to be equally sensitive but more specific than hrHPV testing in detecting squamous lesions, including CIN2 and worse (CIN2+; Peeters et al., 2019; Voidăzan et al., 2022). DS has a high negative predictive value for CIN2+ lesions that is similar to that of the hrHPV test and has been shown to predict the outcome better than cytology and cytology combined with hrHPV genotyping among hrHPV-positive patients (Clarke et al., 2019; Uijterwaal et al., 2014; Uijterwaal et al., 2015; Wright et al., 2022).

DNA methylation, the epigenetic mechanism for the control of gene expression for various cellular functions, has also been widely studied in association with cervical cancer carcinogenesis. Several host cell and HPV targets have been identified. In general, for most of the investigated genes, methylation rates are higher in CIN2+ lesions than in LSIL and benign samples (Bowden et al., 2019; El Aliani et al., 2021; Kelly et al., 2019; Kremer et al., 2021). As a triage test, DNA methylation has been reported to be more specific than cytology in categories ASC-US or worse and as equally specific but more sensitive than HPV16/18 genotyping (Kelly et al., 2019).

Among the most promising targets is the combination of FAM19A4/miR124-2 genes, which have been shown to be positive in 98.3% to 100% of SCC and EAC and even positive in 93.8% of rare EAC subtypes and HPV-independent cancers (De Stooper et al., 2018; Vink et al., 2020). The validated FAM19A4/miR124-2 assays have a good sensitivity rate, from 71.3% to 77.2%, and a specificity of 78.3% for CIN3 lesions and worse (CIN3+; Kremer et al., 2021). Importantly, if the

FAM19A4/miR124-2 methylation test is negative, the likelihood of developing cervical cancer seems to be very low (De Stooper et al., 2018; Vink et al., 2020). In addition, methylation of EPB41L3 and viral targets HPV16 L1/2 have been shown to have good sensitivity and specificity for CIN2+ lesions but only in HPV16-positive cases (Bowden et al., 2019; Kelly et al., 2019).

Studies directly comparing the performance of DS and methylation tests in cervical cancer diagnostics have not yet been published. An advantage of the methylation tests is that they are not subjected to interpretation like DS is. Additionally, they can be performed on different types of samples, including self-collected samples and even urine, in addition to the usual cervical scrape (Kremer et al., 2021). On the other hand, DS is cheap and, in many laboratories, already available as an established method for histological samples. From the point of view of cost-effectiveness, it is not unequivocal which combination of diagnostic methods performs the best in triage of hrHPV-positive patients (Leesona et al., 2021).

Several other markers and methods have been proposed for the more specific detection of cervical cancer or precancer in the context of proven hrHPV positivity. So far, they have only been studied on a limited basis or the published results have been controversial, and further studies are awaited.

7.2 Vaccines

Currently, three HPV vaccines are available in many countries throughout the world. The bivalent vaccine covers the hrHPV genotypes 16 and 18 and the quadrivalent vaccine additionally covers the low-risk HPV genotypes 6 and 11. In addition to HPV genotypes 16, 18, 6 and 11, the nonavalent vaccine provides protection against the hrHPV genotypes 31, 33, 45, 52 and 58.

Vaccines against HPV16/HPV18 have been shown to significantly decrease the incidence of CIN2+ and CIN3+ lesions, AIS and invasive cancer among vaccinated girls and younger women (Arbyn et al., 2018; Ault et al., 2011; Drolet et al., 2019; Lei et al., 2020; Kjaer et al., 2021). The protective effect has been the highest against HPV16- and HPV18-positive lesions and among females proven to be hrHPV-negative before the first vaccine (Arbyn et al., 2018; Ault et al., 2011). The vaccines against HPV16 and HPV18 have significantly decreased the prevalence of HPV16 and HPV18 infections, but have also substantially decreased the prevalence of infections with hrHPV genotypes 31, 33 and 45 (Drolet et al., 2019; Mesher et al., 2016; Tsang et al., 2020; Wheeler et al., 2012). Likewise, at least some level of cross-

protection against genotypes 35, 51 and 58 has been observed. (Tsang et al., 2020; Wheeler et al., 2012).

In light of this, the amount of cervical SCC and EAC can be expected to decrease substantially in the future, at least in developed countries that have implemented the HPV vaccine as part of a national vaccination programme. However, there have been worries about whether vaccinating against HPV16 and HPV18 can lead to HPV type replacement in cervical cancer. This was implicated in a meta-analysis reporting a slight increase in the prevalence of hrHPV genotypes 39 and 52 and possible high-risk genotypes 53 and 73 in the vaccinated population (Mesher et al., 2016). Further epidemiologic studies are needed to answer this question in due time. According to a recent study, the HPV16/18 vaccine also reduces the number of CIN2+ and CIN3+ lesions caused by non-vaccine-targeted and non-cross-protected hrHPV genotypes other than 16, 18, 31, 33 and 45 (Sing et al., 2022). Obviously, HPV vaccines will not be effective against HPV-independent EAC subtypes, which will probably lead to an increase in their relative prevalence.

8 SUMMARY AND CONCLUSIONS

In summary, we investigated the cytomorphological features associated with EAC and AIS, the cytomorphological features obscuring their diagnosis and the significance of hrHPV status in predicting the outcome of cytological endocervical glandular atypia.

Our primary original findings are as follows:

1. A combination of three cytomorphological features comprising palisading cell borders, nuclear pleomorphism and the lack of single atypical cells were found to be associated with EAC and AIS.

2. The reproducibility of the preneoplastic/neoplastic cytopathological diagnoses was only moderate in terms of both inter- and intraobserver agreement, but better than that of atypia and NILM. In EAC and AIS Pap smears, nuclear size > 2 times the normal and nuclear pleomorphism were the most common features in Pap smears, with good consensus of the neoplastic nature and of the endocervical glandular origin of the lesion. In turn, the lack of nuclear enlargement and degenerative changes were the most frequent features encountered in samples with low consensus.

3. No neoplastic lesions were found among hrHPV-negative patients with AEC-NOS in cytology in our study cohort. One case of hrHPV-negative, gastric-type mucinous adenocarcinoma missed by the HPV primary screening was found by matched LIS analysis. Among the hrHPV-positive cases with AEC-NOS or AEC-FN in cytology, 68.4% (13/19) of the neoplastic lesions were purely squamous. Of the originally hrHPV-positive patients, 25.8% (16/62) dropped out of the screening protocol, with a persistent hrHPV infection as their last test.

4. Squamous metaplasia, significant mixed inflammation, tubal metaplasia and microglandular hyperplasia were the most common histological findings among cases with AEC-NOS in cytology and no evidence of neoplastic lesions in histological samples or during the follow-up period. Lack of

degeneration and nuclear crowding were the best cytomorphological features in separating these benign proven AEC-NOS cases from those representing EAC or AIS in histology. However, cytomorphology alone is not sufficient to reliably separate mild glandular atypias related to benign pathologies from those harbouring malignancy. All the benign proven AEC-NOS cases were either hrHPV negative at the study baseline or turned negative during the study period; thus, hrHPV negativity is the best indicator of benign atypia.

9 REFERENCES

Abi-Raad, R., Alomari, A., Hui, P., Buza, N., 2014. Mitotically active microglandular hyperplasia of the cervix: a case series with implications for the differential diagnosis. Int J Gynecol Pathol. 2014;33(5):524-30. https://doi.org/10.1097/PGP.0000000000000086.

Ajit, D., Gavas, S., Joseph, S., Rekhi, B., Deodhar, K., Kane, S., 2013. Identification of atypical glandular cells in pap smears: is it a hit and miss scenario? Acta Cytol. 2013;57(1):45-53. https://doi.org/10.1159/000342744.

Alvarez-Santín, C., Sica, A., Rodríguez, M., Feijó, A., Garrido, G., 1999. Microglandular hyperplasia of the uterine cervix. Cytologic diagnosis in cervical smears. Acta Cytol. 1999;43(2):110-3. https://doi.org/10.1159/000330961.

An, H.J., Kim, K.R., Kim, I.S., Kim, D.W., Park, M.H., Park, I.A., Suh, K.S., Seo, E.J., Sung, S.H., Sohn, J.H., Yoon, H.K., Chang, E.D., Cho, H.I., Han, J.Y., Hong, S.R., Ahn, G.H., 2005 Prevalence of human papillomavirus DNA in various histological subtypes of cervical adenocarcinoma: a population-based study. Mod Pathol 2005; 18:528–534. https://doi.org/10.1038/modpathol.3800316.

Anttila A., Kotaniemi-Talonen, L., Leinonen, M., Hakama, M., Laurila, P., Tarkkanen, J., Malila, N., Nieminen, P., 2010. Rate of cervical cancer, severe intraepithelial neoplasia, and adenocarcinoma in situ in primary HPV DNA screening with cytology triage: randomised study within organised screening programme. BMJ. 2010;340:c1804. https://doi.org/10.1136/bmj.c1804.

Arbyn, M., Gultekin, M., Morice, P., Nieminen, P., Cruickshank, M., Poortmans, P., Kelly, D., Poljak, M., Bergeron, C., Ritchie, D., Schmidt, D., Kyrgiou, M., Van den Bruel, A., Bruni, L., Basu, P., Bray, F., Weiderpass, E., 2021. The European response to the WHO call to eliminate cervical cancer as a public health problem. Int J Cancer. 2021;148(2):277-284. https://doi.org/10.1002/ijc.33189.

Arbyn, M., Raifu, A.O., Weiderpass, E., Bray, F., Anttila, A., 2009. Trends of cervical cancer mortality in the member states of the European Union. Eur J Cancer. 2009; 45:2640-2648. https://doi.org/10.1016/j.ejca.2009.07.018.

Arbyn, M., Weiderpass, E., Bruni, L., de Sanjosé, S., Saraiya, M., Ferlay, J., Bray, F., 2020. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health 2020; 8:191–203. https://doi.org/10.1016/S2214-109X(19)30482-6. .

Arbyn, M, Xu, L., Cindy Simoens, C., Martin-Hirsch, P.P.I., 2019. Prophylactic vaccination against human papillomaviruses to prevent cervical cancer and its precursors. Cochrane Database Syst Rev. 2018;5(5):CD009069. https://doi.org/10.1002/14651858.CD009069.pub3.

Ault, K.A., Joura, E.A., Kjaer, S.K., Iversen, O.E., Wheeler, C.M., Perez, G., Brown, D.R., Koutsky, L.A., Garland, S.M., Olsson, S.E., Tang, G.W.K., Ferris, D.G., Paavonen, J., Steben, M., Bosch, F.X., Majewski, S., Munos, N., Sings, H.L., Harkins, K., Rutkowski, M.A., Haupt, R.M., Garner, E.I.O., 2011. Adenocarcinoma in situ and

associated human papillomavirus type distribution observed in two clinical trials of a quadrivalent human papillomavirus vaccine. Int J Cancer. 2011;128(6):1344-53. https://doi.org/10.1002/ijc.25723.

Austin, R.M., Onisko, A., Zhao, C., 2018. Enhanced Detection of Cervical Cancer and Precancer Through Use of Imaged Liquid-Based Cytology in Routine Cytology and HPV Cotesting. Am J Clin Pathol. 2018; 150:385-392. https://doi.org/10.1093/AJCP/AQY114.

Boddington, M.M., Spriggs, A.I., R H Cowdell, R.H., 1976. Adenocarcinoma of the uterine cervix: cytological evidence of a long preclinical evolution. Br J Obstet Gynaecol.1976;83(11):900-3. https://doi.org/10.1111/j.1471-0528.1976.tb00770.x.

Bosch, F.X., Lorincz, A., Munoz, N., Meijer, C.J., Shah, K.V., 2002 The causal rela- tion between human papillomavirus and cervical cancer. J Clin Pathol 2002; 55:244–65. https://doi.org/10.1136/jcp.55.4.244.

Bouvard, V., Baan, R., Straif, K., Grosse, Y., Secretan, B, El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Freeman, C., Galichet, L., Cogliano, V., 2009. A review of human carcinogens--Part B: biological agents. Lancet Oncol. 2009;10(4):321-2. https://doi.org/10.1016/s1470-2045(09)70096-8.

Bowden, S.J., Kalliala, I., Veroniki, A.A., Arbynf, M., Mitra, A., Lathourasb, K., Mirabellog, L., Chadeau-Hyama, M., Paraskevaidish, E., Flanagana, J.M, Kyrgioua, M., 2019. The use of human papillomavirus DNA methylation in cervical intraepithelial neoplasia: A systematic review and meta-analysis. EBioMedicine. 2019; 50:246-259 https://doi.org/10.1016/j.ebiom.2019.10.053.

Boyd, J., Takahashi, H., Waggoner, S.E., Jones, L.A., Hajek, R.A., Wharton, J.T., Liu, F.S., Fujino, T., Barrett, J.C., McLachlan, J.A., 1996. Molecular genetic analysis of clear cell adenocarcinomas of the vagina and cervix associated and unassociated with diethylstilbestrol exposure in utero. Cancer. 1996; 77:507–13. https://doi.org/10.1002/(SICI)1097-0142(19960201)77:3<507::AID-CNCR12>3.0.CO;2-8.

Bray, F., Carstensen, B., Moller, H., Zappa, M., Zakelj. M.P., Lawrencw, G., Hakama, M., Weiderpass, E., 2005. Incidence trends of adenocarcinoma of the cervix in 13 European countries. Cancer Epidemiol Biomarkers Prev. 2005; 14:2191-21. https://doi.org/10.1158/1055-9965.EPI-05-0231.

Bray, F., Jemal, A., Grey, N., Ferlay, J., Forman, D., 2012. Global cancer transitions according to the Human Development Index (2008-2030): a population-based study. Lancet Oncol 2012;13(8):790-801. https://doi.org/10.1016/S1470-2045(12)70211-5.

Bray, F., Loos, A.H., McCarron, P., Weiderpass, E., Arbyn, M., Moller, H., Hakama, M., Parkin, D.M., 2005.Trends in cervical squamous cell carcinoma incidence in 13 European countries: changing risk and the effects of screening. Cancer Epidemiol Biomarkers Prev 2005;14(3):677-86. https://doi.org/10.1158/1055-9965.EPI-04-0569.

Bulk, S., Visser, O., Rozendaal L., Verheijen, R.H., Meijer, C.J.L.M., 2005. Cervical cancer in the Netherlands 1989–1998: decrease of squamous cell carcinoma in older women, increase of adenocarcinoma in younger women. Int. J. Cancer 2005; 113; 1005–1009. https://doi.org/10.1002/ijc.20678.

Burja, I.T., Thompson, S.K., Sawyer Jr, W.L., Shurbaji, M.S., 1999. Atypical Glandular Cells of Undetermined Significance on Cervical Smears. Acta Cytol.1999;43:351-356. https://doi.org/10.1159/000331080.

Castellsagué, X., Díaz, M., de Sanjosé, S., Muñoz, N., Herrero, R., Franceschi, S., Peeling, R.W., Ashley, R., Smith, J.S., Snijders, P.J.F., Meijer, C.J.L.M., Bosch, F.X., 2006. Worldwide Human Papillomavirus Etiology of Cervical Adenocarcinoma and Its Cofactors: Implications for Screening and Prevention. J Natl Cancer Inst. 2006;98(5):303-15. https://doi.org/10.1093/jnci/djj067.

Chen, L., Yang, B., 2008. Assessment of Reflex Human Papillomavirus DNA Testing in Patients With Atypical Endocervical Cells on Cervical Cytology. Cancer Cytopathol. 2008; 114:236-241. https://doi.org/10.1002/cncr.23639.

Chen, W., Molijn, A., Enqi, W., Zhang, X., Jenkins, D., Yu, X., Quint, W., Schmidt, J.E., Li, J., Pirog, E., Liu, B., Li, Q., Liu, X., Li, L., Qiao, Y., 2016. The variable clinicopathological categories and role of human papillomavirus in cervical adenocarcinoma: a hospital based nation-wide multi-center retrospective study across China. Int J Cancer. 2016; 139:2687-2697. https://doi.org/10.1002/ijc.30401.

Chrysostomou, A.C., Stylianou, D.C., Constantinidou, A. Kostrikis, L.G., 2018. Cervical Cancer Screening Programs in Europe: The Transition Towards HPV Vaccination and Population-Based HPV Testing. Viruses. 2018;10(12):729. https://doi.org/10.3390/v10120729.

Clarke, M.A., Cheung, L.C., Castle, P.E., Schiffman, M., Tokugawa, D., Poitras, N., Lorey, T., Kinney, W., Wentzensen, N., 2019. Five-Year Risk of Cervical Precancer Following p16/Ki-67 Dual-Stain Triage of HPV-Positive Women. JAMA Oncol. 2019;5(2):181-186. https://doi.org/10.1001/jamaoncol.2018.4270.

Classes in oncology: George Nicholas Papanicolaou's new cancer diagnosis presented at the Third Race Betterment Conference, Battle Creek, Michigan, January 2–6, 1928, and published in the Proceedings of the Conference. CA Cancer J Clin. 1973;23(3):174–9.

Cleveland, A.A., Gargano, J.W., Park, I.U., Griffin, M.R., Niccolai, L.M., Powell, M., Bennet, N.M., Saadeh, K., Pemmaraju, M., Higgins, K., Ehlers, S., Scahill, M., Johnson Jones, M.L., Querec, T., Markowitz, L.E., Unger, E.R., 2020. Cervical adenocarcinoma in situ: Human papillomavirus types and incidence trends in five states, 2008–2015 Int J Cancer. 2020;146(3):810-818. https://doi.org/10.1002/ijc.32340.

Confortini, M., Di Bonito, L., Carozzi, F., Ghiringhello, B., Motanari, G., Parisio, F., 2006 Interlaboratory Reproducibility of Atypical Glandular Cells of Undetermined Significance: a National Survey. Cytopathology 2006; 17:353–360. https://doi.org/10.1111/j.1365-2303.2006.00372.x.

Conrad RD, Liu AH, Wentzensen N, Zhang RR, Dunn ST, Wang SS, Schiffman, M., Gold, M.A., Walker, J.L., Zuna, R.E., 2018. Cytologic Patterns of Cervical Adenocarcinomas with Emphasis on Factors Associated with Underdiagnosis. Cancer Cytopathol. 2018; 121:950-958. https://doi.org/10.1002/cncy.22055.

Costa S, Negri G, Sideri M, Santini D, Martinelli G, Venturoli S, Pelusi, C., Syrjanen, S., Syrjanen K., Pelusi, G., 2007. Human Papillomavirus (HPV) Test and PAP Smear as Predictors of Outcome in Conservatively Treated Adenocarcinoma in Situ (AIS) of the Uterine Cervix. Gynecol Oncol.2007; 106:170-176. https://doi.org/10.1016/j.ygyno.2007.03.016.

Dahlström, L.A., Ylitalo, N., Sundström, K., Palmgren, J., Ploner, A., Eloranta, S., Sanjeevi, C.B., Andersson, S., Rohan, T., Dillner, J., Adami, H.O., Sparén, P., 2010. Prospective study of human papillomavirus and risk of cervical adenocarcinoma. Int J Cancer. 2010;127(8):1923-30. https://doi.org/10.1002/ijc.25408.

Demarco, M., Hyun, N., Carter-Pokras, O., Raine-Bennett, T.R., Cheung, L., Chen, X., Hammer, A., Campos, N., Kinney, W., Gage, J.C., Befano, B., Perkins, R.E., He, H., Dallal, C., Chen, J., Poitras, N., Mayrand, M.H., Coutlee, F., Burk, R.D., Lorey, T., Castle, P.E., Wentzensen, N., Schiffman, M., 2020. A study of type-specific HPV natural history and implications for contemporary cervical cancer screening programs. ECinicalMedicine. 2020; 22:100293. https://doi.org/10.1016/j.eclinm.2020.100293.

Doulgeraki, T., Bowden, S., Athanasiou, A., Kechagias, K., Lathouras, K., Kalliala, I., Kyrgiou, M., 2022. 280 Environmental and modifiable risk factors for cervical cancer: An umbrella review. Eur J Obstet Gynecol Reprod Biol. 2022; 270:83-e83 https://doi.org/10.1016/j.ejogrb.2021.11.275.

Drolet, M., Bénard, E., Pérez, N., Brisson, M.Population-level impact and herd effects following the introduction of human papillomavirus vaccination programmes: updated systematic review and meta-analysis. Lancet. 2019;394(10197):497-509. https://doi.org/10.1016/S0140-6736(19)30298-3.

El Aliani, A., El-Abid, H., El Mallali, Y., Attaleb, M., Ennaji, M.M., El Mzibri, M., 2021. Association between Gene Promoter Methylation and Cervical Cancer Development: Global Distribution and A Meta-analysis. Cancer Epidemiol Biomarkers Prev. 2021;30(3):450-459. https://doi.org/ 10.1158/1055-9965.EPI-20-0833.

Elfström, M., Arnheim-Dahlström, L., von Karsa, L., Dillner, J., 2015. Cervical cancer screening in Europe: Quality assurance and organisation of programmes. J Cancer. 2015;51(8):950-68. https://doi.org/10.1016/j.ejca.2015.03.008.

Eun, T.J., Perkins, R.B., 2020.Screening for Cervical Cancer. Med Clin North Am. 2020;104(6):1063-1078. https://doi.org/10.1016/j.mcna.2020.08.006.

Farnsworth, A., 2016.Cervical cancer screening in Australia: Past and present. Cancer Cytopathol. 2016 Apr;124(4):231-4. https://doi.org/10.1002/cncy.

Ferlay, J., Colombet, M., Soerjomataram, I., Dyba, T., Randi, G., Bettio, M., Gavin, A., Visser, O., Bray, F., 2018. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. Eur J Cancer. 2018; 103:356-387. https://doi.org/ 10.1016/j.ejca.2018.07.005.

Gage, J.C., Schiffman, M., Katki, H.A., Castle, P.E., Fetterman, B., Wentzensen, N., Poitras, N.E., Lorey, T., Cheung, L.C., Kinney, W.K., 2014. Reassurance against future risk of precancer and cancer conferred by a negative human papillomavirus test. J Natl Cancer Inst 2014;106(8):1–4. https://doi.org/10.1093/jnci/dju153.

Garg, S., Nagaria, T.S., Clarke, B., Freedman, O., Khan, Z., Schwock, J., Bernardini, M.Q., Oza, A.M., Han, K., Smith, A.C., Stockley, T.L., Rouzbahman, M., 2019. Molecular characterization of gastric-type endocervical adenocarcinoma using next-generation sequencing. Mod Pathol. 2019; 32:1823–33. https://doi.org/10.1038/s41379-019-0305-x.

GCO. The Global Cancer Observatory. (Referred September 9, 2022). Available online at: https://gco.iarc.fr.

Geldenhuys, L., Murray, M.L., 2007. Sensitivity and Specificity of the Pap Smear for Glandular Lesions of the Cervix and Endometrium.Acta Cytol. 2007; 51:47–50. https://doi.org/10.1159/000325682.

Ghorab, Z., Mahmood, S., Schinella, R., 2000. Endocervical Reactive Atypia: A Histologic-Cytologic Study. Diagn Cytopathol. 2000;22(6):342-346. https://doi.org/10.1002/(sici)1097-0339(200006)22:6<342::aid-dc3>3.0.co;2-0.

Grenko, R.T., Abendroth, C.S., Frauenhoffer, E.E., Ruggiero, F.M., Zaino, R.J., 2000. Variance in the interpretation of cervical biopsy specimens obtained for atypical squamous cells of undetermined significance. Am J Clin Pathol. 2000; 114:735-40. https://doi.org/10.1309/K7C9-X5P0-001B-2HK5.

Gunnell, A.S., Ylitalo, N., Sandin, S., Sprén, P., Adami, H.-O., Ripatti, S. 2007. A Longitudinal Swedish Study on Screening for Squamous Cell Carcinoma and Adenocarcinoma: Evidence of Effectiveness and Overtreatment. Cancer Epidemiol Biomarkers Prev 2007; 16 (12): 2641–2648. https://doi.org//10.1158/1055-9965.EPI-07-0278

Gustinucci, D., Ciccocioppo, L., Coppola, L., Negri, G., Zannoni, G., Passamonti, B., Cesarini, E., Ianzano, C., Andreano, T., Pireddu, A., Giorgi-Rossi, P., 2021. Multicentre Evaluation of Hepika Test Clinical Accuracy in Diagnosing HPV-Induced Cancer and Precancerous Lesions of Uterine Cervix. Diagnostics 2021; 11:619. https://doi.org/10.3390/diagnostics11040619

Halec, G., Schmitt, M., Dondog, B., Sharkhuu, E., Wentzensen, N., Gheit, T., Tommasino, M., Kommoss, F., Bosch, F.X., Franceschi, S., Clifford, G., Gissmann, L., Pawlita, M., 2013. Biological activity of probable/possible high-risk human papillomavirus types in cervical cancer. Int. J. Cancer 2013; 132:63–71. https://doi.org/10.1002/ijc.27605.

Hare, A.A., Duncan, A.R., Sharp, A.J., 2003. Cytology suggestive of glandular neoplasia: outcomes and suggested management. Cytopathology 2003;14(1):12-18. https://doi.org/10.1046/j.1365-2303.2003.01020.x.

zur Hausen, H., 2000. Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. J Natl Cancer Inst. 2000;92(9):690-8. https://doi.org/10.1093/jnci/92.9.690.

Hodgson, A., Howitt, B.E., Park, K.J., Lindeman, N., Nucci, M.R., Parra-Herran, C., 2020. Genomic Characterization of HPV-related and Gastric-type Endocervical Adenocarcinoma: Correlation With Subtype and Clinical Behavior. Int J Gynecol Pathol. 2020; 39:578-586. https://doi.org/10.1097/PGP.0000000000000665.

Hodgson, A., Park, K.J., Djordjevic, B., Howitt, B., Nucci, M.R., Oliva, E., Stolnicu, S., Xu, B., Soslow, R., Parra-Herran, C., 2019. International Endocervical Adenocarcinoma Criteria and Classification: Validation and Interobserver Reproducibility. Am J Surg Pathol. 2019; 43: 75–83. https://doi.org/10.1097/PAS.0000000000001095.

Holl, K., Nowakowski, A.M., Powell, N., McCluggage, W.G., Pirog, E.C., Collas De Souza, S., Tjalma, W.A., Rosenlund, M., Fiander, A., Castro Sánchez, M., Damaskou, V., Joura, E.A., Kirschner, B., Koiss, R., O'Leary, J., Quint, W., Reich, O., Torné, A., Wells, M., Rob, L., Kolomiets, L., Molijn, A., Savicheva, A., Shipitsyna, E., Rosillon, D., Jenkins, D., 2015. Human papillomavirus prevalence and type-distribution in cervical glandular neoplasias: Results from a European multinational epidemiological study. Int J Cancer. 2015;137(12):2858-2868. https://doi.org/10.1002/ijc.29651.

Horn, J., Denecke, A., Luyten, A., Rothe, B., Reinecke-Lüthge, A., Mikolajczyk, R., Petry, K.U., 2019. Reduction of cervical cancer incidence within a primary HPV screening pilot project (WOLPHSCREEN) in Wolfsburg, Germany. Br J Cancer. 2019 May;120(10):1015–1022. https://doi.org/10.1038/s41416-019-0453-2.

van der Horst, J., Siebers, A.G., Bulten, J. Massuger, L.F., de Kok I.M.C.M., 2017. Increasing incidence of invasive and in situ cervical adenocarcinoma in The Netherlands during 2004-2013. Cancer Med 2017; 6:416–23. https://doi.org/10.1002/cam4.971.

IARC. The International Agency for Research on Cancer. (Referred September 9, 2022). Available online at: https://www.iarc.who.int/cancer-type/cervical-cancer.

Jenkins, D., Molijn, A., Kazem, S., Pirog, E.C., Alemany, L., de Sanjosé, S., Dinjens, W., Quint, W., 2020. Molecular and pathological basis of HPV-negative cervical adenocarcinoma seen in a global study. Int J Cancer. 2020; 147:2526–2536. https://doi.org/10.1002/ijc.33124.

Joste, N.E., Rushing, L., Granados, R., Zitz, J.S., Genest, D.R., Crum, C.P., Cibas, E.S., 1996. Bethesda classification of cervicovaginal smears: reproducibility and viral correlates. Hum Pathol. 1996; 27:581-585. https://doi.org/10.1016/s0046-8177(96)90165-3.

Kares, S., Veijalainen, O., Kholová, I., Tirkkonen, M., Vuento, R., Huhtala, H. Tuimala, V., Mäenpää, J., Kujala, P., 2019. HIGH-RISK HPV testing as the primary screening method in an organized regional screening program for cervical cancer: the value of HPV16 and HPV18 genotyping? APMIS. 2019; 127:710–716. https://doi.org/10.1111/apm.12990

Katki, H.A., Kinney, W.K., Fetterman, B., Lorey, T., Poitras, N.E., Cheung, L., 2011. Cervical Cancer Risk for 330,000 Women Undergoing Concurrent HPV Testing and Cervical Cytology in Routine Clinical Practise at a Large Managed Care Organization. Lancet Oncol. 2011; 12(7):663-672. https://doi.org/10.1016/S1470-2045(11)70145-0.

Kawakami, F., Mikami, Y., Sudo, T., Fujiwara, K., Hirose, T., Itoh, T., 2015. Cytologic Features of Gastric-Type Adenocarcinoma of the Uterine Cervix. Diagn Cytopathol.201; 43(10):791-796. https://doi.org/10.1002/dc.23304.

Kawano, K., Yamaguchi, T., Nasu, H., Nishio, S., Ushijima, K., 2020. Subcategorization of atypical glandular cells is useful to identify lesion site. Diagn Cytopathology 2020; 48:1224–1229. https://doi.org/ 10.1002/dc.24549

Kim, H.S., Underwood, D., 1991. Adenocarcinoma in the Cervicovaginal Papanicolau Smear: Analysis of a 12-year Experience. Diagn Cytopathol. 1991; 7:119-24. https://doi.org/10.1002/dc.2840070203.

Kim, M.K., Lee, Y.K., Hong, S.R., Lim, K.T., 2017. Clinicopathological significance of atypical glandular cells on cervicovaginal Pap smears. Diagn Cytopathol. 2017; 45:867–872. https://doi.org/10.1002/dc.23777.

Kitchener, H.C., Almonte, M., Gilham, C., Dowie, R., Stoykova, B., Sargent, A., Roberts, C., Desai, M., Peto, J., 2009. ARTISTIC: a randomised trial of human papillomavirus (HPV) testing in primary cervical screening. Health Technol Assess. 2009; 13(51):1-150. https://doi.org/10.3310/hta13510.

Kitchener HC, Gilham C, Sargent A, Bailey A, Albrow R, Roberts C, Desai, M., Mather, J., Turner, A., Moss, S., Peto, J., 2011. A comparison of HPV DNA testing and liquid based cytology over three rounds of primary cervical screening: extended follow up in the ARTISTIC trial. Eur J Cancer 2011;47(6): 864–7118. https://doi.org/10.1016/j.ejca.2011.01.008

Kotaniemi-Talonen, L. Nieminen, P., Anttila, A., Hakama, M., 2005. Routine cervical screening with primary HPV testing and cytology triage protocol in a randomised setting. Br J Cancer. 2005; 93:862-7. https://doi.org/10.1038/sj.bjc.6602799.

Krane, J.F., Granter, S.R., Trask, C.E., Hogan, C.L., 2001. Papanicolaou Smear Sensitivity for the Detection of Adenocarcinoma of the Cervix: a Study of 49 Cases. Cancer. 2001; 93:8–15. https://doi.org/10.1002/1097-0142(20010225)93:1%3C8::AID-CNCR9001%3E3.0.CO;2-K.

Kremer, W.W., Steenbergen, R., Heideman, D., Kenter, G.G., Meijer, C.J.L.M., 2021. The use of host cell DNA methylation analysis in the detection and man-agement of women with advanced cervical intraepithelial neoplasia: a review. BJOG. 2021; 128(3):504-514. https://doi.org/ 10.1111/1471-0528.16395.

Kumar, N., Bongiovanni, M., Molliet, M.-J., Pelte, M.-F., Egger, J.-F., Pache, J.-C., 2009. Diverse Glandular Pathologies Coexist with High-grade Squamous Intraepithelial Lesion in Cyto-histological Review of Atypical Glandular Cells on ThinPrep Specimens. Cytopathology 2009; 20(6):351–358. https://dx.doi.org/10.1111/j.1365-2303.2008.00568.x

Kurtycz, D.F.I., Staats, P.N., Chute, D.J., Russell, D., Pavelec, D., Monaco, S.E., Friedlander, M.A., Wilbur, D.W., Nayar, R., 2017. Bethesda Interobserver Reproducibility Study-2 (BIRST-2): Bethesda System 2014. J Am Soc Cytopathol. 2017;6(4):131–144. https://doi.org/10.1016/j.jasc.2017.03.003.

Käypähoito. Kohdunkaulan, emättimen ja ulkosynnytinten solumuutokset. Current Care Guidelines. Working group set up by the Finnish Medical Society Duodecim and the Finnish Colposcopic Society. Helsinki: The Finnish Medical Society Duodecim, 2021 (referred September 8, 2022). Available online at: https://www.kaypahoito.fi.

Laara, E., Day, N.E., Hakama, M., 1998. Trends in mortality from cervical cancer in the Nordic countries: association with organised screening programmes. Lancet. 1987; 1:1247-1249. https://doi.org/10.1016/s0140-6736(87)92695-x.

Lai, C.-R., Hsu, C.-Y., Tsay, S.-H., Li, A., 2008. Clinical Significance of Atypical Glandular Cells by the 2001 Bethesda System in Cytohistologic Correlation. Acta Cytol. 2008; 52:563-567. https://doi.org/10.1159/000325598.

Landis, J.R., Koch, G.G., 1997. The measurement of observer agreement for categorical data. Biometrics.1977; 33:159-174. https://doi.org/10.2307/2529310.

Layfield, L., Wang, G., Yang, Z., Gomez-Fernandez, C., Esebua, M., Schmidt, R., 2020. Interobserver Agreement for the International Academy of Cytology Yokohama System for Reporting Breast Fine-Needle Aspiration Biopsy Cytopathology. Acta Cytol. 2020; 64:413-419. https://doi.org/10.1159/000506757.

Lee K.R., Darragh, T.M., Joste, N.E., Krane, J.F., Sherman, M.E., Hurley, L.B., Allred, E.M., Manos, M.M., 2002. Atypical Glandular Cells of Undetermined Significance (AGUS): Interobserver Reproducibility in Cervical Smears and Corresponding Thin-Layer Preparations. Am J Clin Pathol. 2002; 117: 96–102. https://doi.org/10.1309/HL0B-C7Y6-AC77-ND2U.

Lee, K.R., Flynn, C.E., 2000. Early invasive adenocarcinoma of the cervix. Cancer 2000; 89:1048-55. https://doi.org/10.1002/1097-0142(20000901)89:5%3C1048::AID-CNCR14%3E3.0.CO;2-S

Lee, K.R., Manna, E.A., St. John, T., 1995. Atypical Endocervical Glandular cells: Accuracy of Cytologic Diagnosis. Diagn Cytopathol. 1995; 13:202-208. https://doi.org/10.1002/dc.2840130305

Lee, K.R., Minter, L.J., Granter, S.R., 1997. Papanicolaou Smear Sensitivity for Adenocarcinoma In Situ of the Cervix:A Study of 34 Cases. Am J Clin Pathol. 1997; 107(1):30-5. https://doi.org/10.1093/ajcp/107.1.30.

Leesona, S., Alaladea, R., Singha, N., Nieminen, P., Cruickshankc, M., Carcopinod, X., Bergeron, C., 2021. Options for triage and implications for colposcopists within European HPV-based cervical screening programmes. Eur J Obstet Gynecol Reprod Biol. 2021; 258:332-342. https://doi.org/10.1016/j.ejogrb.2020.12.061.

Lei, J., Ploner, A., Elfström, K.M., Wang, J., Roth, A., Fang, F., Sundström, K., Dillner, J., Pär Sparén, P., 2020. HPV Vaccination and the Risk of Invasive Cervical Cancer. N Engl J Med.E 2020; 383(14):1340-1348. https://doi.org/10.1056/NEJMoa1917338.

Leinonen. M., Nieminen, P., Kotaniemi-Talonen L., Malila, N., Tarkkanen, J., Laurila, P., Anttila, A., 2009. Age-specific evaluation of primary human papillomavirus screening vs conventional cytology in a randomized setting. J Natl Cancer Inst. 2009; 101:1612–23. https://doi.org/10.1093/jnci/djp367.

Leinonen, M.K., Nieminen, P., Lönnberg, S., Malila, N., Hakama, M., Pokhrel, A., Laurila, P., Tarkkanen, J., Anttila, A., 2012. Detection rates of precancerous and cancerous cervical lesions within one screening round of primary human papillomavirus DNA testing: prospective randomised trial in Finland. BMJ. 2012; 345: e7789. https://doi.org/10.1136/bmj.e7789.

Lepe, M., Eklund, C.M., Quddus, M.R., Paquette, C., 2018. Atypical Glandular Cells: Interobserver Variability According to Clinical Management. Acta Cytol. 2018; 62:397–404. https://doi.org/10.1159/000489968.

Levine, P.A., Elgert, P.A., Sun, P., Simsir, A., 2005.Atypical repair on Pap smears: clinicopathologic correlates in 647 cases. Diagn Cytopathol. 2005; 33(3):214-7. https://doi.org/10.1002/dc.20333.

Loopik, D.L., Bentley, H.A., Eijgenraam, M.N., IntHout, J., Bekkers, R.L.M., Bentley, J.R., 2021. The Natural History of Cervical Intraepithelial Neoplasia Grades 1, 2, and 3: A Systematic Review and Meta-analysis. J Low Genit Trac Dis. 2021; 25(3):221–231. https://doi.org/10.1097/LGT.0000000000000604.

Lou, H., Villagran, G., Boland, J.F., Im, K.M., Polo, S., Zhou, W., Odey, U., Juárez-Torres, E., Medina-Martínez, I., Roman-Basaure, E., Mitchell, J., Roberson, D., Sawitzke, J., Garland, L., Rodríguez-Herrera, M., Wells, D., Troyer, J., Castillo Pinto, F., Bass, S., Zhang, X., Castillo, M., Gold, B., Morales, H., Yeager, M., Berumen, J., Alvirez, E., Gharzouzi, E., Dean, M., 2015. Genome analysis of Latin American cervical cancer: frequent activation of the PIK3CA pathway. Clin Cancer Res. 2015; 21:5360–70. https://doi.org/10.1158/1078-0432.CCR-14-1837.

Lönnberg, S., Anttila, A., Luostarinen, T., Nieminen, P., 2012. Age-Specific Effectiveness of the Finnish Cervical Cancer Screening Programme. Cancer Epidemiol. Biomarkers Prev. 2012; 21:1354-1361. https://doi.org/10.1158/1055-9965.EPI-12-0162.

Malila, N., Leinonen, M., Kotaniemi-Talonen, L., Laurila, P., Tarkkanen, J., Hakama, M., 2013. The HPV test has similar sensitivity but more overdiagnosis than the Pap test--a randomised health services study on cervical cancer screening in Finland. Int J Cancer. 2013; 132:2141-7. https://doi.org/10.1002/ijc.27850.

Mancini, S., Ravaioli, A., Giuliani, O., Vattiato, R., Falcini, F., Ferretti, S., Costa, S., Bucchi, L., 2017. Incidence and survival trends of cervical adenocarcinoma in Italy: Cytology screening has become more effective in downstaging the disease but not in detecting its precursors. Int J Cancer. 2017; 140(1):247-248. https://doi.org/10.1002/ijc.30435.

Mariani, R., Grace, C., Hughes, K., Dietrich, R.M., Cabay, R.J., David, O., 2014. Can We Improve the Positive Predictive Value of Atypical Glandular Cells Not Otherwise Specified? Diagn Cytopathol. 2014; 42:200-204. https://doi.org/10.1002/dc.22991.

Maver, P.J., Poljak, M., 2020. Primary HPV-based cervical cancer screening in Europe: implementation status, challenges, and future plans. Clin Microbiol Infect. 2020; 26(5):579-583 https://doi.org/10.1016/j.cmi.2019.09.006.

McBride, A.A., 2022. Human papillomaviruses: diversity, infection and host interactions. Nat rev Microbiol. 2022; 20(2):95-108. https://doi.org/10.1038/s41579-021-00617-5.

McCluggage, W.G., Walsh, M.Y, Thornton, C.M., Hamilton, P.W., Date, A., Caughley, L.M., Bharucha, H., 1998. Inter- and intra-observer variation in the histopathological reporting of cervical squamous lesions using a modified Bethesda grading system. Br J Obstet Gynaecol. 1998; 105:206-10. https://doi.org/10.1111/j.1471-0528.1998.tb10054.x.

Mesher, D. Soldan, K., Lehtinen, M., Beddows, S., Brisson, M., Brotherton, J.M.L., Chow, E.P.F., Cummings, T., Drolet, M., Fairley, C.K., Garland, S.M., Kahn, J.A., Kavanagh, K., Markowitz, L., Pollock, K.G., Söderlund-Strand, A., Sonnenberg, P., Sabrizi, S.N., Tanton, C., Unger, E., Thomas, T.L., 2016. Population-Level Effects of Human Papillomavirus Vaccination Programs on Infections with Nonvaccine Genotypes. Emerg Infect Dis. 2016; 22(10):1732-40. https://doi.org/10.3201/eid2210.160675.

Mirkovic, J., Schoolmeester, K.J., Campbell, F., Miron, A., Nucci, M.R., Howitt, B.E., 2017. Cervical mesonephric hyperplasia lacks KRAS/NRAS mutations. Histopathology 2017; 71:1003–5. https://doi.org/10.1111/his.13307.

Mirkovic, J., Sholl, L.M., Garcia, E., Lindeman, N., MacConaill, L., Hirsch, M., Dal Cin, P., Gorman, M., Barletta, J.A., Nucci, M.R., McCluggage, W.G., Howitt, B.E., 2015. Targeted genomic profiling reveals recurrent KRAS mutations and gain of chromosome 1q in mesonephric carcinomas of the female genital tract. Mod Pathol. 2015; 28:1504–14. https://doi.org/10.1038/modpathol.2015.103.

Moljin, A., Jenkins, D., Chen, W., Zhang, X., Pirog, E., Enqi, W., Liu, B., Schmidt, J., Cui, J., Qiao, Y., Quint, W., 2016. The complex relationship between human papillomavirus and cervical adenocarcinoma. Int J Cancer 2016; 138:409-16. https://doi.org/10.1002/ijc.29722.

Moreira, M.A.R., Filho, A.L., Castelo, A., De Barros, M.R.E., Da Silva, A.P., Thormann, P., da Gloria Mattosinho de Castro Ferraz, M., Botacini das Dores, G., 2008. How accurate Is Cytological Diagnosis of Cervical Glandular Lesions? Diagn Cytopathol. 2008; 36:270-274. https://doi.org/10.1002/dc.20799.

Moscicki, A.B., Shiboski, S., Broering, J., Powell, K., Clayton, L., Jay, N., Darragh, T.M., Brescia, R., Kanowitz, S., Miller, S.B., Stone, J., Hanson, E., Palefsky, J., 2008. The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. J Pediatr. 1998; 132(2):277-84. https://doi.org/10.1016/s0022-3476(98)70445-7.

Moscicki, A.B., Shiboski, S., Hills, N.K., Powell, K.J., Jay, N., Hanson, E.N., Miller, S., Canjura-Clayton, L.K., Farhat, S., Broering, J.M, Darragh, T.M., 2004. Regression of low-grade squamous intra-epithelial lesions in young women. Lancet 2004; 364(9446):1678-83. https://doi.org/10.1016/S0140-6736(04)17354-6.

Muñoz, N., Bosch, F. X., de Sanjosé, S., Herrero, R., Castellsagué, X., Shah, K., V., Snijders, P.J.F., Meijer, C.J.L.M., 2003. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003 Feb 6; 348(6):518-27. https://doi.org/ 10.1056/NEJMoa021641.

Murphy, J., Kennedy, E.B., Dunn, S., McLachlin, C.M., Fung Kee Fung, M., Gzik, D. MD, Shier, M., MD, Paszat, L., 2012. HPV testing in primary cervical screening: a systematic review and meta-analysis. J Obstet Gynaecol Can. 2012; 34(5):443-452. https://doi.org/10.1016/S1701-2163(16)35241-0.

Nasu, I., Meurer, W., Fu, Y.S., 1993. Endocervical glandular atypia and adenocarcinoma: A correlation of cytology and histology. Int J Gynecol Pathol. 1993; 12:208–218. https://doi.org/10.1097/00004347-199307000-00002.

Naucler, P., Ryd, W., Törnberg, S., Strand, A., Wadell, G., Elfgren, K., Rådberg, T., Strander, B., Johansson, B., Forslund, O., Hansson, B.-G., Rylander, E., Dillner, J., 2007. Human Papillomavirus and Papanicolaou Tests to Screen for Cervical Cancer. Engl J Med. 2007; 357(16):1589-97. https://doi.org/10.1056/NEJMoa073204.

Nayar, R., Wilbur, D.C., 2015. The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria and Explanatory Notes, Third Edition. Springer International Publishing. https://doi.org/10.1007/978-3-319-11074-5.

Negri, G., Macciocu, E., Cepurnaite, R., Kasal, A., Troncone, G., Steinkasserer, M., Vittadello, F., 2021. Non-human papilloma virus associated adenocarcinomas of the cervix uteri. Cytologic features and diagnostic agreement using whole slide digital cytology imaging. Diagn Cytopathol. 2021; 49(2):316-321. https://doi.org/10.1002/dc.24652.

Ng, W.K., Li, A.S.M., Cheung, L.K.N., 2003. Significance of atypical repair in liquid-based gynecologic cytology: a follow-up study with molecular analysis for human papillomavirus. Cancer. 2003;99(3):141-8. https://doi.org/10.1002/cncr.11101.

Nicolás, I., Marimon, L., Barnadas, E., Saco, A., Rodríguez-Carunchio, L., Fusté, P., Martí, C., Rodriguez-Trujillo, A., Torne, A., Del Pino, M., Ordi, J., 2019. HPV-negative tumors of the uterine cervix. Mod Pathol. 2019; 32(8):1189-1196. https://doi.org/10.1038/s41379-019-0249-1.

Niu, S., Molberg, K., Thibodeaux, J., Rivera-Colon, G., Hinson, S., Zheng, W., Lucas, E., 2019. Challenges in the Pap diagnosis of endocervical adenocarcinoma in situ. J Am Soc Cytopathol. 2019; 8:141-148. https://doi.org/10.1016/j.jasc.2018.12.004.

NORDCAN. Association of the Nordic Cancer Registries. (Referred September 9, 2022). Available online at: https://www-dep.iarc.fr/nordcan/fi/frame.asp.

Ogilvie, G.S., van Niekerk, D., Krajden, M., Smith, L.M., Cook, D., Gondara, L., Ceballos, K., Lee, M., Martin, R.E., Gentile, L. Peacock, S., Stuart, G.C.E, Franco, E.L., Coldman, A.J., 2018. Effect of Screening With Primary Cervical HPV Testing vs Cytology Testing on High-grade Cervical Intraepithelial Neoplasia at 48 Months (The HPV FOCAL Randomized Clinical Trial) JAMA 2018;320(1):43-52. https://doi.org/10.1001/jama.2018.7464.

Ojesina, A.I., Lichtenstein, L., Freeman, S.S., Pedamallu, C.S., Imaz-Rosshandler, I., Pugh, T.J., Cherniack, A.D., Ambrogio, L., Cibulskis, K., Bertelsen, B., Romero-Cordoba, S., Treviño, V., Vazquez-Santillan, K., Guadarrama, A.S., Wright, A.A., Rosenberg, M.W., Duke, F., Kaplan, B., Wang, R., Nickerson, E., Walline, H.M., Lawrence, M.S., Stewart, C., Carter, S.L., McKenna, A., Rodriguez-Sanchez, I.P., Espinosa-Castilla, M., Woie, K., Bjorge, L., Wik, E., Halle, M.K., Hoivik, E.A., Krakstad, C., Gabiño, N.B., Gómez-Macías, G.S., Valdez-Chapa, L.D., Garza-Rodríguez, M.L., Maytorena, G., Vazquez, J., Rodea, C., Cravioto, A., Cortes, M.L., Greulich, H., Crum, C.P., Neuberg, D.S., Hidalgo-Miranda, A., Escareno, C.R., Akslen, L.A., Carey, T.E., Vintermyr, O.K., Gabriel, S.B., Barrera-Saldaña, H.A., Melendez-Zajgla, J., Getz, G., Salvesen, H.B., Meyerson, M., 2014. Landscape of genomic alterations in cervical carcinomas. Nature.2014; 506:371–5. https://doi.org/10.1038/nature12881.

Olusola, P., Banerjee, H.N., Philley, J.V., Dasgupta, S., 2019. Human Papilloma Virus-Associated Cervical Cancer and Health Disparities. Cells. 2019;8(6):622. https://doi.org/10.3390/cells8060622.

Orumaa, M., Leinonen, M.K., Campbell, S., Møller, B., Myklebust. T.Å., Nygård, M., 2019. Recent increase in incidence of cervical precancerous lesions in Norway: Nationwide study from 1992 to 2016. Int J Cancer. 2019; 145:2629–2638. https://doi.org/10.1002/ijc.32195.

Papanicolaou, G.N., Traut, H.F., 1941. The Diagnostic Value of Vaginal Smears in Carcinoma of the Uterus. Am J Obstet Gynecol. 1941; 42: 193–206.

Park, E., Kim, S.W., Kim, S., Kim, H.S., Lee, J.Y., Kim, Y.T., Cho, N.H., 2021. Genetic characteristics of gastric-type mucinous carcinoma of the uterine cervix. Mod Pathol. 2021; 34:637-646. https://doi.org/10.1038/s41379-020-0614-0.

Park, J.S, Kim, Y.T., Lee, A., Lee, Y., Kim, K.T., Cho, C.H., Choi, H.S., Jenkins, D., Pirog, E.C., Molijn, A.C., Ramakrishnan, G., Chen, J., 2013. Prevalence and type distribution of human papillomavirus in cervical adenocarcinoma in Korean women. Gynecol Oncol. 2013 Jul; 130(1):115-20. https://doi.org/10.1016/j.ygyno.2013.02.026.

Peeters, E., Wentzensen, N., Bergeron, C., Arbyn, M., 2019. Meta-analysis of the accuracy of p16 or p16/Ki-67 immunocytochemistry versus HPV testing for the detection of CIN2+/CIN3+ in triage of women with minor abnormal cytology. Cancer Cytopathol. 2019; 127(3):169-180. https://doi.org/10.1002/cncy.22103.

Petry, K.U., Horn, J., Luyten, A., Mikolajczyk, R.T., 2018. Punch biopsies shorten time to clearance of high-risk human papillomavirus infections of the uterine cervix. BMC Cancer 2018; 18(1):318. https://doi.org/10.1186/s12885-018-4225-9.

Pimenta, J.M. Galindo, C., Jenkins, D., Taylor, S.M., 2013. Estimate of the global burden of cervical adenocarcinoma and potential impact of prophylactic human papillomavirus vaccination. BMC Cancer 2013; 13: 553. https://doi.org/10.1186/1471-2407-13-553.

Pirog, E.C., Lloveras, B., Molijn, A., Tous, S., Guimerà, N., Alejo, M., Clavero, O., Klaustermeier, J., Jenkins, D., Quint, W.G.V., Bosch, F.X., Alemany, L., de Sanjose, S., 2014. HPV Prevalence and Genotypes in Different Histological Subtypes of Cervical Adenocarcinoma, a Worldwide Analysis of 760 Cases. Mod Pathol 2014; 27:1559–1567. https://doi.org/10.1038/modpathol.2014.55.

Plaxe, S.C., Saltzstein, S.L., 1999. Estimation of the duration of the preclinical phase of cervical adenocarcinoma suggests that there is ample op- portunity for screening. Gynecol Oncol 1999; 75:55-61. https://doi.org/10.1006/gyno.1999.5524.

Polat, A.Y., Tepeoglu, M., Tunca, M.Z., Ayva, E.S., Ozen, O., 2021. Atypical glandular cells in Papanicolaou test: Which is more important in the detection of malignancy, architectural or nuclear features? Cytopathology 2021; 32:344–352. https://doi.org/10.1111/cyt.12957.

Pradhan, D., Li, Z., Ocque, R., Patadji, S., Zhao, C., 2016. Clinical Significance of Atypical Glandular Cells in Pap Tests: An Analysis of More Than 3000 Cases at a Large Academic Women's Center. Cancer Cytopathol. 2016; 124(8):589-595. https://doi.org/10.1002/cncy.21724.

Pulkkinen, J., Huhtala, H., Kholová, I., 2021. The Role of Pap Smear in the Diagnostics of Endocervical adenocarcinoma. APMIS. 2021; 129: 195–203. https://doi.org/10.1111/apm 13115.

Pulkkinen, J., Huhtala, H., Krogerus, L.A., Hollmén, S., Laurila, M., Kholová, I., 2022. Endocervical Cytology: Inter- and Intra-Observer Variability in Conventional Pap Smears. Acta Cytol. 2022;66(3):206-215. https://doi.org/10.1159/000522212.

Pulkkinen, J., Kares, S., Huhtala, H., Kholová, I., 2021. Detection and Outcome of Endocervical Atypia in Cytology in Primary HPV Screening Programme. Diagnostics (Basel). 2021; 11:2402. https://doi.org/10.3390/diagnostics11122402.

Raab, S.S., Isacson, C., Layfield, L.J., Lenel, J.C., Slagel, D.D., Thomas, P.A., 1995. Atypical glandular cells of undetermined significance. Cytologic criteria to separate clinically significant from benign lesions Am J Clin Pathol.1995; 104(5):574-82. https://doi.org/10.1093/ajcp/104.5.574.

Rabelo-Santos, S.H., Derchain, S.F.M., Do Amaral Westin, M.C., Angelo-Andrade, L.A.L., Sarian, L.O.Z., Oliveira, E.R.Z.M., et al. 2008. Endocervical Glandular Cell Abnormalities in Conventional Cervical Smears: Evaluation of the Performance of Cytomorphological Criteria and HPV Testing in Predicting Neoplasia. Cytopathol. 2008; 19:34-43. https://doi.org/10.1111/j.1365-2303.2007.00466.x.

Radomska, A., Lee, D., Neufeld, H., Korte, N., Torlakovic, E., Agrawal, A., Chibbar, R., 2021, A retrospective study on incidence, diagnosis, and clinical outcome of gastric-type endocervical adenocarcinoma in a single institution. Diagn Pathol. 2021; 16:68. https://doi.org/10.1186/s13000-021-01129-9.

Reich, O., Regauer, S., Kashofer, K., 2020. Possibly carcinogenic HPV subtypes are a cause of HSIL and negative clinical HPV tests - A European prospective single center study. Gynecol Oncol. 2020; 158:112-116. https://doi.org/10.1016/j.ygyno.2020.04.685.

Reimers, L.L., Anderson W.F., Rosenberg, P.S., Henson, D.E., Castle, P.E., 2009. Etiologic Heterogeneity for Cervical Carcinoma by Histopathologic Type, Using Comparative Age-Period-Cohort Models. Cancer Epidemiol Biomarkers Prev. 2009; 18(3):792-800. https://doi.org/10.1158/1055-9965.EPI-08-0965.

Rijkaart, D.C., Berkhof, J., Rozendaal, L., van Kemenade, F.J., Bulkmans, N.W.J., Heideman, D.A.M, Kenter, G.G., Cuzick, J., Snijders, P.J.F., Chris J L M Meijer, C.J.L.M., 2012. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomized controlled trial. Lancet Oncol. 2012; 13: 78–88 https://doi.org/10.1016/S1470-2045(11)70296-0.

Rimm, D.L., Gmitro, S., Frable, W.J., 1996. Atypical reparative change on cervical/vaginal smears may be associated with dysplasia. Diagn Cytopathol. 1996;14(4):374-9. https://doi.org/10.1002/(SICI)1097-0339(199605)14:4<374::AID-DC17>3.0.CO;2-H.

Roiguez-Carunchio, L., Soveral, I., Steenbergen, R.D.M., Torne, A., Martinez, S., Fuste, P. Pahisa, J., Marimon, L., Ordi, J., Del Pino, M., 2015. HPV-negative carcinoma of the uterine cervix: a distinct type of cervical cancer with poor prognosis. BJOG. 2015; 122 (1):119-127 https://doi.org/ 10.1111/1471-0528.13071 .

Ronco, G., Dillner, J., Elfström, K.M., Tunesi, S., Snijders, P.J.F., Arbyn, M., Kitchener, H., Segnan, N., Gilham, C., Giorgi-Rossi, P. Berkhof, P.J., Peto, J., Meijer, C.J.L.M, 2014. International HPV screening working group. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet. 2014; 383(9916):524-532. https://doi.org/10.1016/S0140-6736(13)62218-7.

Ronco, G., Giorgi-Rossi, P., Carozzi, F., Confortini, M., Dalla Palma, P., Del Mistro, A., Ghiringhello, B., Girlando, S., Gillio-Tos, A., De Marco, L., Naldoni, C., Pierotti, P., Rizzolo, R., Schincaglia, P., Zorzi, M., Zappa, M., Segnan, N., Cuzick, J., 2010. Efficacy of human papillomavirus testing for the detection of invasive cervical

cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncol. 2010; 11(3):249-57. https://doi.org/10.1016/S1470-2045(09)70360-2.

Ronnet, B.M., Manos, M.M., Ransley, J.E., Fetterman, B.J., Kinney, W.K., Hurley, L.B., Shieh Ngai, J., Kurman, R.J., Sherman, M.E., 1999. Atypical glandular cells of undetermined significance (AGUS): Cytopathologic features, histopathologic results, and human papillomavirus DNA detection. Human Pathol. 1999; 30:816-825. https://doi.org/10.1016/S0046-8177(99)90143-0.

Ryu, A., Nagata, S., Kubo, C., Ueda, T., Tanada, S., Idota, A., Kamiura, S., Honma, K., Yamasaki, T., 2021. Conventional Direct Smear Yields Diagnostic Indicators of Gastric-Type Mucinous Carcinoma Compared with Cytomorphological Features Identified by Liquid-Based Cervical Cytology. Acta Cytol. 2021;65(2):150-157. https://doi.org/10.1159/000511337.

Salciccioli, I., Zhoum C.D., Okonji, E.C., Shalhoub, J., Salciccioli, J.D., Marshall, D., 2021. European trends in cervical cancer mortality in relation to national screening programs, 1985-2014. Cancer Edemiol. 2021; 74:102002. https://doi.org/10.1016/j.canep.2021.102002.

Salo, H., Nieminen, P., Kilpi, T., Auranen, K., Leino, T., Vänskä, S., Tiihonen, P., Lehtinen, M., Anttila, A., 2004. Divergent Coverage, Frequency and Costs of Organised and Opportunistic Pap Testing in Finland. Int J Cancer 2014; 135:204-213. https://doi.org/10.1002/ijc.28646

de Sanjose, S., Quint, W.G., Alemany, L., Geraets, D.T., Klaustermeier, J.E., Lloveras, B., Tous, S., Felix, A., Bravo, L.E., Shin, H.-R., Vallejos, C.S., Alonso de Ruiz, P., Lima, M.A., Guimera, N., Clavero, O., Alejo, M., Llombart-Bosch, A., Cheng-Yang, C., Tatti, S.A., Kasamatsu, E., Iljazovic, E., Odida, M., Prado, R., Seoud, M., Grce, M., Usubutun, A., Jain, A., Hernandez Suarez, G.A., Lombardi, L.E., Banjo, A., Menéndez, C., Domingo, E.J., Velasco, J., Nessa, A., Bunnag Chichareon, S.C., Qiao, Y. L., Lerma, E., Garland, S.M., Sasagawa, T., Ferrera, A., Hammouda, D., Mariani, L., Pelayo, A., Steiner, I., Oliva, E., Meijer, C.J.L.M, Al-Jassar, W.F., Cruz, E., Wright, T.C., Puras, A, Ladines Llave, C., Tzardi, M., Agorastos, T., Garcia-Barriola, V., Clavel,C., Ordi, J., Andújar, M., Castellsagué, X., Sánchez, G.I., Nowakowski, A.M., Bornstein, J., Muñoz, N., Bosch, F.X., 2010. Human Papillomavirus Genotype Attribution in Invasive Cervical Cancer: a Retrospective Cross-Sectional Worldwide Study. Lancet Oncol. 2010; 11:1048-1056. https://doi.org/10.1016/S1470-2045(10)70230-8.

Sasieni, P., Adams, J., 2001. Changing rates of adenocarcinoma and adenosquamous carcinoma of the cervix in England. Lancet 2001; 357; 1490–1493. https://doi.org/10.1016/S0140-6736(00)04646-8.

Schiffman, M., Kinney, W.K., Cheung, L.C., Cage, J.C., Fetterman, B., Poitras, N.E., Lorey, T.S, Wentzensen, N., Befano, B., Schussler, J., Katki, H.A., Castle, P.E., 2018. Relative Performance of HPV and Cytology Components of Cotesting in Cervical Screening. J Natl Cancer Inst. 2018; 110(5):501-508. https://doi.org/10.1093/jnci/djx225

Schindler, S, Pooley Jr, R.J., De Frias, D.V., Yu, G.H., Bedrossian, C.W., 1998. Follow-up of atypical glandular cells in cervical-endocervical smears. Ann Diagn Pathol. 1998; 2(5):312-7. https://doi.org/10.1016/s1092-9134(98)80024-5.

Schnatz, P.F., Guile, M., O'Sullivan, D.M., Sorosky J.I., 2006. Clinical significance of atypical glandular cells on cervical cytology. Obstet Gynecol. 2006; 107(3):701-8. https://doi.org/10.1097/01.AOG.0000202401.29145.

Schoolland, M., Segal, A., Allpress, S., Miranda, A., Frost, F.A., Sterrett, G.F., 2002. Adenocarcinoma in Situ of the Cervix - Sensitivity of Detection by Cervical Smear. Cancer. 2002; 96:330–337. https://doi.org/10.1002/cncr.10886.

Selenica, P., Alemar, B., Matrai, C., Talia, K.L., Veras, E., Hussein, Y., Oliva, E., Beets-Tan, R.G.H., Mikami, Y., McCluggage, W.G., Kiyokawa, T., Weigelt, B., Park, K.J., Murali, R., 2021. Massively parallel sequencing analysis of 68 gastric-type cervical adenocarcinomas reveals mutations in cell cycle-related genes and potentially targetable mutations. Mod Pathol. 2021; 34:1213-1225. https://doi.org/10.1038/s41379-020-00726-1.

Sellors, J.W., Nieminen, P., Vesterinen, E., Paavonen J., 1990 Observer variability in the scoring of colpophotographs. Obstet Gynecol.1990; 76:1006-8.

Selvaggi, S.M., 1994. Cytologic features of squamous cell carcinoma in situ involving endocervical glands in cervical cytobrush specimen. Acta Cytol. 1994; 38:687-692 https://doi.org/10.1002/dc.10061.

Selvaggi, S.M., 2016. Glandular Epithelial Abnormalities on Thinprep® Pap Tests: Clinical and Cytohistologic Correlation. Diagn Cytopathol. 2016; 44:389-393. https://doi.org/10.1002/dc.23452.

Selvaggi, S.M., 2002. Cytologic Features of High-grade Squamous Intraepithelial Lesions Involving Endocervical Glands on ThinPrep® Cytology. Diagn Cytopathol. 2002; 26:181-185. https://doi.org/10.1002/dc.10061.

Selvaggi, S.M., Haefner, H.K., 1997. Microglandular Endocervical Hyperplasia and Tubal Metaplasia: Pitfalls in the Diagnosis of Adenocarcinoma on Cervical Smears. Diagn Cytopathol. 1997; 16(2):168-73. https://doi.org/ 10.1002/(sici)1097-0339(199702)16:2<168::aid-dc15>3.0.co;2-k.

Sherman, M.E., Dasgupta, A., Schiffman, M., Nayar, R., Solomon D., 2007 The Bethesda Interobserver Reproducibility Study (BIRST). Cancer Cytopathol. 2007; 111:15-25. https://doi.org/10.1002/cncr.22423.

Shing, J.Z., Shangying Hu, S., Herrero, R., Hildesheim, A., Porras, C., Sampson, J.N., Schussler, J., Schiller, J.T., Lowy, D.R., Sierra, M.S., Carvajal, L., Kreimer, A.R., 2022. Precancerous cervical lesions caused by non-vaccine- preventable HPV types after vaccination with the bivalent AS04-adjuvanted HPV vaccine: an analysis of the long-term follow-up study from the randomised Costa Rica HPV Vaccine Trial. Lancet Oncol 2022; 23: 940–49. https://doi.org/10.1016/ S1470-2045(22)00291-1.

Simsir, A., Hwang, S., Cangiarella, J., Elgert, P., Levine, P., Sheffield, M.V., Roberson, J., Talley, L., Chhieng, D.C., 2003. Glandular Cell Atypia on Papanicolaou Smears: Interobserver Variability in the Diagnosis and Prediction of Cell of Origin. Cancer 2003; 99:323–330. https://doi.org/10.1002/cncr.11826.

Smith, H.O., Tiffany, M.F., Qualls, C.R., Key, C.R., 2000.The rising incidence of adenocarcinoma relative to squamous cell carcinoma of the uterine cervix in the United States – a 24 year population- based study. Gynecol. Oncol. 2000; 78; 97–105. https://doi.org/10.1006/gyno.2000.5826.

Smith, J.S., Lindsay, L., Hoots, B., Keys, J., Franceschi, S., Winer, R., Clifford, G.M., 2007. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer. 2007; 121(3):621-32. https://doi.org/10.1002/ijc.22527.

Soofer, S.M., Sidawy, M.K., 2000. Atypical glandular cells of undetermined significance: clinically significant lesions and means of patient follow-up. Cancer. 2000; 90(4):207-

14. https://doi.org/10.1002/1097-0142(20000825)90:4%3C207::AID-CNCR2%3E3.0.CO;2-H.

Stoler, M., 2000. Human papillomaviruses and cervical neoplasia: a model for carcinogenesis. Int J Gynecol Pathol 2000; 19:16– 28. https://doi.org/ 10.1097/00004347-200001000-00004.

Stolnicu, S., Barsan, J., Hoang, L., Patel, P., Terinte, C., Pesci, P., Aviel-Ronen, S., Kiyokawa, T., Alvarado-Cabrero, I., Pike, M.C., Oliva, E., Park, K.J., Soslow, R.A., 2018. International Endocervical adenocarcinoma criteria and classification (IECC): a new pathogenetic classification for invasive adenocarcinomas of the endocervix am J Surg. Pathol. 2018:214–26. https://doi.org/10.1097/PAS.0000000000000986.

Stolnicu, S., Park, K.J., Kiyokawa, T., Oliva, E., McCluggage, W.G., Soslow, R.A., 2021. Tumor Typing of Endocervical Adenocarcinoma: Contemporary Review and Recommendations From the International Society of Gynecological Pathologists. Int J Gynecol Pathol. 2021; 40: S75-S91. https://doi.org/10.1097/PGP.0000000000000751.

De Strooper L.M.A., Berkhof, J., Steenbergen, R.D.M., Lissenberg-Witte, B.I., Snijders, P.J.F., Meijer, C.J.L.M, Heideman, D.A.M., 2018. Cervical cancer risk in HPV-positive women after a negative FAM19A4/mir124-2 methylation test: a post hoc analysis in the POBASCAM trial with 14 year follow-up. Int J Cancer 2018:143:1541–8. Int J Cancer. 2018; 143(6):1541-1548. https://doi.org/ 10.1002/ijc.31539.

Syöpärekisteri. Syöpä 2020: Tilastoraportti Suomen syöpätilanteesta. (Referred September 9, 2022). Available online at: https://syoparekisteri.fi/assets/files/2022/06/Syopa-2020-raportti_fin.pdf.

Tjalma, W.A., Fiander, A., Reich, O., Powell, N., Nowakowski, A.M., Kirschner, B., Koiss, R., O'Leary, J., Joura, E.A., Rosenlund, M., Colau, B., Schledermann, D., Kukk, K., Damaskou, V., Repanti, M., Vladareanu, R., Kolomiets, L., Savicheva, A., Shipitsyna, E., Ordi, J., Molijn, A., Quint, W., Raillard, A., Rosillon, D., Collas De Souza, S., Jenkins, D., Holl, K., 2013. Differences in human papillomavirus type distribution in high-grade cervical intraepithelial neoplasia and invasive cervical cancer in Europe. Int J Cancer. 2013; 132:854-867. https://doi.org/10.1002/ijc.27713.

Tornesello, M.L., Annunziata, C., Buonaguro, L., Losito, S., Greggi, S., Buonaguro, F.M., 2014. TP53 and PIK3CA gene mutations in adenocarcinoma, squamous cell carcinoma and high-grade intraepithelial neoplasia of the cervix. J Transl Med. 2014; 12:255. https://doi.org/10.1186/s12967-014-0255-5.

Torous V.F., Pitman M.B., 2021. Interpretation pitfalls and malignant mimics in cervical cytology. J Am Soc Cytopathol. 2021; 10(2):115-127. https://doi.org/10.1016/j.jasc.2020.06.005.

Torres, J.C.C., Derchain, S.F.M., Gontijo, R.C., Do Amaral Westin, M.C, Zeferino, L.C., Angelo-Andrade, L.A.L., Rabelo-Santos, S.H., 2005. Atypical glandular cells: criteria to discriminate benign from neoplastic lesions and squamous from glandular neoplasia Cytopathology 2005; 16(6):295-302. https://doi.org/ 10.1111/j.1365-2303.2005.00300.x.

Trzeszcz, M., Mazurec, M., Jach, R., Mazurec, K., Jach, Z., Kotkowska-Szeps, I., Kania, M., Wantuchowicz, M., Prokopyk, A., Barcikowski, P., Przybylski, M., Wach, J., Halon, A., 2021. Liquid-Based Screening Test Results: HPV, Liquid-Based Cytology, and P16/Ki67 Dual-Staining in Private-Based Opprtunistic Cervical Cancer Screening. Diagnostics 2021; 11:1420. https://doi.org/10.3390/diagnostics11081420.

Tsang, S.H., Sampson, J.N., Schussler, J., Porras, C., Wagner, S., Boland, J., Cortes, B., Lowy, D.R., Schiller, J.T., Schiffman, M., Kemp, T.J., Rodriguez, A.C., Quint, W., Gail, H.G., Pinto, L.A., Gonzalez, P., Hildesheim, A., Kreimer, A.R., Herrero, R., 2020. Durability of Cross-Protection by Different Schedules of the Bivalent HPV Vaccine: The CVT Trial. J Natl Cancer Inst. 2020; 112(10):1030-1037. https://doi.org/10.1093/jnci/djaa010.

Uijterwaal, M.H., Polman, N.J., Witte, B.I., van Kemenade, F.J., Rijkaart, D., Berkhof, J., Balfoort-van der Meij, G.A.M.A., Ridder, R., Snijders, P.J.F., Meijer, C.J.L.M., 2015. Triaging HPV-positive women with normal cytology by p16/Ki-67 dual-stained cytology testing: baseline and longitudinal data. Int J Cancer. 2015; 136(10):2361-8. https://doi.org/ 10.1002/ijc.29290.

Uijterwaal, M.H., Witte, B.I., Van Kemenade, F.J., Rijkaart, D., Ridder, R., Berkhof, J., Balfoort-van der Meij, G.A.M.A, Bleeker, M.C.G., Snijders, P.J.F, Meijer, C.J.L.M., 2014. Triaging borderline/mild dyskaryotic Pap cytology with p16/Ki-67 dual-stained cytology testing: cross-sectional and longitudinal outcome study. Br J Cancer. 2014 Mar 18; 110(6):1579-86. https://doi.org/10.1038/bjc.2014.34.

Ullal, A., Roberts, M., Bulmer, J.N., Mathers, M.E., Wadehra, V., 2009. The Role of Cervical Cytology and Colposcopy in Detecting Cervical Glandular Neoplasia. Cytopathology 2009; 20:359-366. https://doi.org/ 10.1111/j.1365-2303.2008.00566.x.

Vaccarella, S., Franceschi, S., Engholm, G., Lonnberg, S., Khan, S., Bray, F., 2014. 50 years of screening in the Nordic countries: quantifying the effects on cervical cancer incidence. Br J Cancer. 2014; 111:965-969. https://doi.org/10.1038/bjc.2014.362.

Vallikad, E., Siddartha, P.T., Kulkarni, K.A., Firtion, C., Keswarpu, P., Vajinepalli, P., Naik, S., Gupta, L., 2017. Intra and Inter-Observer Variability of Transformation Zone Assessment in Colposcopy: A Qualitative and Quantitative Study. J Clin Diagnostic res. 2017; 11(1): XC04-XC06. https://doi.org/10.7860/JCDR/2017/21943.9168

Veijalainen, O., Kares, S., Kotaniemi-Talonen, L., Kujala, P., Vuento, R., Luukkala, T., Kholová, I., Mäenpää, J., 2021. Primary HPV screening for cervical cancer: Results after two screening rounds in a regional screening program in Finland. Acta Obstet Gynecol Scand. 2021; 100:403–409. https://doi.org/10.1111/aogs.14021.

Veijalainen, O., Kares, S., Kujala, P., Tirkkonen, M., Vuento, R., Kholová, I., Luukkaala, T., Osuala, V., Mäenpää, J., 2016. Human papillomavirus test with cytology triage in organized screening for cervical cancer. Acta Obstet Gynecol Scand. 2016; 95:1220-1227. https://doi.org/10.1111/aogs.13013.

Veijalainen, O., Kares, S., Kujala, P., Vuento, R., Osuala, V., Tirkkonen, M., Luukkaala, T., Kholová, I., Mäenpää, J., 2019. Implementation of HPV based cervical cancer screening in an organised regional screening programme: 3 years of experience. Cytopathology 2019; 30:150–156. https://doi.org/10.1111/cyt.12652.

Vink, F.J., Meijer, C.J.L.M., Clifford, G.M., Poljak, M., Oštrbenk, A., Petry, K.U., Rothe, B., Bonde, J., Pedersen, H., de Sanjosé, S., Torres, M., del Pino, M., Quint, W.G.V., Cuschieri, K., Boada, E.A., van Trommel, N.E., Lissenberg-Witte B.I., Floore, A.N., Hesselink, A.T., R. D.M., Bleeker, M.C.G., Heideman, D.A., 2020. FAM19A4/miR124-2 methylation in invasive cervical cancer: A retrospective cross-sectional worldwide study. Int J Cancer. 2020; 147(4):1215-1221. https://doi.org/ 10.1002/ijc.32614.

Viswanathan, K., Patel, A., Abdelsayed, M., Rosado, L., Soong, L., Margolskee, E., Heymann, J.J., Goyal, A., Rao, R.A., 2020. Interobserver Variability Between

Cytopathologists and Cytotechnologists Upon Application and Characterization of the Indeterminate Category in the Milan Sytstem for Reporting Salivary Gland Cytopathology. Cancer Cytopathol. 2020; 128:828-839. https://doi.org/10.1002/cncy.22312.

Voidăzan, S.T., Dianzani, C., Husariu, M.A., Geréd, B., Turdean, S.G., Uzun, C.C., Kovacs, Z., Rozsnyai, F.F., Neagu, N., 2022. The Role of p16/Ki-67 Immunostaining, hTERC Amplification and Fibronectin in Predicting Cervical Cancer Progression: A Systematic Review. Biology (Basel). 2022; 11(7):956. https://doi.org/10.3390/biology11070956.

Vu, M., Yu, J., Awolude, O.A., Chuang, L., 2018. Cervical cancer worldwide. Curr Probl Cancer. 2018;42(5):457-465. https://doi.org/10.1016/j.currproblcancer.2018.06.003.

Walboomers, J.M.M., Jacobs, M.V., Manos, M.M., Bosch, F.X., Kummer, J.A., Shah, K.V., Snijders, P.J., Peto, J., Meijer, C.L.M., Muñoz, N., 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999; 189:12-19. https://doi.org/10.1002/(SICI)1096-9896(199909)189:1<12::AID-PATH431>3.0.CO;2-F.

Wang, J., Andrae, B., Sundström, K., Ström, P., Ploner, A., Elfström, K.M. Arnheim-Dahlström, L., Dillner, J., Sparén, P., 2016. Risk of invasive cervical cancer after atypical glandular cells in cervical screening: nationwide cohort study BMJ. 2016; 352: i276. https://doi.org/10.1136/bmj.i276.

Wang, J., Elfström, M., Andrae, B., Nordqvist Kleppe, S., Ploner, A., Lei, J., Dillner, J., Sundström, K., Sparén, P., 2020. Cervical cancer case–control audit: Results from routine evaluation of a nationwide cervical screening program Int J Cancer. 2020; 146(5):1230-1240. https://doi.org/10.1002/ijc.32416.

Wang, S.S., Sherman, M.E., Hildesheim, A., Lacey, J.V. Jr., Devesa, S., 2004 Cervical adenocarcinoma and squamous cell carcinoma incidence trends among white women and black women in the United States for 1976-2000. Cancer 2004; 100:1035–44. https://doi.org/10.1002/cncr.20064.

Wheeler, C.M., Castellsagué, X., Garland, S.M., Szarewski, A., Paavonen, J., Naud, P., Salmerón, J., Chow, S.N., Apter, D., Kitchener, H., Teixeira, J.C., Skinner, S.R., Jaisamrarn, U., Limson, G., Romanowski, B., Aoki, F.Y., Schwarz, T.F., Poppe, W.A.J., Bosch, F.X., Harper, D.M., Huh, W., Hardt, K., Zahaf, T., Descamps, D., Struyf, F., Dubin, G., Lehtinen, M., 2012. Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Lancet Oncol. 2012; 13(1):100-10. https://doi.org/10.1016/S1470-2045(11)70287-X.

WHO Classification of Tumours Editorial Board, 2020. Female Genital Tumours, 5th Edition. International Agency for Research on Cancer. https://publications.iarc.fr/592.

Westin, M.C., Derchain, S.F., Rabelo-Santos, S.H., Angelo-Andrade L.A., Sarian, L.O., Oliveira, E., Zeferino., L.C., 2008. Atypical Glandular Cells and Adenocarcinoma in Situ According to the Bethesda 2001 Classification: Cytohistological Correlation and Clinical Implications. J Obstet Gynecol Reprod Biol. European Journal of Obstetrics and Gynecology 2008; 139:79-85. https://doi.org/10.1016/j.ejogrb.2007.08.017

Wilbur, D.C., 2016. Practical issues related to uterine pathology: in situ and invasive cervical glandular lesions and their benign mimics: emphasis on cytology–histology

correlation and interpretive pitfalls. Mod Pathol. 2016; 29 Suppl 1: S1-11. https://doi.org/10.1038/modpathol.2015.138.

Wood, M.D., Horst, J.A, Bibbo, M., 2007. Weeding atypical glandular cell look-alikes from the true atypical lesions in liquid-based Pap tests: a review. Cytopathology 2007; 35(1):12-7. https://doi.org/10.1002/dc.20589.

Wright, A.A., Howitt, B.E., Myers, A.P., Dahlberg, S.E., Palescandolo, E., Van Hummelen, P., MacConaill, L.E., Shoni, M., Wagle, N., Jones, R.T., Quick, C.M., Laury, A., Katz, I.T., Hahn, W.C., Matulonis, U.A., Hirsch, M.S., 2013. Oncogenic mutations in cervical cancer: genomic differences between adenocarcinomas and squamous cell carcinomas of the cervix. Cancer. 2013; 119:3776-83. https://doi.org/10.1002/cncr.28288.

Wright Jr, T.C., Stoler, M.H., Ranger-Moore, J., Fang, Q., Volkir, P., Safaeian, M., Ridder, R., 2022. Clinical validation of p16/Ki-67 dual-stained cytology triage of HPV-positive women: Results from the IMPACT trial. Int J Cancer. 2022; 150(3):461-471. https://doi.org/10.1002/ijc.33812.

Wright, T.C., Stoler, M.H., Behrens, C.M., Sharma, A., Zhang, G., Wright, T., 2015. Primary cervical cancer screening with human papillomavirus: End of study results from the ATHENA study using HPV as the first line screening test. Gynecol Oncol. 2015; 136(2):189-97. https://doi.org/10.1016/j.ygyno.2014.11.076.

Yahr, L.J., Lee, K.R., 1991. Cytologic findings in microglandular hyperplasia of the cervix. Diagn Cytopathol. 1991; 7(3):248. https://doi.org/10.1002/dc.2840070308.

Zaino, R.J., 2002. Symposium part I: adenocarcinoma in situ, glandular dysplasia, and early invasive adenocarcinoma of the uterine cervix. Int J Gynecol Pathol. 2002; 21:314–26. https://doi.org/10.1097/00004347-200210000-00002

Zardo, L.M.G., Thuler, L.C.S., Zeferino, L.C., Horta, N.M.S.R., Fonseca, R.C.S.P., 2009. Performance of the Cytologic Examination for the Diagnosis of Endocervical Adenocarcinoma in Situ. Acta Cytologica 2009; 53:558-564. https://doi.org/10.1159/000325384.

Zhang, X., Qingle Zeng, Q., Cai, W., Ruan, W., 2021.Trends of cervical cancer at global, regional, and national level: data from the Global Burden of Disease study 2019. BMC Public Health 2021; 21(1):894. https://doi.org/10.1186/s12889-021-10907-5.

Zhao, C., Florea, A., Onisko, A., Austin, R.M., 2009. Histologic follow-up results in 662 patients with Pap test findings of atypical glandular cells: Results from a large academic womens hospital laboratory employing sensitive screening methods. Gynecol Oncol. 2009; 114(3):383-389. https://doi.org/10.1016/j.ygyno.2009.05.019.

Zeferino, L.C., Rabelo-Santos, S.H., Villa, L.L., Sarian, L.O., Costa, M.C., do Amaral Westin, M.C., de Ângelo-Andrade, L.A.L., Derchain, S., 2011. Value of HPV-DNA test in women with cytological diagnosis of atypical glandular cells (AGC) Eur J Obstet Gynecol Reprod Biol. 2011; 159:160-4. https://doi.org/10.1016/j.ejogrb.2011.05.023.

PUBLICATION I

The role of Pap smear in the diagnostics of endocervical adenocarcinoma

Pulkkinen J, Huhtala H, Kholová I.

APMIS. 2021; 129(4):195–203.
doi:10.1111/apm.13115.

APMIS JOURNAL OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY

APMIS 129: 195–203 © *2021 The Authors. APMIS* published by John Wiley & Sons Ltd on behalf of Scandinavian Societies for Medical Microbiology and Pathology.

DOI 10.1111/apm.13115

The role of Pap smear in the diagnostics of endocervical adenocarcinoma

JOHANNA PULKKINEN,[1] HEINI HUHTALA[2] and IVANA KHOLOVÁ[1,3]

[1]Pathology, Fimlab Laboratories, Tampere, Finland; [2]Faculty of Social Sciences, Tampere University, Tampere, Finland; and [3]Department of Pathology, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

Pulkkinen J, Huhtala H, Kholová I. The role of Pap smear in the diagnostics of endocervical adenocarcinoma. APMIS. 2021; 129: 195–203.

In the high-income countries, the amount of cervical adenocarcinomas is on the rise. The pap smear sampling has a low sensitivity and a low specificity for endocervical malignancies, and there are only a few cytomorphological features, that are specifically associated with glandular atypia. In this study, 298 pap smears of 60 patients with endocervical adenocarcinoma or adenocarcinoma in situ (AIS) and 30 patients with high-grade intraepithelial lesion (HSIL) in histology were reviewed. The pap smear type (screening/clinical), the HPV status and the time from sampling to the histological confirmation of diagnosis were recorded for each case. Despite that no cytomorphological features could be associated with adenocarcinoma statistically, 70% of the pap smears were initially correctly diagnosed as an endocervical glandular lesion. Palisading cell borders, nuclear pleomorphism and the lack of single atypical cells present simultaneously were found to be associated with adenocarcinoma and AIS with the corresponding ORs of 5.89 (95% CI 1.96–17.70), 3.71 (95% CI 1.14–12.02) and 10.76 (95% CI 1.20–59.50). This combination of features was seen in smears taken up to 5 years before the histological diagnosis. Of all our screening samples, 10.9% were HPV-positive. There were no HPV-negative samples among patients with adenocarcinoma.

Key words: Endocervical adenocarcinoma; AIS; pap smear; cytological features; HPV; pathology.

Ivana Kholová, Department of Pathology, Fimlab Laboratories, P.O. Box 66, FIN 33101 Fimlab, Finland.
e-mail: ivana.kholova@tuni.fi

Worldwide cervical cancer is the fourth most frequent cancer in women (1). Since the cervical cancer screening programme in Finland started in the 1960s, the amount of cervical cancer deaths has decreased to one-fifth of its original number (2). In Finland, in addition to the national cervical cancer screening programme, there are also symptom-based and opportunistic (3) pap smears. While about 90% of the deaths caused by cervical cancer occur in low- and middle-income countries, in the high-income countries the total amount of cervical cancers has decreased (4). Yet, in the high-income countries, the relative and total amount of cervical adenocarcinomas seems to be rising, especially among the younger age groups (4).

While the diagnostic cytological and histological features of endocervical adenocarcinoma (EAC) and endocervical adenocarcinoma in situ (AIS) are defined by The Bethesda System for Reporting Cervical Cytology (5) and the WHO Classification of the Tumours of Female Reproductive Organs (6), respectively, there are currently no accepted lower grade precursor lesions for adenocarcinoma like there are the intraepithelial neoplasias for cervical squamous cell carcinoma (5–8). In cytological cervical samples, a large amount of the lesions behind glandular diagnoses (atypical endocervical cells, NOS, atypical endocervical cells, favour neoplastic, endocervical adenocarcinoma in situ and endocervical adenocarcinoma) are non-neoplastic or of squamous or endometrial origin or other carcinomas leading to a low screening specificity for EAC and AIS (9–17).

Cytological diagnoses of atypical endocervical cells, NOS (AEC, NOS), atypical endocervical cells, favour neoplastic (AEC, FN) and adenocarcinoma in situ (AIS) were shown to have a progressively better association with neoplasia (10–11,17–18) and

Received 15 June 2020. Accepted 8 January 2021

Table 1. Cohort characteristics

	Number of patients	Age ± SD (range)	Number of pap smears	Average number of smears	Number of screening pap smears	Number of clinical pap smears	Abnormal[1]			Diagnostic[2]		
							Total	Screening pap smear	Clinical pap smear	Total	Screening pap smear	Clinical pap smear
				n (range)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
AC total	60	43.0 ± 14.6 (22–83)	201	3.4 (1–10)	55 (27.4%)	146 (72.6%)	106 (52.7%)	36 (34.0%)	70 (66.0%)	60 (29.5%)	21 (35.0%)	39 (65.0%)
AC only	37	46.0 ± 16.0 (23–83)	109	2.9 (1–9)	27 (24.8%)	82 (75.2%)	57 (52.3%)	18 (31.6%)	39 (68.4%)	37 (33.3%)	2 (5.4%)	35 (94.6%)
AC + LSIL/ HSIL	23	37.5 ± 9.9 (22–56)	92	4.0 (1–10)	28 (30.4%)	64 (69.6%)	49 (53.3%)	18 (36.7%)	31 (63,3%)	23 (25.0%)	6 (26.1%)	17 (73.9%)

[1]Abnormal pap smear sample was defined as sample other than Negative for Intraepithelial Lesion or Malignancy (NILM).
[2]Diagnostic pap smear sample was defined as sample after which histology confirmed the diagnosis of adenocarcinoma or adenocarcinoma in situ.

also specifically to cervical glandular malignancies (19). The reported sensitivity of a single pap smear for neoplasia in previous studies ranged from 15.3% up to 100% depending on the sample selections in the studies. Generally, it was lower in the studies that included also previous pap smears diagnosed as AEC, NOS, Atypical endometrial cells, NOS, atypical glandular cells, NOS, normal or insufficient on contrary to inclusion of only samples with possible or definite high-grade epithelial features (11–12,14–16,20–21).

In the studies, with pap smear samples diagnosed as AIS or EAC in cytology, in histology AIS was found in 13%–28.3% of the cases and EAC in 13% to 38.3% of the cases, although there was a clinically significant lesion in 98.3%–100% of the samples (11,16). When the pap smear samples of histologically confirmed EAC, AIS and AIS + HSIL were reanalysed a positive predictive value for a pap smear for AIS was reported to vary from 47.6% to 54.3% and for EAC from 45 to 76% (20,21).

There were only a few cytomorphological features associated specifically with AEC and AIS (9,10).

In previous studies, feathering was the cytological feature showing the strongest association specifically with endocervical glandular neoplasia, but also pseudostratified strips, rosettes, palisading borders and even papillary groups were reported (9,10).

The aim of the present study was to find out what type of cytological features and samples lead to the diagnosis of EAC or AIS and what are the reasons leading to sampling in these cases. In addition, we traced the first abnormal samples and the cytological progression of endocervical adenocarcinoma to find potential precursor cytological features.

MATERIALS AND METHODS

A laboratory information system (LIS) search for histopathological diagnosis was made to find the patients operated for EAC or AIS at the Tampere University Hospital during the years 2008–2014. In total, 60 patients were found in the 7-year-study period, all of whom had pap smears taken prior to the diagnosis. The pap smear samples were conventional pap smears. All patients had histologically confirmed diagnosis either by conization or hysterectomy. The average age of the patients was 43.0 years (SD ± 14.6, range 22–83) (Table 1). The patients had altogether 201 pap smear samples taken, with the average of 3.4 samples per patient (Table 1).

This group of patients was further divided into the cases with EAC only or AIS only in the final histology, and to the cases with EAC or AIS together with a low or a high-grade squamous intraepithelial lesion (LSIL/HSIL) in the final histology. The first group had 37 patient-cases and 109 pap smear samples and the latter group 23 cases and 92 pap smears (Table 1). All the available information was retrieved from the clinical referral accompanying the pap smear samples.

A control group of patients with the diagnosis of high-grade intraepithelial lesion (HSIL) only in the final histology was retrieved from the LIS. Of the 83 samples signed out as HSIL during the year 2014, those cases lacking diagnostic pap smears or histological confirmation, either by conization or by biopsy, were excluded. Of the remaining cases, 30 were randomly selected for the study. Altogether, 97 pap smear samples were available in the control group. The patients in this group were between the ages of 21 and 54 with the average of 36.7 years (SD ± 10.0).

Altogether, the 90 patients included in the study, had 298 pap smears samples available for the revision. The number of pap smears per patient in this study varied from 1 to 10 with the average amount of samples being 3.3.

In some older samples, the diagnosis was given according to the Papanicolaou Classification System and those were converted to the corresponding diagnosis according to the Bethesda System for Reporting Cervical Cytology 2014 (5).

All pap smears were blindly reviewed by a senior cytopathologist in search for 38 different cytological features, consisting of background features (clean, inflammatory, necrotic, apoptotic debris, necrotic debris, bloody), cellular features (columnar cell shape, cuboidal cell shape, irregular cell borders, increased nuclear/cytoplasmic ratio, regeneration, degeneration, atrophy), nuclear features (enlarged nuclei, nuclear hyperchromasia, irregular nuclear membranes, oval nuclei, elongated nuclei, mitotic figures, nuclear pleomorphism, nucleoli, macronucleoli, finely granular chromatin, coarsely granular chromatin, chromatin clearing, nuclear vacuoles) and architectural features (high cellularity, scant cellularity, single atypical cells, nuclear crowding, loss of polarity, loss of honeycomb pattern, pseudostratified strips, palisading borders, papillary groups, rosettes, feathering). The time from cytological sampling to the histological confirmation of diagnosis was calculated for each pap smear. For statistical analyses, samples that had both a glandular and a squamous lesion in the final histopathology were excluded. Altogether, features of 256 pap smears representing 37 cases with EAC or AIS and 30 cases with HSIL in the final histopathology were analysed.

The statistical analysis was performed by an experienced statistician and the programme used was SPSS version 25 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) A further analysis of the cytomorphological features to define the combination of features associated with the occurrence of EAC and AIS was made with a forward stepwise multivariable logistic regression analysis using probability values of <0.05 for the entry of features.

The study was approved by the Ethical committee of Pirkanmaa Health Care District (R16022), and informed content of each individual was not requested. The study was conducted according to the Declaration of Helsinki.

RESULTS

The oldest sample in this study was taken 19 years and 10 months before the cancer diagnosis and for 16 patients the first sample taken was already diagnostic for EAC or AIS.

Our material consisted of clinical and screening programme samples. Out of the 201 pap smear samples of the patients with EAC or AIS, 27.4% (55/201) were taken in the cervical screening programme and 72.6% (146/201) were clinical samples (Table 1). During the study period, in 2012, the city of Tampere started HPV-screening programme in the age group of 35–60 years (22,23). Of the screening samples, 10.9% (6/55) had a positive HPV-result representing genotypes HPV16 (2 samples), HPV18 (2 samples) and other high-risk HPV types (2 samples) (22). There were no negative HPV cases among patients with EAC or AIS in the final histology.

Of the screening samples, 65.5% (36/55) had an abnormal finding (Table 1) and of all diagnostic samples in our series 35.0% (21/60) were screening samples. In 45.0% (27/60) of the cases, screening lead to biopsies or follow-ups, which eventually lead to the diagnosis of EAC or AIS.

Of the clinical pap smear samples, 12.3% (18/146) were taken because of an abnormal finding in a previous screening pap smear sample (Table 2).

Follow-ups of a previous abnormal clinical cytological or histological finding represented 19.2% (28/146) of the clinical samples. Testing in the association of general check-ups accounted for 13.7% (20/146) of the samples and 5.5% (8/146) were taken in association of birth control-related issues. In 4.8 % (7/146) of the samples, there was a macroscopic tumour, either clinical or radiological. Symptoms reported by patients that led to sampling included abnormal bleeding, abdominal pain or symptoms related to infection like abnormal vaginal discharge, itching, burning sensation or a macroscopic condyloma.

In our material, issues related to infection were the most common symptoms, occurring in 62.5% (10/16) of the cases. They were also the most common non-specific cause leading to diagnosis as they were seen at a wide time range from one sample taken at the time of the final diagnosis to samples taken up to 106 months before the final histological diagnosis (average 53.8 months). The one case with serious infection symptoms at the time of the diagnosis also turned out to have a macroscopic tumour in the clinical check-up.

The second most common cause to symptom-based sampling was abnormal uterine bleeding, which was seen in 37.5% (6/16) of the cases. It was the most specific symptom leading straight to cancer diagnosis in 83% of the reported cases. In our material, only one patient presented with a coital bleeding. Abdominal pain was reported by 3 patients, and in one case, pain-following sampling lead to the cancer diagnosis.

Altogether, only 11.0% (16/146) of the clinical pap smears were taken because of a patient-reported symptom, two of those smears also presenting with a macroscopic tumour in the clinical check-up. Notably, 70.0% of the patients with adenocarcinoma or AIS did not report any symptoms during their history. Unfortunately, 34.9% (51/146) of the clinical samples had no clinical information given in their referral to the pathologist (Table 2).

In the cytomorphological analysis, coarsely granular nuclear chromatin and inflammatory debris were the only features associated specifically with HSIL with respective p-values of <0.001 and 0.024 (Table 3). Other studied features were seen also in EAC and AIS pap smears when all samples were taken into account. However, in HSIL pap smears certain features were recognized earlier. Cuboidal

Table 2. Indications of clinical pap smear sampling

	AC		AC only		AC + LSIL/HSIL	
Number of clinical samples	146		82		64	
Number of indications	149		85		64	
	n	%	n	%	n	%
Gynaecological check-up related	20	13.7	15	18.3	5	7.8
Follow-up[1]	28	19.2	17	20.7	11	17.2
Previous abnormal screening result	18	12.3	5	6.1	13	20.3
Birth control-related	8	5.5	4	4.9	4	6.3
Abnormal uterine bleeding	8[2]	5.5	4	4.9	4[2]	6.3
Macroscopic tumour	7	4.8	6	7.3	1	1.6
Abdominal pain	3	2.1	3	3.7	0	
Infection related symptoms	6	4.1	2	2.4	4	6.3
No data available	51	34.9	29	35.4	22	34.4

[1]Follow-up of a an abnormal finding in a previous clinical sample.
[2]Including 1 coital bleeding.

Table 3. Analysis of cytomorphological features

	Cytopathological feature	Time before histopathological diagnosis (years)	Total p-value
HSIL only[1]	Coarsely granular chromatin	1	0.001
	Inflammatory debris	1	0.024
HSIL earlier[2]	Cuboidal cell shape	3	0.002
	Single atypical cells	3	0.002
	High cellularity	1	0.007
	Papillary groups	1	<0.001
	Rosettes	1	0.006
AC/AIS earlier[3]	Palisading cell borders	2	<0.001

There were no cytopathological features associated with adenocarcinoma or AIS. The following features showed a similar association with squamous and endocervical glandular lesions: feathering, loss of polarity, loss of honeycomb pattern, nuclear crowding, irregular cell borders, columnar cell shape, elongated nuclei. The following features showed no association to squamous or endocervical glandular lesions: clean background, necrotic background, bloody background, apoptotic debris, necrotic debris, increased nuclear/cytoplasmic ratio, regeneration, degeneration, atrophy, enlarged nuclei, nuclear hyperchromasia, irregular nuclear membranes, oval nuclei, mitotic figures, nuclear pleomorphism, nucleoli, macronucleoli, finely granular chromatin, chromating clearing, nuclear vacuoles, scant cellularity and pseudostratified strips.
[1]Features seen in pap smear samples in HSIL group only.
[2]Features seen in both groups, but presenting earlier in HSIL group compared to AC/AIS group.
[3]A feature seen in both groups, but presenting earlier in AC/AIS group compared to HSIL group.

cell shape and single atypical cells could be seen up to 36 months before histopathological diagnosis of HSIL ($p = 0.002$ and 0.002, respectively). High cellularity ($p = 0.007$), inflammatory debris ($p = 0.024$) and traditionally to glandular neoplasia-associated features as papillary groups ($p < 0.001$) and rosettes ($p = 0.006$) were seen up to 12 months before the diagnosis in the HSIL group. However, in the retrospective review of the conizates of the HSIL group, 26 out of 30 conizates showed HSIL extending to the endocervical glands, although in 4 cases the extension was very superficial.

In summary, feathering, palisading cell borders, nuclear crowding, loss of polarity, loss of honeycomb pattern, irregular cell borders, elongated nuclei and columnar cell shape were all seen in both squamous and glandular abnormalities in pap smear samples. Of those features, palisading cell borders were the only feature seen earlier among the adenocarcinoma and AIS samples, as it presented in samples up to two years before histological diagnosis. Among HSIL samples, palisading cell borders were seen a year before the histological diagnosis. Nucleoli did not show an association with adenocarcinoma or AIS in the present study.

Rest of the studied features including clean background, necrotic background, bloody background, apoptotic debris, necrotic debris, increased nuclear/cytoplasmic ratio, regeneration, degeneration, atrophy, enlarged nuclei, nuclear hyperchromasia, irregular nuclear membranes, oval nuclei, mitotic figures, nuclear pleomorphism, nucleoli, macronucleoli, finely granular chromatin, chromatin clearing, nuclear vacuoles, scant cellularity and pseudostratified strips did not show any association with squamous or glandular lesions.

Table 4. The cytopathological diagnoses in the cohort[1]

		NILM	Glandular abnormality			
			AEC, NOS	AEC-FN	AIS	AC
AC total	N	95	27	29	2	4
	Time[1] ± SD (range)	84.9 ± 57.4 (0–229)	23.8 ± 29.4 (0–121)	4.2 ± 10.4 (0–41)	0 ± NA (NA)	0 ± NA (NA)
Ac only	n	53	17	16	1	4
	Time[1] ± SD (range)	93.4 ± 66.3 (0–229)	24.8 ± 32.5 (0–121)	3.7 ± 10.9 (0–41)	0 ± NA (NA)	0 ± NA (NA)
AC + LSIL/ HSIL	n	42	10	13	1	0
	Time[1] ± SD (range)	74.3 ± 42.2 (12–170)	22.1 ± 24.7 (0–69)	4.5 ± 10.1 (0–25)	0 ± NA (NA)	NA

		Squamous abnormality			
		ASC-US	LSIL	ASC-H	HSIL
AC total	n	27	6	10	13
	Time[1] ± SD (range)	41.2 ± 38.2 (0–173)	20 ± 17.2 (0–51)	8.7 ± 16.8 (0–48)	8.1 ± 15.6 (0–54)
Ac only	n	16	0	4	5
	Time[1] ± SD (range)	44.6 ± 44.5 (0–173)	NA	16 ± 24.0 (0–48)	13.8 ± 23.4 (0–54)
AC + LSIL/ HSIL	n	11	6	6	8
	Time[1] ± SD (range)	36.3 ± 25.4 (6–92)	20 ± 17.2 (0–51)	6.5 ± 12.6 (0–30)	4.5 ± 8.0 (0–22)

NA, not applicable.
[1]The average time of cytological diagnosis in months before the histological confirmation of adenocarcinoma or adenocarcinoma in situ.

Even though no single cytomorphological feature could be associated specifically with adenocarcinoma or AIS, the pap smears of patients with only EAC or AIS in histopathology were signed out as a glandular neoplasia more than twice as often as a squamous neoplasia (21 vs. 9), which means that 70% of the neoplastic diagnoses given in this group were glandular (Table 4). In the EAC/AIS + LSIL/HSIL – group, 41% of the neoplastic diagnosis given were glandular (14 vs. 20) while in the HSIL-only group, all the neoplastic diagnosis were squamous (0% glandular, data not shown).

The same although slightly in favour to glandular diagnoses ascending trend was seen in the pap smear samples taken within five years and during the last 12 months before the histological diagnosis. In the first mentioned group 71% of the neoplastic diagnoses given were glandular (22 vs. 9) in samples with AIS or EAC only in histology and 42% (11 vs. 15) in EAC/AIS + LSIL/HSIL –group (Table 5). During the last 12 months before the histological diagnosis, the corresponding figures were 76% (19 vs. 6) and 43% (10 vs. 13) (Table 5).

In the further analysis of the cytomorphological features, the combination of palisading cell borders, nuclear pleomorphism and the lack of single atypical cells were associated with EAC and AIS. An analysis was made with pap smear samples taken a year before the histological diagnosis and another analysis with samples taken 5 years before the histological diagnosis. In the 1-year-analysis, the OR for palisading cell borders was 5.89 (95% CI 1.96–17.70), the OR for nuclear pleomorphism 3.71 (95% CI 1.14–12.02) and the OR for the lack of single atypical cells 10.76 (95% CI 1.20–59.50). The corresponding p-values for the features were 0.002, 0.034 and 0.005.

In the analysis of the samples taken within the preceding 5 years of the histological diagnosis, the OR for palisading cell borders was 4.98 (95% CI 1.78–13.88), the OR for nuclear pleomorphism 3.24 (95% CI 1.09–9.62) and the OR for the lack of single atypical cells 10.70 (95% CI 2.01–56.89). The corresponding p-values for the features were 0.02, 0.34 and 0.05.

The earliest single neoplastic diagnosis in AEC/AIS only group was HSIL, and it was given 54 months before the cancer diagnosis. The first diagnosis of AEC, NOS, that with current guidelines, would lead to colposcopy and histological sampling immediately was signed out as early as 121 months before the diagnosis. Also in the AEC/AIS + LSIL/HSIL– group, the earliest diagnosis that nowadays would lead to histological sampling, was AEC, NOS, and it was given 69 months before diagnosis. The first neoplastic diagnosis in this latter group was LSIL seen in a sample 51 months

Table 5. The cytopathological diagnoses in the cohort 12 months before the cancer diagnosis (A) and 5 years before the cancer diagnosis (B)[1]

		NILM	Glandular abnormality			
			AEC, NOS	AEC,FN	AIS	AC
(A)						
AC total	n	7	13	23	2	4
	Time[1] ± SD (range)	8.1 ± 4.3 (0–12)	2.8 ± 4.1 (0–12)	0.1 ± 0.4 (0–2)	0 ± 0 (0)	0 ± 0 (0)
AC only	n	4	9	14	1	4
	Time[1] ± SD (range)	5.5 ± 3.9 (0–9)	3.4 ± 4.5 (0–12)	0 ± 0 (0)	0 ± NA (NA)	0 ± 0 (0)
AC + LSIL/ HSIL	n	3	4	9	1	0
	Time[1] ± SD (range)	11.7 ± 0.6 (11–12)	1.5 ± 3.0 (0–6)	0.2 ± 0.7 (0–2)	0 ± NA (NA)	NA
(B)						
AC total	n	39	23	27	2	4
	Time[1] ± SD (range)	33.8 ± 18.0 (0–60)	11.8 ± 13.8 (0–44)	6.2 ± 14.8 (0–60)	0 ± 0 (0)	0 ± 0 (0)
AC only	n	19	15	17	1	4
	Time[1] ± SD (range)	30.2 ± 19.1 (0–60)	11.7 ± 14.6 (0–44)	7.0 ± 17.3 (0–60)	0 ± NA (NA)	0 ± 0 (0)
AC + LSIL/ HSIL	n	20	8	10	1	0
	Time[1] ± SD (range)	36.6 ± 16.8 (11–59)	11.9 ± 13.3 (0–38)	4.8 ± 10.1 (0–25)	0 ± NA (NA)	NA

		Squamous abnormality			
		ASC-US	LSIL	ASC-H	HSIL
(A)					
AC total	n	6	1	8	10
	Time[1] ± SD (range)	4.5 ± 5.3 (0–12)	0 ± NA (NA)	1.1 ± 3.2 (0–9)	1.4 ± 3.5 (0–11)
AC only	n	4	0	3	3
	Time[1] ± SD (range)	2.25 ± 4.5 (0–9)	NA	0 ± 0 (0)	0 ± 0 (0)
AC + LSIL/ HSIL	n	2	1	5	7
	Time[1] ± SD (range)	9 ± 4.2 (6–12)	0 ± NA (NA)	1.8 ± 4.0 (0–9)	2.3 ± 4.1 (0–11)
(B)					
AC total	n	21	6	11	13
	Time[1] ± SD (range)	27.8 ± 17.6 (0–60)	23.3 ± 17.6 (0–51)	7.9 ± 16.1 (0–48)	8.1 ± 15.6 (0–54)
AC only	n	12	0	4	5
	Time[1] ± SD (range)	25.2 ± 17.3 (0–54)	NA	12.0 ± 24.0 (0–48)	13.8 ± 23.4 (0–54)
AC + LSIL/ HSIL	n	9	6	7	8
	Time[1] ± SD (range)	30.7 ± 19.6 (6–60)	23.3 ± 20.1 (0–51)	5.6 ± 11.3 (0–30)	4.5 ± 8.0 (0–22)

NA, not applicable.
[1]Average time of cytological diagnosis in months before the histological confirmation of adenocarcinoma or adenocarcinoma in situ.

before the histological diagnosis of endocervical neoplasia.

DISCUSSION

In conclusion, although no single of the 38 cytomorphological features analysed could be associated specifically with EAC or AIS, the present study showed, that pathologists are, in fact, often able to differentiate neoplastic squamous lesions from neoplastic glandular lesions in cytology. There was not a single pap smear signed out with the diagnosis of atypical endocervical cells, favour neoplastic, AIS or adenocarcinoma among histologically approved HSIL-only samples. Yet, in the series there were significant glandular extension of HSIL in 73% of the cases, which is known to be a common cause of false positive glandular diagnosis (24). Furthermore, among patients with EAC or AIS only in the final histology, the neoplastic diagnosis given was glandular in 70% of the cases.

In the further analysis of the cytomorphological features, a combination of palisading cell borders, nuclear pleomorphism and the lack of single atypical cells showed a positive association with EAC and AIS in pap smear samples taken up to five years before the histological diagnosis. A similar finding of a combination of cytological features predicting a positive finding in pap smears

diagnosed as AEC, NOS was also previously reported. In the study by Mariani et al. the combination of features included nuclear pleomorphism, as in the present study. In addition, nuclear enlargement, increased nuclear to cytoplasmic ratio and cells occurring in sheets and strips with cell crowding and nuclear overlap were described (25). In the study by Conrad et al., abundant tumour cellularity, nuclear size from 3 to 6 times normal, abundant 3-dimensional tumour cell groups, round cell shape and cytoplasmic neutrophils were reported in cases correctly diagnosed as adenocarcinoma in cytology (26).

In the present study, papillary groups and rosettes were seen in HSIL samples a year before the histological diagnosis. In a previous study by Rabelo-Santos et al., (9) papillary groups were reported to have a predictive value of 80 % for glandular neoplasia. In a study by Burja et al., (10) papillary groups were significantly associated with AIS in comparison to benign glandular lesions but did not differentiate AIS from squamous lesions. In the association of HSIL involving endocervical glands, round to oval clusters of abnormal cells with slightly irregular to smooth cell borders were described (27,28). Since there was a significant glandular extension of HSIL in the majority of our HSIL conizates, perhaps these were the cellular clusters interpreted as papillary. According to the Bethesda Criteria, papillary groups are not typical for HSIL (7). Rosettes associated with necrosis in HSIL extending into glands have been described (24), but in general, rosettes are not considered as a feature of HSIL involving glands (7,27–28).

In the studies with LBC samples cytologically diagnosed as glandular neoplasia, the PPV for any high-grade disease was reported to vary from 95.3% to 100% (29,30) and the PPV for endocervical neoplasia was reported to be 74.4% (29), which are in the same range as the best results seen with conventional pap smears.

In the study by Burnley et al., (29) LBC samples also showed higher PPVs in comparison to conventional smears both for high-grade lesions in general and also for endocervical malignancies, although the differences were not significant. The authors of the study described thin pseudostratified strips as a feature of glandular lesions often seen in LBC samples and also reported chromatin abnormalities to be more easily recognized in LBC samples.

Of the above studies by Conrad et al. and by Mariani et al., (26,25) studies were based on LBC samples. In the studies by Rabelo-Santos et al. and Burja et al. (9,10) on conventional pap smears, cytological features reported to be associated with adenocarcinoma included pseudostratified strips, rosettes, palisading borders and papillary groups.

In summary, pseudostratified strips seem to be a feature recognized both in conventional and LBC samples. The 3-dimensional tumour cell groups can be interpreted as papillary groups and cells occurring in sheets and strips with cell crowding and nuclear overlap understood either as pseudostratified strips or fragments with palisading borders. In general, features recognized in conventional smears seem to be mainly architectural while in LBC samples also more cellular and nuclear features are seen.

In 25.5% of the cases in this study, there was a combined lesion of EAC or AIS and a squamous intraepithelial lesion, which is expected since most EACs and cervical squamous cell carcinomas share the same high-risk HPV-related aetiology (31–33).

During the study period, there was a change in the guidelines resulting in atypical glandular cells, NOS in cytology being sent straight to colposcopy (34). The colposcopy is known to have its limitations in the diagnostics of endocervical malignancies. Its sensitivity in detecting endocervical lesions has been reported to be even as low as 9.8% and the probability of significant lesion after a normal colposcopy as high as 87.5% (35,36).

Combining HPV testing to cytological sampling was shown to improve the diagnostic accuracy in cases with atypical endocervical cells in cytology and also to predict the outcome in conservatively treated in situ cases better than the pap smear only (36,37). Chen et al. reported a sensitivity of 91.0% and a specificity of 91.2% in diagnosing high-grade intraepithelial lesions and AIS or EAC among women with atypical endocervical cells in cytology and a positive high-risk HPV DNA result (37). Importantly, the combination of these two tests showed a high negative predictive value of 98.4% for the same lesions. In the study by Costa et al., (36) the combination of Pap smear and HPV test had a sensitivity of 90.0% and a negative predictive value of 88.9% at the first follow-up visit among patients treated for AIS by conization, and a sensitivity of 100% and a negative predictive value of 100% at the second follow-up.

In our study, the samples with high-grade cytological features were placed in the right diagnostic category according to the cell of origin in 100% of the cases in the HSIL group and in 70% of the cases in the EAC/AIS group. In the clinical practice, it can be argued that it does not matter whether the neoplastic cells are deemed of squamous or glandular origin as long as the patient is sent to a colposcopy. Recognizing and reporting atypical glandular cells, though, could guide the

colposcopist to specifically pay attention to the endocervical canal and lead to endocervical curettings and perhaps to an earlier diagnosis.

In our study, AEC, NOS was reported in 17 pap smears among patients with EAC/AIS only in the final histology and in 5 pap smears among patients with HSIL only in the final histology. The time range for those diagnoses varied from 121 months before the histological cancer diagnosis to the diagnostic '0 months' samples. Since before the change in the guidelines this diagnosis of AEC, NOS led only to a control pap smear sample instead of a colposcopy, it can only be speculated which of these prediagnostic pap smears already harboured a significant clinical lesion. Nevertheless, considering this very wide time range of presentation and also the above described challenges in this cytological diagnostic group, it would seem sensible with current guidelines, to accompany a pap smear with atypical glandular cells, NOS with a reflex HPV testing to avoid unnecessary procedures. On the other hand, among older women a large proportion of malignancies behind cytological glandular abnormalities are of endometrial origin, and in their diagnostics, HPV testing is not helpful (12–14).

As mentioned earlier, in Finland, there is a national screening programme for cervical cancer including women between ages of 30 and 60 and, in some municipalities, also women aged 25 and/or 65 years (2). Yet, of all the pap smear samples taken for screening purposes, only 40% were samples taken in the organized programme resulting in opportunistic screening accounting for 71% of the total screening costs annually (3). Since 55% of the EAC/AIS cases in the present study were diagnosed by clinical samples, of which only 11% were taken because of a patient-reported symptom, it is clear that screening is necessary in order to diagnose the endocervical glandular malignancies when they still are curable. Based on the previous studies from Finland, a national organized screening programme seems to be the most cost-effective way to do the screening (3,38).

IK was supported by VTR grant.

REFERENCES

1. https://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/ (cited 05.03.2020).
2. https://cancerregistry.fi/screening/cervical-cancer-screening/ (cited 05.03.2020).
3. Salo H, Nieminen P, Kilpi T, Auranen K, Leino T, Vänskä S, et al. Divergent coverage, frequency and costs of organised and opportunistic pap testing in Finland. Int J Cancer. 2014;135:204–13.
4. Shiliang L, Semenciw R, Mao Y. Cervical cancer: the increasing incidence of adenocarcinoma and adenosquamous carcinoma in younger women. Can Med Assoc J. 2001;164:1151–2.
5. Wilbur DC, Chhieng DC, Guidos B, Mody DR. Epithelial abnormalities: glandular. In: Nayar R, Wilbur DC, editors. The Bethesda system for reporting cervical cytology, 3rd ed. Cham: Springer, 2015.
6. Wilbur DC, Colgan TJ, Ferenczy AS, Hirschowitz L, Loening T, McCluggage WG, et al. Glandular tumors and precursors. In: Kurman RJ, Carcangiu ML, Herrington CS, Young RH, editors. WHO Classification of Tumours of Female Reproductive Organs, 4th edn, vol. 6. Lyon: International Agency for Research on Cancer, 2014;183–189.
7. Henry MR, Russel DK, Luff RD, Prey MU, Wright TC Jr, Nayar R. Epithelial abnormalities: squamous. In: Nayar R, Wilbur DC, editors. The Bethesda System for Reporting Cervical Cytology, 3rd ed. Cham: Springer, 2015.
8. Stoler M, Bergeron C, Colgan TJ, Ferenczy AS, Herrington CS, Kim KR, et al. Squamous cell tumors and precursors. In: Kurman RJ, Carcangiu ML, Herrington CS, Young RH, editors. WHO Classification of Tumours of Female Reproductive Organs, 4th ed., vol. 6. Lyon: International Agency for Research on Cancer, 2014;172–182.
9. Rabelo-Santos SH, Derchain SFM, Do Amaral Westin MC, Angelo-Andrade LAL, Sarian LOZ, Oliveira ERZM, et al. Endocervical glandular cell abnormalities in conventional cervical smears: evaluation of the performance of cytomorphological criteria and HPV testing in predicting neoplasia. Cytopathology. 2008;19:34–43.
10. Burja IT, Thompson SK, Sawyer WL Jr, Shurbaji MS. Atypical glandular cells of undetermined significance on cervical smears. Acta Cytol. 1999;43:351–6.
11. Zardo LMG, Thuler LCS, Zeferino LC, Horta NMSR, Fonseca RCSP. Performance of the cytologic examination for the diagnosis of endocervical adenocarcinoma in situ. Acta Cytol. 2009;53:558–64.
12. Kim M-K, Lee YK, Hong SR, Lim KT. Clinicopathological significance of atypical glandular cells on cervicovaginal pap smears. Diagn Cytopathol. 2017;45:867–72.
13. Pradhan D, Li Z, Ocque R, Patadji S, Zhao C. Clinical significance of atypical glandular cells in pap tests: an analysis of more than 3000 cases at a large academic women's center. Cancer Cytopathology. 2016;124:589–95.
14. Zhao C, Florea A, Onisko A, Austin RM. Histologic follow-up results in 662 patients with pap test findings of atypical glandular cells: results from a large academic womens hospital laboratory employing sensitive screening methods. Gynecol Oncol. 2009;114:383–9.
15. Kim HS, Underwood D. Adenocarcinoma in the cervicovaginal papanicolau smear: analysis of a 12-year experience. Diagn Cytopathol. 1991;7:119–24.
16. Geldenhuys L, Murray ML. Sensitivity and specificity of the pap smear for glandular lesions of the cervix and endometrium. Acta Cytol. 2007;51:47–50.

17. Lai C-R, Hsu C-Y, Tsay S-H, Li A. Clinical significance of atypical glandular cells by the 2001 Bethesda system in cytohistologic correlation. Acta Cytol. 2008;52:563–7.
18. Westin MC, Derchain SF, Rabelo-Santos SH, Angelo-Andrade LA, Sarian LO, Oliveira E, et al. Atypical glandular cells and adenocarcinoma in situ according to the Bethesda 2001 classification: cytohistological correlation and clinical implications. Eur J Obstet Gynecol. 2008;139:79–85.
19. Selvaggi SM. Glandular epithelial abnormalities on Thinprep® pap tests: clinical and cytohistologic correlation. Diagn Cytopathol. 2016;44:389–93.
20. Schoolland M, Segal A, Allpress S, Miranda A, Frost FA, Sterrett GF. Adenocarcinoma in situ of the cervix – sensitivity of detection by cervical smear. Cancer Cytopathol. 2002;96:330–7.
21. Krane JF, Granter SR, Trask CE, Hogan CL. Papanicolaou smear sensitivity for the detection of adenocarcinoma of the cervix: a study of 49 cases. Cancer Cytopathol. 2001;93:8–15.
22. Kares S, Veijalainen O, Kholová I, Tirkkonen M, Vuento R, Huhtala H, et al. High-risk HPV testing as the primary screening method in an organized regional screening program for cervical cancer: the value of HPV16 and HPV18 genotyping? APMIS. 2019;127:710–6.
23. Veijalainen O, Kares S, Kujala P, Tirkkonen M, Vuento R, Kholová I, et al. Human papillomavirus test with cytology triage in organized screening for cervical cancer. Acta Obstet Gynecol Scand. 2016;95:1220–7.
24. Kumar N, Bongiovanni M, Molliet M-J, Pelte M-F, Egger J-F, Pache J-C. Diverse glandular pathologies coexist with high-grade squamous intraepithelial lesion in cyto-histological review of atypical glandular cells on ThinPrep specimens. Cytopathology. 2009;20:351–8.
25. Mariani R, Grace C, Hughes K, Dietrich RM, Cabay RJ, David O. Can we improve the positive predictive value of atypical glandular cells not otherwise specified? Diagn Cytopathol. 2014;42:200–4.
26. Conrad RD, Liu AH, Wentzensen N, Zhang RR, Dunn ST, Wang SS, et al. Cytologic patterns of cervical adenocarcinomas with emphasis on factors associated with underdiagnosis. Cancer Cytopathol. 2018;121:950–8.
27. Selvaggi SM. Cytologic features of high-grade squamous intraepithelial lesions involving endocervical glands on ThinPrep® cytology. Diagn Cytopathol. 2002;26:181–5.
28. Selvaggi SM. Cytologic features of squamous cell carcinoma in situ involving endocervical glands in cervical cytobrush specimen. Acta Cytol. 1994;38:687–92.
29. Burnley C, Dudding N, Parker M, Parsons P, Whitaker CJ, Young W. Glandular neoplasia and borderline endocervical reporting rates before and after conversion to the Surepath™ liquid-based cytology (LBC) system. Diagn Cytopathol. 2011;39:869–74.
30. Thiryayi SA, Marshall J, Rana DN. An audit of liquid-based cervical cytology screening samples (ThinPrep and SurePath) reported as glandular neoplasia. Cytopathology. 2010;21:223–8.
31. Walboomers JMM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189:12–9.
32. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol. 2010;11:1048–56.
33. Pirog EC, Lloveras B, Molijn A, Tous S, Guimerà N, Alejo M, et al. HPV prevalence and genotypes in different histological subtypes of cervical adenocarcinoma, a worldwide analysis of 760 cases. Mod Pathol. 2014;27:1559–67.
34. Current Care Working Group. Cytological changes in the cervix, vagina and vulva (online). Current Care Guideline. Working group set by the Finnish Medical Society Duodecim and the Finnish Colposcopy Association. Published 17.04.2019 [cited 05.03.2020]. Available at: www.kaypahoito.fi
35. Ullal A, Roberts M, Bulmer JN, Mathers ME, Wadehra V. The role of cervical cytology and colposcopy in detecting cervical glandular neoplasia. Cytopathology. 2009;20:359–66.
36. Costa S, Negri G, Sideri M, Santini D, Martinelli G, Venturoli S, et al. Human papillomavirus (HPV) test and PAP smear as predictors of outcome in conservatively treated adenocarcinoma in Situ (AIS) of the uterine cervix. Gynecol Oncol. 2007;106:170–6.
37. Chen L, Bin Y. Assessment of reflex human papillomavirus DNA testing in patients with atypical endocervical cells on cervical cytology. Cancer Cytopathol. 2008;114:236–41.
38. Lonnberg S, Anttila A, Luostarinen T, Nieminen P. Age-specific effectiveness of the Finnish cervical cancer screening programme. Cancer Epidemiol Biomark Prev. 2012;21:1354–61.

PUBLICATION II

Endocervical Cytology: Inter- and Intra-Observer Variability in Conventional Pap Smears

Pulkkinen J, Huhtala H, Krogerus LA, Hollmén S, Laurila M, Kholová I.

Acta Cytol. 2022; 66(3):206–215.
doi:10.1159/000522212.

Acta Cytologica

Gynecologic Cytopathology

Acta Cytologica 2022;66:206–215
DOI: 10.1159/000522212

Received: September 21, 2021
Accepted: January 24, 2022
Published online: February 28, 2022

Endocervical Cytology: Inter- and Intra-Observer Variability in Conventional Pap Smears

Johanna Pulkkinen[a] Heini Huhtala[b] Leena Anneli Krogerus[c]
Sinikka Hollmén[a, d] Marita Laurila[a] Ivana Kholová[a, e]

[a]Department of Pathology, Fimlab Laboratories, Tampere, Finland; [b]Faculty of Social Sciences, Tampere University, Tampere, Finland; [c]Department of Pathology, Vita Laboratories, Helsinki, Finland; [d]Department of Pathology, Satasairaala, Pori, Finland; [e]Department of Pathology, Faculty of Medicine and University, Tampere, Finland

Keywords
Pap smear · Cervical cytology · Reproducibility · Interobserver · Endocervical cells

Abstract
Introduction: Although the cytological diagnostic criteria for cervical squamous and glandular lesions are established by the Bethesda System for Reporting Cervical Cytology, the reproducibility of the diagnosis of these lesions has been shown to be variable in previous studies. At best, occasional good kappa (κ) values were reached both inter- and intra-observerly. Generally, consensus on high-grade lesions has been better compared to milder changes. ***Methods:*** Altogether, 167 conventional Pap smears from 50 patients with histologically confirmed endocervical adenocarcinomas (EAC) and adenocarcinomas in situ (AIS) and from 28 patients with histologically proven high-grade intraepithelial lesions were analyzed by four cytopathologists. Twenty of the smears were later re-evaluated by the same cytopathologists. κ-values between cytopathologists in the categories of squamous versus glandular, negative for intraepithelial lesion or malignancy (NILM), atypical, and preneoplastic/neoplastic were calculated. The diagnostic Pap smears of EAC and AIS with best and worst consensus between observers were then morphologically analyzed. ***Results:*** The reproducibility ranged from poor to substantial. The overall κ-values between the four cytopathologists were 0.412, 0.314, 0.272, and 0.082, respectively, in the categories of preneoplastic/neoplastic, squamous versus glandular, NILM, and atypical. Overall intra-observer κ-values were correspondingly 0.491, 0.616, 0.345, and 0.241. In the diagnostic smears of AIS and EAC, the nuclear size >2 times the normal and nuclear pleomorphism were the commonest features associated with good diagnostic consensus and the lack of nuclear enlargement and degenerative changes were associated with poor consensus. ***Conclusions:*** The reproducibility of preneoplasia/neoplasia diagnoses was better than that of atypia and NILM both in the inter- and intra-observer part in this study. In the smears from AIS and EAC patients, general neoplasia-associated features were more common in samples with good agreement by the four cytopathologists of the neoplastic nature and the endocervical origin of the lesion.

Karger@karger.com
www.karger.com/acy

Correspondence to:
Johanna Pulkkinen, johanna.pulkkinen@fimlab.fi

Introduction

The cervical cytology-based screening programs have been efficient in reducing cervical cancer mortality [1]. The diagnostic cytopathological criteria for both squamous and glandular lesions are well described in the Bethesda System for Reporting Cervical Cytology (TBSRCC) [2–4]. Nevertheless, in practice, separating atypical endocervical cells from atypical squamous cells as well as from atypical endometrial cells in preneoplastic/neoplastic and non-neoplastic conditions can be challenging [5–7].

The reproducibility of the cytological diagnoses has been low in most previous studies, regarding both interobserver and intra-observer variability [8–11]. The level of agreement was better in diagnosing the more severe lesions, both squamous and glandular, but milder cellular changes have been a common cause of misinterpretation [9, 10, 12]. Importantly, mild cellular changes are more likely to be missed leading to a lack of appropriate follow-up, giving the precursor lesions a chance to develop into an invasive carcinoma.

The aim of this study was to evaluate inter- and intra-observer reproducibility in the categories of NILM, atypical, and preneoplastic/neoplastic endocervical cells and in the category of squamous versus endocervical cells. In addition, we focused on the samples with the highest and the lowest concordances to find morphological features that they have in common and those that can tell them apart.

Materials and Methods

A randomly selected subset of 167 conventional Pap smear slides from a previously analyzed cohort from the Pathology department of Fimlab Laboratories Oy was studied [13]. The slides represented Pap smears from 27 patients with endocervical adenocarcinomas (EAC), 23 patients with adenocarcinomas in situ (AIS), and 28 patients with high-grade squamous lesions (HSIL). All the diagnoses had been histologically confirmed.

In Finland, the conventional Pap smear is still the main method used in cervical cytology. No liquid-based Pap tests are included in this study.

The Pap tests were from the years 1994 to 2014. Altogether 10 of the Pap tests were taken during the 1990s, 71 of the Pap tests between the years 2000 and 2009, and 86 of the Pap tests after the year 2010. The slides represented samples from 50 patients with EAC or AIS and 28 patients with histological HSIL. The diagnoses were histologically confirmed either by conization or by hysterectomy, except for one inoperable adenocarcinoma case with a biopsy diagnosis only.

Table 1. Number of diagnoses given in routine practice and in the interobserver part of the study

	Routine practice	Observers 1–4
NILM	67	126
ASC-US	16	40
LSIL	8	21
ASC-H	11	24
HSIL	21	51
SCC	0	3
AEC-NOS or AGC-NOS	18	131
AEC-FN	12	107
AIS	2	58
EAC	0	35
Other*	0	6
ASC-US + AEC-NOS	2	13
ASC-US + AEC-FN	0	1
ASC-US + AIS	0	2
LSIL + AEC-NOS	1	5
LSIL + AIS	0	1
ASC-H + AEC-NOS	2	5
ASC-H + AEC-FN	3	2
ASC-H + EAC	0	1
ASC-H + other	0	1
HSIL + AEC-NOS	2	11
HSIL + AEC-FN	1	19
HSIL + AIS	0	3
SCC + AEC-NOS	0	2
Endometrial adenocarcinoma	1	0
Total	167	668

NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells, undetermined significance; LSIL, low-grade intraepithelial lesion; ASC-H, atypical squamous cells, cannot exclude an HSIL; HSIL, high-grade intraepithelial lesion; SSC, squamous cell carcinoma; AEC-NOS, atypical endocervical cells, NOS; AGC-NOS, atypical glandular cells, NOS; AEC-FN, atypical endocervical cells, favor neoplastic; AIS, adenocarcinoma in situ; EAC, endocervical adenocarcinoma. * Diagnoses not specified by the observes.

The original diagnoses of the samples are summarized in Table 1. All the Pap smear slides were re-analyzed by four senior cytopathologists with a Pap smear diagnostics experience of >30 years (n = 3) and >20 years (n = 1). The cytopathologists represented three different institutions located in three different health care districts. Three of the cytopathologists were university-based pathology department representatives and one was a regional hospital pathologist.

The cytopathologists re-analyzed the Pap smears individually and without knowledge of the original diagnoses of the samples or their corresponding histological diagnoses. The cytopathologists knew they were participating in a study concerning cervical malignancies, but they were not provided any clinical information along with the slides. One of the cytopathologists was involved in the study design and knew the total number of the final histological diagnoses. All diagnoses were given using TBSRCC 2014 [2–4].

In the statistical analyses, the original diagnosis was marked as observer 0 and the four cytopathologists were each named correspondingly as observer 1, observer 2, observer 3, and observer 4. Observer 1 was the observer involved in the study design. Kappa (κ) values for each of the four pairs of observers were calculated in four categories: (1) to find out the level of agreement on whether the lesion is squamous or glandular, (2) to find out the level of agreement on samples that are NILM (TBSRCC category negative for intraepithelial lesion or malignancy), (3) to find out the level of agreement on what is atypical only (TBSRCC categories: atypical squamous cells, undetermined significance [ASC-US], atypical endocervical cells, NOS [AEC-NOS], and atypical glandular cells, NOS [AGC-NOS]), and finally (4) to find out the level of agreement on what is preneoplastic/neoplastic or not (TBSRCC categories: low-grade squamous intraepithelial lesion [LSIL], high-grade squamous intraepithelial lesion [HSIL], atypical squamous cells, cannot exclude an HSIL [ASC-H], atypical endocervical cells, favor neoplastic [AEC-FN], adenocarcinoma in situ [AIS], and endocervical adenocarcinoma [EAC]).

The subgrouping was based on the national treatment protocol in Finland during the time of the study design. The lesions with the same clinical follow-up or intervention level were grouped together [14]. Briefly, there was a follow-up strategy with new Pap smear tests in the atypical categories and a colposcopy referral in the preneoplastic/neoplastic categories. Now, according to the national guidelines, diagnoses AEC-NOS and AGC-NOS all lead to colposcopy, to a gynecologic ultrasound, to a high-risk HPV test, and to a repeated Pap test. Also, the cases with repeated ASC-US in women over 30 years are now routinely tested for high-risk HPV infection [14]. The strength of association was defined as proposed by Landis and Koch: 1 for perfect agreement, 0.81–0.99 for almost perfect agreement, 0.61–0.80 for substantial agreement, 0.41–0.60 for moderate agreement, 0.21–0.40 for fair agreement, 0–0.20 slight agreement, and <0 poor agreement [15].

To perform an intra-observer analysis, 20 smears from the 167 samples analyzed in the first round were selected for an intra-observer re-evaluation. The chosen samples consisted partly of samples with an agreement of three observers in the first round (n = 12) and partly of samples with disagreement of all five observers in the first round (n = 8). These 20 smears included eight smears from AIS or adenocarcinoma-only patients as diagnosed in the final histology, six smears from adenocarcinoma or AIS and HSIL patients as diagnosed in the final histology, and of six smears from patients with HSIL only in the final histology. The twenty samples were once again blindly and independently re-analyzed by the four cytopathologists and the κ-values were calculated for each observer as described above.

From the original 167 Pap smear slides, all the slides of patients with EAC or AIS only in the final histology were retrieved together with diagnostic samples with agreement of ≥3 observers (n = 8) and the diagnostic samples with disagreement of ≥3 observers (n = 14) for further descriptive morphological analysis. The statistical analyses were performed by with SPSS program, version 25 (IBM SPSS Statistics for Windows, Version 25.0: IBM Corp., Armonk, NY, USA)

The study was approved by the Ethical committee of Pirkanmaa Health Care District (R16022) without informed consent of each individual. The study was conducted according to the Declaration of Helsinki.

Table 2. Agreement of three observers

Diagnosis	No
NILM	39
ASC-US	4
LSIL	3
HSIL	5
ASC-H	2
AEC-NOS	8
AEC-FN	11
AIS	7
EAC	3
	82

NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells, undetermined significance; LSIL, low-grade intraepithelial lesion; ASC-H, atypical squamous cells, cannot exclude an HSIL; HSIL, high-grade intraepithelial lesion; AEC-NOS, atypical endocervical cells, NOS; AEC-FN, atypical endocervical cells, favor neoplastic; AIS, adenocarcinoma in situ; EAC, endocervical adenocarcinoma.

Results

The patients in the adenocarcinoma group were aged from 22 to 83 years with a mean age of 43.1 (SD = 14.4) years, and the patients in the HSIL group were 21 to 54 years old with a mean age of 36.3 (10.1) years. The mean age of all patients included in the study was 40.7 (13.1) years. Some of the Pap smears in the study were cytologically preneoplastic or neoplastic and were taken at the time of the histological confirmation of the malignancy. Others were older smears and represented cytological diagnoses of NILM, ASC-US, and AEC-NOS. On average, the Pap smears included in the study were taken 0–104 months (mean 26.2 (31.3) months) before the histological diagnosis of a malignancy was made.

The diagnoses given by observers 1–4 are listed in the Table 1. Out of the 167 analyzed samples, agreement of three observers was reached in 82 (46.5%) of the cases, nearly half of them (39/82, 47.5%) representing samples diagnosed as NILM (Table 2). If in the patient group with both squamous and glandular diagnoses, only one of them was taken into account and the number of samples with agreement of three observers reached 104/167 (62.3%) cases (data not shown). The best κ-values between observer pairs in this study were achieved in recognizing squamous and glandular features (Table 3). All the κ-values among observers 0, 2, 3, and 4 were above 0.503, with the highest value being 0.689 between observers 2 and 3.

Table 3. The kappa values between the observers in the categories of recognizing negative, atypical, and preneoplastic/neoplastic samples and differentiating between squamous and glandular features

	Observer 1	Observer 2	Observer 3	Observer 4	Overall κ
Kappa values between observer pairs in recognizing NILM samples					
Observer 0	−0.018	0.633	0.357	0.444	0.272
Observer 1	–	0.036	0.035	−0.047	
Observer 2	–	–	0.474	0.469	
Observer 3	–	–	–	0.159	
Kappa values between observer pairs in recognizing atypia*					
Observer 0	−0.050	0.203	−0.088	0.169	0.082
Observer 1	–	0.072	0.200	0.080	
Observer 2	–	–	0.092	0.259	
Observer 3	–	–	–	−0.037	
Kappa values between observer pairs in recognizing preneoplasia/neoplasia**					
Observer 0	0.229	0.456	0.400	0.379	0.412
Observer 1	–	0.376	0.356	0.351	
Observer 2	–	–	0.483	0.536	
Observer 3	–	–	–	0.496	
Kappa values between observer pairs in differentiating atypical or preneoplastic/neoplastic squamous and glandular features					
Observer 0	−0.073	0.503	0.620	0.613	0.314
Observer 1	–	−0.062	−0.027	0.052	
Observer 2	–	–	0.689	0.631	
Observer 3	–	–	–	0.664	

ASC-US, atypical squamous cells, undetermined significance; LSIL, low-grade intraepithelial lesion; ASC-H, atypical squamous cells, cannot exclude an HSIL; HSIL, high-grade intraepithelial lesion; AEC-NOS, atypical endocervical cells, NOS; AGC-NOS, atypical glandular cells, NOS; AEC-FN, atypical endocervical cells, favor neoplastic; AIS, adenocarcinoma in situ; EAC, endocervical adenocarcinoma. *Includes diagnoses ASC-US, AEC-NOS, and AGC-NOS. **Includes diagnoses LSIL, HSIL, ASC-H, AEC-FN, AIS, and EAC.

The least dispersion among the κ-values between the observer pairs was seen in the category of recognizing preneoplasia/neoplasia with an overall κ-value of 0.401, which was also the highest overall κ-value reached in the interobserver part of the study (Table 3). The highest κ-value in this category was 0.536 between observers 2 and 3. Four pairs of observers reached κ-values varying from 0.400 to 0.496 and another four values varying from 0.351 to 0.379.

In the category of samples considered to be NILM, the best and only κ-value above 0.500 was 0.633 between observers 0 and 2 (Table 3). Only 3 observer pairs reached κ-values above 0.4 and the overall κ-value was only 0.272. In the category of atypia only (ASC-US, AEC-NOS and AGC-NOS), the diagnostic reproducibility turned out to be poor with the highest κ-value in this group being 0.259 between observers 2 and 4 and the overall κ-value of only 0.082.

The highest κ-values ranging from 0.581 to 0.696 were reached in differentiating squamous and glandular features in the intra-observer part of this study (Table 4). The only substantial overall κ-value of 0.616 in the study was also reached in this category. Reproducibility of recognizing preneoplasia/neoplasia ranged from moderate to substantial with κ-values from 0.300 to 0.625 and an overall moderate κ-value of 0.491. The overall κ value for samples diagnosed as NILM was 0.345 and for atypical samples 0.241.

In the further analysis of the diagnostic Pap smear samples from patients with EAC or AIS only in the final histology, consensus diagnosis of ≥3 observers was reached in 35/40 (87.5%) of the smears. All the diagnoses given by the observers were neoplastic (AEC-FN, or HSIL or worse among them). In all, 37/40 (92.5%) of the diagnoses were of glandular origin and 3/40 (7.5%) of squamous origin. All the consensus diagnoses of ≥3 in this group were glandular and AEC-FN or worse. No samples were considered NILM. No combinations of squamous and glandular diagnoses were given.

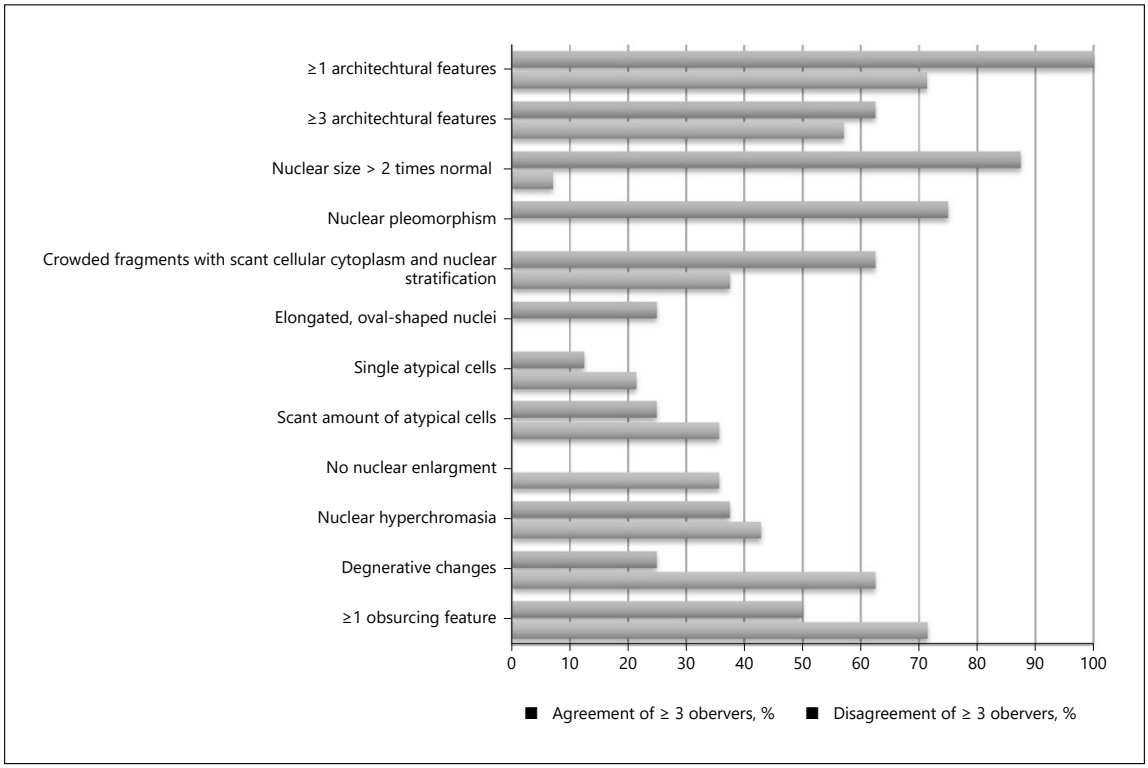

Fig. 1. Cytomorphological features among diagnostic Pap smears of histologically confirmed EAC and AIS cases with diagnostic agreement of ≥3 observers and disagreement ≥3 observers. *Architectural features include rosettes, feathering, palisading cell borders, and papillary groups.

Table 4. The intra-observer kappa values

	General	NILM	Atypical*	Preneoplastic/ neoplastic**	Sq/ glandular
Observer 1	0.045	0.000	0.043	0.300	0.598
Observer 2	0.480	0.412	0.524	0.615	0.581
Observer 3	0.328	0.444	0.216	0.625	0.636
Observer 4	0.178	0.385	−0.087	0.400	0.696
Overall	0.269	0.345	0.241	0.491	0.616

ASC-US, atypical squamous cells, undetermined significance; LSIL, low-grade intraepithelial lesion; ASC-H, atypical squamous cells, cannot exclude an HSIL; HSIL, high-grade intraepithelial lesion; AEC-NOS, atypical endocervical cells, NOS; AGC-NOS, atypical glandular cells, NOS; AEC-FN, atypical endocervical cells, favor neoplastic; AIS, adenocarcinoma in situ; EAC, endocervical adenocarcinoma. * Includes diagnoses ASC-US, AEC-NOS, and AGC-NOS. ** Includes diagnoses LSIL, HSIL, ASC-H, AEC-FN, AIS, and EAC.

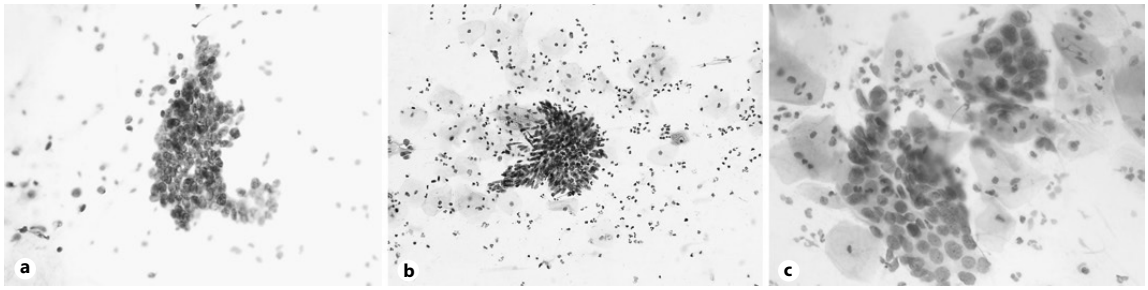

Fig. 2. a, **b** Representation of the diagnostic Pap smear of an invasive adenocarcinoma case with agreement of ≥3 observers. The sample was abundant with crowded fragments with scant cellular cytoplasm and nuclear stratification. **a** An endocervical glandular fragment showing feathering and rosettes (with elongated nuclei). **b** The diagnoses given by the observers were three times AIS, EAC, and AEC-FN. **c** Representation of the diagnostic Pap smear of an invasive adenocarcinoma case with disagreement of ≥3 observers showing only a few clusters of cells with degenerated nuclei. Nuclear enlargement >2 times the normal size was seen. All observers agreed on the neoplastic nature of the lesion but there was no consensus on the cellular origin. The diagnoses given were ASC-H + AEC-FN, two times AEC, FN, HSIL, and HSIL + AEC-FN.

Among the diagnostic samples with disagreement of ≥3 observers, a combination of squamous and glandular diagnoses was seen in 16/70 (22.9%) of the smears. A glandular diagnosis only was given in 42/70 (60.0%) of the smears, a squamous diagnosis in 10/70 (14.3%) of the smears, and in addition, 2/70 (2.9%) of the smears were considered NILM. When combination diagnoses and cases diagnosed as ASC-H were included in the calculations, 58/70 (82.9%) of the diagnoses were neoplastic.

The cytomorphological features observed in the descriptive analysis of the smears are shown in Figure 1. Among the smears with agreement of ≥3 observers, at least focally enlarged nuclei were seen in all smears. A substantial number of cells with markedly (>2 × normal size) enlarged nuclei were seen in 87.5% (7/8) of the smears (shown in Fig. 1). Nuclear pleomorphism was observed in 75% (6/8) of the smears and crowded fragments with scant cellular cytoplasm and nuclear stratification were seen in 62.5% (5/8) of the smears. On the other hand, elongated, oval-shaped nuclei were encountered in 2/8 (25%) smears.

Among smears with disagreement of ≥3 observers, there was only one of the 14 smears (7.1%) with a substantial number of cells with markedly enlarged nuclei. In 57.1% (8/14) of the smears only focal or mild (≤2 × normal size) nuclear enlargement was seen. In five of the 14 (35.7%) smears no nuclear enlargement was observed at all. In three of those cases there was nuclear hyperchromasia and the other two presented with degenerative changes. Nuclear pleomorphism or elongated, oval-shaped nuclei were not seen among samples with disagreement of ≥3. Crowded fragments with scant cellular cytoplasm and nuclear stratification were encountered in 37.5% (5/14) of the smears.

Degenerative changes were more common in Pap smears with disagreement of ≥3 observers than in samples with an agreement of ≥3 observers (62.5% vs. 25%) (shown in Fig. 2). In the first group there was at least one significant background or cellular feature (blood, inflammation, regeneration, degeneration) interfering with the interpretation in 71.4% (10/14) of the samples. In the latter group, obscuring features were seen in 50% (4/8) of the smears. Also, the number of atypical cells was slightly more often scant in the group of disagreement of ≥3 observers in comparison to the group of an agreement of ≥3 observers (35.7% vs. 25%).

At least one of the architectural features previously described to be associated with EAC or AIS (rosettes, feathering, palisading cell borders, papillary groups) [16, 17] was seen in all (8/8) of the smears among the smears with agreement of ≥3 observers and in 71.4% (10/14) of the smears with disagreement of ≥3 observers. The corresponding figures for three or more architectural features for each group were 62.5% (5/8) and 57.1% (8/14).

Discussion

In TBSRCC, in addition to the diagnoses "Insufficient" and "NILM," there are five different diagnoses in the squamous cell category and altogether 10 different diagnoses in the glandular cell category [2–4]. In routine prac-

Acta Cytologica 2022;66:206–215
DOI: 10.1159/000522212

tice, the combinations of squamous and glandular diagnoses are common resulting into an even larger pool of diagnostic possibilities.

In the present study, 26 different diagnoses or combinations of diagnoses are given (Table 1). For the statistical analyses, all the components of the diagnoses had to match exactly to be considered equal. In the BIRST study, the exact agreement of participants with the panel was 55.1%, but the agreement improved to 82.3% at the level of Negative versus non-Negative [18]. In the BIRST-2 study, the respondents agreed with the panel in 62.8% of the cases. In this study, the agreement on samples with NILM and on the samples in the squamous cell categories form ASC-US to SCC was relatively good and varied from 60% to 86%. Yet, in the category of glandular atypia agreement of only 33% was reached [19]. In the present study, the exact agreement was reached only in 47.5% of the smears. In comparison to some previously published studies, with fewer diagnostic categories given to choose from [8, 9, 11, 12], the amount of diagnostic options itself can be considered as partially responsible for the diagnostic diversity observed in this study.

However, in comparison to some previous studies [11, 20], the cytological squamous and glandular features were recognized surprisingly well with five out of ten observer pairs reaching substantial κ-values of 0.613 and above in this category in our study. Also, in the intra-observer part of the study half of the observers reached substantial κ-values in the category with an overall κ-value of 0.616. The variation between the observer pairs in our study was a lot larger than in the abovementioned previous studies, though, with three negative κ-values leading to an only fair overall κ-value of 0.314, which is in accordance with ranges reported earlier by Niu et al. [21].

Interestingly, in the study by Moreira et al. [20] the interobserver agreement recognizing glandular features was very similar in both conventional and liquid-based cytology (LBC) samples [20], although in LBC preparations the cellular features are presumed to be more easily recognizable and the artifacts interfering with the interpretation of features fewer in comparison to the conventional smears. Moreira et al. reported κ-values between 0.27 and 0.61 in conventional Pap smear samples and κ-values between 0.18 and 0.70 in LBC samples. In LBC samples 0.70 was the only κ-value between the observer pairs, which was above 0.45 [20]. It is worth mentioning that in that study, the materials for the LBC and conventional samples were collected sequentially instead of using only residual material of the conventional smears for preparing the LBC slides [20].

On the other hand, in the study by Lee et al. [10] the κ-values between the observer pairs were slightly higher in LBC samples compared to the conventional smears in recognizing glandular atypia and high-grade lesions in general. In LBC samples, the corresponding κ-values ranged from 0.13 to 0.36 and from 0.34 to 0.58 and in conventional smears, respectively, from 0.08 to 0.28 and from 0.21 to 0.51 [10].

In the present study, the results are very similar to ones reported by Lee et al. [10] and Lepe et al. [11] in the preneoplastic/neoplastic category. The agreement among the observers was moderate with the overall κ-value of 0.412 and the variation of κ-values between the observer pairs ranging from 0.229 to 0.536. In the intra-observer part of the study, two of the observers reached substantial κ-values, but the overall κ-value was only a moderate 0.491. The highest interobserver κ-value in this category was 0.67 reported by Niu et al. [21] in the study of patients with AIS in histology and their Pap smear samples from the year preceding the histological diagnosis. In the setting of LSIL versus HSIL, Joste et al. [12] reported interobserver κ-values from 0.40 to 0.63 and substantial intra-observer κ-values from 0.63 to 0.74 for high-grade lesions.

In our study, the samples diagnosed as ASC-US, AEC-NOS or AGC-NOS were grouped together since during the study design according to the Finnish national guidelines these diagnoses led only to a control Pap smear instead of colposcopy and histological sampling. It is worth noticing that, even with the current guidelines, the Finnish follow-up and treatment protocols for Pap smears with atypical glandular cells differ from the treatment algorithms applied in the USA right now [22]. The agreement between observers in this category was poor with an overall κ-value of only 0.082. The variation of κ-values from −0.088 to 0.259 was slightly worse than that reported by Lee et al. [10] on atypical glandular cells in conventional smears. In this category, the intra-observer overall κ-value in the present study was also only a fair 0.241.

In the category of NILM samples, the overall κ-value was a fair 0.272 in the interobserver part of the study with large variation between the observer pairs from two negative values to one substantial value of 0.633. The overall κ-value in the present study was lower than the 0.370 previously reported by Simsir et al. [8] and the 0.46 reported by Confortini et al. [9]. In the intra-observer part of the study, two of the observers reached moderate κ-values of 0.41 and 0.44 but the overall κ-value was only a fair 0.345.

Very similar results have been observed with supposed "gold standard" cervical histological samples. The repro-

ducibility between pathologists in previous studies based on hematoxylin and eosin slides alone was only fair with average κ-values ranging from 0.30 to 0.358 in the interobserver studies [23–25]. The agreement among observers was progressively better on cervical intraepithelial lesion grade 3 (κ = 0.496) and invasive cancer (κ = 0.832) compared to cervical intraepithelial lesion grades 1 (κ = 0.172) and 2 (κ = 0.175) [22]. In the intra-observer part of the study by McCluggage et al. [23], a moderate κ-value of 0.47 was reached. Also, in the interobserver study by Sellors et al. [26] in which the macroscopic characteristics of atypical transformation zone were assessed on colpophotographs, the agreement between colposcopists varied from poor to good depending on the feature in question. Thus, at all levels of diagnostics on cervical lesions, the interobserver variability must be reckoned with.

The variation in diagnostic reproducibility is not unique for cervical samples, though. Very similar results ranging from only fair to moderate agreement between observers was reported for other organs, including cytological diagnoses. For example, in the fine-needle aspirations of salivary glands and breast, the overall κ-values have been reported to be 0.314 and 0.50 and the variation of values, respectively, from 0.091 to 0.539 and from 0.41 to 0.62 [27, 28].

Concerning the cervical cytological samples, the most important thing, though, is to find the patients requiring immediate treatment or intensive follow-up and to separate the women requiring a follow-up from those who do not. In that sense, it does not matter if an atypia or a prenoplastic/neoplastic finding in cytology is named a squamous or a glandular lesion if the follow-up is the same. Recognizing glandular atypia, though, guides the colposcopist to pay special attention to the endocervical canal and its sampling. Keeping the goals of cervical cytological sampling in mind, it is also reassuring that the preneoplastic/neoplastic features are the features cytopathologists have the best overall consensus on. The NILM samples and the diagnoses in the atypical group are problematic and the low reproducibility in these categories probably leads to some unnecessary follow-up and diagnostic procedures and maybe also to some missed malignancies.

As mentioned earlier, now a cytological AEC-NOS or AGC-NOS in all age groups and a repeated ASC-US in women over 30 years in Finland leads to a high-risk HPV test, which is helpful in the risk stratification and management of these patients. Of course, there is also the p16/Ki67 dual stain available for the LBC samples, the use of which has shown to lead to a higher consensus and a better specificity on samples with an outcome of HSIL or worse [29, 30]. The high-risk HPV test as an additional tool is already widely used in clinical practice and the use of special stains will probably become more common when transition from conventional smears to LBC sampling is finalized.

In the morphological analysis of the diagnostic Pap smears from EAC and AIS patients with a good and a poor consensus between the observers, degeneration was one of the two most common features obscuring the diagnosis. It was seen in 62.5% of the smears with poor consensus and in only 25% of the smears with good consensus. The lack of enlarged nuclei was encountered in 37.5% of the smears with poor consensus and in none of the smears with good consensus.

Nuclear size >2 times the normal and nuclear pleomorphism were the most common features observed in smears correctly diagnosed as a glandular neoplasia with good agreement on the specific diagnosis among observers. In addition, crowded fragments with scant cellular cytoplasm and at least one architectural feature (rosettes, feathering, palisading cell borders, papillary groups) were more commonly encountered among samples with good diagnostic consensus. Marked nuclear enlargement and nuclear pleomorphism were a combination of features associated with adenocarcinoma and AIS also in previous studies [13, 31, 32]. Similar features of increased nuclear to cytoplasmic ratio and cells occurring in sheets and strips with cell crowding and nuclear overlap have also earlier been described [31].

This study consisted of conventional Pap smears only. Some of the samples were old with suboptimal preservation possibly interfering with the evaluation of cytological features. There was also an unfortunate change in the national guidelines during the study, which produced difficulties in grouping the Pap smears according to their Bethesda diagnoses and their clinical management. During the first years of the study, all the "glandular atypia, not otherwise specified" patients were treated with a control Pap smear only, but during the last years with a colposcopy. Thus, we had no cytological referee diagnoses, which would have been beneficial for the comparisons, but instead we used the majority diagnoses of three observers.

One of the observers in the study constantly agreed less with the others. The observer was the one involved in the study design. The observer knew that the smears with AIS and EAC were overrepresented in the final histology compared to the smears with HSIL. In addition, the ob-

server knew there were no samples with a negative final histology included. The knowledge might have influenced the observer's interpretation of cytological features in favor of glandular diagnoses and in favor of malignancies. Despite the reason behind the lack of agreement, this result is a good reminder of the fact that maintaining an adequate level of skills in the Pap diagnostics requires constant surveillance and that even competent, experienced pathologists can shift in their standards.

In conclusion, the diagnostic reproducibility throughout the diagnostic categories in cervical cytology is variable and, at its best, only occasional substantial κ-values are reached. The agreement on high-grade lesions is generally better than on milder changes and negative samples. Mostly, the agreement on endocervical glandular cytological features is only modest compared to the agreement on squamous differentiation, although the individual cytopathologists seem to have a relatively consistent approach to this differential diagnosis. Maybe we are aided in the future by artificial intelligence to reach higher κ-values.

The cytological features in Pap smears that lead to good or poor consensus among pathologists have been previously investigated very little. In the present study, we could show that the general neoplasia-associated features of marked nuclear enlargement and nuclear pleomorphism are more commonly encountered in EAC and AIS smears with good consensus on the neoplastic nature and the endocervical glandular origin of the lesion. Also at least one of the architectural features (rosettes, feathering, palisading cell borders, papillary groups), which are among the most important features differentiating glandular lesions from the squamous, was more commonly seen in these samples. We also showed that the most common features in the smears obscuring the diagnosis of endocervical glandular neoplasia are degeneration and the lack of enlarged nuclei. These are the features that should be emphasized in future educational programs.

Statement of Ethics

The study protocol was reviewed and approved by the Ethical committee of Pirkanmaa Health Care District (the Ethical committee of Pirkanmaa Health Care District, contact person secretary Minna Lahtinen, email: minna.maa.lahtinen@pshp.fi, telephone: +358 3 311 66910, address: Eettinen toimikunta, PL 2000, 33521 TA, Finland). The approval number is R16022. The study was based on archive samples and, therefore, had no impact on the treatment or on the follow-up of the patients. The study was conducted without informed consent of each individual, which was approved by the Ethical Committee of Pirkanmaa Health care district. The study was conducted according to the Declaration of Helsinki.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

The work was supported by grant from the Competitive Research Funding of the Pirkanmaa Hospital Distric to Ivana Kholová. The funding source had no role in the preparation of data or the manuscript.

Author Contributions

Johanna Pulkkinen: data curation, formal analysis, investigation, project administration, visualization, validation, and writing – original draft and editing. Heini Huhtala: data curation, statistical analyses, supervision, and writing – original draft. Leena Krogerus, Sinikka Hollmen, and Marita Laurila: data curation, investigation, and writing – original draft. Ivana Kholová: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, visualization, supervision, validation, and writing – original draft and editing.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1 Jansen EEL, Zielonke N, Gini A, Anttila A, Segnan N, Vokó Z, et al. Effect of organised cervical cancer screening on cervical cancer mortality in Europe: a systematic review. Eur J Cancer. 2020;127:207–23.
2 Abdul-Karim FW, Powers CN, Bererk JS, Sherman ME, Tabbara SO, Sidawy MK. Atypical squamous cells. In: Nayar R, Wilbur DC, editors. Bethesda system for reporting cervical cytology. 3rd ed. Springer; 2015. p. 103–34.
3 Henry MR, Russel DK, Luff RD, Prey MU, Wright TC Jr, Nayar R. Epithelial abnormalities: squamous. In: Nayar R, Wilbur DC, editors. Bethesda system for reporting cervical cytology. 3rd ed. Springer; 2015. p. 135–92.
4 Wilbur DC, Chhieng DC, Guidos B, Mody DR. Epithelial abnormalities: glandular. In: Nayar R, Wilbur DC, editors. Bethesda system for reporting cervical cytology. 3rd ed. Springer; 2015. p. 193–240.
5 Pradhan D, Li Z, Ocque R, Patadji S, Zhao C. Clinical significance of atypical glandular cells in pap tests: an analysis of more than 3000 cases at a large academic women's center. Cancer Cytopathol. 2016;124:589–95.
6 Geldenhuys L, Murray ML. Sensitivity and specificity of the pap smear for glandular lesions of the cervix and endometrium. Acta Cytol. 2007;51:47–50.

7 Gupta N, Srinivasan R, Nijhawan R, Rajwanshi A, Dey P, Suri V, et al. Atypical squamous cells and low grade squamous intraepithelial lesion in cervical cytology: cytohistological correlation and implication for management in a low resource setting. Cytopathol. 2011;22: 189–94.
8 Simsir A, Hwang S, Cangiarella J, Elgert P, Levine P, Sheffield MV, et al. Glandular cell atypia on papanicolaou smears: interobserver variability in the diagnosis and prediction of cell of origin. Cancer. 2003;99:323–30.
9 Confortini M, Di Bonito L, Carozzi F, Ghiringhello B, Montanari G, Parisio F, et al. Interlaboratory reproducibility of atypical glandular cells of undetermined significance: a national survey. Cytopathology. 2006;17: 353–60.
10 Lee KR, Darragh TM, Joste NE, Krane JF, Sherman ME, Hurley LB, et al. Atypical Glandular cells of undetermined significance (AGUS): interobserver reproducibility in cervical smears and corresponding thin-layer preparations. Am J Clin Pathol. 2002;117:96–102.
11 Lepe M, Eklund CM, Quddus MR, Paquette C. Atypical glandular cells: interobserver variability according to clinical management. Acta Cytol. 2018;62:397–404.
12 Joste NE, Rushing L, Granados R, Zitz JS, Genest DR, Crum CP, et al. Bethesda classification of cervicovaginal smears: reproducibility and viral correlates. Hum Pathol. 1996;27: 581–5.
13 Pulkkinen J, Huhtala H, Kholová I. The role of pap smear in the diagnostics of endocervical adenocarcinoma. APMIS. 2021;129(4): 195–203.
14 Available from: www.kaypahoito.fi (Accessed 2021 Feb 1).
15 Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159–74.
16 Rabelo Santos SH, Derchain SF, do Amaral Westin MC, Angelo-Andrade LA, Sarian LO, Oliveira ER, et al. Endocervical glandular cell abnormalities in conventional cervical smears: evaluation of the performance of cytomorphological criteria and HPV testing in predicting neoplasia. Cytopathol. 2008;19:34–43.
17 Burja IT, Thompson SK, Sawyer WL Jr, Shurbaji MS. Atypical glandular cells of undetermined significance on cervical smears. A study with cytohistologic correlation. Acta Cytol. 1999;43:351–6.
18 Sherman ME, Dasgupta A, Schiffman M, Nayar R, Solomon D. The bethesda interobserver reproducibility study (BIRST). Cancer Cytopathol. 2007;111:15–25.
19 Kurtycz DFI, Staats PN, Chute DJ, Russell D, Pavelec D, Monaco SE, et al. Bethesda interobserver reproducibility study-2 (BIRST-2): bethesda system 2014. J Am Soc Cytopathol. 2017;6(4):131–44.
20 Moreira MAR, Filho AL, Castelo A, De Barros MRE, Da Silva AP, Thormann P, et al. How accurate is cytological diagnosis of cervical glandular lesions? Diagn Cytopathol. 2008; 36:270–4.
21 Niu S, Molberg K, Thibodeaux J, Rivera-Colon G, Hinson S, Zheng W, et al. Challenges in the pap diagnosis of endocervical adenocarcinoma in situ. J Am Soc Cytopathol. 2019; 8:141–8.
22 Perkins RB, Guido RS, Castle PE, Chelmow D, Einstein MH, Garcia F, et al. ASCCP risk-based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2020;24:102–31.
23 McCluggage WG, Wlsh MY, Thornton CM, Hamilton PW, Date A, Caughley LM, et al. Inter- and intra-observer variation in the histopathological reporting of cervical squamous lesions using a modified Bethesda grading system. Br J Obstet Gynaecol. 1998;105: 206–10.
24 Ismail SM, Colclough AB, Dinnen J, Eakins D, Evans DMD, Gradwell E, et al. Observer variation in histopathological diagnosis and grading of cervical intraepithelial neoplasia. Br Med J. 1989;298:707–10.
25 Hodgson A, Park K, Djordjevic B, Howitt B, Nucci MR, Oliva E, et al. International endocervical adenocarcinoma criteria and classification: validation and interobserver reproducibility. Am J Surg Pathol. 2019;43: 75–83.
26 Sellors JW, Nieminen P, Vesterinen E, Paavonen J. Observer variability in the scoring of colpophotographs. Obstet Gynecol. 1990;76: 1006–8.
27 Viswanathan K, Patel A, Abdelsayed M, Rosado L, Soong L, Margolskee E, et al. Interobserver variability between cytopathologists and cytotechnologists upon application and characterization of the indeterminate category in the milan system for reporting salivary gland cytopathology. Cancer Cytopathol. 2020;128:828–39.
28 Layfield LJ, Wang G, Yang ZJ, Gomez-Fernandez C, Esebua M, Schmidt RL. Interobserver agreement for the international academy of cytology yokohama system for reporting breast fine-needle aspiration biopsy cytopathology. Acta Cytol. 2020;64:413–9.
29 Wentzen N, Fetterman B, Tokugawa D, Schiffman M, Castle PE, Wood SN, et al. Interobserver reproducibility and accuracy of p16/Ki-67 dual-stain cytology in cervical cancer screening. Cancer Cytopathol. 2014;122: 914–20.
30 Luttmer R, Dijkstra MG, Snijders PJF, Berkhof J, van Kemenade FJ, Rozendaal L, et al. p16/Ki-67 dual-stained cytology for detecting cervical (pre)cancer in a HPV-positive gynecologic outpatient population. Mod Pathol. 2016;29:870–8.
31 Mariani R, Grace C, Hughes K, Dietrich RM, Cabay RJ, David O. Can we improve the positive predictive value of atypical glandular cells not otherwise specified? Diagn Cytopathol. 2014;42:200–4.
32 Conrad RD, Liu AH, Wentzensen N, Zhang RR, Dunn ST, Wang SS, et al. Cytologic patterns of cervical adenocarcinomas with emphasis on factors associated with underdiagnosis. Cancer Cytopathol. 2018;121:950–8.

PUBLICATION III

Detection and Outcome of Endocervical Atypia in Cytology in Primary HPV Screening Programme

Pulkkinen J, Kares S, Huhtala H, Kholová I

Diagnostics (Basel). 2021; 11(12):2402.
doi:10.3390/diagnostics11122402.

diagnostics

MDPI

Article

Detection and Outcome of Endocervical Atypia in Cytology in Primary HPV Screening Programme

Johanna Pulkkinen [1], Saara Kares [1], Heini Huhtala [2] and Ivana Kholová [1,3,*]

[1] Pathology, Fimlab Laboratories, Arvo Ylpön katu 4, 33520 Tampere, Finland; johanna.pulkkinen@fimlab.fi (J.P.); saara.kares@fimlab.fi (S.K.)
[2] Faculty of Social Sciences, Tampere University, Arvo Ylpön katu 34, 33520 Tampere, Finland; heini.huhtala@tuni.fi
[3] Faculty of Medicine and Health Technology, Tampere University, Arvo Ylpön katu 34, 33520 Tampere, Finland
* Correspondence: ivana.kholova@tuni.fi; Tel.: +358-3311-74851

Abstract: Most endocervical adenocarcinomas (EAC) are associated with high-risk HPV (hrHPV) infection, with HPV genotypes 16, 18 and 45 accounting for >90% of the cases. Among endocervical glandular lesions, screening with hrHPV test has previously shown to predict the outcome better than cytology, although around one-fifth of the EAC remain negative both in hrHPV testing and cytology. The study consists of two consecutive HPV-primary screening rounds, conducted in 2012–2015 and 2017–2020. Of the 87 women aged 35 to 60 years of age diagnosed with Atypical endocervical cells, NOS or Atypical endocervical cells, favor neoplastic cytology during the first screening round, 63 (72.4%) were hrHPV positive and 24 (27.6%) were hrHPV negative. Among hrHPV positive patients, three EAC, two adenocarcinomas in situ (AIS), one AIS + high-grade intraepithelial lesion (HSIL) and 13 HSIL were found. Of the histologically verified lesions, 68.4% (13/19) were purely of squamous origin. All the EAC and AIS were HPV16 or HPV 18 positive. No high-grade histological lesions were found among the hrHPV negative patients with cytological glandular atypia. A later database search revealed one HPV-negative, gastric-type mucinous EAC that was missed by the HPV primary screening.

Keywords: HPV; screening; cytology; endocervical adenocarcinoma; adenocarcinoma in situ; atypical endocervical cells

Citation: Pulkkinen, J.; Kares, S.; Huhtala, H.; Kholová, I. Detection and Outcome of Endocervical Atypia in Cytology in Primary HPV Screening Programme. *Diagnostics* **2021**, *11*, 2402. https://doi.org/10.3390/diagnostics11122402

Academic Editor: Lukasz Wicherek

Received: 22 November 2021
Accepted: 8 December 2021
Published: 20 December 2021

1. Introduction

In Finland, national cervical cancer screening has been organized since the 1960s. Women from the age of 30 to the age of 60 are invited to participate every fifth year, and in some municipalities 25- and 65-year-olds are also included. Since the beginning of the organized screening, the number of cervical cancer deaths in Finland has decreased to one-fifth of its original number [1]. Nowadays, Finland is among the European countries with the lowest rates of cervical cancer incidence [2].

Traditionally, the conventional Pap smear has been the primary test. In 2012, the city of Tampere, and later all municipalities in Pirkanmaa region, started HPV primary screening with high-risk HPV (hrHPV) test and cytological smear as a triage in women aged $\geq$35 years [3–6].

The important role of hrHPV infection leading to the development of invasive cervical squamous cell carcinoma (SCC) and behind the most of the endocervical adenocarcinomas (EAC) has been well documented [7–10]. In the previous studies, based on DNA detection of whole-tissue sections, 62% to 75% of the EAC were reported to be HPV positive with HPV genotypes 16, 18 and 45 accounting for 90% to 94.1% of the positive cases [8–10]. Of the EAC subtypes, the usual subtype is the most common, with the reported relative portion of 59% to 74.6% of all EAC [9,10]. The usual subtype also presents with the strongest association with HPV infection, with 60% to 82% of the tumors found to be positive [9,10].

In the previous studies, the clear cell adenocarcinomas (AC) accounted for 3.9% to 4%, the serous AC for 3% to 3.2%, the endometrioid AC for 1.4% to 3%, the minimal deviation AC 1.6% to 6% and the AC, not otherwise specified, for 4.7% to 8% of the EAC [9,10]. These less common EAC subtypes showed significantly lower prevalence for hrHPV, with only 13% to 20% of the clear cell AC, 0% to 25% of the serous AC, 13% to 27.3% of the endometrioid AC, 0% to 8.3% of the minimal deviation AC and 13.9% to 24% of the AC, not otherwise specified as positive [9,10].

In previous studies, HPV testing identified earlier more women with cancer or precancerous lesions than cytology alone [11–16]. Especially among endocervical glandular lesions, the HPV test predicted the outcome better than the cytology. Of the EAC 78% and 79.0% and of the adenocarcinoma in situ (AIS) cases 80% and 82.2% were detected by positive HPV screening test in comparison to detection of only 15% and 45.4% of EAC and 40% and 53.2% of AIS by cytology alone [11,12]. A negative HPV test result also predicted a negative end result better than a negative cytological sample alone [11–13]. Yet, 22% of the EAC and around 15% of the AIS have been reported to remain negative both in hrHPV testing and cytology [11,12].

The aims of the present study are to evaluate the detection and outcome of endocervical atypia in an HPV primary screening programme.

2. Materials and Methods

The study cohort represents women participating in an HPV primary screening programme organized by Fimlab Laboratories during 2012–2015 in the Pirkanmaa region, Finland. During these years, altogether 93.439 women aged 35 to 60 years were invited to cervical cancer screening, which included both the hrHPV test and the conventional pap test. All the pap tests of the hrHPV positive women were analyzed and, as a quality assurance, 10% of the HPV negative patients were assessed cytologically in the 2012–2016 period.

In total, 66.147 (70.8%) of the invited women participated in the screening. Out of the participants, 87 (0.13%) were diagnosed with endocervical glandular atypia (Atypical Endocervical Cells, NOS or Atypical Endocervical Cells, Favor Neoplastic) with or without a squamous atypia (Atypical Squamous Cells, Undetermined Significance, Low-Grade Squamous Intraepithelial Lesion, High-Grade Squamous Intraepithelial Lesion, Atypical Squamous Cells, Cannot Exclude HSIL).

At the beginning of the study, according to the screening protocol and the national guidelines, women with a positive hrHPV test result and/or a cytological diagnosis of Atypical Squamous Cells, Undetermined Significance (ASC-US) and/or Atypical Endocervical Cells, NOS (AEC, NOS) were referred to repeat sampling after 12 months [17]. The repeat test included both the hrHPV test and the conventional Pap test. If the repeated hrHPV test was found to be positive and/or there was a cytological atypia, the woman was referred for a colposcopy. If the hrHPV test was negative and there was no cytological atypia, the patient received an invitation to the next screening round in five years from the original invitation.

In 2016, there was a change in the national guidelines after which patients with cytological diagnosis of AEC, NOS with or without a positive hrHPV test result were referred for a colposcopy immediately.

Women with a cytological diagnosis of Atypical Endocervical Cells, Favor Neoplastic (AEC, FN) and/or a squamous cytological diagnosis of Low-Grade Squamous Intraepithelial Lesion (LSIL) or worse were always immediately referred for a colposcopy despite the hrHPV status of the patient. After the colposcopy, the patient was treated according to the national guidelines [17]. Despite the colposcopic findings or the procedures they lead in to, all women received an invitation to the next screening round after five years from the original invitation.

At the first screening round in 2012–2015, the patients were between 35 to 60 years old, and at the second screening round in 2017–2020, they were between the ages of 40 to 65 years.

All diagnoses on cervical cytological samples were provided according to the Bethesda Classification for Reporting Cervical Cytology 2014 [18–20]. The Abbot RealTime hrHPV PCR assay (RealTime; Abbot, Wiesbaden, Germany) was used for the detection of the hrHPV DNA. The test recognizes 14 hrHPV genotypes, including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. The hrHPV genotypes 16 and 18 were reported separately and the rest of the genotypes were reported as "other hrHPV than 16 or 18".

The HPV status, genotype, cytological and histological data were retrieved from the Laboratory Information System (LIS) of the Fimlab Laboratories Oy. Later, an additional LIS- search was conducted to find possible EACs missed by the primary HPV screening during the study period 2012 to 2020.

For the statistical analysis, SPSS version 25 was used (IBM SPSS Statistics for Windows, version 25.0, IBM Corporation, Armonk, NY, USA).

Because the samples of the study include those produced by the national cervical cancer screening protocol and its follow-ups, the individual consent of each participant was not requested. The study was approved by the Ethical committee of Pirkanmaa Health Care District (R13094 and R16022). The study was conducted according to the Declaration of Helsinki.

3. Results

After the first screening rounds in 2012–2015, 61 patients were diagnosed as AEC, NOS on cytology (Figure 1). Of those patients, 37 were hrHPV positive and presented with one adenocarcinoma in situ (AIS) and six high-grade intraepithelial lesions (HSIL) on follow-ups in the first screening rounds. During the second screening rounds in 2017–2020, a patient with HSIL-histology on the first screening round was diagnosed with an additional AIS. The diagnosis of AIS was reached after 5 years and 9 months from the first screening sample and from the first cytological diagnosis of endocervical glandular atypia. The patient was initially positive with hrHPV genotypes 16, 18 and an hrHPV type other than 16 or 18 (Table 1). At the time of the AIS diagnosis, HPV16 persisted.

Table 1. The high-grade histological lesions detected on the two screening rounds during 2012–2015 and 2017–2020, including the hrHPV genotypes and the initial cytological diagnoses.

HPV Genotype [1]	Cytological Diagnosis [2]	Histological Lesion [3]
other	AEC, NOS	HSIL
other	AEC, NOS	HSIL
other	AEC, NOS	HSIL
other	AEC, NOS	HSIL
16	AEC, NOS	AIS
16	AEC, NOS	HSIL
16, 18, other	AEC, NOS	HSIL, later AIS
other	AEC, NOS + ASC-US	HSIL
16, other	AEC, NOS + ASC, US	HSIL
16	AEC, NOS + ASC-H	HSIL
16, 18	AEC, NOS + ASC-H	HSIL
16, other	AEC, NOS +HSIL	HSIL
16	AEC, NOS + HSIL	HSIL
16	AEC, NOS +HSIL	EAC + LSIL
18	AEC, NOS + HSIL	EAC + LSIL
16	AEC, FN	HSIL

Table 1. *Cont.*

HPV Genotype [1]	Cytological Diagnosis [2]	Histological Lesion [3]
other	AEC, FN	HSIL
16	AEC, FN	EAC
18	AEC, FN	AIS

[1] In the screening hrHPV genotypes 16 and 18 were reported separately and the rest of the 14 genotypes were recognized by The Abbot RealTime hrHPV PCR assay as "other hrHPV than HPV16 or HPV18". [2] AEC, NOS (Atypical Endocervical Cells, NOS), AEC, FN (Atypical Endocervical Cells, Favor Neoplastic), AIS (Adenocarcinoma in Situ), EAC (Endocervical Adenocarcinoma), ASC-US (Atypical Squamous Cells, Undetermined Significance), LSIL (Low-Grade Squamous Intraepithelial Lesion), HSIL (High-Grade Squamous Intraepithelial Lesion), ASC-H (Atypical Squamous Cells, Cannot Exclude an HSIL). [3] AIS (Adenocarcinoma in Situ), EAC (Endocervical Adenocarcinoma), LSIL (Low-Grade Squamous Intraepithelial Lesion) including Cervical Intraepithelial Lesion (CIN) 1 and condyloma, HSIL (High-Grade Squamous Intraepithelial Lesion) including CIN2 and CIN3.

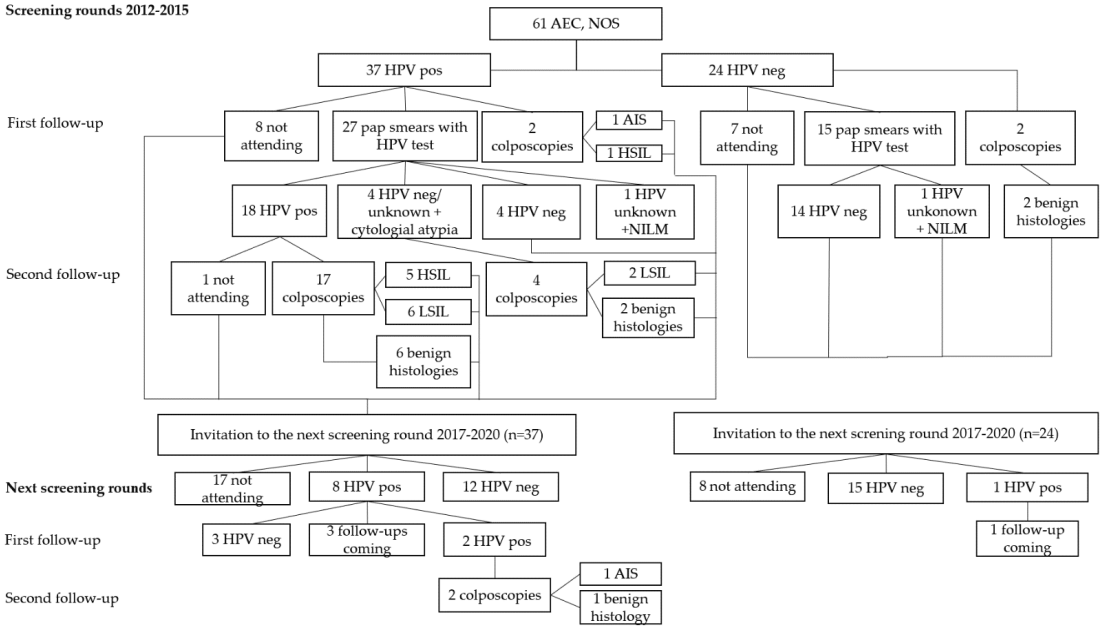

Figure 1. The outcome of two consecutive HPV primary cervical cancer screening rounds of patients with atypical endocervical cells, NOS in cytology. Abbreviations representing cytological diagnoses: AEC, NOS, Atypical Endocervical Cells, NOS; NILM, Negative for Intraepithelial Lesion or Malignancy. Abbreviations representing histological diagnoses: AIS, Adenocarcinoma in Situ; HSIL, High-Grade Intraepithelial Lesion; LSIL, Low-Grade Intraepithelial Lesion; Other abbreviations: HPV, human papillomavirus; pos, positive; neg, negative.

In 2012–2015 a total of 17 patients were diagnosed with AEC, NOS in combination with a squamous cytological diagnosis (Figure 2). Out of these patients, 16 were hrHPV positive. During the follow-ups, two histological HSIL were found behind the diagnoses of AEC, NOS + ASC-US. The combination of AEC, NOS + HSIL/Atypical Squamous Cells, Cannot Exclude an HSIL (ASC-H) resulted in four histological HSIL and to two combinations of EAC and LSIL. No additional high-grade histological lesions were found during the next screening rounds in 2017–2020.

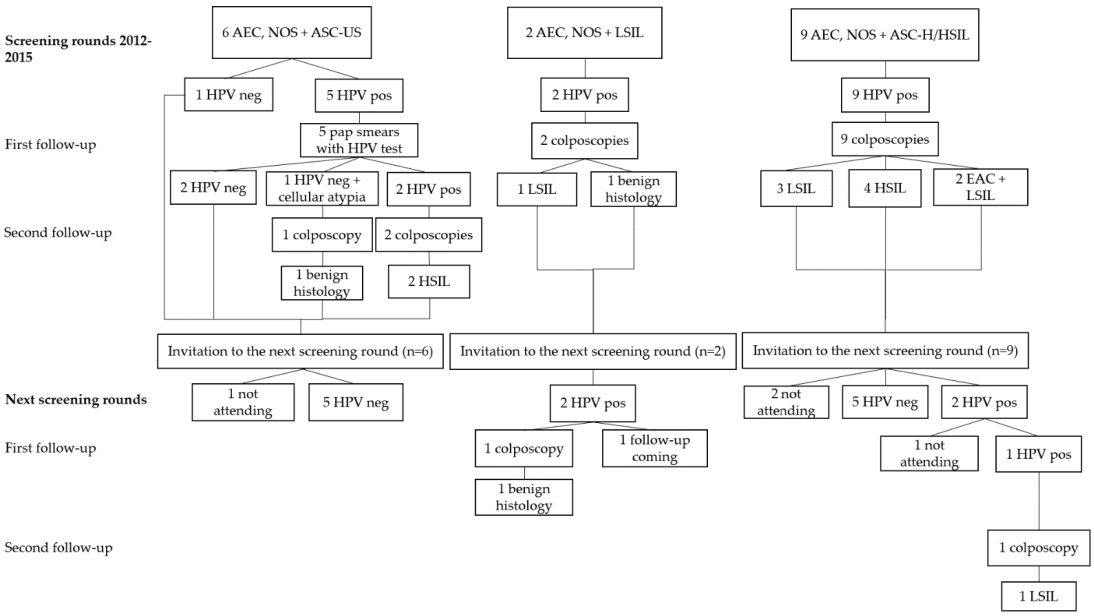

Figure 2. The outcome of two consecutive HPV primary cervical cancer screening rounds of patients with atypical endocervical cells, NOS and a squamous cell atypia in cytology. Abbreviations: AEC, NOS, Atypical Endocervical Cells, NOS; EAC, Endocervical Adenocarcinoma; ASC-US, Atypical Squamous Cells, Undetermined Significance; LSIL, Low-Grade Squamous Intraepithelial Lesion; HSIL, High-Grade Squamous Intraepithelial Lesion; ASC-H, Atypical Squamous Cells, Cannot Exclude an HSIL; HPV, human papillomavirus; pos, positive; neg, negative. Combinations of abbreviations representing cytological diagnoses: AEC, NOS + ASC-US; AEC, NOS + LSIL; AEC, NOS + ASC-H/HSIL. Abbreviations representing histological diagnoses: LSIL; HSIL; EAC + LSIL.

A cytological diagnosis AEC, FN was provided seven times in the first screening rounds in 2012–2015 and AEC, FN + LSIL was diagnosed two times (Figure 3). All patients were hrHPV positive. In the first group, two HSIL and one EAC was diagnosed during the follow-ups at the first screening rounds and one AIS on the follow-ups during the second screening rounds. The diagnosis of AIS was reached 6 years and 11 months after the first screening sample, with cytological suspicion of endocervical glandular neoplasia. During the time, the patient presented with persistent HPV18 infection (Table 1). In the latter group, no high-grade histological lesions were found in either the first or the second screening round.

Altogether, behind the 87 cytological endocervical glandular diagnoses with or without combined squamous cytological atypia, one EAC, two AIS, two EAC + LSIL, one AIS + HSIL and 13 HSIL were found reflecting that 68.4% (13/19) of the high-grade lesions verified histologically were purely of squamous origin (Table 1). There are still six follow-ups planned and yet to be conducted among patients initially positive with hrHPV and with a persistent infection.

Of the endocervical malignancies, 33.3% (2/6) were HPV18 positive and 66.7% (4/6) were HPV 16 positive (Table 1). Among HSIL, 42.9% (6/14) represented hrHPV genotypes other than HPV16 or HPV18, 28.7% (4/14) HPV16 and 28.7% (4/14) a combination of HPV16 and/or HPV18 and hrHPV type other than HPV16 or HPV18. The investigated HPV types (16, 18 or hrHPV other than 16 or 18), or a combination of them, showed no statistical association (p-value > 0.05) to endocervical malignancies or to squamous neoplasias.

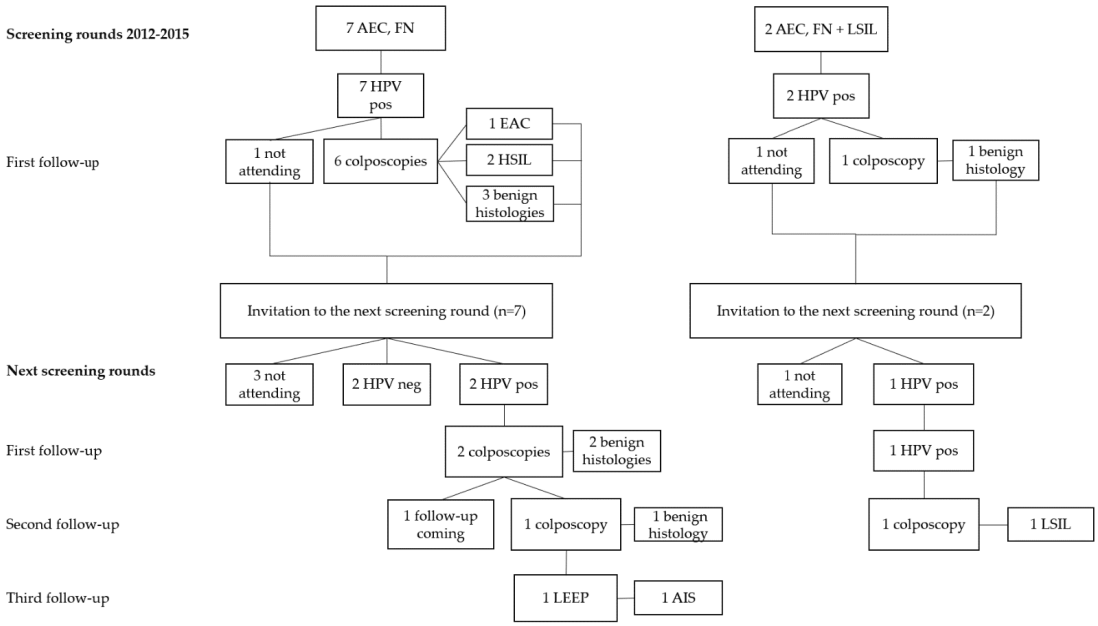

Figure 3. The outcome of two consecutive HPV primary cervical cancer screening rounds of patients with atypical endocervical, favoring neoplastic with or without a squamous cell atypia in cytology. Abbreviations representing cytological diagnoses: AEC, FN, Atypical Endocervical Cells, Favor Neoplastic; AEC, FN + LSIL, Low-Grade Squamous Intraepithelial Lesion. Abbreviations representing histological diagnoses: EAC, Endocervical Adenocarcinoma; LSIL, Low-Grade Squamous Intraepithelial Lesion; HSIL, High-Grade Squamous Intraepithelial Lesion. Other abbreviations: HPV, human papillomavirus; pos, positive; neg, negative; LEEP, loop electrosurgical excision procedure.

Aside for the two above-described AIS cases with the wide time lag before the histological diagnosis, all the high-grade histological lesions were diagnosed during the first screening round. No high-grade histological lesions were found among patients with cytological endocervical glandular atypia and a negative hrHPV test result (Figures 1–3, Table 2). There was one HPV-negative gastric-type mucinous EAC missed by the HPV primary screening. The pap test of the patient was not analyzed, that is to say, the patient was not included in the 10% of the HPV-negative cases assessed cytologically as a quality assurance.

The diagnosis of this gastric-type mucinous EAC was made two years after the negative HPV primary screening result. The diagnosis was immunohistochemically confirmed, and the patient was treated with hysterectomy, combined with salpingo-oophorectomy and lymphadenectomy. There were no metastases. The tumor itself was not tested for hrHPV, but the vaginal hrHPV tests have remained negative since. A 5-year follow-up has been conducted, and no additional tumors were observed.

During the first screening round, 17.5% (11/63) of the hrHPV positive patients and 29.2% (7/24) of the hrHPV negative patients left follow-ups unattended (Table 2). Of the originally hrHPV-negative women in the first screening round in 2012–2015, 33.3% (8/24) did not attend the second screening round in 2017–2020. Of the originally hrHPV-positive women in 2012–2015, 38.1% (24/63) did not attend the next screening round in 2017–2020 or left a follow-up during this screening round unattended. Of these patients, 8 (12.7%) had a negative hrHPV test result at the last follow-up they attended but 16 (25.4%) were still hrHPV positive when they dropped out of the screening protocol (data not shown).

Table 2. Cytological diagnoses with HPV status and the follow-up data on two cervical cancer screening rounds (2012–2015 and 2017–2020) among patients with cytological endocervical glandular diagnosis at the first screening round.

	AEC, NOS +/− ASC-US/LSIL *	**AEC, NOS +/− ASC-H/HSIL ****	**AEC, FN *** +/− ASC-US/LSIL**	**TOTAL**
ATTENDANCE	HPV+/HPV− (n = 44/n = 25)	HPV+/HPV− (n = 9/n = 0)	HPV+/HPV− (n = 9/n = 0)	HPV+/HPV− (n = 62/n = 25)
Not attending a follow-up during the 1st screening round	9/7	0/NA ****	2/NA	11/7
Not attending a follow-up during the 2nd screening round	18/8	2/NA	4/NA	24/8
HPV negative on a follow-up during the 1st screening round	8/15	0/NA	0/NA	8/15
HPV negative on a follow-up during the 2nd screening round	16/15	5/NA	2/NA	23/15
FINAL HISTOLOGY				
Adenocarcinoma (EAC)	0/0	0/NA	1/NA	1/0
Adenocarcinoma in situ (AIS)	1/0	0/NA	1/NA	2/0
High-grade intraepithelial lesion (HSIL)	7/0	4/NA	2/NA	13/0
Low-grade intraepithelial lesion (LSIL)	9/0	4/NA	1/NA	13/1
EAC + LSIL	0/0	2/NA	0/NA	2/0
AIS + HSIL	1/0	0/NA	0/NA	1/0
Benign histology	12/2	0/NA	5/NA	17/2
Follow-up coming	3/1	0 NA	0/NA	3/1

* AEC, NOS (Atypical Endocervical Cells, NOS), ASC-US (Atypical Squamous Cells, Undetermined Significance), LSIL (Low-Grade Intraepithelial Lesion) including condyloma and Cervical Intraepithelial Lesion 1 (CIN1). ** ASC-H (Atypical Squamous Cells, Cannot Exclude an HSIL), HSIL (High-Grade Intraepithelial Lesion. *** AEC, FN (Atypical Endocervical Cells, Favor Neoplastic). **** NA (not applicable). There were no combination diagnoses of AEC, FN + ASC-H/HSIL.

4. Discussion

The results of the present study are in agreement with the previously published series in the sense that no endocervical glandular malignancies were found among patients with endocervical cell atypia and a negative hrHPV result, reflecting that the majority of the endocervical adenocarcinomas are associated with hrHPV infection [8–10]. In our study, 0.13% of the pap tests were reported as endocervical glandular atypia, which is in the lower range compared to the previously published incidence rates of between 0.1% and 1.84% [21–24]. This variation in reported incidence is not surprising, since the interobserver agreement between pathologists, especially regarding mild cytological changes, is known to be relatively poor, and differentiating between cytological glandular features and squamous features has also proved to be challenging [25,26]. In the present study, 37.3% of the cases diagnosed with mild (not otherwise specified) endocervical glandular atypia were hrHPV negative, which is significantly less than the 79.8% reported by Chen et al. [23].

In addition to the lack of glandular malignancies, in the present study, there were no histological HSIL or SCC among the cases with endocervical glandular atypia and a negative hrHPV result. This is supporting the previous findings, according to which over 60% of the mild cytological glandular atypias with or without mild squamous atypia represent benign changes, or low-grade squamous histological lesions [22,27–29].

In our study, behind the cytological endocervical glandular diagnoses, with or without combined squamous cytological atypia, 68.4% of the clinically significant lesions (HSIL, AIS or worse) were purely of squamous origin. This might be caused by the extension of HSIL to endocervical glands, which is known to be a common cause of false cytological glandular diagnoses, or simply the result of the everyday struggle pathologists have in separating the cytological glandular features from squamous features [25,26,30]. Nevertheless, the amount of squamous lesions behind cytological endocervical diagnoses in our study, was roughly in the same range as the 73% and 77% previously reported [24,31].

In previous studies, the hrHPV test has been more often positive for cancer and precancerous lesions than cytology, and the difference in performance has been more pronounced among cases subsequently diagnosed with AIS or EAC than CIN3 or SCC [11,12,15,16]. Additionally,

the implementation of hrHPV testing has led to an earlier detection of CIN2 lesions or worse, and reduced the detection of CIN3 lesions and cervical cancer during follow-ups in comparison to cytology alone [13,14,31–33]. In the ARTISTIC study, however, the routine HPV testing did not significantly improve the recognition of CIN3 lesions compared to liquid-based cytology [34]. Nevertheless, a negative hrHPV test result during screening was shown to predict a negative outcome better than the cytology [11,13], which was also conversely seen in our study, since there were no high-grade histological lesions among hrHPV negative samples with cytological atypia [13,33].

Similarly, in the present study, only two malignancies were found during the second screening rounds. Both lesions represented AIS, and their histological diagnoses were not reached until 5 years and 9 months and 6 years and 11 months after the initial hrHPV positivity and cytological glandular atypia at the study baseline.

Although the participation rate for screening (70.8%) in the present study was similar to that previously reported in Finland [2], the high drop-out rate in the study was surprising, and possibly affected the end results. Of the originally hrHPV-positive patients, 25.4% dropped out of the screening protocol with a positive hrHPV test result at their last follow-up. This might have led to some cancers or precancerous lesions left undiagnosed. There was one death due to other reasons. Since different regional and private practice databases in Finland do not communicate, we do not know if the other patients dropping out the screening protocol have moved, were treated elsewhere or if they simply chose not to participate.

Our database search revealed one hrHPV-negative mucinous, gastric type adenocarcinoma case missed by the primary HPV screening during the study years 2014–2021. Since the hrHPV test at the screening was negative, no follow-ups were scheduled for the patient. The cancer was later diagnosed on a gynecological check-up for other reasons.

Since, in previous studies, 22% of the EAC and around 15% of the AIS were negative both in cytology and in hrHPV test, it can be only speculated if including the cytological sample to the screening protocol would have led to an earlier diagnosis in this case [11,12]. It has been calculated that adding a cytological sample to hrHPV screening would lead to an earlier detection of, at most, five cases per million women in a year [12]. Adding a p16/Ki67 dual-stain to screening has been reported to increase the detection rate of histological HSIL among hrHPV positive and cytology negative cases [35]. A positive Hepika test on a cytological sample seems to have a high sensitivity for invasive carcinoma, both squamous and glandular, in comparison to precursor lesions [36]. Since p16 and Hepika tests are both surrogate markers for hrHPV infection, adding them to the screening protocol in selected cases would probably increase the rate of, or at least provide an earlier detection for endocervical adenocarcinomas. Since the gastric type endocervical adenocarcinomas are practically always hrHPV negative, these tests are not likely to be of benefit, in their diagnosis [9,10].

In our series, all AIS and EAC were positive with either HPV16 or HPV18. Out of the HSIL, 42.9% were positive with an hrHPV type other than HPV16 or HPV18. In the statistical analyses, the HPV types (16, 18 or other) or combinations of them did not provide a prediction specifically relating to endocervical malignancies or to squamous lesions. This may have been due to the small number of positive cases, which may also have been the reason why the positive cases were not analysed further.

5. Conclusions

In conclusion, in this study of two consecutive HPV-primary screening rounds, among 87 women diagnosed with AEC, NOS or AEC, FN in cytology, three EAC, two AIS, one AIS + HSIL and 13 HSIL in were found in histology. All the EAC and AIS were either HPV16 or HPV 18 positive. No high-grade histological lesions were found among the hrHPV-negative patients diagnosed with cytological endocervical cell atypia. A later database search revealed one HPV-negative gastric-type mucinous adenocarcinoma that was missed by the primary HPV screening.

Author Contributions: Conceptualization, I.K. and J.P.; methodology, I.K. and J.P.; software, H.H. and S.K.; validation, I.K. and J.P.; formal analysis, H.H., S.K., J.P. and I.K.; investigation, H.H., J.P. and I.K.; resources, I.K.; data curation, H.H., S.K., J.P. and I.K.; writing—original draft preparation, J.P.; writing—review and editing, H.H., S.K., J.P. and I.K.; visualization, H.H., S.K., J.P. and I.K.; supervision, I.K.; project administration, I.K. and J.P.; funding acquisition, I.K. All authors have read and agreed to the published version of the manuscript.

Funding: The work was supported by grant from the Competitive Research Funding of the Pirkanmaa Hospital District to Ivana Kholová, grant number VTR X5211. The funding source had no role in the preparation of data or the manuscript.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Pirkanmaa Health Care District (protocol codes R13094 and R16022, dates of approval 25 June 2013 and 9 February 2016).

Informed Consent Statement: Since the samples of the study consist of samples produced by the national cervical cancer screening protocol and its follow-ups, patient consent was waived.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Finnish Cancer Registry, Institute for Statistical and Epidemiological Cancer Research. Available online: https://cancerregistry.fi/screening/cervical-cancer-screening/ (accessed on 11 October 2021).
2. Elfström, K.M.; Arnheim-Dahlström, L.; von Karsa, L.; Dillner, J. Cervical cancer screening in Europe: Quality assurance and organisation of programmes. *Eur. J. Cancer* **2015**, *51*, 950–968. [CrossRef] [PubMed]
3. Kares, S.; Veijalainen, O.; Kholová, I.; Tirkkonen, M.; Vuento, R.; Huhtala, H.; Tuimala, V.; Mäenpää, J.; Kujala, P. HIGH-RISK HPV testing as the primary screening method in an organized regional screening program for cervical cancer: The value of HPV16 and HPV18 genotyping? *APMIS* **2019**, *127*, 710–716. [CrossRef] [PubMed]
4. Veijalainen, O.; Kares, S.; Kujala, P.; Tirkkonen, M.; Vuento, R.; Kholová, I.; Luukkaala, T.; Osuala, V.; Mäenpää, J. Human papillomavirus test with cytology triage in organized screening for cervical cancer. *Acta Obstet. Gynecol. Scand.* **2016**, *95*, 1220–1227. [CrossRef] [PubMed]
5. Veijalainen, O.; Kares, S.; Kujala, P.; Vuento, R.; Osuala, V.; Tirkkonen, M.; Luukkaala, T.; Kholová, I.; Mäenpää, J. Implementation of HPV-based cervical cancer screening in an organised regional screening programme: 3 years of experience. *Cytopathology* **2018**, *30*, 150–156. [CrossRef] [PubMed]
6. Veijalainen, O.; Kares, S.; Kotaniemi-Talonen, L.; Kujala, P.; Vuento, R.; Luukkaala, T.; Kholová, I.; Mäenpää, J. Primary HPV screening for cervical cancer: Results after two screening rounds in a regional screening program in Finland. *Acta Obstet. Gynecol. Scand.* **2020**, *100*, 403–409. [CrossRef]
7. Walboomers, J.M.M.; Jacobs, M.V.; Manos, M.M.; Bosch, F.X.; Kummer, J.A.; Shah, K.V. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J. Pathol.* **1999**, *189*, 12–19. [CrossRef]
8. José, F.X.B.; Quint, W.G.; Alemany, L.; Geraets, D.T.; Klaustermeier, J.E.; Lloveras, B.; Tous, S.; Felix, A.; Bravo, L.E.; Shin, H.-R.; et al. Human papillomavirus genotype attribution in invasive cervical cancer: A retrospective cross-sectional worldwide study. *Lancet Oncol.* **2010**, *11*, 1048–1056. [CrossRef]
9. Pirog, E.C.; on behalf of the RIS HPV TT study group; Lloveras, B.; Molijn, A.; Tous, S.; Guimerà, N.; Alejo, M.; Clavero, O.; Klaustermeier, J.; Jenkins, D.; et al. HPV prevalence and genotypes in different histological subtypes of cervical adenocarcinoma, a worldwide analysis of 760 cases. *Mod. Pathol.* **2014**, *27*, 1559–1567. [CrossRef]
10. Molijn, A.; Jenkins, D.; Chen, W.; Zhang, X.; Pirog, E.; Enqi, W.; Liu, B.; Schmidt, J.; Cui, J.; Qiao, Y.; et al. The complex relationship between human papillomavirus and cervical adenocarcinoma. *Int. J. Cancer* **2015**, *138*, 409–416. [CrossRef]
11. Katki, H.A.; Kinney, W.K.; Fetterman, B.; Lorey, T.; Poitras, N.E.; Cheung, L.; Demuth, F.; Schiffman, M.; Wacholder, S.; Castle, P.E. Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: A population-based study in routine clinical practice. *Lancet Oncol.* **2011**, *12*, 663–672. [CrossRef]
12. Schiffman, M.; Kinney, W.K.; Cheung, L.C.; Gage, J.C.; Fetterman, B.; E Poitras, N.; Lorey, T.S.; Wentzensen, N.; Befano, B.; Schussler, J.; et al. Relative Performance of HPV and Cytology Components of Cotesting in Cervical Screening. *J. Natl. Cancer Inst.* **2017**, *110*, 501–508. [CrossRef]
13. Ogilvie, G.S.; Van Niekerk, D.; Krajden, M.; Smith, L.W.; Cook, D.; Gondara, L.; Ceballos, K.; Quinlan, D.; Lee, M.; Martin, R.E.; et al. Effect of Screening with Primary Cervical HPV Testing vs. Cytology Testing on High-grade Cervical Intraepithelial Neoplasia at 48 Months. *JAMA* **2018**, *320*, 43–52. [CrossRef]

14. Naucler, P.; Ryd, W.; Törnberg, S.; Strand, A.; Wadell, G.; Elfgren, K.; Rådberg, T.; Strander, B.; Johansson, B.; Forslund, O.; et al. Human Papillomavirus and Papanicolaou Tests to Screen for Cervical Cancer. *N. Engl. J. Med.* **2007**, *357*, 1589–1597. [CrossRef]
15. Wright, T.C.; Stoler, M.H.; Behrens, C.M.; Sharma, A.; Zhang, G.; Wright, T.L. Primary cervical cancer screening with human papillomavirus: End of study results from the ATHENA study using HPV as the first-line screening test. *Gynecol. Oncol.* **2015**, *136*, 189–197. [CrossRef]
16. Ronco, G.; Rossi, P.G.; Carozzi, F.; Confortini, M.; Palma, P.D.; Del Mistro, A.; Ghiringhello, B.; Girlando, S.; Gillio-Tos, A.; De Marco, L.; et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: A randomised controlled trial. *Lancet Oncol.* **2010**, *11*, 249–257. [CrossRef]
17. Cervical Cancer Screening Current Guidelines, Working Group set by the Finnish Medical Society Duodecim and The Finnish Colposcopic Society 2019. Available online: www.kaypahoito.fi (accessed on 28 September 2021).
18. Abdul-Karim, F.W.; Powers, C.N.; Bererk, J.S.; Sherman, M.E.; Tabbara, S.O.; Sidawy, M.K. Atypical Squamous Cells. In *Bethesda System for Reporting Cervical Cytology*, 3rd ed.; Nayar, R., Wilbur, D.C., Eds.; Springer: Berlin/Heidelberg, Germany, 2015; pp. 103–134.
19. Nayar, R.; Wilbur, D.C. The Bethesda System for Reporting Cervical Cytology: A Historical Perspective. *Acta Cytol.* **2017**, *61*, 359–372. [CrossRef]
20. Wilbur, D.C.; Chhieng, D.C.; Guidos, B.; Mody, D.R. Epithelial Abnormalities: Glandular. In *Bethesda System for Reporting Cervical Cytology*, 3rd ed.; Nayar, R., Wilbur, D.C., Eds.; Springer: Berlin/Heidelberg, Germany, 2015; pp. 193–240.
21. Selvaggi, S.M. Glandular epithelial abnormalities on thinprep®pap tests: Clinical and cytohistologic correlation. *Diagn. Cytopathol.* **2016**, *44*, 389–393. [CrossRef]
22. Lee, K.R.; Manna, E.A.; John, T.S. Atypical endocervical glandular cells: Accuracy of cytologic diagnosis. *Diagn. Cytopathol.* **1995**, *13*, 202–208. [CrossRef]
23. Chen, L.; Yang, B. Assessment of reflex human papillomavirus DNA testing in patients with atypical endocervical cells on cervical cytology. *Cancer* **2008**, *114*, 236–241. [CrossRef]
24. Nasu, I.; Meurer, W.; Fu, Y.S. Endocervical glandular atypia and adenocarcinoma: A correlation of cytology and histology. *Int. J. Gynecol. Pathol.* **1993**, *12*, 208–218. [CrossRef]
25. Lee, K.R.; Darragh, T.M.; Joste, N.E.; Krane, J.F.; Sherman, M.E.; Hurley, L.B.; Allred, E.M.; Manos, M.M. Atypical Glandular Cells of Undetermined Significance (AGUS). *Am. J. Clin. Pathol.* **2002**, *117*, 96–102. [CrossRef]
26. Moreira, M.A.R.; Filho, A.L.; Castelo, A.; de Barros, M.R.E.; da Silva, A.P.; Thomann, P.; Ferraz, M.D.G.M.D.C.; das Dores, G.B. How accurate is cytological diagnosis of cervical glandular lesions? *Diagn. Cytopathol.* **2008**, *36*, 270–274. [CrossRef]
27. Burja, I.T.; Thompson, S.K.; Sawyer, J.W.L.; Shurbaji, M.S. Atypical Glandular Cells of Undetermined Significance on Cervical Smears. *Acta Cytol.* **1999**, *43*, 351–356. [CrossRef]
28. Kim, M.-K.; Lee, Y.K.; Hong, S.R.; Lim, K.T. Clinicopathological significance of atypical glandular cells on cervicovaginal Pap smears. *Diagn. Cytopathol.* **2017**, *45*, 867–872. [CrossRef]
29. Pradhan, D.; Li, Z.; Ocque, R.; Patadji, S.; Zhao, C. Clinical significance of atypical glandular cells in Pap tests: An analysis of more than 3000 cases at a large academic women's center. *Cancer Cytopathol.* **2016**, *124*, 589–595. [CrossRef]
30. Kumar, N.; Bongiovanni, M.; Molliet, M.-J.; Pelte, M.-F.; Egger, J.-F.; Pache, J.-C. Diverse glandular pathologies coexist with high-grade squamous intraepithelial lesion in cyto-histological review of atypical glandular cells on ThinPrep specimens. *Cytopathology* **2009**, *20*, 351–358. [CrossRef]
31. Rabelo-Santos, S.H.; Derchain, S.F.M.; Westin, M.C.D.A.; Angelo-Andrade, L.A.L.; Sarian, L.O.Z.; Oliveira, E.R.Z.M.; Morais, S.S.; Zeferino, L.C. Endocervical glandular cell abnormalities in conventional cervical smears: Evaluation of the performance of cytomorphological criteria and HPV testing in predicting neoplasia. *Cytopathology* **2008**, *19*, 34–43. [CrossRef]
32. Rijkaart, D.C.; Berkhof, J.; Rozendaal, L.; van Kemenade, F.J.; Bulkmans, N.W.J.; Heideman, D.A.M. Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: Final results of the POBASCAM randomized controlled trial. *Lancet Oncol.* **2012**, *13*, 78–88. [CrossRef]
33. Horn, J.; Denecke, A.; Luyten, A.; Rothe, B.; Reinecke-Lüthge, A.; Mikolajczyk, R.; Petry, K.U. Reduction of cervical cancer incidence within a primary HPV screening pilot project (WOLPHSCREEN) in Wolfsburg, Germany. *Br. J. Cancer* **2019**, *120*, 1015–1022. [CrossRef]
34. Kitchener, H.; Almonte, M.; Gilham, C.; Dowie, R.; Stoykova, B.; Sargent, A.; Roberts, C.; Desai, M.; Peto, J. ARTISTIC: A randomised trial of human papillomavirus (HPV) testing in primary cervical screening. *Health Technol. Assess.* **2009**, *13*. [CrossRef] [PubMed]
35. Trzeszcz, M.; Mazurec, M.; Jach, R.; Mazurec, K.; Jach, Z.; Kotkowska-Szeps, I.; Kania, M.; Wantuchowicz, M.; Prokopyk, A.; Barcikowski, P.; et al. Liquid-Based Screening Tests Results: HPV, Liquid-Based Cytology, and P16/Ki67 Dual-Staining in Private-Based Opportunistic Cervical Cancer Screening. *Diagnostics* **2021**, *11*, 1420. [CrossRef] [PubMed]
36. Gustinucci, D.; Ciccocioppo, L.; Coppola, L.; Negri, G.; Zannoni, G.; Passamonti, B.; Cesarini, E.; Ianzano, C.; Andreano, T.; Pireddu, A.; et al. Multicentre Evaluation of Hepika Test Clinical Accuracy in Diagnosing HPV-Induced Cancer and Precancerous Lesions of the Uterine Cervix. *Diagnostics* **2021**, *11*, 619. [CrossRef] [PubMed]

MANUSCRIPT
IV

False-positive atypical endocervical cells in conventional Pap smears: Cyto-histological correlation and analysis

Pulkkinen J, Huhtala H, Kholová I

Submitted 28.11.2022.