UiT The Arctic University of Norway

Faculty of Health Sciences

Department of Community Medicine & Department of Clinical Medicine

HPV mRNA and HPV DNA Tests in Cervical Cancer Screening

Amir Rad

A dissertation for the degree of Philosophiae Doctor - June 2023

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Table of Contents

Preface and Acknowledgements

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List of Papers

My PhD thesis is based on three papers:

Paper I: Amir Rad, Sveinung Wergeland Sørbye, Greta Dreyer, Siri Hovland, Bente Marie Falang, Melanie Louw, Finn Egil Skjeldestad. HPV types in cervical cancer tissue in South Africa: A head-to-head comparison by mRNA and DNA tests. Medicine (Baltimore). 2017; 96(47):e8752. https://doi.org/MD.0000000000008752.

Paper II: Amir Rad, Sveinung Wergeland Sørbye, Sweta Tiwari, Maja-Lisa Løchen, Finn Egil Skjeldestad. Risk of intraepithelial neoplasia grade 3 or worse (CIN3+) among women examined by a 5-type HPV mRNA test during 2003 and 2004, followed through 2015. Cancers. 2023; 15, 3106. https://doi.org/10.3390/cancers15123106.

Paper III: Amir Rad, Sveinung Wergeland Sørbye, Tormod Brenn, Sweta Tiwari, Maja-Lisa Løchen, Finn Egil Skjeldestad. 13-type HPV DNA test versus 5-type HPV mRNA test in triage of women aged 25-33 years with minor cytological abnormalities - 6 years of follow-up. International Journal of Environmental Research and Public Health. 2023; 20(5):4119. https://doi.org/10.3390/ijerph20054119.

Abbreviations

ADC	adenocarcinoma
AGUS	atypical glandular cells of undetermined significance
AIS	adenocarcinoma *in situ*
ASC-H	atypical squamous cells favor high-grade
ASC-US (+)	atypical squamous cells of undetermined significance (or worse)
bp	base pair
CIN	cervical intraepithelial neoplasia
CIN1(+)	cervical intraepithelial neoplasia grade 1 (or worse)
CIN2(+)	cervical intraepithelial neoplasia grade 2 (or worse)
CIN3(+)	cervical intraepithelial neoplasia grade 3 (or worse)
CRN	Cancer Registry of Norway
E	early
EIA	enzyme immunoassay
HC2	hybrid capture II
HIV	human immunodeficiency virus
HPV	human papillomavirus
HSIL	high-grade squamous intraepithelial lesion
ICC	invasive cervical cancer
ISH	sequencing, *in situ* hybridization
L	late
LBC	liquid-based cytology
LSIL	low-grade squamous intraepithelial lesion
NA	not applicable/not provided
NASBA	nucleic acid sequence base amplification
NCCSP	Norwegian Cervical Cancer Screening Programme
NCI	United States National Cancer Institute
NPV	negative predictive value
PCR	polymerase chain reaction
PPV	positive predictive value
RLB	reverse line blot
SAR	screening algorithm-recommended
SCC	squamous cell carcinoma
SPF10	short PCR fragment primers 10
WHO	World Health Organization

Abstract in Norwegian

Bakgrunn: På verdensbasis er livmorhalskreft den fjerde mest utbredte kreftformen blant kvinner. HPV-vaksine og screening (ved hjelp av celleprøve eller HPV-test) kan redusere forekomst og dødelighet av livmorhalskreft. Siden celleprøvebasert screening har lav sensitivitet for å oppdage høygradige celleforandringer (CIN3+), har flere land erstattet celleprøve med HPV-test. Mens HPV DNA-tester oppdager tilstedeværelse av virus, som kan indikere en forbigående infeksjon, undersøker HPV mRNA-tester for onkogen aktivitet som medfører økt risiko for høygradige celleforandringer og kreft.

Formål: I denne doktorgradsavhandlingen hadde vi som mål å: Artikkel I) sammenligne ytelsen til en HPV-mRNA- og en HPV-DNA-test for påvisning av ulike HPV-typer i vevsprøver fra kvinner med livmorhalskreft; Artikkel II) undersøke evnen til en HPV-mRNA-test til å forutsi langsiktig risiko for høygradige celleforandringer (CIN3+) blant kvinner med normal celleprøve ved screening; og Artikkel III) sammenligne ytelsen til en HPV-mRNA-test og en HPV-DNA-test i triage av unge kvinner med lavgradige celleforandringer.

Materialer og metoder: I artikkel I ble det utført tester på 167 livmorhalskreftprøver fra Sør-Afrika ved hjelp av en 9-typer HPV mRNA-test og en 45-typer HPV DNA-test, og deres evne til å påvise ulike HPV-typer i kreftvev. Data som ble brukt i artikkel II og III ble hentet fra Kreftregisteret. Artikkel II inkluderte 9582 norske kvinner med normal celleprøve som ble screenet med en 5-typer HPV mRNA-test. Kvinnene ble deretter fulgt opp i opptil 11 år og 8 måneder med tanke på forekomst av CIN3+. Artikkel III inkluderte 4115 kvinner mellom 25 og 33 år med lavgradig celleprøve, og de ble triagert ved hjelp av enten en 5-typer HPV mRNA-test (n=1559) eller en 13-typer HPV DNA-test (n=2556). Resultatene som ble evaluert inkluderte positivitetsrate, henvisningsrate til kolposkopi/biopsi og andel med gjentatt testing, samt forekomst av CIN3+ blant kvinner med positiv og negativ HPV-test opptil 6 år etter triage.

Resultater: Artikkel I viste tilsvarende evne til å påvise ulike typer HPV både for HPV-mRNA- og HPV-DNA-testene (91,6 %). Totalt sett var 83,8 % av prøvene positive for de samme HPV-typene med begge testene. I Artikkel II ble 20,8 % av HPV mRNA-positive kvinner og 1,1 % av HPV mRNA-negative kvinner diagnostisert med CIN3+ ved senere oppfølging. Det var ingen signifikant forskjell i forekomst av CIN3+ for ulike HPV-typer. I Artikkel III var 23,3 % av kvinnene HPV-mRNA-positive og 52,8 % var HPV-DNA-positive ved triage ($p<0,001$). Henvisningsrater til kolposkopi/biopsi (24,9 % vs. 18,3 %) og gjentatt

cytologi (27,9 % vs. 5,1 %), samt CIN3+-deteksjonsrater (13,1 %/ vs. 6,9 %; p<0,001) var signifikant høyere hos kvinner triagert av HPV DNA-testen. Av de 10 tilfellene av livmorhalskreft som ble diagnostisert i løpet av oppfølgingsperioden, oppsto åtte hos kvinner som ble analysert med HPV DNA-testen.

Konklusjoner: Evnen til HPV-mRNA- og HPV-DNA-testene til å oppdage HPV-typer i livmorhalskreftprøver var lik. Langtidsrisiko for CIN3+ var høy blant HPV mRNA-positive kvinner og lav blant HPV mRNA-negative kvinner. Dette gjør en 5-typer HPV mRNA test godt egnet i screening av kvinner uavhengig av alder. I triage av unge kvinner med lavgradig celleprøve viste mRNA-testen tilsvarende effekt som HPV DNA-testen i kreftforebygging, samtidig som den krevde betydelig mindre bruk av helsetjenester.

Abstract

Background: Cervical cancer is the fourth most prevalent cancer in women worldwide. Cervical cancer screening (cytology or human papillomavirus [HPV] testing) and HPV vaccination can decrease cervical cancer incidence and mortality. However, because cytology-based screening has a low sensitivity to detect high-grade cervical lesions, several countries have replaced it with HPV test-based screening. While HPV DNA tests detect the presence of the virus, which may indicate a transient infection, HPV mRNA tests examine for oncogenic activity that carries an increased risk of high-grade cell changes and cancer.

Aims: In this PhD thesis, I aimed to: Paper I) compare the performance of an HPV mRNA and an HPV DNA test in the detection of HPV types in cervical cancer specimens; Paper II) examine the ability of an HPV mRNA test to predict the long-term risk of cervical intraepithelial neoplasia grade 3 or worse (CIN3+) among women with normal cytology at screening; and Paper III) compare the performance of an HPV mRNA test and an HPV DNA test in the triage of young women with minor cytological abnormalities at screening.

Materials and Methods: In Paper I, 167 cervical cancer specimens from South Africa were tested with a 9-type HPV mRNA test and a 45-type HPV DNA test, and HPV detection rates were calculated. Papers II and III were based on data obtained from the Cancer Registry of Norway. Paper II included 9582 Norwegian women with normal cytology and results from a 5-type HPV mRNA test at screening. We then followed these women for up to 11 years and 8 months for CIN3+. Paper III included 4115 women aged 25-33 years with minor cytological abnormalities at screening who were triaged with either a 5-type HPV mRNA test (n=1559) or a 13-type HPV DNA test (n=2556). The outcomes were positivity rate, referral rates to colposcopy/biopsy and repeat testing, and CIN3+ detection rate up to 6 years after triage.

Results: Paper I showed equal HPV detection rates for the HPV mRNA and HPV DNA tests (91.6%). Overall, 83.8% of specimens were positive for the same HPV types with both tests. In Paper II, 20.8% of HPV mRNA-positive women and 1.1% of HPV mRNA-negative women were diagnosed with CIN3+ during follow-up. There was no difference in CIN3+ incidence by HPV type. In Paper III, 23.3% of women were HPV mRNA-positive and 52.8% were HPV DNA-positive at triage ($p<0.001$). Referral rates to colposcopy/biopsy (24.9% vs. 18.3%) and repeat cytology (27.9% vs. 5.1%), as well as CIN3+ detection rates (13.1%/ vs. 6.9%; $p<0.001$) were significantly higher in women triaged by the HPV DNA test. Of the 10

cancers diagnosed during the study period, eight occurred in women triaged by the HPV DNA test.

Conclusions: The ability of the HPV mRNA and HPV DNA tests to detect HPV types in cervical cancer specimens was similar. The risk of CIN3+ during our long-term follow-up was low among HPV mRNA-negative women, and high among HPV mRNA-positive women. This adds to and strengthens existing evidence on the appropriateness of using the 5-type HPV mRNA in the screening of women, regardless of age. In the triage of young women with minor cytological abnormalities, the mRNA test demonstrated similar efficacy as the HPV DNA test in cancer prevention, while requiring significantly less healthcare utilization.

1. Introduction

1.1. Human Papillomavirus and Other Papillomaviruses

Human papillomavirus (HPV) has a single-stranded circular DNA containing about 8000 base pairs (bp) [1]. The HPV genome has eight open reading frames and one upstream regulatory region [1]. HPV genes are classified according to their expressions in the early (E) or late (L) differentiation stage of the epithelium [1]. For example, E1, E2, E5, E6, and E7 are genes involved in the early differentiation stage, while the E4 gene is expressed during the differentiation stage, and L1 and L2 genes are involved throughout the final differentiation stage [1]. The DNA of HPV is maintained at the basal layer of the epithelium, where HPV infection occurs [1].

Papillomaviruses have been discovered in birds and most mammals [2]. They are vastly diverse, and their isolations are labelled as ‘types’ [2, 3]. Throughout decades of research, thousands of papillomavirus types have been sequenced and isolated to form a classification system [2]. The open reading frame of the L1 gene is the most conserved region of HPV DNA. Therefore, in order for any newly discovered HPV type to be truly considered new and recorded as such at the International HPV Reference Center in Heidelberg, Germany, the nucleotide sequences of its L1 gene should have a variation of at least 10% from the corresponding sequences of the nearest identified HPV types [2, 3]. If the variation is less than 2%, it is defined as a ‘variant’, and if it is between 2% and 10%, it is defined as a ‘subtype’ [2].

1.2. Cervical Cancer and Precancerous Lesions

1.2.1. Anatomy of Involved Organs

The cervix is the lower fibromuscular part of the uterus [4, 5] and is located between the lower and upper genital tract [6]. The cervix permits spermatozoa to travel to the genital tract and protects the uterus and upper genital tract against bacterial infection [7]. The cervix is usually between 3 and 4 cm in length and 2.5 cm in diameter, but its size and shape vary depending on women’s age, parity, and menstrual status [4]. Embryologically, the cervix originates from the lower segment of the fused Mullerian ducts [6]. While the upper half of cervix remains above vagina, its lower half (the portio vaginalis), extends into the vagina

through an orifice called the external os [4].The most visible part of the cervix is the ectocervix, which is covered by pink stratified squamous epithelium composed of multi-layered cells, whereas the endocervix is the portion that is proximal to the external os and covered by red columnar epithelium made of a single, mostly invisible, layer of cells [4]. The endocervical canal crosses the endocervix and ties the uterine cavity with the vagina from the internal to the external os, where it opens into the vagina [4]. The length and width of this canal varies depending on a woman's age and hormonal status, with widths reaching 6-8 mm in women of reproductive age [4]. Two types of epithelium, stratified nonkeratinizing squamous and columnar epithelium, cover the cervix [4]. These two epithelia join at the squamocolumnar junction [4]. The cervical transformation zone is a circle of mucosa where the stratified squamous epithelium of the ectocervix gradually replaces the mucus-producing glandular epithelium of the ectocervix [1]. This zone changes its position as women age, moving towards and into the endocervical canal [1].

1.2.2. Cytological and Histological Classifications and Features

During the last decades, the scientific community has gained a deeper understanding of the pathogenesis and natural history of cervical cancer, which had led to changes in the cytological and histological classifications of cervical cancer and its precursors [8]. A new classification for reporting cervical cytology results, the Bethesda System, was invented after two workshops organized by the United States National Cancer Institute (NCI) held in 1988 and 1991 [4]. The main aspect of the Bethesda System was the establishment of the term squamous intraepithelial lesion, and the classification of this term into two-levels: low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL) [4]. The histological nomenclature of dysplasia and carcinoma *in situ* are being phased out [9] in favor of the World Health Organization (WHO) system (WHO 2014), including a two-level histological classification of LSIL and HSIL, which is biologically more related and reproducible than the former three-level classification of cervical intraepithelial neoplasia (CIN) [10].

The cytological features of low-grade CIN include cellular enlargement, multinucleation, nuclear hyperchromasia, nuclear irregularity, and perinuclear halos presence [8]. The cytological and histological features of high-grade CIN include immature basaloid-type cells with a high ratio of nucleus to cytoplasm, immature basaloid-type cells in the upper part of the

epithelium, mitoses in the upper part of the epithelium, irregular and hyperchromatic nuclei, and abnormal mitotic figures [8].

1.3. Etiology of Cervical Cancer and Precancerous Lesions

1.3.1. HPV Infection and Carcinogenesis

HPV can infect all types of cervical epithelia [10]. It is assumed that HPV accesses basal cells through microabrasions in the cervical epithelium [11], which allows HPV to interact with cervical basal cells, after which the virus is transferred to the nucleus, where the HPV genome discharges few episomes [10]. Infection is followed by E1, E2, E4, E5, E6, and E7 expression and viral DNA replication from episomal DNA [11]. DNA replication continues in the midzone and superficial zone of the epithelium, where L1, L2, and E4 are expressed [11]. L1 and L2 encapsulate the viral genomes to shape offspring virions in the nucleus so that a new infection can be initiated by the shed virus [11]. HPV DNA integration into the host chromosomes is associated with the loss or disruption of E2. This, followed by upregulated expression of the E6 and E7 oncogenes, can lead to microinvasive and invasive cancer [11].

High-risk HPV infection is separated into three phases: latent, permissive, and transforming [10, 12]. The latent phase occurs immediately after the infection and discharge of the viral genome, when the virus has no genetic activity and remains clinically hidden [12]. The permissive phase is characterized by low viral genome activity, low virus replication, expansion of the infection to cervical squamous basal cells, and the presence of CIN grade 1(CIN1) [12]. In the transforming phase, especially in the metaplastic epithelium of the transformation zone, viral gene activity changes from replication to transformation, with high E6/E7 gene expression followed by overexpression of the p16-inhibiting cyclin-dependent kinase-4 gene [10, 12]. This makes the host genome unstable [10, 12] and is followed by aberrant mitosis and proliferation of atypical basaloid cells [10]. The nuclei of the host cells are then customized to permit local expansion of HPV-transformed epithelial cells, i.e., CIN grade 2 or worse (CIN2+) [12]. While E gene expression is restricted to basal cells, L gene expression is involved in the packing of viral genome copies [10]. At this stage, morphologic changes, such as LSIL, can be detected [10]. Transforming infections can result in HSIL and adenocarcinoma *in situ* (AIS), some of which may progress to invasive cervical cancer (ICC) [10].

The expression patterns of HPV genes are identical during CIN1 (LSIL) and CIN grade 2 (CIN2) [1], but the expression of genes involved during CIN2 and CIN grade 3 (CIN3) is delayed, limiting the effects of the infection to a small zone around the epithelial surface [1]. These changes take place when the HPV genome integrates into the host DNA and begins to cause alterations in E7 gene expression, damage to E1 proteins, and replication of E2 proteins [1]. Once the lesion has progressed to cervical cancer, the productive phases of the viral life cycle are no longer effective, and the episomes of virus are mostly lost [1].

Most women contract at least one HPV infection throughout their life, but few women actually develop ICC [11]. A better understanding of the natural history of HPV infection and type-specific HPV infection is needed to identify associations with, and better markers of, disease progression [11]. Another issue to consider is the importance of HPV host genome integration in carcinogenesis, as after this integration, the negative feedback of oncogene expression and E2 gene regulation are disrupted [11]. HPV host genome integration can be determined from an mRNA test; a negative test may reflect an episomal viral state [13]. Logically, HPV host genome integration should confer a higher risk of progression to high-grade lesions and cervical cancer, whereas an infection in the episomal state would be more likely to clear spontaneously [13]. Women positive for both HPV DNA and HPV mRNA tests at screening have been reported to have a higher chance of persistent infection [14].

Moreover, the prevalence of integrated HPV forms differs by HPV type [11]. Contrary to HPV16, HPV18 host genome integration is almost complete in women with CIN3+ or ICC [11]. The integrated form of HPV16 is more frequently detected in women with severe cervical neoplasia, and the episomal form is rare in women with ICC [11]. On the other hand, in women with high-grade CIN and ICC, HPV18 is almost always detected in its integrated form only [11]. Viral load is too complicated to measure and is not clinically useful due to its variation by HPV type, the physical state of HPV, and the heterogeneity of cervical lesions [11].

It is common to detect concurrent or sequential infections of more than one HPV type, and there is evidence that the life cycles of different HPV types are not independent [11]. HPV oncogenes can stimulate methylation in the cells, and these methylation patterns change with the life cycle of the virus, and the occurrence of disease [11]. Numerous epigenetic changes, such as aberrant methylation of CpG islands in the promoter regions of tumor suppressor genes, can contribute to carcinogenesis [11]. Thus, if we can detect epigenetic alterations in

exfoliated cervical cells, we can enhance the efficiency of cervical cancer screening programs [11].

1.3.2. Persistence of HPV Infection and Progression of Cervical Lesions

HPV infections can be transient or persistent, the latter can manifest either as a constant low/high amount of viral activity or as progressive, periodic latent and active viral phases [11]. Although it has been reported that persistent HPV infection is necessary for the development of high-grade CIN and ICC [1, 10, 15], the definition of persistence varies. Woodman et al. defined persistent infection as detecting the same HPV type twice, with at least 6 months between tests [11]. In a systematic review from 2008, 78% of included studies defined HPV persistence as positivity at more than two visits (two-test setting), 19.5% of studies defined it as positivity at more than three visits, 4.9% defined it as the proportion of positive visits, and one study each defined it as HPV positivity through follow-up and as time to clearance of HPV infection (i.e., duration of HPV infection) [16]. The different HPV detection methods, research methods, HPV classifications, and definitions of clearance used in the aforementioned studies make the definition of persistent infection even more complicated [16].

A two-test setting is not perfect for identifying persistence and clearance (Figure 1). Indeed, if a single infection is detected in the first test but not the second test in several individuals, it would only show HPV prevalence (Figure 1, Part A). If the first test is negative and the second is positive, it becomes as a measure of HPV incidence (Figure 1, Part B). Another possibility is that the two-test setting detects a persistent infection without determining duration or clearance (Figure 1, Part C). Most studies evaluate HPV infection as a combination of the aforementioned scenarios (Figure 1, Part D). Studies that use this scenario do not give enough information on length of persistence, duration, or clearance of HPV infection. Instead, they use cytology and histology results to provide measures of cumulative risk of high-grade cervical lesions after an incident or persistent HPV infection.

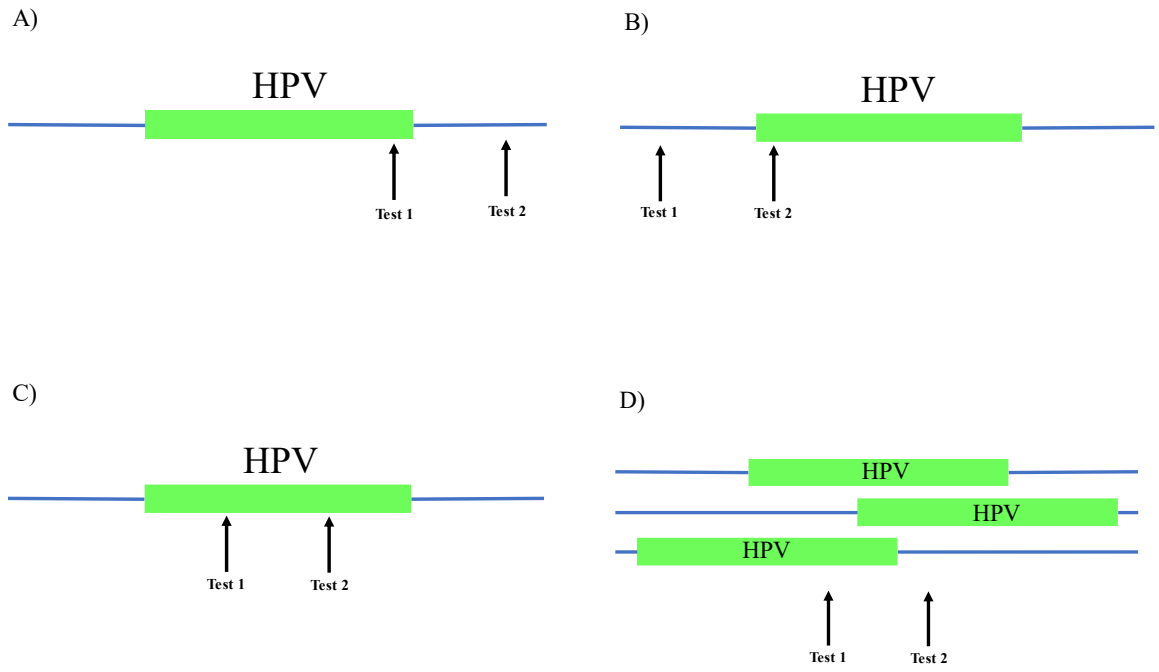

Figure 1. *Two-test setting scenarios to detect HPV infection and persistence. The green rectangles represent the period of HPV detection. Derived from Woodman et al. [11].*

As the number of tests performed increases, the certainty of detecting a persistent infection may also increase; however, choosing the proper interval is important. In a two-test setting, performing more tests with short intervals may detect persistence but miss initiation and clearance (Figure 2, Part A). Ho et al. showed a median duration of an incident infection of 8 months, with 70% and 91% of these infections clearing after 12 and 24 months, respectively [17]. The median duration of high-risk HPV infections is longer: 11 and 12 months for HPV16 and 18, respectively [17]. Therefore, a more desirable test setting is one that is long enough and performs more HPV tests at appropriate intervals (Figure 2, Part B). Woodman et al. tested 1075 women at 6-month intervals over 3 years [18], with an average of four HPV tests per women. Only two had four positive test results during the 3-year study period; 15 had three; 81 had two; and 407 had one positive test result [18].

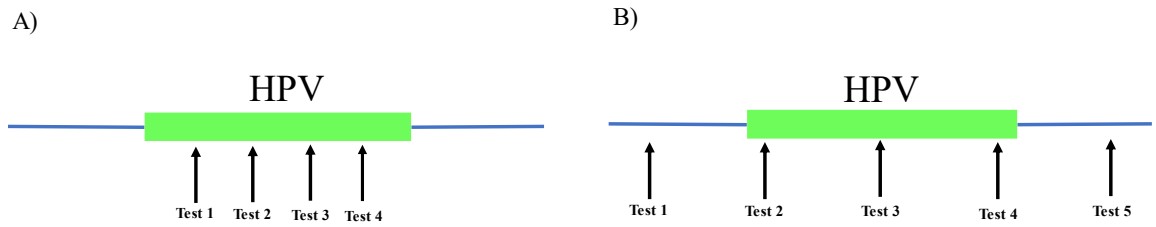

Figure 2. *Possibilities for improved HPV test settings to determine persistent HPV infection. Derived from Woodman et al. [11]. A) Method with several tests at short intervals, but that may miss infection initiation and clearance. B) Ideal method with several tests at proper intervals which provides information on persistent HPV infection as well as infection initiation and clearance (if it regressed). The green rectangles represent the period of HPV detection.*

Observations at unknown times during the natural history of HPV infection make it difficult to distinguish transient and persistent infections [18]. The definition of transient and persistent infections is dependent on the timing of sample collection with respect to the natural history of the infection, and on the sampling intervals [18]. Most studies do not have information on the history of HPV infection in participating women, but some restrict their analyses to HPV-negative women and/or women with normal cytology/histology at screening. For example, Kim et al. reported a 48-month cumulative incident rate for HPV16 and 18 of 25% and 13.6%, respectively [19] in a cohort of Norwegian women who were HPV DNA/serology-negative at screening and had tests performed every 6 months [19].

The risk of cervical lesions in follow-up studies can vary greatly between women with incident and persistent infections [20]; phylogenetic group has been found to predict both the persistence and carcinogenicity of individual HPV types. HPV types in the alpha 9 group (HPV16, 31, 33, 35, 52, and 58) are reported to be more likely to persist and to progress when they persist [20]. HPV16 was reported to be the most prevalent and persistent type, and had the highest potential to progress when it persisted [20], with a 12-year cumulative risk of CIN3+ of 17.3% for women who were negative at screening and positive 2 years thereafter (incident infection). This risk increased to 47.4% in women who were positive at both tests (persistent infection) [20].

Chan et al. used a three-test setting: one at enrolment, one at 3 months, and one at 6 months before CIN diagnosis [21]. Persistent infection was defined as HPV DNA positivity both at enrollment and at 6 months [21]. The majority of patients had only one persistent HPV type, but a few tested positive for multiple HPV types [21].

Despite the wide range of definitions and research methods, the reported associations between HPV persistence and CIN2-3/HSIL are consistent and strong, though duration of infection and test intervals change the magnitude of the associations [16]. Table 1 summarizes and compares the definition of persistence across studies [14, 17, 18, 20-33].

The amount of time needed for high-grade lesions to regress to low-grade lesions/normal is dependent on HPV type. It takes longer for lesions induced by oncogenic HPV types than non-oncogenic types to regress [34]. Although about 40% of undiagnosed CIN2 will regress within 2 years, regression of HPV16-induced CIN2 is less probable than that induced by other oncogenic HPV types [35]. Of CIN1 lesions, 60% regress, 30% persist, 10% progress to CIN3, and only 1% progress to ICC [36]. The corresponding measures for CIN2 lesions are 40%, 40%, 20%, and 5%, respectively [36]. Of CIN3 lesions, 33% regress and 12% progress to ICC [36].

Table 1. *A summary of published definitions of persistent HPV infection.*

Reference	**Test Interval/ Length of Study (Months)**	**Definition of Persistence**	**Information on Background/ Persistence/ Clearance of HPV Infection**	**Test Types**	**Endpoints**
Koutsky et al., 1992 [22]	4/24	Not provided	Not provided	HPV DNA	CIN2/3
Ho et al., 1995 [23]	3/15	Positive at 2 visits	Not provided	HPV DNA	Squamous intraepithelial lesions
Giuliano et al., 1997 [24]	3/6	Positive at both tests	Not provided	HPV DNA	Antioxidant nutrient
Ho et al., 1998 [17]	6/36	At least one of the types continued to be positive at next visits	Negative at screening//median 8 months	HPV DNA	Squamous intraepithelial lesions
Nobbenhuis et al., 1999 [25]	3-4/72	If not incidence (-/+) or clearance (+/-), it is persistence.	HPV test and histological characteristics at screening, number of negative, persistent, clearance	HPV DNA	CIN3
Hopman et al., 2000 [26]	6/48	Positive at t_0 and t_1	Not provided	HPV DNA	CIN1+
Ahdieh et al., 2001 [27]	6/36	Repeated positivity of same HPV type at consecutive visits 6-36 months apart	Not provided	HPV DNA	HPV positivity
Woodman et al., 2001 [18]	6/36	An uninterrupted sequence of one or more HPV-positivity	Not provided	HPV DNA	High-grade CIN
Kjær et al., 2002 [28]	24/?	Positivity of same type at both visits	Not provided	HPV DNA	HSIL
Chan et al., 2003 [21]	3/6	Any HPV positivity both at screening and 6 months	Not provided	HPV DNA	Regression of CIN2/3
Giuliano et al., 2003 [29]	4/12	2 or more consecutive tests positive for the same HPV type	Not provided	HPV DNA	Dietary intake
Cuschieri et al., 2004 [14]	24/24	Positivity of an HPV DNA type both at	Not provided	HPV DNA	Dyskaryosis

		screening and follow up		HPV RNA	
Piyathilake et al., 2004 [30]	6/24	Repeated HC-2 positivity	Not provided	HPV DNA	Folate
Lillo et al., 2005 [31]	6-12/?(61)	Positivity of same types at T0 T1, and T2 or at T1 and T2	Not provided	HPV DNA	High-grade CIN
Elfgren et al., 2005 [32]	19 (12-54)/54	Repeated HPV DNA positivity	Not provided	HPV DNA	CIN2/3
Castle et al., 2009 [33]	12 (9-21)/36	Positivity at enrollment and after about a year	Not provided	HPV DNA	CIN2+
Kjæer et al., 2010 [20]	24	Positivity of same type at both visits	Not provided	HPV DNA	CIN3+

1.3.3. HPV and Other Cancers

HPV has also been associated with other cancers, including vaginal [37, 38], vulvar [38, 39], head and neck [38, 40, 41], anal [38, 42, 43], penile [38, 44, 45], prostate [46], and breast cancers [47]. Vulvar intraepithelial neoplasia [39] and vaginal intraepithelial neoplasia [37], precursors of vulvar and vaginal cancer, respectively, are associated with high-risk HPV infection. One study detected HPV in 75% of invasive vaginal cancer samples; HPV16 was the most prevalent type (55%) followed by HPV33 (18.3%) [48]. However, a systematic review of 22 studies in the United States revealed that HPV16 and 18 (72.7%) are the most common types in invasive vaginal cancer, while HPV16 and 33 (55.5%) are the most prevalent types in invasive vulvar cancer [49]. According to a global meta-analysis, HPV16 (53.7%), 18 (7.6%), and 31 (5.6%) are the most frequent types found in vaginal cancer [50]. The most prevalent HPV types in vulvar carcinoma were HPV16 (32.2%), 33 (4.5%), and 18 (4.4%) [50]. Yet another study reported that HPV16 is the most prevalent type in all vulvar and vaginal cancers and precursor lesions (vulvar cancer: 29.3%, vaginal cancer: 55.4%, vulvar intraepithelial neoplasia grades 2/3: 71.2%, vaginal intraepithelial neoplasia grades 2/3: 65.8%) [51].

High-grade anal intraepithelial neoplasia is a precursor of anal cancer and is associated with HPV16 and 18 infections [42]. A study found 88% of anal tumors in both sexes were positive for HPV [43]. Similar to HPV type-specific prevalence in cervical cancer, HPV16 and 18 were the most prevalent HPV types: 73% for men and 6.9% for women [43]. HPV prevalence

in men with anal cancer is as high as 97.7% [43]. A global meta-analysis showed that the most prevalent HPV types in anal cancer were HPV16 (73.4%), 18 (5.2%), and 33 (4.8%) [50]. Due to the high detection of HPV in anal tumors, it has been suggested that, similar to cervical cancer, HPV infection is a necessary cause of anal cancer [43].

A worldwide meta-analysis studied type-specific HPV prevalence in vulvar, vaginal, and anal precancerous lesions and cancers, and revealed that the most prevalent types in vulvar, vaginal, and anal cancers are HPV16 and 18, in that order [50].

It is now clear that HPV can also cause a portion of head and neck squamous cell carcinoma (SCC) [40], with the most prevalent types being HPV16 (40.0%) and 18 (11.9%) [52]. A systematic review reported overall HPV positivity of 25.9% in head and neck SCC (23.5% in oral cavity cancer, 35.6% in oropharyngeal cancer, and 24.0% in laryngeal and hypopharyngeal cancer) [41]. HPV16 and 18 were found in 16% and 8% of oral cavity cancer, 30.1% and 1.0% of oropharyngeal cancer, and 16.6% and 3.9% of laryngeal and hypopharyngeal cancers [41].

A review of penile cancer studies revealed that infection with high-risk HPV was a common among males with penile intraepithelial neoplasia [44]. Penile cancers are classified as HPV-positive and HPV-negative [45], with prevalence ranging from 70-100%, and a strong association between basaloid and warty penile SCC and high-risk HPV infection [45]. The prevalence of high-risk HPV positivity in other penile SCC was 30% [45]. A systematic review of 31 worldwide studies containing 1466 penile carcinomas showed on overall HPV prevalence of 46.9%, with the most frequent types being HPV16 (60.23%), 18 (13.35%), 6/11 (8.13%), 31 (1.16%), 45 (1.16%), 33 (0.97%), 52 (0.58%), and other types (2.47%) [53].

A review of 46 studies showed that overall HPV prevalence in prostate cancer was 19% on average [46]. The most frequent types were HPV16 (13.68%), 31 (11.82%), 33 (8.39%), 18 (6.60%), 58 (3.55%), 11 (2.34%), and 6 (1.02%) [46].

Summary odd ratios in a recent meta-analysis on 22 case-control studies showed that HPV infection increases the risk of breast cancer (summary odd ratio=4.02) [47]. A global review of 29 studies stated a 23.0% average overall HPV prevalence in breast cancer samples [54].

1.4. Cervical Cancer Incidence and Mortality

In 2018, there were 569 847 incident cases of cervical cancer (3.54% of all incident cancer cases in both sexes, ranked 9th) and 311 365 deaths (3.52% of all cancer deaths in both sexes, ranked 9th) worldwide [55]. The global cervical cancer incidence rate was 13.1 per 100 000 woman-years, with the highest incidence rates observed in the Southern Africa (43.1 per 100 000) and the lowest in Western Asia (4.1 per 100 000) [55]. In 2020, cervical cancer was ranked third in terms of cancer incidence and mortality among women worldwide [56, 57]. And in 2021, the incidence rate in Northern Europe was 9.5 per 100 000 [55].

The global cervical cancer mortality rate in 2018 was 3.5 per 100 000, with the highest rates recorded in Eastern Africa (16 per 100 000) and the lowest in Australia and New Zealand (0.86 per 100 000) [55]. The mortality rates in Northern, Southern, and Western Europe were the same (1.1 per 100 000) [55].

In 2016, Norway had a cervical cancer mortality rate of 3.4 per 100 000 [58]. In 2017, the country recorded 316 new cases of cervical cancer, and 74 women who died from the disease [59]. In 2021, the incidence rate in Northern Europe and in Norway alone was 9.5 per 100 000 [55], and 12.6 per 100 000 [58], respectively.

1.5. Cervical Cancer Screening

Cervical cancer screening is meant to detect cervical cancer in its early stages, when it is more treatable and has high survival rates [60, 61], in patients with no signs or symptoms. A proper screening test must be accurate (i.e., give correct results), reproducible, affordable, easy to perform, acceptable for patients and providers, and safe (no or minimal side effects) [60, 61]. Health care systems are always looking to improve screening programs by using tests with higher sensitivity and specificity [62]. Indeed, tests with higher sensitivity will find more early-stage morbidity/precancerous lesions and disease, which will subsequently lower disease-specific mortality. Screening tests with high specificity will reduce referrals, overtreatment, economic costs, and stress for those screened. When the cut-off level for a positive test is set at a point that increases disease detection, sensitivity will increase, but specificity may decrease; inversely, when the cut-off level shifts to a point that increases the exclusion of disease, specificity will increase, but sensitivity may decrease [63].

However, the screening test alone does not have definitive preventive value; it should be paired with follow-up and treatment [60, 61] into an organized cervical cancer screening program in order to decrease morbidity and mortality in the population. The overall quality of a screening program is dependent upon the efficacy of the combination of screening for and treatment of precancerous lesions [60, 61]. A poor screening test could lead to overdiagnosis and overtreatment, or false-negative screening results that could preclude early treatment [60, 61].

1.5.1. Visual Inspection

For visual inspections with acetic acid, acetic acid is applied to the cervical epithelium, which turns abnormal cervical tissue white (i.e., acetowhite) making it possible for the practitioner or gynecologist to perform a rapid assessment of the tissue [4, 60]. This acetowhite change is due to the higher concentration of abnormal protein in the nuclei of abnormal tissue and to the existence of numerous dysplastic cells in the superficial layers of the epithelium [4]. For visual inspections with Lugol's iodine, the iodine turns precancerous/cancerous lesions a thick mustard or saffron-yellow color [60]. When iodine is applied to the squamous epithelium, it turns brown or black [60]. Iodine does not change the normal pink color of columnar epithelium [60].

1.5.2. Cytology

In 1943, George Papanicolaou suggested a diagnostic method that classified normal and abnormal vaginal and cervical epithelium (cytology). Through a test called the Pap smear, cells were scraped from the cervical surface with a spatula and fixed on a glass slide with 95% ethanol [64-66]. The examination was not expensive and became globally attractive. The Pap smear later became the basis of the Bethesda System [67] (section 1.2.2). The terms Pap test, Pap smear, cytology test, cytology specimen, and cervical cytology are often used interchangeably. However, sometimes authors distinguish between conventional cytology and liquid-based cytology (LBC). In conventional cytology, cervical cells are collected and fixed on a slide for microscopic assessment [68, 69]. LBC was introduced in the mid-1990s, and preserves cervical cells in a liquid medium [68, 69]. Nowadays, all hospitals in Norway use LBC.

1.5.3. HPV Testing

Given the etiological association between HPV and cervical cancer, HPV testing represents a viable alternative to cytology in screening programs [70]. HPV tests differ in their clinical performance, sensitivity, specificity [71-75], the nucleic acids they target (DNA or RNA), the genes they target in the HPV genome, and in their ability to distinguish different genotypes [76].

The different methods available to detect HPV DNA include polymerase chain reaction (PCR), reverse line blot (RLB), sequencing, *in situ* hybridization (ISH), and enzyme immunoassay (EIA). However, PCR is the most common. PCR-based methods apply either consensus PCR primers that detect a broad spectrum of DNA types, or type-specific PCR primers. The size of the PCR-amplified fragment changes according to the applied PCR primers; the PCR product of MY09/11 is nearly 450 bp, while the PCR product of GP5/6 is about 140 bp [77]. The aim of short PCR fragment primers (SPF10) is the universal detection of HPV, so they target only 65 bp of the L1-open reading frame for more than 43 HPV types [78, 79]. The sensitivity of SPF10 primers is higher than that of other primers, especially in the presence of multiple HPV types [79, 80]. As the PCR primers in the HPV DNA test are designed to be shorter (fewer bp), the ability of the test to detect HPV DNA in tumor tissues increases, along with test sensitivity [81]. However, the specificity decreases, and the test gives less information on oncogenic properties [81].

There are different commercial diagnostic kits available for HPV DNA testing. The digene hybrid capture 2 (HC2) test is based on a nucleic acid hybridization assay for detecting HPV, *Chlamydia trachomatis*, and *Neisseria gonorrhea* [82]. The digene HC2 High-Risk HPV DNA Test, also known as the Digene HPV Test, detects 13 high-risk HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) by applying full genome probes complementary to HPV DNA, hybridization, antibody capture, and signal amplification using qualitative chemiluminescent analyzing [82, 83]. The cobas HPV test is an automated qualitative *in vitro* test based on the PCR amplification of HPV DNA and nucleic acid hybridization that detects 14 high-risk HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in a single analysis [84]. It reports results on HPV16 and 18 individually, and a gives a pooled result for all other types [85].

HPV mRNA tests target the E6 and E7 expression of HPV and are based on the real-time multiplex nucleic acid sequence base amplification (NASBA) assay, branded as PreTect

HPV-Proofer [86]. The other HPV mRNA test, the Aptima HPV assay, also targets E6/E7 mRNA and identifies 14 high-risk HPV types [87].

1.5.4. Implementation of HPV Testing in Screening Algorithms

European guidelines recommend HPV testing every 5 years until age 30 years and cytology thereafter until age 60-65 years [88-90]. However, European countries have implemented HPV testing in different ways. Sweden used conventional cytology/LBC for women aged 23-29 years and HPV testing for those aged 30-64 years [91] until 2019, when screened by HPV testing was adopted for both age groups [92]. The screening program in Denmark uses LBC for women aged 23-59 years and HPV testing for those aged 60-64 years [88]; in the case of a negative HPV test, these older women can stop screening for the rest of their life [85, 88, 93, 94]. Denmark is currently considering expanding HPV test-based screening to women aged over 50 years [85, 94]. At present, HPV DNA testing is used to triage younger women with abnormal cytology at screening, while women who are HPV-positive at screening are triaged by either repeat HPV testing or cytology [85]. Finland is currently working towards the implementation of high-risk HPV testing for women aged 30-60 years at 5-year intervals [95, 96].

In the Netherlands, all cervical cancer screening is done with HPV testing. Screening begins at age 30 at continues at 5-year intervals until age 60; screening at earlier ages is not recommended [88, 97]. If an HPV test is positive at age 60, the test is repeated at age 65, if the test is negative at age 40 or 50, the screening interval widens to 10 years [97].

By 2019, high-risk HPV testing had replaced cytology in the United Kingdom for all women aged 25-64 years, with 3-year screening intervals for women aged under 50 years, and 5-year intervals for those aged 50 years and older [88, 98]. If the HPV test is positive, cytology is performed as a triage test; if that is normal, repeat HPV testing is done after 12 months [99]. HPV-positive women and those with abnormal cytology are referred to colposcopy [99] and HPV16/18 testing if available [100, 101]. Women who are HPV16/18-positive at screening and have normal cytology at triage attend repeat HPV testing in 12 months. HPV16/18-positive women with abnormal cytology at triage are referred to colposcopy [100, 101].

Turkey adopted a new screening algorithm in December 2012, applying HPV testing for women aged 30-65 at 5-year intervals [102]. Triage of positive women consists of HPV genotyping and cytology [101]. Women positive for HPV16/18 (regardless of cytology) or

atypical squamous cells of undetermined significance (ASC-US) or worse (ASC-US+) are referred to colposcopy [101]. Women positive for an HPV type other than HPV16/18 and normal cytology are sent to high-risk HPV testing within 3-6 months as a second triage [101].

Screening guidelines in the United States recommend high-risk HPV testing, either alone or in combination with cytology, for women aged 25-65 years [90, 101, 103]. HPV testing is also used for triage, by genotyping specific HPV types, including HPV16/18 [90, 103]. When women are screened by HPV testing alone, those positive for HPV16/18 are referred to colposcopy, those positive for other high-risk HPV types are referred to reflex cytology. Women with ASC-US+ at reflex cytology are referred to colposcopy/biopsy and those with normal reflex cytology are referred to repeat co-testing in 12 months as a second triage [101, 103].

In Australia and New Zealand, a partial-genotyping HPV test has been implemented in screening at 5-year intervals for women aged 25-74 years; reflex cytology is used as a triage test [90, 101, 104]. Women positive for HPV16/18 are referred to colposcopy, while those positive for other HPV types are referred based on reflex cytology; women with high-grade cytology are referred to colposcopy, and all others are followed-up with repeat HPV testing after 12 months [90, 101, 104].

In Italy, cytology/LBC or HPV testing (in some regions only) is used to screen women aged 25-30/35 years at 3-year intervals, and HPV testing is used to screen women aged 30/35-64 years at 5-year intervals [88, 93].

1.6. HPV Vaccination

There are two HPV vaccines currently available: Gardasil, made by Merck in the United States; and Cervarix™, made by Glaxo-SmithKline in the United Kingdom. Cervarix is a bivalent vaccine composed of L1 virus-like particles from HPV16 and 18 [105]. Gardasil is a quadrivalent vaccine that contains virus-like particles from HPV16, 18, 6, and 11 [106]. Merck has also created another, nonavalent version of Gardasil, Gardasil9, which includes virus-like particles from HPV16, 18, 31, 33, 45, 52, 58, 6, and 11 [106]. Gardasil9 has three times the antigenic load for HPV16 and 18 that Cervarix does [106].

Gardasil9 and Cervarix have equal efficacy against CIN2+ [106], although Cervarix has the highest cost-effectiveness, with proven efficiency after one dose. Still, the WHO recommends

two doses of Gardasil9 or Cervarix among women younger than 15 years old, and three doses for women 15 years of age or older [106]. Both Gardasil and Cervarix showed 98% efficacy against HPV16/18-related CIN2+ [106]. The term efficacy is defined as the prevention of persistent, type-specific HPV infection [106]. A global review carried out after the first decade of vaccine availability showed efficacies related to CIN2+ for Gardasil9, Cervarix, and Gardasil of 63%, 62%, and 22%, respectively [106]. The efficacies of HPV vaccines against CIN3+ were reported at 93% for Cervarix and 43% for Gardasil [106].

A Finnish study followed 98 561 women randomized to HPV16/18-vaccinated/unvaccinated arms for 10 years and reported 75 CIN3 and four ICC cases in the unvaccinated arm, while there were only four CIN3 cases, and no ICC cases, in the vaccinated arm [107]. The efficacy of HPV vaccination against CIN3+ related to any HPV type in the Finnish study was 66% [107]. Another randomized controlled trial with HPV16/18-vaccinated/unvaccinated arms reported 100% efficacy of vaccination against HPV16/18-related CIN during 4.5 years of follow-up [108].

1.7. Cervical Cancer Screening and HPV Vaccination in the Norwegian Setting

1.7.1. The Norwegian Cervical Cancer Screening Programme and the Cancer Registry of Norway

The organized Norwegian Cervical Cancer Screening Programme (NCCSP) aims to screen women aged 25-69 years and is managed by the Cancer Registry of Norway (CRN) [109], which was established in 1951 and collects data on cancer prevalence in Norway [110]. From 1953, all cytology and pathology departments in Norway have had to report all cancer and precancerous lesions to the CRN [110]. Cervical cytology results are available from 1991; cervical histology from 2002, and HPV test results from 2005 [110].

Cytology samples obtained within the framework of the NCCSP are analyzed by local cytology laboratories according to national guidelines, and results are recorded in the CRN following the Bethesda System classification as normal (i.e., negative for intraepithelial lesions or malignancy), unsatisfactory, ASC-US, LSIL, atypical squamous cells where a high grade squamous intraepithelial lesion cannot be excluded (ASC-H), HSIL, atypical glandular cells of undetermined significance (AGUS), AIS, and cervical cancer [111]. Histology

samples are reviewed by qualified pathologists, and outcomes are recorded following the WHO criteria on CIN: CIN1, CIN2, CIN3, AIS, and cervical cancer [60]. At birth or immigration, all Norwegian citizens or residents are assigned an 11-digit personal identification number, which can be used to merge all data reported to the CRN.

1.7.2. Cervical Cancer Screening Algorithms

In 2009, a working group was appointed by the Norwegian Directorate of Health to investigate the possibility of transitioning from cytology-based screening to HPV-based screening in the NCCSP [112]. Following their report in February 2014, a new screening algorithm was introduced in July 2018 [113]. This algorithm classified targeted women into two age groups: 25-33 years old and 34-69 years old [113]. Cytology was specified as the screening method for the younger age group (Figure 3); and women with normal cytology continue screening at the standard 3-year interval [113]. For women with ASC-US/LSIL, the LBC sample is used to perform an HPV test (reflex testing) [113]. Those with negative results are returned to the standard 3-year screening interval, while those with positive results are referred to repeat HPV testing in 12 months' time. If results are still positive at repeat HPV testing, the woman is sent to colposcopy/biopsy. Women with high-grade cytology are immediately referred to colposcopy/biopsy (Figure 3) [113].

In the older age group, HPV testing is used as the screening method, with reflex cytology performed in HPV-positive women [113]. HPV-negative women continue screening at the standard 5-year interval. HPV16/18-positive women with abnormal/unknown cytology are referred to colposcopy/biopsy (Figure 3) [113]. This HPV test-based algorithm was implemented for all women in the older age group in 2021 [114].

Regardless of the age group, women with a combination of HPV16/18 positivity and low-grade cytology are referred to colposcopy/biopsy [113]. However, women positive for HPV types other than HPV16/18 are only referred to colposcopy/biopsy if they also have high-grade cytology; in all other cases, women are referred to repeat testing [113].

The screening algorithm described above changed again in 2023, when Norway started screening women aged 25-33 years with HPV testing every 3 years [115].

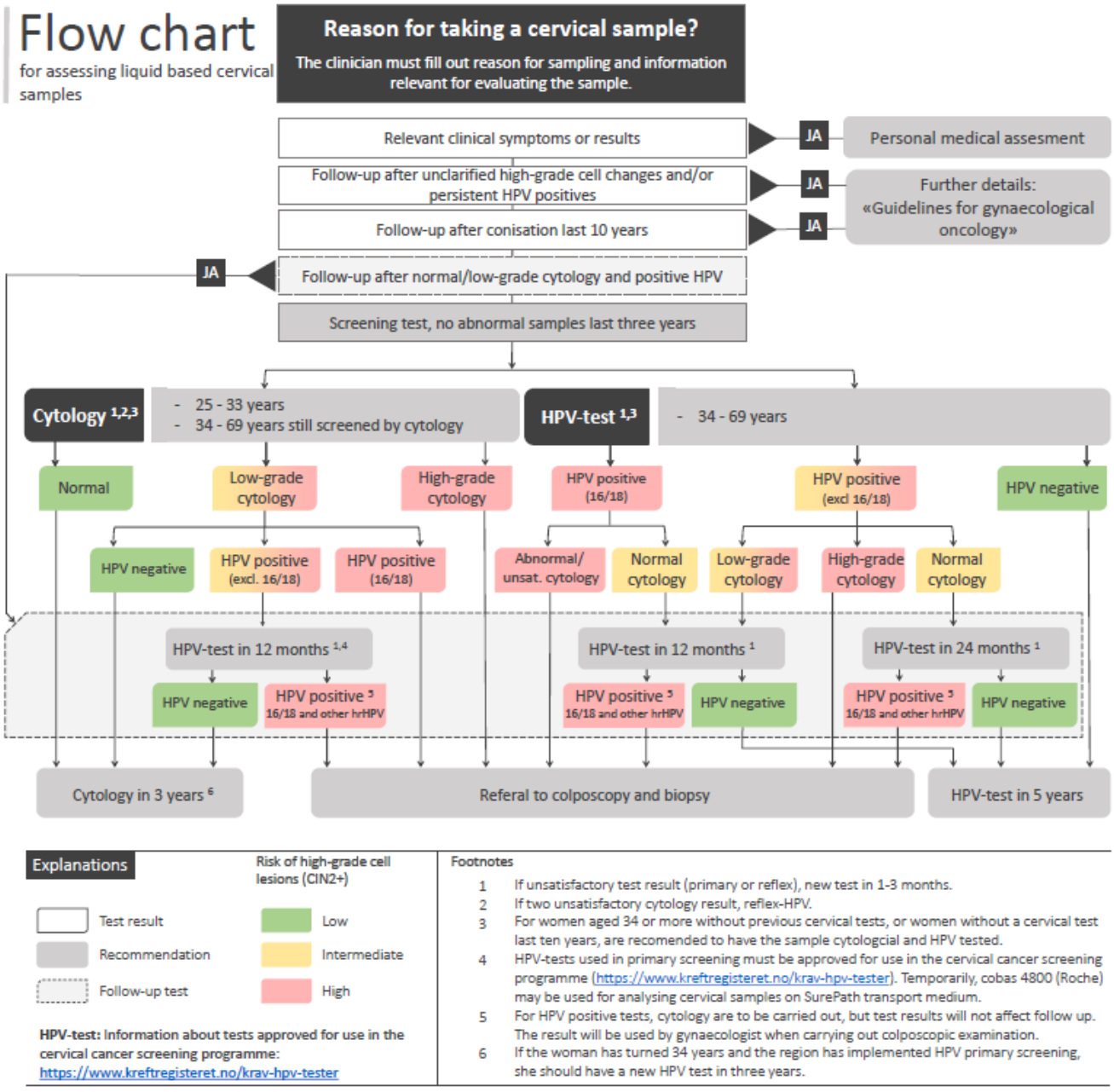

Figure 3. *The NCCSP flowchart for evaluating LBC, valid from July 2018, revised in March 2020 (available in Norwegian on the official website of the CRN) [113].*

1.7.3. HPV Vaccination

In Norway, the National Institute of Public Health is responsible for HPV vaccination [116]. From 2009, the quadrivalent Gardasil vaccine was offered to seventh-grade girls through the Childhood Immunization Programme [117, 118]. In November 2016, a 2-year catch-up program offered free HPV vaccination to all women born in/after 1991 [117-119]. Almost 45 000 girls were vaccinated through the Childhood Immunization Programme by December

2018 [118]. Since autumn 2018, Norway has been using a two-dose regimen of Cervarix and has extended vaccination to boys [117-119].

2. Aims

In order to improve HPV-based screening strategies, it is important to compare the performance of HPV tests applied in cervical cancer screening and triage.

Therefore, in this PhD thesis, we aimed to:

1) Compare the performance of an HPV mRNA and an HPV DNA test in the detection of HPV types in cervical cancer specimens.

2) Examine the ability of an HPV mRNA test to predict the long-term risk of CIN3+ among women with normal cytology at screening.

3) Compare performance of an HPV mRNA test and an HPV DNA test in the triage of young women with minor cytological abnormalities at screening.

3. Materials and Methods

3.1. Paper I

3.1.1. Study Sample

This comparative study included 188 women aged 18 years or older who were diagnosed with cervical cancer from January 2008 through July 2011 at the Gynecologic Oncology Unit, Department of Obstetrics and Gynecology, University of Pretoria, South Africa. Three women who did not have a validated histological diagnosis of cervical cancer in the slides adjacent to those collected for HPV testing, and 18 women with negative intrinsic sample control at mRNA/ DNA detection, were excluded, leaving a final analytical sample of 167 women with cervical cancer.

3.1.2. Data Collection

Cervical tumor tissue biopsies were collected from each woman, conserved in formalin, and sent to the Department of Anatomical Pathology at the University of Pretoria for histological diagnosis and HPV mRNA and DNA analyses. All histological diagnoses were reviewed by two pathologists until consensus was achieved.

3.1.3. HPV Testing

PreTect HPV Proofer was used to detect the mRNA of nine HPV types (HPV16, 18, 31, 33, 45, 35, 51, 52, and 58). GP5+/6+ PCR using RLB was employed to detect the DNA of 45 HPV types (HPV6, 11, 16, 18, 26, 30, 31, 32, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82/MM4, 82/IS39, 83, 84, 85, 86, CP6108, and JC9710) [120, 121].

3.1.4. Data Analysis

Statistical analyses were performed in SPSS (versions 24.0 through 29.0), applying a two-tailed, two-proportion Z-test to compare positivity rates between the HPV tests, with the significance level set at $p<0.05$. Multiple-type infections were assigned to a single HPV type

in hierarchical fashion, according to the decreasing prevalence order in our own data (hierarchical analyses).

3.2. Paper II

3.2.1. Study sample

The study included 19 153 women aged 13-87 years who were screened by both cytology (conventional or LBC) and HPV mRNA from May 2003 through December 2004 (baseline) identified in the NCCSP dataset. Women outside the target screening age (<25 and >69 years), with a history of CIN1+ or ASC-US+, with unsatisfactory cytology or no cytology at baseline, or with no follow-up information, were excluded. This rendered a final study sample of 9582 women.

3.2.2. Data Collection

Women screened by conventional cytology had an extra sample taken for HPV mRNA testing; for women who received LBC, the LBC sample was used for HPV mRNA testing. All data on cytology, HPV testing, and histology were taken from the CRN.

3.2.3. HPV Testing

PreTect HPV-Proofer was used to detect the mRNA of five HPV types (HPV16, 18, 31, 33, and 45), based on real-time NASBA technology. All HPV testing at baseline was performed in a single laboratory.

3.2.4. Follow-up

We followed women in the CRN through December 2015 for histologically confirmed CIN3+. Only cervical cancer cases that were validated by the CRN against hospital pathology reports were considered true cases of cancer. Cases less severe than CIN3+ were not considered.

3.2.5. Data Analysis

Statistical analyses were performed in SPSS (version 29.0), applying the Pearson Chi-square test and the Wilcoxon (Gehan) statistical method to make pairwise comparisons for categorical variables with the significance level set at $p<0.05$. Multiple-type infections were assigned to a single HPV type in hierarchical fashion, according to oncogenicity: HPV16, HPV18, and HPV31/33/45 (hierarchical analyses).

3.3. Paper III

3.3.1. Study Sample and Screening Algorithm during the Study Period

The study sample included 4115 women (1559 tested by HPV mRNA and 2556 tested by HPV DNA) aged 25-33 years with a screening result of ASC-US or LSIL recorded in the NCCSP in 2005-2010 and no previous diagnosis of CIN1+ or HSIL. Women with no follow-up, direct biopsy, no or inconclusive HPV test, and with ASC-H/HSIL at triage were excluded.

The NCCSP screening algorithm during the study period recommended that women with ASC-US/LSIL at screening attend triaged 6-12 months thereafter. At triage women were to receive both HPV testing and repeat cytology. The results of triage tests determined whether women should attend further follow-up or be returned to the 3-year screening interval (routine screening). To reflect best practices at during the study period, we categorized the post-triage study sample into three screening algorithm-recommended (SAR) groups: SAR referral to colposcopy/biopsy, SAR referral to repeat testing (HPV and cytology), or SAR return to routine screening. However, it is important to note that actual clinical practice may not always follow the screening algorithm. To increase study power, we expanded the window for triage to 3-18 months after screening results.

3.3.2. Data Collection

All data on cytology, HPV testing, and histology were taken from the CRN.

3.3.3. HPV Testing

HPV mRNA testing was done with PreTect HPV-Proofer, which detects five HPV types (16, 18, 31, 33, and 45). HPV DNA testing was done with HC2, which detects 13 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).

3.3.4. Follow-up and Outcomes

We followed women through December 2013 in the CRN for HPV positivity at triage, referral to colposcopy/biopsy or repeat testing after triage, and CIN3+. We used this data to calculate the following outcomes: HPV positivity rates at triage, SAR referral rates for colposcopy/biopsy or repeat testing after triage, and CIN3+ detection rates from triage to the end of follow-up. We also explored CIN3+ detection rates at 42 (one screening interval: 36 months + 6 months) and 78 months (two screening intervals: 72 + 6 months) post-screening.

3.3.5. Data Analysis

Statistical analyses were performed in SPSS (version 29.0), applying Pearson Chi-square test with $p<0.05$ as the significance level.

3.4. Ethical Permissions

3.4.1. South Africa

The study protocol (27/2008, 108/2008, 189/2012) was reviewed and approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Pretoria, and all participants gave written informed consent.

3.4.2. Norway

The study reported in Paper II was reviewed by the Regional Committee for Medical Research Ethics, Region East, Oslo, Norway. Data on HPV, cytology, and histology from the CRN was linked without informed consent from the participants, as was approved by the committee (REK Sør-Øst 2010/2858).

The study reported in Paper III was done following Norwegian law with regard to national health registries. The law states that national health registries are exempted from informed

consent because participants are informed about their rights to withhold information on demand. We worked with anonymous data, with no access to laboratory identification nor any personal data except for age. The ethical committee approved the research (REK 2016/2584).

4. Results

4.1. Paper I

Of the 167 women diagnosed with cervical cancer in our final study sample, 77.2% were older than 40 years (age range: 25-89 years), 41.3% were human immunodeficiency virus (HIV)-positive, 92.2% were diagnosed with SCC, 4.8% with adenocarcinoma (ADC), and 3.0% with adenosquamous carcinoma. Overall, 95.2% (159/167) of specimens were either HPV mRNA- or DNA-positive, while the individual positivity rates for the HPV mRNA and DNA tests were identical at 91.6% (153/167). The same number of specimens were positive by only one of the HPV tests: 13 each for the HPV mRNA and DNA tests (7.8%). While eleven double infections were detected by the HPV mRNA test, only four were identified by the HPV DNA test.

In hierarchical analyses restricted to the 9 high-risk HPV types in the mRNA test, 91.6% (153/167), and 88.0% (147/167) of specimens were positive by the HPV mRNA and DNA tests (P=0.28), respectively. In hierarchical analyses that considered the 9 HPV types included in both the HPV mRNA and DNA tests, concordance was 83.8% (140/167), while discordance was observed in 26 specimens. Type-specific concordance across the two tests was 66/67, 25/27, 19/21, and 33/45 for HPV16, HPV18, HPV45, and for HPV31, 33, 35, 51, 52 and 58 combined, respectively. While eight specimens were HPV-negative by both tests, the individual tests each detected six HPV-negative biopsies. The positivity rates by both tests for HPV16, 18, and 45 combined ranged from 66-68%; for HPV16, 18, 45, 33, and 35 combined, this range was 80-83%.

4.2. Paper II

The 9582 women with normal cytology included in our study population were followed up to 12 years. Overall, 3.2% were positive for HPV mRNA at baseline; 1.5% for HPV16, 15% for HPV18, and 1.2% for HPV31/33/35. About one-third of our study population was aged 25-33 years at screening and two-thirds were aged 34-69 years (27.2% vs. 72.8%). HPV mRNA positivity was 2.6 times higher in the younger age group.

The cumulative incidence of CIN3+ during follow-up was 20.8%. The cumulative incidence of CIN3+ during follow-up was 2.2% and 1.6% in the younger and older age groups, respectively (p=0.028), and no significant difference in cumulative incidence was observed by

HPV type. The overall cervical cancer incidence rate was 5 per 100 000 woman-years; 99 and 2 per 100 000 woman-years for HPV-positive and HPV-negative women, respectively.

4.3. Paper III

We observed considerable similarities in age, cytology result at screening, most recent cytology result before screening, and adherence to the screening algorithm between mRNA-tested and DNA-tested women (Table 2).

Table 2. *Screening adherence between most recent cytology before screening and screening.*

		HPV DNA-tested women N=2556 (%)	**HPV mRNA-tested women N=1559 (%)**
Adherence to the screening algorithm between most recent cytology results before screening and screening	No Previous Test	35.5	36.0
	Compliant	18.8	17.3
	Non-Compliant	45.7	46.8

HPV positivity rates at triage were 52.8% and 23.3% among HPV DNA-tested and HPV mRNA-tested women, respectively ($p<0.001$). The SAR referral rate to colposcopy/biopsy (24.9% vs. 18.3%) and repeat testing (27.9% vs. 5.1%) after triage was higher in DNA-tested than mRNA-tested women ($p<0.001$).

Overall, CIN3+ detection rates were 13.1% and 8.3% in DNA-tested and mRNA tested women, respectively ($p<0.001$). CIN3+ detection rates at 42 months and 78 months post-screening among women with SAR referrals to colposcopy/biopsy after triage were 31.7% vs. 30.5% ($p=0.72$) and 33.4% vs. 32.3%; ($p=0.73$), in DNA-tested and mRNA-tested women, respectively. Eight cancer cases were diagnosed during follow-up in DNA-tested women and two in mRNA-tested women.

5. General Discussion of Papers

In my research, the detection ability of a 9-type HPV mRNA test was compared to that of a 45-type HPV DNA test. Then, we evaluated the long-term ability of a 5-type HPV mRNA test to predict CIN3+ within the framework of the NSSCP. We observed a high positive predictive value of the HPV mRNA test for CIN3+ and a low risk of developing CIN3+ among women who were HPV mRNA-negative at screening. Finally, we compared a 5-type HPV mRNA test with a 13-type HPV DNA test in the triage of young women with minor cytological abnormalities. While the HPV DNA test had a higher CIN3+ detection rate, it also had significantly higher referral rates, and follow-up examinations, and consequently led to more utilization of health resources.

5.1. Summary of Main Findings

5.1.1. Paper I

In our direct comparison of two HPV tests in cervical cancer specimens, we found:

- An HPV detection rate of 95.2% in cervical cancer specimens when results of both the 9-type HPV mRNA test and the 45-type HPV DNA test were considered.
- When considered separately, both the 9-type HPV mRNA test and the 45-type HPV DNA test showed equal HPV detection rates (91.6%).
- Overall, 83.8% of cervical cancer specimens were positive for the same HPV types by both tests.
- Hierarchical analyses restricted to the 9 high-risk types included in HPV mRNA test reduced the HPV DNA detection rate to 88.0%.
- Hierarchical analyses of the 9 HPV types included in both the HPV mRNA and DNA tests showed concordance in 66/67 specimens for HPV16, 25/27 specimens for HPV18, 19/21 specimens for HPV45, and in 33/45 specimens for HPV31, 33, 35, 51, 52, and 58.
- The positivity rate for 3 high-risk HPV types (16, 18, and 45) was 66% by the HPV mRNA test and 68% by the HPV DNA test. When considering 5 high-risk types (16, 18, 45, 33, and 35), these values added up to 80% and 83%, respectively.

5.1.2. Paper II

In our longitudinal study on the performance of a 5-type HPV mRNA test in the screening of Norwegian women, we found:

- 3.2% HPV positivity; 1.5% for HPV16, 0.5% for HPV18, and 1.2% for HPV31/33/45.
- Significantly higher positivity in the younger age group (25-33 years).
- Of the 303 women who were HPV-positive at baseline, the cumulative incidence of CIN3+ was 20.8% for HPV-positive and 1.1% for HPV-negative women.
- No difference in the cumulative incidence of CIN3+ by HPV type.
- A higher 10-year cumulative incidence of CIN3+ in the younger age group, but no difference after adjustment for HPV positivity.
- Five cervical cancer cases in 100 000 woman-years.

5.1.3. Paper III

In our comparison of the HPV mRNA and HPV DNA tests in the triage of young women with minor cytological abnormalities, we found:

- HPV positivity rates of 23.3% for mRNA-tested and 52.8% for DNA-tested women at triage ($p<0.001$).
- Significantly higher SAR referral rates to direct colposcopy (24.9% vs. 18.3%) and to repeat cytology (27.9% vs. 5.1%) when the HPV DNA test was used at triage compared to the HPV mRNA test.
- Higher overall CIN3+ detection rates for the HPV DNA test compared to the HPV mRNA test (13.1% vs. 6.9%; $p<0.001$).
- More cancer cases in DNA-tested women (8/2556) than mRNA-tested women (2/1559) during the study period.

5.2. Methodological Considerations

5.2.1. Study Design

Paper I was a direct comparison of two tests applied to the same samples. Paper II was a nationwide register-based cohort study in which only one test was applied in the study population. Paper III had two different study groups, with a different test applied in each.

5.2.1.1. Cancer Registries

There are two main types of cancer registries: hospital-based and population-based [122]. Hospital-based cancer registries seek to record information on the cancer cases diagnosed in a particular hospital [122]; population-based cancer registries aim to collect, classify, consolidate, and link data on the occurrence of cancer in a particular population and to provide a framework for scientific and clinical analyses and policy assessments [122, 123]. Sources of information for population-based cancer registries can be hospital reports, medical records, pathology reports, hospital discharge abstracts, death certificates, etc. [123].

Over time, population-based cancer registries generate more valuable data, which can be used to identify distributions, patterns, and trends of cancers in different populations [123]. Cancer registries collect information about both exposures and outcomes that can sometimes be linked to other population data to explore associations between hypothetical risk factors and outcomes. Indeed, it would be difficult to investigate the cause of cancers without any exposure or outcome data in the years prior to cancer diagnosis.

Population-based cancer registries can also be used to describe the nature of the cancer burden in a community and assist in the organization of public health policies; they can serve as a data source for biomedical and epidemiological studies; and they can offer opportunities to monitor and assess the effectiveness of cancer controlling activities [122, 123]. The data included in population-based cancer registries are collected independently over time, which minimizes selection bias in the studies that use these data [124]. However, cancer registries may have some missing data, such as information on confounders [124]. Moreover, in its first years, a cancer registry may overestimate incident cases, as there will be individuals who had history of disease before the registry was established [124].

5.2.2. Validity (Systematic Error)

Validity refers to the deviation of a measurement from its true value that originates from systematic error [125]. Systematic error is mainly divided into selection bias, information bias, and confounding (although confounding is sometime categorized separately from bias). A number of other biases have also been discussed in different epidemiological sources, but in this chapter, I will elaborate on the main and most relevant biases in the papers included in this PhD thesis.

5.2.2.1. Selection Bias

Bias that originates from the selection of a study population is called selection bias [126], which reduces as the study population increases. In Paper I, selection bias might be higher than in Papers II and III, as the initial study sample was chosen from a single department in South Africa and was limited to 188 women. The selection bias was increased by our exclusion of three women without a validated histological diagnosis of cervical cancer in adjacent slides, and of an additional 18 women with intrinsic sample control-negative DNA/mRNA detection. The final study sample comprised 167 cases of cervical cancer.

There is a debate on whether a study sample must be representative or highly selected [126]. Representativeness is required in studies that are trying to comprehend causality in health phenomena [126], but in some studies, a representative population might not be important or even necessary [126]. Indeed, in some cases it may be beneficial to perform smaller, more focused, quicker, and cheaper studies, especially when it is unlikely that the results will be generalizable [126]. In Paper I, we aimed to compare the performances of an HPV mRNA test and an HPV DNA test to detect HPV types in cervical cancer specimens. We did not aim to investigate causality or to generalize our findings. Therefore, the representativeness of the study sample may not be important. In addition, it is very time-consuming and expensive to collect and test cervical cancer specimens.

5.2.2.2. Information Bias

Information bias refers to any error or systematic differentiation from the truth in the collection, recall, recording, and handling of study information [127]. Information bias has subclasses, including observer bias, misclassification bias, and recall bias [127]. Observer bias occurs when there is systematic error from true values due to observer variation [128]. In Papers II and III, observer bias may have occurred, as different pathologists observed and reported cytology and histology results. It may also have occurred in DNA analyses in Paper III, as HC2 was performed in different laboratories across Norway. On the other hand, in Paper I, all DNA analyses were done in a central laboratory. The DNA analyses in Paper III were done in local hospitals. However, all mRNA analyses in Papers I, II, and III were performed by the PreTect laboratory.

Misclassification bias is a systematic error that happens when individuals are categorized incorrectly [128]. This can happen at any stage of the research, and it can be derived during

the collection of exposure or outcome data [128]. If individuals are misclassified with equal likelihood between study groups, it is called non-differential misclassification [129]. If misclassification occurs with a different probability between study groups, it is called differential misclassification [129]. Our research could be affected both by differential and non-differential misclassification. We used HPV test results in all our papers, and any false-positive and false-negative results could be regarded as misclassification. This misclassification would be non-differential in a study that used one type of HPV test, like in Paper II, but the misclassification would be differential if it occurred in a study where more than one HPV test was applied, such as in Papers I and III. We used SAR referrals in Paper III, which was dependent on the results of HPV testing and cytology. Similar to HPV testing, misclassification would have been non-differential if a single pathologist assigned cytology and histology results, and differential if this was done by several pathologists, which was the case in Papers II and III. Misclassification bias can be reduced if another pathologist checks and confirms the diagnoses assigned by of the first one, as was done in Paper I, in which two pathologists reviewed all histological diagnosis until consensus was achieved.

In Papers II and III, we selected the study sample based on cytology results prior to study start and at screening. In Paper II, we selected women with normal cytology, and in Paper III, we selected women with ASC-US/LSIL. However, cervical cytology has poor accuracy [130]. One Norwegian study that evaluated the accuracy of cytology results assigned by four pathologists for the same samples showed that the sensitivity and specificity for CIN2+ varied between 68.8-93.8% and 70.6-95.6%, respectively [130]. Moreover, there was a trade-off between sensitivity and specificity; the pathologist with the highest sensitivity for CIN2+ had the highest false-positive rate and the lowest specificity [130]. The accuracy of cytology for CIN2+ was between 74.1-83.8% [130]. Therefore, our studies could be affected by non-differential misclassification of cytology results.

Papers II and III also looked at histological outcomes. During the study periods covered in Papers II and III, histological segments were visually assessed by pathologists. Because visual assessment carries a high chance of misclassification, in the case of high-grade dysplasia (CIN2+), two independent pathologists usually confirmed the diagnosis. This could reduce the chance of misclassification for CIN2+, but, if one pathologist classified a biopsy as CIN1, there was no second pathologist to confirm it. Hence, in our studies, the misclassification of CIN2+ as normal/CIN1 is more likely than misclassification of normal/CIN1 as CIN2+. Therefore, it is possible that there was differential misclassification between the diagnoses of

normal/CIN1 and CIN2+. Automated histopathological analysis may result in more accurate CIN classification [131].

5.2.2.3. Verification Bias

In Paper II, one HPV test was performed, and this was done at screening. The lack of a second test performed later in the study period may be considered as verification bias for HPV infection at screening. Such verification bias limits our knowledge about the persistence of HPV infection (discussed in section 1.3.3.).

5.2.3. Confounding

Confounding is defined as an alteration in the measurement of the effect of an exposure on an outcome because that exposure is also associated with other factors that affect the outcome [129]. There is a strong causal association between persistent infection with high-risk HPV types and ICC [16], but prior to the discovery of this association, sexual behavior was associated with this cancer [132]. Indeed, sexual behavior is a confounder in this PhD thesis, as it influences both the exposure (high-risk HPV infection) and outcomes (precancerous cervical lesions and ICC). Therefore, any risk factors that are related to or affect sexual behavior (e.g., age, parity, number of sexual partners, etc.) may also be considered confounders, and it is wise to consider these confounders in studies that evaluate causality or that include different populations. In our papers, we did not aim to assess the causal association between exposures and outcomes. Moreover, Papers I and II each had a single population. When considering age and HPV prevalence, adjustment for age can give a good overview of the confounding effect of age on HPV prevalence and outcomes. In Paper I, the majority of patients (77.2%) were older than 40 years, while nearly 23% were younger than 40 years. Moreover, women over 40 were more often HPV mRNA- and DNA-negative. We also observed an HIV prevalence of 81.6% among women younger than 40 years in Paper I. Adjustment for age in Paper II showed that age was a confounder for CIN3+, and the cumulative incidence of CIN3+ was higher in the younger age group. After stratifying for HPV status in Paper II, there was no difference in the cumulative incidence of CIN3+ between the age groups throughout the study period. Paper III included two different study populations, and evaluated characteristics like age, cytology results at screening, the most recent cytology results prior to screening, and screening adherence in the most recent cytology

result prior to screening. We observed high similarity in the characteristics of both study groups, indicating that these confounding factors did not affect the comparison of our outcomes in these two populations.

5.2.4. Overscreening, Overdiagnosis, and Overtreatment

Overscreening (unnecessary screening among individuals in the target population who are likely to be free of the disease), overdiagnosis (unnecessary diagnosis of individuals whose disease status is likely to regress from high/low-grade to a lower grade or normal status [133]), and overtreatment (unnecessary treatment of falsely diagnosed or overdiagnosed individuals) have always been issues of concern for health care systems. Overdiagnosis and overtreatment can cause psychological, behavioral, and physical harms and side effects. Moreover, they may negatively affect people's quality of life and waste health care resources [134]. Screening of very young women can end in overscreening, overdiagnosis, and overtreatment, so the recommended age to begin cervical cancer screening is 25 years old [135]. In Norway, cervical cancer screening is offered to women aged 25-69 years [110].

Other important concerns are HPV prevalence, infection clearance, incidence of high-grade cervical lesions, and progression or regression of cervical lesions, which may not be similar in all age groups. Choosing the proper tests for specific age groups is important to avoid overmanagement. The prevalence of genital HPV infection in Norwegian women younger than 30 years is as high as 32% [136]. Furthermore, 70% of HPV infections in young women clear within 1 year, and 91% clear within 2 years [17]. Moreover, it has been shown that the incidence rate of high-grade cervical lesions is lower [137], and the regression rate of high-grade lesions is higher [138], among young women compared to adolescents. Considering all of the above-mentioned issues, the use of an HPV DNA test for screening in women younger than 30 years may cause overscreening and overtreatment, thus cytology may still be regarded as a better screening method [139]. On the other hand, the high rate of cytological abnormalities in young women raises concerns about management strategies [114]. Another shortcoming of cytology-based screening is its low sensitivity for CIN3+ [70]. Therefore, some countries, like the United Kingdom and Australia, have replaced cytology with HPV testing in cervical cancer screening, even for young women [104, 140]. The European guidelines recommend the use of HPV testing in cervical cancer screening with 5-year intervals for women aged 30-60 years [88-90]. Since 2015, some counties of Norway have

applied HPV DNA testing for screening in women 34-69 years old at 5-year intervals, while cytology-based screening is still in place for women aged 25-33 years at 3-year intervals [113]. Since 2023, The HPV testing is also applied for women aged 25-33 years at 5-year intervals [141]. Table 3 shows types of testing in cervical cancer screening in some example countries.

Table 3. *Cervical cancer screening strategies in selected countries that have implemented HPV testing in screening.*

	Cytology screening		**HPV screening**	
Country/Region	Ages (years)	Interval (years)	Ages (years)	Interval (years)
Sweden [92]	-	-	23-50	3
			51-70	7
Finland* - selected counties from 2016 [96]	(25) 30-60 (65)	5	30 (25)-60 (65)	5
Norway – selected counties from 2023 [141]	-	-	25-69	5
Netherlands – nationally from 2017 [142]	-	-	30-40	5
			40-60	10
UK – nationally from 2020 [140]	-	-	25-50	3
			51-64	5
Turkey – nationally from 2012 [102]	-	-	30-65	5
US [90]	21-25	3	25-65[1]	3
	21-65	3	30-65[2]	5
Australia/New Zealand – nationally from 2017 [104]	-	-	25-74	5
Tuscany (Italy) – from 2013 [143]	25-34	3	35-64	5

[1]Only HPV test. [2]Co-testing. * Screening test is cytology but it can be also HPV [96].

In Paper II, we analyzed the performance of a 5-type HPV mRNA test at screening with 10 years of follow-up. The cumulative incidence of CIN3+ we observed for the HPV mRNA test was similar to that reported in screening studies that used HPV DNA tests. We also observed low HPV mRNA detection rates and a similar cumulative incidence of CIN3+ among mRNA-positive women in both age groups (25-33 years and 34-69 years). This suggests that the HPV mRNA test may not lead to overdetection, overreferral, or overmanagement when used to screen young women, and maintains the same ability to predict CIN3+ as HPV mRNA or DNA tests in older women.

Management of screening outcomes is also important in preventing overreferral, overdiagnosis, and overtreatment. Most minor cytological lesions regress spontaneously [144], therefore careful triage is important to prevent overmanagement. Direct referral of all women with ASC-US or LSIL to colposcopy represents overreferral and increases overtreatment [145]. In Paper III, we compared an HPV mRNA and an HPV DNA test in the delayed triage of ASC-US/LSIL among women aged 25-33 years. We evaluated HPV positivity rates, SAR referral rates, and CIN3+ detection rates from triage to the end of follow-up. The HPV DNA group had a higher positivity rate, higher referral rates, and more follow-up examinations compared to the HPV mRNA group. However, the overall CIN3+ detection rate was higher in the HPV DNA group. This underlines the trade-off that exists between utilization of health resources and detection of CIN3+.

5.3. Interpretation of Main Results of Papers

5.3.1. Paper I

In Paper I, we directly compared the ability of two tests to detect HPV infection overall, as well as type-specific HPV detection in cervical cancer specimens. However, it is difficult to compare the HPV positivity rates we observed in ICC specimens with other studies of different sample sizes and from different regions (**Table 4**).

5.3.1.1. HPV Prevalence in Cervical Cancer Specimens

We obtained an overall HPV positivity rate of 95.1% in cervical cancer specimens, while the positivity rate for the individual HPV tests was 91.6% each. The HPV positivity rate in African countries is usually higher than the global positivity rate [146-149], and our overall positivity rate was slightly higher, but in line with overall HPV positivity rates reported for African countries in recent updates of the global meta-analyses by Clifford et al., in which 19 HPV types (94.0%) [147] and 27 HPV types (94.2%)[148] were considered. Clifford et al. have performed four global meta-analyses on HPV type distribution in ICC: in 2003 [146], 2007 [147], 2011 [148], and 2012 [149]; all of which used HPV DNA data from PCR testing. We observed a higher positivity rate than those reported in other African studies from Ghana, Nigeria, and South Africa (90.4%) [150]. Our overall positivity by both HPV DNA and HPV

mRNA tests was higher than that in European countries (95.1% vs. 91.8%) [151], but if we consider only HPV DNA positivity, they were similar (91.6% vs. 91.8%) (**Table 4**).

In a global context [146-149], the sample size in Paper I (n=167) may appear small; however, on a regional and national scale, our sample size was above average (**Table 4**). For example, the meta-analyses by Clifford et al. included six studies from Africa, which considered nine countries. The average sample size in each of these nine countries was 67, and the average sample size in each of the six studies from Africa was 101 [146]. The meta-analysis by Ogembo et al. included 4067 ICC specimens from 71 studies in 23 African countries, which included an average of 168 samples per country and 57 samples per study [152].

Table 4. *A comparison of some different studies on HPV prevalence and type distribution in cervical cancer specimens.*

Country, Year of Publication	N of Specimens	HPV mRNA Test (Types included)	HPV mRNA Positivity Rate (%)	HPV DNA Test (Types included)	HPV DNA Positivity Rate (%)	Total Positivity Rate (%)	Common Types (Decreasing Order)
South Africa, 2017 [81] (Paper I)	167	9-Type PreTect Proofer16, 18, 31, 33, 35, 45, 51, 52, 58	91.6	45 –type GP5^{+}/6^{+} PCR /RLB (6, 11, 16,18, 26, 30, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82/MM4, 82/IS39, CP6108,32, 83, 84, 85, 86, JC9710)	91.6	95.2	Both tests: 16, 18, 45, 35, 33, 52, 31, 58, 51, DNA only: 82, 30, 56, 69, 73
Meta-analysis (23 African countries), 2015 [152]	4067	NA	NA	Different methods 16 and 18s	67.7	67.7	16, 18
Ghana, Nigeria, South Africa, 2014 [150]	570	NA	NA	25-type SPF10 PCR-DEIA-LiPA25 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 6, 11, 34, 40, 42, 43, 44, 53, 54, 70, 74	90.4	90.4	16, 18, 45, 35, 33, 52, 51, 31, 68, 73, 56, 59, 39, 11, 54, 66, 6, 44, 53, 70, 74
Austria, Czech Republic, Denmark, Estonia, Greece, Hungary,	3162	NA	NA	25-type SPF10 PCR-DEIA-LiPA2516, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 6, 11, 34, 40,	91.8	91.8	16, 18, 45, 33, 31

Country, Year of Publication	N of Specimens	HPV mRNA Test (Types included)	HPV mRNA Positivity Rate (%)	HPV DNA Test (Types included)	HPV DNA Positivity Rate (%)	Total Positivity Rate (%)	Common Types (Decreasing Order)
Ireland, Norway, Poland, Portugal, Romania, Russia, Spain, Belgium, Germany, Scotland, Wales, 2013 [151]				42, 43, 44, 53, 54, 70, 74			
Malaysia, Vietnam, Singapore, South Korea, the Philippines, 2013 [153]	500	NA	NA	25-type SPF10 PCR16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73, 6, 11, 34, 40, 42, 43, 44, 53, 54, 70, 74	93.8	93.8	16, 18, 52, 45
Global meta-analysis, 2012 [149]	36374	NA	NA	13-type PCR-based16, 18, 31, 33, 35, 39, 45, 51,52, 56, 58, 59, 68	89	89	16, 18, 33, 31, 58, 52, 35, 45, 59, 39, 51, 56, 68
Africa, 2012 [149]	2402	NA	NA	13-type PCR-based16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	90	90	NA
Global meta-analysis, 2011 [148]	30848	NA	NA	27-type PCR-based16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56,	89.9	89.9	16, 18, 58, 33, 45, 31, 52, 35, 59, 39

Country, Year of Publication	N of Specimens	HPV mRNA Test (Types included)	HPV mRNA Positivity Rate (%)	HPV DNA Test (Types included)	HPV DNA Positivity Rate (%)	Total Positivity Rate (%)	Common Types (Decreasing Order)
				58, 59, 66, 67, 68, 69, 70, 73, 82, 85, 97, 6, 11			
Africa (Algeria, Benin, Ethiopia, Guinea, Kenya,1 Mali, Morocco, Mozambique, Senegal, South Africa, Tanzania, Uganda, Zimbabwe), 2011 [148]	2011	NA	NA	27-type PCR-based16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82, 85, 97, 6, 11	94.2	94.2	16, 18, 45, 33, 35, 52, 51, 31, 58, 68
Thailand, 2011 [154]	120	NA	NA	$GP5^{+}/6^{+}$ PCR not provided	93.3	93.3	16, 18
India, 2009 [155]	278	5-Type PreTect Proofer 16, 18, 31, 33, 45	83.3	My09/My11 PCR Not provided.	83.4	91.7	16, 18, 31, 33, 45, 56, 52, 53, 59, 62, 67, 69, 73
Global meta-analysis, 2007 [147]	14595	NA	NA	19-type PCR-based 6, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70, 73, 82, 11	87.0	87.0	16, 18, 33, 45, 31, 58, 52, 35

Country, Year of Publication	N of Specimens	HPV mRNA Test (Types included)	HPV mRNA Positivity Rate (%)	HPV DNA Test (Types included)	HPV DNA Positivity Rate (%)	Total Positivity Rate (%)	Common Types (Decreasing Order)
Africa (Algeria, Benin, Ethiopia, Guinea, Ivory Coast, Kenya, Mali, Morocco, Mozambique, Senegal, South Africa, Tanzania, Uganda, Zimbabwe), 2007 [147]	1339	NA	NA	19-type PCR-based (6, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70, 73, 82, 11	94.0	94.0	16, 18, 33, 45, 35, 31, 58, 52
Norway, 2006 [156]	204	5-Type PreTect Proofer & 3-type real-time multiplex 16, 18, 31, 33, 45, 35, 52, 58	92.0	My09/My11 & PPF1/ CP5 PCR & $GP5^{+}/6^{+}$ PCR-EIA/RLB & ISH Not provided.	92.0	97.0	16, 18, 45, 33, 31, 52, 35, 58
Global meta-analysis, 2003 [146]	10058	NA	NA	18-type PCR-based (6, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70, 73, 82	86.9	86.9	16, 18, 45, 31, 33, 58, 52, 35, 59, 56, 6, 51, 68, 39, 82, 73, 66, 70

Country, Year of Publication	N of Specimens	HPV mRNA Test (Types included)	HPV mRNA Positivity Rate (%)	HPV DNA Test (Types included)	HPV DNA Positivity Rate (%)	Total Positivity Rate (%)	Common Types (Decreasing Order)
Africa (Algeria, Benin, Guinea, Mali, Morocco, Senegal, South Africa, Tanzania, Uganda), 2003 [146]	609	NA	NA	18-type PCR-based (6, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70, 73, 82	88.8	88.8	16, 18, 45, 33, 31, 52, 56, 58, 35, 51, 68, 73, 59, 6, 82, 66, 39, 70

The blue highlighted row represents the results of Paper I. The green highlighted rows are the results of meta-analyses from African countries. NA: not applicable/not provided.

5.3.1.2. HPV Type Distribution in Cervical Cancer Specimens

We used hierarchical analyses in Paper I, in which multiple-type infections were assigned to only one HPV type by order of decreasing prevalence. The six most prevalent HPV types we observed (in decreasing order: HPV16, 18, 45, 35, 33, and 52) was exactly the same as the most prevalent types in sub-Saharan Africa [150]. The six most prevalent HPV types in African countries reported in the meta-analysis by Li et al. were also very similar to ours, except for HPV33, which was more common than HPV35 [148]. Smith et al. did not include a hierarchical analysis, and instead counted each HPV type once; thus multiple-type infections could have been counted twice or three times if they were double or triple infections [147]. However, we observed a lower HPV16 prevalence, a similar HPV18 prevalence, and a higher HPV45, 33, and 52 prevalence than did Smith et al. [147] or another study from sub-Saharan Africa [150]. We found a lower prevalence of HPV16, and relatively a higher prevalence of HPV45, 35, and 52, than was reported in a European study [151]. The five most prevalent HPV types in the European study (16, 18, 45, 33, and 31) [151] were similar to ours, except for HPV35, which was replaced by HPV31 in Europe. The eight most prevalent HPV types in Norway (16, 18, 45, 33, 31, 52, 35, and 58) [156] were also similar to ours, except for the order after the third most prevalent type (**Table 4**). HPV16, 18, 45, and 33 were among the

five most prevalent HPV types in our study, as well as in most of other global studies [146-148], studies from Africa [146-148, 150], studies from Europe [151, 156], or studies from other regions [153, 155].

5.3.1.3. HPV Detection Rate by HPV mRNA Test vs. HPV DNA Test

Generally, when more than one HPV test is applied to the same specimens, the positivity rates for the individual tests are lower than when their results are combined to determine overall HPV positivity. Similar to our study, other research found equal HPV positivity rates by HPV mRNA testing and HPV DNA testing on the same ICC specimens [155, 156] (**Table 4**). A Norwegian study that performed an 8-type E6/E7 HPV mRNA test and a consensus GP5+/6+ PCR HPV DNA test reported the same, individual positivity rate (92%), but the overall HPV positivity rate when both tests were combined was 97% [156]. Likewise, another study from India used a 5-type E6/E7 HPV mRNA test and a MY09/11 L1 consensus PCR HPV DNA test and reported an individual positivity rate for each test of 83%, but an overall HPV positivity rate of 91.7% [155]. These studies suggest that HPV mRNA tests have a detection rate that is at least as high as that of HPV DNA tests.

5.3.2. Paper II

NCCSP guidelines categorize women into two age groups: 25-33 and 34-69 years [113]. HPV testing is used to screen women in the older age group, but cytology screening is still used in younger women [113]. Many countries have recently substituted HPV testing for cytology in cervical cancer screening, and there are many validated HPV tests available for this purpose. Their main differences are the molecular pieces of HPV they identify (genes or gene transcripts) and the HPV types they detect [76]. It is known that persistent infection with high-risk HPV types is a necessary cause of cervical cancer. The central dogma in molecular biology is that DNA produces mRNA and mRNA produces protein. Gene expression of a virus in its host takes place after the integration of HPV DNA into the genome [11]. Therefore, viral gene expression may imply that the virus has been present in the host longer. Moreover, cervical cancer is not caused by HPV infection *per se*, but by the constant overexpression of the viral E6/E7 oncogenes of high-risk HPV types [11]. The expression of the E6/E7 oncogenes of HPV16, 18, 31, 33, and 45 have been detected in most cervical

carcinomas [156], and HPV mRNA tests are able to detect continuous transcription of viral E6/E7 oncogenes [81].

The risk of high-grade cervical lesions in women screened by different tests has been evaluated in many studies. However, when it comes to HPV, most studies have looked at DNA-based screening; few have analyzed the long-term risk of high-grade lesions in women screened by HPV mRNA. Paper II was an effort to fill this knowledge gap, and represented an update of research published in 2016 [157]. In addition to the extended length of follow-up from 72 to 120 months, the selection criteria in the updated study excluded women with undetermined cytology results at screening, thus the study sample was smaller than that in the 2016 study. **Table 5** compares some recent longitudinal studies on women screened by HPV testing with Paper II.

Both the HPV DNA and HPV mRNA tests showed high sensitivity for CIN3+; however, the HPV mRNA test had higher specificity [158]. A highly specific screening test will produce fewer false-positive results and thus will result in fewer unnecessary referrals to follow-up. All of these factors can improve the efficacy of screening programs and help avoid overmanagement.

We obtained 3.2% positivity by HPV mRNA at screening in Paper II. This positivity was slightly lower than that reported in a Dutch study that used a 5-type HPV DNA test (3.8%) [159] (**Table 5**), and was much lower than what was reported in two studies from the United States: the Kaiser Permanente study, which used HPV DNA (HC2, positivity rate 5.1%) [160], and another study that used a 14-type HPV DNA test (Cobas/Amplicor/Linear array, positivity rate 8.3%) [161] (**Table 5**). The overall detection rate of HPV DNA in a European meta-analysis was 9.4% [162]. The HPV DNA positivity rates were 9.4% in 46 680 women in Italy, 4.9% in 21 996 women in the Netherlands, 9.5% in 6238 women in Sweden, and 15.7% in 18 386 women in England [162].

Table 5. *A comparison of recent studies on women screened by HPV testing and the risk of high-grade cervical lesions during long-term follow-up.*

Country and Year of Publication, Study Population (N)	Screening Cytology Inclusions	Screening Test	Positivity Rate at Screening (%)	Follow-up Duration (Months)	Cumulative Incidence of CIN3+ in women who were HPV-Positive at Screening (%)	Cumulative Incidence of CIN3+ in women who were HPV-Negative at Screening (%)
Norway 2023, (N=9582) [163]	Normal	5-type HPV mRNA (PreTect)	3.2	120	20.8	1.1
Norway 2021, (N=642) [164]	Genital/cervical/vulvar symptoms	6-type HPV DNA (ONCOR) (MY09/MY11 PCR)	34.7	360	46.6	10.5
Netherlands 2017, (N=19 286) [159]	Normal	5-type HPV DNA (GP5+/6+ PCR)	3.8	60	3.5	0.2
Norway 2016, (N=11 220) [157]	Normal Undetermined	5-type HPV mRNA (PreTect)	3.6	72	19.7	0.62
The United States 2015, (N=38 284) [161]	Normal	14-type HPV DNA (Cobas, Amplicor, Linear array)	8.3	36	6.1	0.3
The United States 2011, (N=331 818) [160]	Normal	HPV DNA (HC2)	5.1	60	5.9	0.16

The blue highlighted row represents the results of Paper II. The green highlighted row represents the results of our previous research in 2016. NA: not applicable/not provided.

In Paper II, the cumulative incidence of CIN3+ in HPV mRNA-positive women was much higher than that reported in any other study, and that difference increased with increasing follow-up time. The cumulative incidence of CIN3+ in Paper II was 20.8%, 15.8%, and 10.9% within 10, 5, and 3 years of HPV mRNA testing. The 5-year cumulative incidence of CIN3+ in Paper II was more than four times higher than the rate (3.5%) observed in a Dutch study [159] and more than two times higher than the rate (5.9%) in an American study [160].

One issue to consider is that HPV negativity may be less reassuring when it comes from a 5-type test than from a test that includes more high-risk HPV types. However, the considerably low long-term cumulative incidence of CIN3+ after a negative 5-type HPV mRNA test at screening may provide more reassurance and trust. The 10-year cumulative incidence of CIN3+ in HPV mRNA-negative women in our study was 1.1%, which is considered a very low risk. This may also imply that frequent screening of HPV mRNA-negative women might not be necessary.

Another important issue is choosing which HPV types should be included in an HPV test. The strong association between persistent infection with the five HPV types included in the HPV mRNA test (HPV16, 18, 31, 33, and 45) and the risk of ICC is well-known [20, 33, 165-171], as they are attributed to 84.3% of ICC [172]. A Swedish study showed that six high-risk HPV types (HPV16, 18, 31, 33, 45, or 52) were detected in 85.3% of ICCs [173]. The inclusion of HPV types 35, 39, 51, 56, 58, 59, 66, or 68 to the 6-type HPV test would only increase HPV prevalence by 1.5% [173]. Therefore, restricting screening to the HPV types in the 6-type test could considerably enhance the specificity of screening programs [173]. Moreover, 60.6% of ICCs were positive for HPV16 infection alone, and 70.8% were positive for HPV16 and/or HPV18 infections [172]. HPV16 and 18 are included in both the Cervarix and Gardasil9 vaccines, though the latter also includes HPV6, 11, 16, 18, 31, 33, 45, 52, and 58. Therefore, women vaccinated with Gardasil9 should be screened by tests limited to the seven high-risk HPV types (16, 18, 31, 33, 45, 52, and 58). Indeed, screening for more HPV types can create a suboptimal balance of harms and benefits [174]. The results from Paper II suggest that the five HPV types included in the HPV mRNA test might be sufficient for cervical cancer screening.

Before applying HPV testing in the screening of young women, the prevalences of HPV and high-grade lesions in the target population should be considered. Applying an HPV test with a high detection rate in a population with a high prevalence of transient HPV infections can result in overdiagnosis and overmanagement. A global meta-analysis reported an HPV DNA

positivity as high as 13.9% in women aged 25-34 years [175]. Although cytology is neither highly sensitive nor highly specific, the high HPV DNA positivity rate in young women pushed the healthcare system in Norway to continue with cytology-based screening of women aged 25-33 years, in order to avoid overreferrals and overmanagement. On the other hand, the HPV mRNA positivity rates for women aged 25-33 and 34-69 years in Paper II were 5.7% and 2.2%, respectively. Although the rate was higher in the younger age group, it was still low by HPV mRNA test standards, and much lower than positivity rates for HPV DNA in this age group. Moreover, although the cumulative incidence of CIN3+ remained higher in the younger age group throughout our study period, there was no difference in incidence between age groups after adjustment for HPV status. In other words, the cumulative incidence of CIN3+ remained similar in both age groups, regardless of HPV mRNA positivity. This implies that the 5-type HPV mRNA test is an appropriate candidate screening test for both age groups.

Another important aspect when implementing HPV testing in screening is the incidence rate of ICC. To eliminate cervical cancer, the WHO aims state that all countries must achieve and maintain a cervical cancer incidence rate below 4 per 100 000 woman-years [176]. The incidence rate of ICC in HPV mRNA-negative women was 2 per 100 000 woman-years, which is in line this initiative.

5.3.3. Paper III

As was mentioned in previous sections, Norway still uses cytology-based cervical cancer to screen women aged 25-33 years [113]. However, starting in 2023, the use of HPV test-based screening was implemented for all women aged 25 years and older [115].

According to the follow-up guidelines in effect from 2005 through 2013, women with normal cytology were returned to the 3-year screening interval. Women with unsatisfactory, ASC-US, or LSIL cytology were triaged in 6-12 months by repeat cytology and HPV testing. Women with normal repeat cytology and positive HPV test at triage were referred to follow-up cytology and HPV testing within 12 months, and women with unsatisfactory, normal, ASC-US, or LSIL repeat cytology and HPV negative results at triage were returned to 3-year screening. Women with HSIL, or with unsatisfactory, ASC-US, or LSIL repeat cytology and a positive HPV-test at triage, were referred to colposcopy/biopsy examination, applying CIN2+ as threshold for treatment.

The performance of HPV testing at triage in young age groups in Norway is a matter of concern. In a systematic review of 40 studies on screening, the sensitivity of LBC to predict CIN2+ ranged from 52-94% while the sensitivity of HC2 was 61-100% [70]. HC2 had the highest pooled sensitivity to predict CIN2+ (89.9%), followed by LBC (72.9%), and conventional cytology (62.5%) [70]. However, the corresponding estimates for pooled specificity were 89.9%, 90.3%, and 96.6% [70].

Another review evaluated the use of cytology and HPV DNA testing in secondary screening for low-grade cervical lesions, and concluded that the pooled sensitivity of HC2 to detect high-risk HPV types was higher than the sensitivity of repeat cytology to detect CIN2/3+ (relative sensitivity 1.27 and 1.23, respectively) [177]. There was no significant difference in the pooled specificity of these methods to triage women with ASC-US [177]. However, the specificity of HC2 was considerably lower than that of cytology in the triage of women with LSIL (relative specificity 0.66) [177].

Another study randomly assigned women with LSIL to one of three screening groups and followed them for 2 years using 6-month testing intervals. The screening groups were: immediate colposcopy, in which all women were referred to colposcopy; HPV triage, in which women were referred to colposcopy only if they were HPV DNA-positive or had HSIL at enrollment; and conservative management, in which women were referred to repeat cytology and those with HSIL were referred to colposcopy [178]. Sensitivity for cumulative diagnosis of CIN3+ was 65.9%, 55.9%, and 48.4%, respectively for the HPV triage, immediate colposcopy, and conservative management groups [178]. Although the 2-year cumulative diagnosis of CIN3+ in all arms was equal (15%), the timing of CIN3 diagnosis was significantly different [178]: a much more rapid diagnosis was observed in the HPV triage and immediate colposcopy groups than the conservative management group [178].

In a comparison of the Roche Cobas 4800 DNA test (14 HPV types) and the PreTect HPV-Proofer mRNA test (5 HPV types) in the triage of women with ASC-US/LSIL, both tests were 100% sensitive among CIN3+ cases [158]. Among CIN2+ cases, the sensitivity of the HPV DNA test was higher than that of the HPV mRNA test (100% vs. 79%), but the specificity of HPV mRNA test was higher (91% vs. 84%) [158]. This higher specificity of the HPV mRNA test in the triage of women with ASC-US/LSIL was also shown in another study [179].

However, an evaluation and comparison of the properties of HPV testing in the triage of low-grade cytology is also needed, and Paper III aimed to fill this knowledge gap. Several studies

have compared triage tests, but the settings and outcomes were not specifically aligned with the Norwegian screening algorithm. In Paper III, adherence to follow-up was shown at screening, and the triage outcomes were analyzed and shown within 42 months and 78 months post-screening.

The HPV DNA positivity rate at triage that we observed in Paper III was twice that of HPV mRNA (52.8% vs. 23.3%), but similar to that reported in other studies [180-183]. The global average HPV DNA positivity rate (59.4%) at triage among women with minor cytological abnormalities at screening visit was reported to be slightly higher [184] (**Table 6**) than what we found in Paper III. The positivity rate of a 13-type HPV DNA test at triage in women aged 25-33 years with low-grade cytology was even higher (65%) in a previous Norwegian study [185] (**Table 6**). The huge difference between the positivity rates of HPV DNA and HPV mRNA testing at triage among women with minor cytological abnormalities at screening was also shown in other Norwegian studies [158, 179, 186] (**Table 6**). In comparisons of triage HPV DNA positivity rates, we should consider that this rate is usually higher in women with LSIL than ASC-US at screening. In a global meta-analysis, the HPV DNA positivity rate at triage was 42.8% for women with ASC-US and 75.9% for women with LSIL at screening [184] (**Table 6**).

The higher positivity rate of the HPV DNA test, both at screening and triage, yields higher referral rates to colposcopy/biopsy [179]. In opportunistic screening programs, the specificity of a screening test is more important than its sensitivity, as the former can affect referral rates to colposcopy/biopsy, and consequently, attendance rates [179]. Moreover, the HPV DNA test resulted in a referral rate to colposcopy/biopsy that was more than twice that of the HPV mRNA test [179]. In the triage of women with ASC-US/LSIL at repeat cytology, the HPV mRNA test was more specific and relevant in clinical applications than the HPV DNA test [179]. In order to achieve a balance in the trade-offs related to sensitivity and specificity, the HPV mRNA test was suggested for use in screening [157].

In Paper III, more HPV DNA-triaged women were referred to direct colposcopy/biopsy (24.9% vs. 18.3%) and to repeat cytology/HPV testing (27.9% vs. 5.1%; $p<0.001$) than mRNA-triaged women, which was in accordance with other studies [158, 179, 187-189] (**Table 6**), including a model-based economic evaluation on the triage of young women with minor cytological abnormalities [190]. In a Danish study of women younger than 30 years of age with ASC-US/LSIL cytology at screening, the referral to biopsy rate was 67% for women triaged with an HPV DNA test (any assay) and 58% when triage was done with a 5-type HPV

mRNA test [145]. This suggests that applying HPV DNA tests in triage may double the workload of gynecologists and laboratories, and increased health care costs, overtreatment, the negative impact of cervical treatment on pregnancy outcomes, and psychological stress for women.

The number of HPV types included in a test may also affect positivity, referral, and detection rates. In Paper III, the five-fold higher referral rate to repeat cytology/HPV testing we observed in DNA-tested women compared to mRNA-tested women could be due to the inclusion of more oncogenic HPV types in the DNA test. Conformingly, two Danish studies showed that the biopsy rates of women with minor cytological abnormalities were higher in those triaged by a 14-type HPV mRNA test compared to those triaged by a 5-type HPV mRNA test [145, 191].

Table 6. *A comparison of Paper III and other studies that used HPV DNA and/or HPV mRNA tests in the triage of women with minor cytological abnormalities at screening [70, 158, 177, 183-201].*

Country and year of publication	Age (years)	Screening with HPV	Screening with Cytology	Triage Interval (Months)	Triage Cytology	Triage DNA	Triage mRNA	Max. Follow-up Time (Months)	Selected and Abstracted Results
2023 Norway [202]	25-33	NA	ASC-US LSIL	3-18	Repeat Adjacent	N=2556 13-type HC2 (study group A)	N=1559 5-type Proofer (study group B)	78	DNA vs. mRNA: Positivity rate: 52.8% vs. 23.3% SAR referral at triage: colposcopy/biopsy 24.9% vs. 18.3% repeat cytology/HPV test 27.9% vs. 5.1% return to screening 47.2% vs. 76.7% CIN3+ within 42 months post-screening, among SAR referrals to: colposcopy/biopsy 31.7% vs. 30.5% repeat cytology/HPV test 18.5% vs. 25.3% return to screening 0.9% vs. 1.8% CIN3+ 78 months post-screening, among SAR referrals to: colposcopy/biopsy 33.4% vs. 32.3% repeat cytology/HPV test 19.9% vs. 27.8% return to screening 1.4% vs. 2.8% Overall CIN3+: 42 months post-screening: 13.1% vs. 8.3% 78 months post-screening: 14.6% vs. 9.4%
Denmark 2021 [145]	<30	NA	ASC-US (N= 19 946) LSIL (N= 19 825)		NA	Any assay	5-type & 14-type	24	DNA vs. 14-type mRNA vs. 5-type mRNA Positivity Rate: Total: 82.5%, 73.5%, 40% Biopsy rate: Total: 67% vs. 77% vs. 58%
Denmark 2019 [191]	23-65	NA	LSIL	6	NA	NA	N=2176 5-type Proofer	36	Aptima vs. Proofer/ 36 months, Positivity Rate: Total: 66.7% vs. 42.8% 23-39: 53.1% vs. 34.7%

Country and year of publication	Age (years)	Screening with HPV	Screening with Cytology	Triage Interval (Months)	Triage Cytology	Triage DNA	Triage mRNA	Max. Follow-up Time (Months)	Selected and Abstracted Results
							N=426 14-type Aptima		Sens. for CIN2+, 94% vs. 77%, spec. 34% vs. 69%. Sens. for CIN2+/ 23-29 yrs., 93% vs. 80%, spec. 19% vs. 64%. Sens. for CIN2+/ 30-39 yrs., 93% vs. 77%, spec. 10% vs. 71%. PPV for CIN2+., 37.6% vs. 54.3% PPV for CIN2+/ 23-29 yrs., 42.3% vs. 55.6% PPV for CIN2+/ 30-39 yrs., 37.6% vs. 58.3% NPV for CIN2+., 5.9% vs. 13.1% NPV for CIN2+/ 23-29 yrs., 13.1% vs. 15.2% NPV for CIN2+/ 30-39 yrs., 7.7% vs. 14.1%
Norway 2018 [185]	23-69	NA	ASC-US LSIL	6-12	Repeat Adjacent	N=6058 13-type HC2	NA	36	DNA: Positivity Rate: Total: 45% 25-33: 65% 34-69: 38% 3-years cumulative CIN3+: Total: 18% 25-33: 26% 34-69: 15%
Norway 2017 [190]	25-33	NA	ASC-US LSIL	0 (reflex) - 12	Dual staining Adjacent (DNA co-test) cytology	N=10 000* Reflux/co-testing with delayed cytology without types Reflux type1 6/18 Reflux 5 types	N=10 000* Reflex 5 types Reflex 14 types	36	Strategies involving HPV mRNA testing required fewer resources, whereas HPV DNA-based strategies detected >50% more precancers, but were more costly and required twice as many colposcopy referrals compared with the current guidelines

Country and year of publication	Age (years)	Screening with HPV	Screening with Cytology	Triage Interval (Months)	Triage Cytology	Triage DNA	Triage mRNA	Max. Follow-up Time (Months)	Selected and Abstracted Results
						Reflux 14 types			
Norway 2016 [158]	25-69	NA	ASC-US LSIL	3-18	Repeat Adjacent	N=564 14-type Cobas	N=564 5-type Proofer	33	DNA vs. mRNA: Positivity Rate: 30.3% vs. 18.6% Referral status at triage: Return to screening 71 % vs. 81 % to colposcopy/biopsy 29 % vs. 19 % Sens. for CIN2+, 100% vs. 79% Spec. for CIN2+ in solved cases, 84% vs. 91% PPV for CIN2+, 34% vs. 39%
Denmark 2016 [187]	30-65	NA	ASC-US	< 3	NA	N=9405 HC2 N=1533 Linear Array (LA) HPV-Genotyping	N=3226 5-type Proofer	114	LA vs. HC2 vs. Proofer Average positivity rate of ASC-US/LSIL of all tests age 25-34: 54.9% After 5 years follow-up, LA vs. HC2 vs. Proofer/ for CIN2+: Sens. 88.7%. vs. 83.5% vs. 37.5% Spec. 66.1% vs. 63.4% vs. 91.0% PPV 40.4% vs. 35.0% vs. 57.2% NPV 97.4% vs. 97.6% vs. 88.8%
Norway 2015 [192] 1999-2001 (N=75852) 2004-08 (N=66616)	25-69	NA	Unsatisfactory ASC-US LSIL	1-18	Repeat only vs. Repeat Adjacent	HC2/Amplicor	Proofer	36	Period 1 (repeat cytology only) vs. period 2 (repeat only) vs. period 2 (repeat cytology & adjacent HPV test): Status at triage: In screening 62.7% vs. 54.5% vs. 65.7% Diagnostic referral 5.2% vs. 3.6% vs. 23.7%
Norway 2014 [179]	25-69	NA	ASC-US LSIL	3-18	Repeat Adjacent	N=311 14-type Cobas	N=311 5-type Proofer	57	DNA vs. mRNA: Positivity Rate: 36.7% vs. 18.3% Referral status at triage: Colposcopy/biopsy: 23.8% vs. 10.0% Cytology: 32.0% vs. 40.2%

Country and year of publication	Age (years)	Screening with HPV	Screening with Cytology	Triage Interval (Months)	Triage Cytology	Triage DNA	Triage mRNA	Max. Follow-up Time (Months)	Selected and Abstracted Results
									Return to screening 44.1% vs. 49.8% Sens. 100% (reference) vs. 64.3% Spec. 70.8% vs. 89.5% PPV for CIN2+ 21.5% vs. 34.6% Odds ratio for referral to colposcopy 2.8 times higher in DNA than mRNA. Equal CIN3+ detection rate! (One case more in DNA)
Norway 2014 [194]	25-69	NA	Unsatisfactory ASC-US LSIL	3-15	Repeat Adjacent	N=4715 13-type AMPLICOR N=9162 13-type HC2	N=5188 5-type Proofer	36	Amplicor vs. HC2 vs. Proofer Positivity Rate: 57.3% vs. 69.9% vs. 32.6%. Referral: Cytology/HPV f/u for HPV positives in 3 yrs.: 95% vs. 95% vs. 95% Cytology/HPV f/u for HPV negatives in 3 yrs.: 64% vs. 64% vs. 88% Biopsy f/u for HPV negatives in 3 yrs.: 12% vs. 12% vs. 19% 3-year risk for CIN2+: HPV-positive: 48.1% vs. 43% vs. 48.2% HPV-negative: 2.1% vs. 4% vs. 7.2%
Norway 2013 [195]	15-69	NA	ASC-US	6-12	N=964 Repeat only vs. N=542 Repeat Adjacent	NA	N=542 5-type Proofer	40	Repeat only vs. repeat cytology and mRNA: Referral status at triage: Colposcopy/Biopsy 3.4% vs. 3.5% In screening 83.2% vs. 91.5% PPV: 85.7% vs. 79.2%
Global Meta-analysis 2013 [188]	NA	NA	ASC-US LSIL	NA	NA	13-type HC2	5-type Proofer	NA	HC2 vs. Proofer: For outcome CIN3+: among ASC-US: Pooled Sens. 95.7% vs. 86.1% Pooled Spec. 35.1% vs. 79.9% among LSIL: Pooled Sens. 99.1% vs. 81%

Country and year of publication	Age (years)	Screening with HPV	Screening with Cytology	Triage Interval (Months)	Triage Cytology	Triage DNA	Triage mRNA	Max. Follow-up Time (Months)	Selected and Abstracted Results
									Pooled Spec. 18.8% vs. 71.5%
Global Meta-analysis 2013 [177]	NA	NA	ASC-US LSIL	NA	Repeat Adjacent	HC2	NA	NA	HC2 vs. repeat cytology (ASC-US+): For outcome CIN2+: Overall: among ASC-US: Relative Sens. 1.27 Relative Spec. 0.99 among LSIL: Relative Sens. 1.23 Relative Spec. 0.66 Among <30 years: among ASC-US: Relative Sens. 0.97 Relative Spec. 0.44 among LSIL: Relative Sens. 0.98 Relative Spec. 0.21
Greece 2012 [203]	>35	NA	ASC-US LSIL	0	NA	N=472 Reflex 35-type Clinical Arrays (Not compared)	Reflex: N=472 5-type NASBA N=472 15-type OncoTest Flowcytometry N=472 16-type NASBA N=472 P16 immunostaining (Not compared)	0	Performance for CIN2+, NASBA5 vs. NASBA16 vs. flowcytometry: Among ASC-US: Sens. 55.6% vs. 27.3% vs. 54.6% Spec. 93.7% vs. 98.7% vs. 83.1% PPV, 37.5% vs. 60% vs. 15.8% NPV, 96.7 vs. 95.1% vs. 96.7% Among LSIL: Sens. 61.3% vs. 45.1% vs. 67.7% Spec. 82.5% vs. 92.2% vs. 67.3% PPV, 33.3% vs. 45.1% vs. 22.1% NPV, 93.7% vs. 92.2% vs. 93.8%
Spain 2012 [196]	25-65	NA	ASC-US	3	NA	N=493 13-type HC2	NA	36	Performance of HC2 for CIN2+: Sens. 97.2% Spec. 68.3% PPV 14.3% NPV 99.6%
Norway 2012 [186]	18-83	NA	ASC-US LSIL	6-12	N=625 Repeat	N=625 Amplicor	N=625 Proofer	36	DNA vs. mRNA vs. repeat cytology Positivity rate ASC-US/LSIL: 51.6% vs. 28.2%

Country and year of publication	Age (years)	Screening with HPV	Screening with Cytology	Triage Interval (Months)	Triage Cytology	Triage DNA	Triage mRNA	Max. Follow-up Time (Months)	Selected and Abstracted Results
									(ASC-US+ threshold) for CIN2+: PPV 41.3% vs. 52.8% vs. 41% NPV 98.2% vs. 89.7% vs. 91.2%
Norway 2011 [197]	25-69	NA	LSIL	6	N=225 Repeat only vs. N=297 Repeat Adjacent	NA	N=297 5-type Proofer	24	Repeat cytology only (ASC-US+) vs. repeat cytology only (ASCH+) vs. mRNA only vs. combined repeat cytology (ASC-US+) and mRNA vs. combined repeat cytology (ASCUH+) and mRNA: Sens. 85.7% vs. 33.9% vs. 94.2% vs. NA vs. 98.6% Spec. 54.4 % vs. 97.6% vs. 86.0% vs. 47.4% vs. 83.8% PPV 38.4% vs. 82.6% vs. 67.0% vs. 36.5% vs. 64.8% NPV 92% vs. 81.7% vs. 98% vs. NA vs. 99.5%
England 2011 [198]	25-64	NA	ASC-US LSIL	6	NA	N=10051 HC2	NA	15	PPV for CIN2+ in ASC-US/LSIL: Among 25-34: 18% Among 35-49: 14.5% Among 50-64: 6.7% Total:16.3%
Italy 2011 [189]	18-83	13-type HC2 29-type MX Bio PCR	ASC-US LSIL (and HPV DNA+)	3	NA	N=912 13-type HC2 and/or 29-type MX Bio PCR	N=912 5-type Proofer	12	Status at triage: 57.3% had colposcopy follow-up DNA vs. mRNA in ASC-US/LSIL for CIN2+: Sens. 93% vs. 67% Spec. 18% vs. 45% PPV 20% vs. 80% NPV 97% vs. 31%
Norway 2010 [199]	25-69	NA	ASC-US LSIL	6	N=1798 Repeat	NA	N=1798 5-type Proofer	36	mRNA for CIN2+ among ASC-US/LSIL: Sens. 81% Spec. 91% PPV 57.5% NPV 97%
Global Meta-analysis2009 [184]	NA	NA	ASC-US LSIL	NA	NA	N=26311 HC2	NA	NA	DNA positivity Overall: among ASC-US: 42.8% among LSIL: 75.9% except in Italy/ In women <30 years among LSIL: > 80% among ASC-US: > 48%
	25-60	NA	ASC-US/AGUS	NA	NA	N=1242	NA	NA	DNA Sensitivity vs. DNA Specificity for CIN2+

Country and year of publication	Age (years)	Screening with HPV	Screening with Cytology	Triage Interval (Months)	Triage Cytology	Triage DNA	Triage mRNA	Max. Follow-up Time (Months)	Selected and Abstracted Results
Italy2007 [183]			LSIL			13-type HC2			among 25-34 years 1 RLU: 96% vs. 44.5% 2 RLU: 96% vs. 47.5% 4 RLU: 91.5% vs. 50% 10 RLU: 87% vs. 53% 20 RLU: 87% vs. 57.5% among 35-60 years 1 RLU: 95% vs. 69% 2 RLU: 95% vs. 74% 4 RLU: 91.5% vs. 76% 10 RLU: 88% vs. 78.5% 20 RLU: 77% vs. 80%
Norway 2005 [200]	>30	NA	ASC-US LSIL	0	NA	N=77 Gp5+/6+ PCR	N=77 5-type Proofer	24	DNA vs. mRNA for CIN2+ among ASC-US/LSIL: Sens. 85.7% vs. 85.7% Spec. 50% vs. 84.9% PPV 15.4% vs. 37.5% NPV 97.1% vs. 98.3% OR 5.7% vs. 69.8%
The United States 2002 [201]	>18	NA	ASC-US LSIL	NA	N=3046 Repeat	N=3046 13-type HC2	NA	NA	HC2 (1 pg./mL) vs. Repeat (ASC-US+): among all ages: Referred to colposcopy at triage: 69.4% vs. 69.8% CIN3+ Sens, 96.4% vs. 88.1% Among <29 years: Referred to colposcopy at triage: 77.7% vs. 72.9% CIN3+ Sens, 97.9% vs. 87% Among >=29 years: Referred to colposcopy at triage: 53% vs. 63.9% Sens. for CIN3+, 88.6% vs. 91.3%

*The blue highlighted row represents results from Paper III. *The study was a simulation. Sens.: Sensitivity; Spec.: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; NA: not applicable/not provided.*

An important issue in screening is adherence to follow-up, which influences the effectiveness of a program to detect precancerous cervical lesions [204]. In Paper III, 99% of women with SAR referral to colposcopy/biopsy, and 95-96% of women with SAR referral to repeat cytology/HPV testing, attended their follow-up visits (**Table 7**). We consider this to be high adherence especially compared to low- and middle-income countries [204, 205], where reported adherence was half that in our study. Of women with SAR referral to repeat cytology/HPV testing, more than 75% adhered to the timing of follow-up. Among women returned to routine screening, a considerable proportion of both DNA-tested and mRNA-tested women did not attend screening according to recommendations. Significantly more mRNA-tested than DNA-tested women attended their next screening round earlier than recommended ($p<0.01$) (**Table 7**), and about two-thirds attended the next screening round within the recommended time interval.

Table 7. *Adherence to follow-up guidelines by triage recommendation and study group.*

SAR Referrals	Adherence to follow-up	HPV DNA	HPV mRNA	p	p
Colposcopy/Biopsy		N=637 %	N=285 %		
	Not met	1.1	0.4	0.29	0.001
	Too early (NA)	0	0	0	
	Adherence (1-6 mo.)	67.2	78.9	0.0003	
	Too late	31.7	20.7	0.0006	
	In total	100.0	100.0	-	
Repeat cytology/HPV testing		N=713 %	N=79 %		
	Not met	5.2	3.8	0.59	0.069
	Too early	7.7	15.2	0.023	
	Adherence (4-18 mo.)	76.6	75.9	0.88	
	Too late	10.5	5.1	0.129	
	In total	100.0	100.0	-	
Return to Screening		N=1,206 %	N=1,195 %		
	Not met	25.5	20.2	0.002	<0.001
	Too early	34.2	49.5	<0.00001	
	Adherence (24-42 mo.)	30.9	22.2	<0.00001	
	Too late	9.3	8.2	0.34	
	In total	100.0	100.0	-	

NA: Not applicable.

Table 8 displays screening and follow-up characteristics of the 10 cervical cancer cases diagnosed within 78 months of cytology screening. Eight cases occurred in DNA-tested women, and two were among mRNA-tested women. Among the four HPV-positive cases that were adherent to follow-up guidelines, average time to diagnosis was 14 months (range 7-19 months), relative to 60 months (range 41-73 months) among the three HPV-positive cases that were non-adherent. Three cases were most likely false-negative at triage, among which two cases were diagnosed more than 5 years after screening, while the third HPV-negative case was diagnosed 19 months after screening, probably due to the appearance of symptoms.

Table 8. *Characteristics of cervical cancer cases.*

Screening visit		**Triage visit**			**Follow-up**				
Age	Cytology	Months to triage[1]	Cytology	HPV outcome	Follow-up visits (N)	Adherence to follow-up	Months to diagnosis [1]	Age at cancer diagnosis	Histology
31	ASC-US	7	Normal	DNA pos.	2	Adherent	17	33	SCC
28	ASC-US	7	ASC-US	DNA pos.	1	Adherent	19	30	SCC
33	ASC-US	5	ASC-US	DNA pos.	2	Adherent	13	34	SCC
27	LSIL	3	Normal	DNA pos.	1	Non-adherent	73	33	SCC
33	ASC-US	5	Normal	DNA pos.	3	Non-adherent	41	37	AC.
30	ASC-US	6	LSIL	DNA pos.	4	Non-adherent	66	35	AC
26	ASC-US	5	ASC-US	DNA neg.	8	Adherent	72	32	SCC
27	ASC-US	10	Normal	DNA neg.	1	Non-adherent	19	29	AC
31	LSIL	6	LSIL	mRNA pos.	1	Adherent	7	31	SCC
32	ASC-US	8	Normal	mRNA neg.	2	Non-adherent	62	37	SCC

[1]*Measured from screening visit.*

6. Conclusions

The ability of the HPV mRNA and HPV DNA tests to detect HPV types in cervical cancer specimens was similar. The risk of CIN3+ during our long-term follow-up was low among HPV mRNA-negative women, and high among HPV mRNA-positive women. This adds to and strengthens existing evidence on the appropriateness of using the 5-type HPV mRNA test in the screening test for women of all ages. Although CIN3+ incidence was high in young women, the HPV mRNA detection rate for CIN3+ was low even among younger women. In the triage young women with minor cytological abnormalities, the mRNA test demonstrated similar efficacy as the HPV DNA test in cancer prevention, while requiring significantly less healthcare utilization. Therefore, the HPV mRNA test can be regarded as an appropriate tool in cervical cancer screening, regardless of age, and as an appropriate triage test for minor cytological abnormalities among younger women.

7. Future Prospective

HPV mRNA tests are based on technologies that are newer than those used in HPV DNA tests. In this PhD thesis, I tried to take a closer look at the abilities, performance, advantages, and flaws of an HPV mRNA test in comparison to an HPV DNA test. These results could help improve cervical cancer screening guidelines in Norway and in other countries, although future research might be needed to determine whether the HPV mRNA test is a proper test for screening and triage.

It may be interesting to examine the long-term ability of the HPV DNA test to predict CIN3+ and compare it with that of the HPV mRNA test in cervical cancer screening. This would only be possible for the study sample included in Paper II, as some diagnostic material still remains and could be used for another test. Although there were no guidelines or funding for HPV mRNA testing, PreTect AS (former NorChip AS) paid for HPV mRNA testing as a part of the screening program in Norway. Therefore, testing on the same population with another HPV DNA test is currently impossible. A future study in which women are screened using both HPV DNA and HPV mRNA tests could provide a good comparison of the predictive abilities of these tests.

Another, more important issue is to understand the persistence of HPV infection across different studies. Additional HPV testing after screening can indicate the proportion of HPV infections that persist and that clear in the population. Another interesting future study would be one that is similar to Paper III, but that includes HPV DNA and HPV mRNA testing at screening. This would provide information on the persistence and clearance of HPV infection at triage, and the long-term ability of HPV testing at screening to predict CIN3+.

References

1. Schiffman, M., Castle, P.E., Jeronimo, J., Rodriguez, A.C., and Wacholder, S., *Human papillomavirus and cervical cancer*. Lancet, 2007. **370**(9590): p. 890-907.
2. de Villiers, E.M., Fauquet, C., Broker, T.R., Bernard, H.U., and zur Hausen, H., *Classification of papillomaviruses.* Virology, 2004. **324**(1): p. 17-27.
3. Lizano, M., Berumen, J., and Garcia-Carranca, A., *HPV-related carcinogenesis: basic concepts, viral types and variants.* Arch Med Res, 2009. **40**(6): p. 428-34.
4. Sellors, J.W., Sankaranarayanan, R., *Colposcopy and treatment of cervical intraepithelial neoplasia: a beginners' manual*. 2003: International Agency for Research on Cancer.
5. Joseph A. Jordan and Albert Singer, *The Cervix*. Second ed. 2006: Blackwell.
6. J.C. Felix, T.C. Wright JR., and Amezcua, C.A., *Cervix*, in *Modern Surgical Pathology*. 2009.
7. Kruk, P.A., *Structure and Function of the Female Reproductive System*, in *xPharm: The Comprehensive Pharmacology Reference*. 2007, Elsevier.
8. Wright, T.C., *Pathology of HPV infection at the cytologic and histologic levels: Basis for a 2-tiered morphologic classification system.* International Journal of Gynecology & Obstetrics, 2006. **94**: p. S22-S31.
9. Girardi, F., Reich, O., Tamussino, K., and Pickel, H., *Burghardt's Colposcopy and Cervical Pathology*. 4th ed. 2015: Thieme.
10. Girardi, F.R., O., Tamussino, K., and Pickel, H., *Burghardt's Colposcopy and Cervical Pathology*. 4th ed. 2015: Thieme.
11. Woodman, C.B., Collins, S.I., and Young, L.S., *The natural history of cervical HPV infection: unresolved issues.* Nat Rev Cancer, 2007. **7**(1): p. 11-22.
12. Doeberitz, M. and Vinokurova, S., *Host factors in HPV-related carcinogenesis: cellular mechanisms controlling HPV infections.* Arch Med Res, 2009. **40**(6): p. 435-42.
13. Cattani, P., Siddu, A., D'Onghia, S., Marchetti, S., Santangelo, R., Vellone, V.G., Zannoni, G.F., and Fadda, G., *RNA (E6 and E7) assays versus DNA (E6 and E7) assays for risk evaluation for women infected with human papillomavirus.* J Clin Microbiol, 2009. **47**(7): p. 2136-41.
14. Cuschieri, K.S., Whitley, M.J., and Cubie, H.A., *Human papillomavirus type specific DNA and RNA persistence--implications for cervical disease progression and monitoring.* J Med Virol, 2004. **73**(1): p. 65-70.
15. Cuschieri, K.S., Cubie, H.A., Whitley, M.W., Gilkison, G., Arends, M.J., Graham, C., and McGoogan, E., *Persistent high risk HPV infection associated with development of cervical neoplasia in a prospective population study.* Journal of Clinical Pathology, 2005. **58**(9): p. 946-50.
16. Koshiol, J., Lindsay, L., Pimenta, J.M., Poole, C., Jenkins, D., and Smith, J.S., *Persistent human papillomavirus infection and cervical neoplasia: A systematic review and meta-analysis.* American Journal of Epidemiology, 2008. **168**(2): p. 123-137.

17. Ho, G.Y., Bierman, R., Beardsley, L., Chang, C.J., and Burk, R.D., *Natural history of cervicovaginal papillomavirus infection in young women.* N Engl J Med, 1998. **338**(7): p. 423-8.

18. Woodman, C.B., Collins, S., Winter, H., Bailey, A., Ellis, J., Prior, P., Yates, M., Rollason, T.P., and Young, L.S., *Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study.* Lancet, 2001. **357**(9271): p. 1831-6.

19. Kim, S., Arduino, J.M., Roberts, C.C., Marsico, M., Liaw, K.L., and Skjeldestad, F.E., *Incidence and predictors of human papillomavirus-6, -11, -16, and -18 infection in young norwegian women.* Sex Transm Dis, 2011. **38**(7): p. 587-97.

20. Kjaer, S.K., Frederiksen, K., Munk, C., and Iftner, T., *Long-term Absolute Risk of Cervical Intraepithelial Neoplasia Grade 3 or Worse Following Human Papillomavirus Infection: Role of Persistence.* Journal of the National Cancer Institute, 2010. **102**(19): p. 1478-1488.

21. Chan, J.K., Monk, B.J., Brewer, C., Keefe, K.A., Osann, K., McMeekin, S., Rose, G.S., Youssef, M., Wilczynski, S.P., Meyskens, F.L., and Berman, M.L., *HPV infection and number of lifetime sexual partners are strong predictors for 'natural' regression of CIN 2 and 3.* Br J Cancer, 2003. **89**(6): p. 1062-6.

22. Koutsky, L.A., Holmes, K.K., Critchlow, C.W., Stevens, C.E., Paavonen, J., Beckmann, A.M., DeRouen, T.A., Galloway, D.A., Vernon, D., and Kiviat, N.B., *A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection.* N Engl J Med, 1992. **327**(18): p. 1272-8.

23. Ho, G.Y., Burk, R.D., Klein, S., Kadish, A.S., Chang, C.J., Palan, P., Basu, J., Tachezy, R., Lewis, R., and Romney, S., *Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia.* J Natl Cancer Inst, 1995. **87**(18): p. 1365-71.

24. Giuliano, A.R., Papenfuss, M., Nour, M., Canfield, L.M., Schneider, A., and Hatch, K., *Antioxidant nutrients: associations with persistent human papillomavirus infection.* Cancer Epidemiol Biomarkers Prev, 1997. **6**(11): p. 917-23.

25. Nobbenhuis, M.A., Walboomers, J.M., Helmerhorst, T.J., Rozendaal, L., Remmink, A.J., Risse, E.K., van der Linden, H.C., Voorhorst, F.J., Kenemans, P., and Meijer, C.J., *Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study.* Lancet, 1999. **354**(9172): p. 20-5.

26. Hopman, E.H., Rozendaal, L., Voorhorst, F.J., Walboomers, J.M., Kenemans, P., and Helmerhorst, T.J., *High risk human papillomavirus in women with normal cervical cytology prior to the development of abnormal cytology and colposcopy.* BJOG, 2000. **107**(5): p. 600-4.

27. Ahdieh, L., Klein, R.S., Burk, R., Cu-Uvin, S., Schuman, P., Duerr, A., Safaeian, M., Astemborski, J., Daniel, R., and Shah, K., *Prevalence, incidence, and type-specific persistence of human papillomavirus in human immunodeficiency virus (HIV)-positive and HIV-negative women.* J Infect Dis, 2001. **184**(6): p. 682-90.

28. Kjaer, S.K., van den Brule, A.J., Paull, G., Svare, E.I., Sherman, M.E., Thomsen, B.L., Suntum, M., Bock, J.E., Poll, P.A., and Meijer, C.J., *Type specific persistence of high risk human papillomavirus (HPV) as indicator of high grade cervical squamous intraepithelial lesions in young women: population based prospective follow up study.* BMJ, 2002. **325**(7364): p. 572.

29. Giuliano, A.R., Siegel, E.M., Roe, D.J., Ferreira, S., Baggio, M.L., Galan, L., Duarte-Franco, E., Villa, L.L., Rohan, T.E., Marshall, J.R., Franco, E.L., and Ludwig-McGill, H.P.V.N.H.S., *Dietary intake and risk of persistent human papillomavirus (HPV) infection: the Ludwig-McGill HPV Natural History Study.* J Infect Dis, 2003. **188**(10): p. 1508-16.

30. Piyathilake, C.J., Henao, O.L., Macaluso, M., Cornwell, P.E., Meleth, S., Heimburger, D.C., and Partridge, E.E., *Folate is associated with the natural history of high-risk human papillomaviruses.* Cancer Research, 2004. **64**(23): p. 8788-93.

31. Lillo, F.B., Lodini, S., Ferrari, D., Stayton, C., Taccagni, G., Galli, L., Lazzarin, A., and Uberti-Foppa, C., *Determination of human papillomavirus (HPV) load and type in high-grade cervical lesions surgically resected from HIV-infected women during follow-up of HPV infection.* Clin Infect Dis, 2005. **40**(3): p. 451-7.

32. Elfgren, K., Rylander, E., Radberg, T., Strander, B., Strand, A., Paajanen, K., Sjoberg, I., Ryd, W., Silins, I., Dillner, J., and Swedescreen Study, G., *Colposcopic and histopathologic evaluation of women participating in population-based screening for human papillomavirus deoxyribonucleic acid persistence.* Am J Obstet Gynecol, 2005. **193**(3 Pt 1): p. 650-7.

33. Castle, P.E., Rodriguez, A.C., Burk, R.D., Herrero, R., Wacholder, S., Alfaro, M., Morales, J., Guillen, D., Sherman, M.E., Solomon, D., Schiffman, M., and Grp, P., *Short term persistence of human papillomavirus and risk of cervical precancer and cancer: population based cohort study.* British Medical Journal, 2009. **339**.

34. Schlecht, N.F., Platt, R.W., Duarte-Franco, E., Costa, M.C., Sobrinho, J.P., Prado, J.C., Ferenczy, A., Rohan, T.E., Villa, L.L., and Franco, E.L., *Human papillomavirus infection and time to progression and regression of cervical intraepithelial neoplasia.* J Natl Cancer Inst, 2003. **95**(17): p. 1336-43.

35. Castle, P.E., Schiffman, M., Wheeler, C.M., and Solomon, D., *Evidence for frequent regression of cervical intraepithelial neoplasia-grade 2.* Obstet Gynecol, 2009. **113**(1): p. 18-25.

36. Ostor, A.G., *Natural history of cervical intraepithelial neoplasia: a critical review.* Int J Gynecol Pathol, 1993. **12**(2): p. 186-92.

37. Hatch, K.D., *A3. Vaginal intraepithelial neoplasia (VAIN).* Int J Gynaecol Obstet, 2006. **94 Suppl 1**: p. S40-S43.

38. Bansal, A., Singh, M.P., and Rai, B., *Human papillomavirus-associated cancers: A growing global problem.* Int J Appl Basic Med Res, 2016. **6**(2): p. 84-9.

39. Hatch, K.D., *A2. Vulval intraepithelial neoplasia (VIN).* Int J Gynaecol Obstet, 2006. **94 Suppl 1**: p. S36-S39.

40. D'Souza, G. and Dempsey, A., *The role of HPV in head and neck cancer and review of the HPV vaccine.* Prev Med, 2011. **53 Suppl 1**: p. S5-S11.

41. Kreimer, A.R., Clifford, G.M., Boyle, P., and Franceschi, S., *Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(2): p. 467-75.

42. Salati, S.A. and Al Kadi, A., *Anal cancer - a review.* Int J Health Sci (Qassim), 2012. **6**(2): p. 206-30.

43. Daling, J.R., Madeleine, M.M., Johnson, L.G., Schwartz, S.M., Shera, K.A., Wurscher, M.A., Carter, J.J., Porter, P.L., Galloway, D.A., and McDougall, J.K., *Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer.* Cancer, 2004. **101**(2): p. 270-80.

44. Kidd, L.C., Chaing, S., Chipollini, J., Giuliano, A.R., Spiess, P.E., and Sharma, P., *Relationship between human papillomavirus and penile cancer-implications for prevention and treatment.* Transl Androl Urol, 2017. **6**(5): p. 791-802.

45. Bleeker, M.C., Heideman, D.A., Snijders, P.J., Horenblas, S., Dillner, J., and Meijer, C.J., *Penile cancer: epidemiology, pathogenesis and prevention.* World J Urol, 2009. **27**(2): p. 141-50.

46. Yang, L., Xie, S., Feng, X., Chen, Y., Zheng, T., Dai, M., Zhou, C.K., Hu, Z., Li, N., and Hang, D., *Worldwide Prevalence of Human Papillomavirus and Relative Risk of Prostate Cancer: A Meta-analysis.* Sci Rep, 2015. **5**: p. 14667.

47. Bae, J.M. and Kim, E.H., *Human papillomavirus infection and risk of breast cancer: a meta-analysis of case-control studies.* Infect Agent Cancer, 2016. **11**: p. 14.

48. Sinno, A.K., Saraiya, M., Thompson, T.D., Hernandez, B.Y., Goodman, M.T., Steinau, M., Lynch, C.F., Cozen, W., Saber, M.S., Peters, E.S., Wilkinson, E.J., Copeland, G., Hopenhayn, C., Watson, M., Lyu, C., and Unger, E.R., *Human papillomavirus genotype prevalence in invasive vaginal cancer from a registry-based population.* Obstet Gynecol, 2014. **123**(4): p. 817-21.

49. Insinga, R.P., Liaw, K.L., Johnson, L.G., and Madeleine, M.M., *A systematic review of the prevalence and attribution of human papillomavirus types among cervical, vaginal, and vulvar precancers and cancers in the United States.* Cancer Epidemiol Biomarkers Prev, 2008. **17**(7): p. 1611-22.

50. De Vuyst, H., Clifford, G.M., Nascimento, M.C., Madeleine, M.M., and Franceschi, S., *Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis.* Int J Cancer, 2009. **124**(7): p. 1626-36.

51. Smith, J.S., Backes, D.M., Hoots, B.E., Kurman, R.J., and Pimenta, J.M., *Human papillomavirus type-distribution in vulvar and vaginal cancers and their associated precursors.* Obstet Gynecol, 2009. **113**(4): p. 917-24.

52. McKaig, R.G., Baric, R.S., and Olshan, A.F., *Human papillomavirus and head and neck cancer: epidemiology and molecular biology.* Head Neck, 1998. **20**(3): p. 250-65.

53. Miralles-Guri, C., Bruni, L., Cubilla, A.L., Castellsague, X., Bosch, F.X., and de Sanjose, S., *Human papillomavirus prevalence and type distribution in penile carcinoma.* Journal of Clinical Pathology, 2009. **62**(10): p. 870-8.

54. Simoes, P.W., Medeiros, L.R., Simoes Pires, P.D., Edelweiss, M.I., Rosa, D.D., Silva, F.R., Silva, B.R., and Rosa, M.I., *Prevalence of human papillomavirus in breast cancer: a systematic review.* International Journal of Gynecological Cancer, 2012. **22**(3): p. 343-7.

55. The Global Cancer Observatory. *Cervix uteri-Source: Globocan 2020*. 2021; Available from: http://gco.iarc.fr/today/data/factsheets/cancers/23-Cervix-uteri-fact-sheet.pdf.

56. The Global Cancer Observatory. *World-Source:Globocan 2020*. 2021; Available from: http://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf.

57. International Agency for Research on Cancer. *Estimated number of incident cases and deaths World, females, ages 0-74 (excl. NMSC)*. 2023; Available from: https://gco.iarc.fr/today/online-analysis-multi-bars?v=2020&mode=cancer&mode_population=countries&population=900&populations=900&key=total&sex=2&cancer=39&type=0&statistic=1&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=14&nb_items=10&group_cancer=1&include_nmsc=0&include_nmsc_other=1&type_multiple=%257B%2522inc%2522%253Atrue%252C%2522mort%2522%253Atrue%252C%2522prev%2522%253Afalse%257D&orientation=horizontal&type_sort=0&type_nb_items=%257B%2522top%2522%253Atrue%252C%2522bottom%2522%253Afalse%257D.

58. Cancer Registry of Norway, *Cancer in Norway 2017-Cancer incidence, mortality, survival and prevalence in Norway*. 2017.

59. Jakobsen, E. *Vil halvere forekomsten av livmorhalskreft i Norge*. 2019; Available from: https://www.kreftregisteret.no/Generelt/Nyheter/2019/vil-halvere-forekomsten-av-livmorhalskreft/.

60. World Health Organization (WHO), *Comprehensive Cervical Cancer Control: A guide to essential practice*, in *Comprehensive Cervical Cancer Control: A Guide to Essential Practice*. 2006: Geneva.

61. Word Health Organization, *Comprehensive Cervical Cancer Control: A guide to essential practice*. 2014: Geneva.

62. Hartmann, K.E., Hall, S.A., Nanda, K., Boggess, J.F., and Zolnoun, D., in *Screening for Cervical Cancer*. 2002: Rockville (MD).

63. Florkowski, C.M., *Sensitivity, specificity, receiver-operating characteristic (ROC) curves and likelihood ratios: communicating the performance of diagnostic tests.* Clin Biochem Rev, 2008. **29 Suppl 1**: p. S83-7.

64. Tan, S.Y. and Tatsumura, Y., *George Papanicolaou (1883-1962): Discoverer of the Pap smear.* Singapore Med J, 2015. **56**(10): p. 586-7.

65. Papanicolaou, G.N., *Diagnostic value of exfoliated cells from cancerous tissues.* J Am Med Assoc, 1946. **131**: p. 372-8.

66. Naib, Z.M., *Pap Test*, in *Clinical Methods: The History, Physical, and Laboratory Examinations*, rd, et al., Editors. 1990: Boston.

67. Nayar, R. and Wilbur, D.C., *The Pap Test and Bethesda 2014. "The reports of my demise have been greatly exaggerated." (after a quotation from Mark Twain).* Acta Cytol, 2015. **59**(2): p. 121-32.

68. Singh, V.B., Gupta, N., Nijhawan, R., Srinivasan, R., Suri, V., and Rajwanshi, A., *Liquid-based cytology versus conventional cytology for evaluation of cervical Pap smears: experience from the first 1000 split samples.* Indian J Pathol Microbiol, 2015. **58**(1): p. 17-21.

69. Karimi-Zarchi, M., Peighmbari, F., Karimi, N., Rohi, M., and Chiti, Z., *A Comparison of 3 Ways of Conventional Pap Smear, Liquid-Based Cytology and Colposcopy vs Cervical Biopsy for Early Diagnosis of Premalignant Lesions or Cervical Cancer in Women with Abnormal Conventional Pap Test.* Int J Biomed Sci, 2013. **9**(4): p. 205-10.

70. Koliopoulos, G., Nyaga, V.N., Santesso, N., Bryant, A., Martin-Hirsch, P.P., Mustafa, R.A., Schunemann, H., Paraskevaidis, E., and Arbyn, M., *Cytology versus HPV testing*

for cervical cancer screening in the general population. Cochrane Database Syst Rev, 2017. **8**: p. CD008587.

71. Szarewski, A., Ambroisine, L., Cadman, L., Austin, J., Ho, L., Terry, G., Liddle, S., Dina, R., McCarthy, J., Buckley, H., Bergeron, C., Soutter, P., Lyons, D., and Cuzick, J., *Comparison of predictors for high-grade cervical intraepithelial neoplasia in women with abnormal smears.* Cancer Epidemiol Biomarkers Prev, 2008. **17**(11): p. 3033-42.

72. Ovestad, I.T., Vennestrom, U., Andersen, L., Gudlaugsson, E., Munk, A.C., Malpica, A., Feng, W.W., Voorhorst, F., Janssen, E.A.M., and Baak, J.P.A., *Comparison of different commercial methods for HPV detection in follow-up cytology after ASCUS/LSIL, prediction of CIN2-3 in follow up biopsies and spontaneous regression of CIN2-3.* Gynecologic Oncology, 2011. **123**(2): p. 278-283.

73. Szarewski, A., Mesher, D., Cadman, L., Austin, J., Ashdown-Barr, L., Ho, L., Terry, G., Liddle, S., Young, M., Stoler, M., McCarthy, J., Wright, C., Bergeron, C., Soutter, W.P., Lyons, D., and Cuzick, J., *Comparison of seven tests for high-grade cervical intraepithelial neoplasia in women with abnormal smears: the Predictors 2 study.* J Clin Microbiol, 2012. **50**(6): p. 1867-73.

74. Cuzick, J., Cadman, L., Mesher, D., Austin, J., Ashdown-Barr, L., Ho, L., Terry, G., Liddle, S., Wright, C., Lyons, D., and Szarewski, A., *Comparing the performance of six human papillomavirus tests in a screening population.* Br J Cancer, 2013. **108**(4): p. 908-13.

75. Cuzick, J., Ahmad, A.S., Austin, J., Cadman, L., Ho, L., Terry, G., Kleeman, M., Ashdown-Barr, L., Lyons, D., Stoler, M., and Szarewski, A., *A comparison of different human papillomavirus tests in PreservCyt versus SurePath in a referral population-PREDICTORS 4.* Journal of Clinical Virology, 2016. **82**: p. 145-151.

76. Arbyn, M., Snijders, P.J.F., Meijer, C.J.L.M., Berkhof, J., Cuschieri, K., Kocjan, B.J., and Poljak, M., *Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening?* Clinical Microbiology and Infection, 2015. **21**(9): p. 817-826.

77. Husnjak, K., Grce, M., Magdic, L., and Pavelic, K., *Comparison of five different polymerase chain reaction methods for detection of human papillomavirus in cervical cell specimens.* J Virol Methods, 2000. **88**(2): p. 125-34.

78. Kleter, B., van Doorn, L.J., ter Schegget, J., Schrauwen, L., van Krimpen, K., Burger, M., ter Harmsel, B., and Quint, W., *Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses.* Am J Pathol, 1998. **153**(6): p. 1731-9.

79. Kleter, B., van Doorn, L.J., Schrauwen, L., Molijn, A., Sastrowijoto, S., ter Schegget, J., Lindeman, J., ter Harmsel, B., Burger, M., and Quint, W., *Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus.* J Clin Microbiol, 1999. **37**(8): p. 2508-17.

80. Micalessi, M.I., Boulet, G.A., Vorsters, A., De Wit, K., Jannes, G., Mijs, W., Ieven, M., Van Damme, P., and Bogers, J.J., *A real-time PCR approach based on SPF10 primers and the INNO-LiPA HPV Genotyping Extra assay for the detection and typing of human papillomavirus.* Journal of Virological Methods, 2013. **187**(1): p. 166-171.

81. Rad, A., Sorbye, S.W., Dreyer, G., Hovland, S., Falang, B.M., Louw, M., and Skjeldestad, F.E., *HPV types in cervical cancer tissue in South Africa: A head-to-head comparison by mRNA and DNA tests.* Medicine (Baltimore), 2017. **96**(47): p. e8752.

82. QIAGEN. *Hybrid Capture 2 Modular System*. 2023; Available from: https://www.qiagen.com/us/products/diagnostics-and-clinical-research/sexual-reproductive-health/cervical-cancer-screening/hybrid-capture-2-modular-system.

83. QIAGEN. *digene HC2 High-Risk HPV DNA Test*. 2023; Available from: https://www.qiagen.com/kr/products/diagnostics-and-clinical-research/sexual-reproductive-health/cervical-cancer-screening/digene-hc2-high-risk-hpv-dna-test-ce#productdetails.

84. F. Hoffmann-La Roche Ltd. *cobas® HPV Test: Delivering confidence with 3-in-1 HPV test results*. 2023; Available from: https://diagnostics.roche.com/global/en/products/params/cobas-hpv.html.

85. Kvernrød, A., Bigaard, J. *Screening for livmoderhalskræft*. 2023; Available from: https://www.cancer.dk/forebyg/screening/livmoderhalskraeft/.

86. Molden, T., Kraus, I., Skomedal, H., Nordstrom, T., and Karlsen, F., *PreTect (TM) HPV-Proofer: Real-time detection and typing of E6/E7 mRNA from carcinogenic human papillomaviruses.* Journal of Virological Methods, 2007. **142**(1-2): p. 204-212.

87. Hologic Inc. *Aptima® HPV Assay*. 2023; Available from: https://hologicwomenshealth.com/products/aptimahpvassay/#:~:text=The%20Aptima%C2%AE%20HPV%20assay,that%20are%20present%20and%20active.&text=Studies%20have%20shown%20mRNA%20identifies,14%20high%2Drisk%20HPV%20types.

88. Chrysostomou, A.C., Stylianou, D.C., Constantinidou, A., and Kostrikis, L.G., *Cervical Cancer Screening Programs in Europe: The Transition Towards HPV Vaccination and Population-Based HPV Testing.* Viruses, 2018. **10**(12).

89. von Karsa, L., Arbyn, M., De Vuyst, H., Dinner, J., Dinner, L., Franceschi, S., Patnick, J., Ronco, G., Segnan, N., Suonio, E., Tornberg, S., and Anttila, A., *European guidelines for quality assurance in cervical cancer screening. Summary of the supplements on HPV screening and vaccination.* Papillomavirus Research, 2015. **1**: p. 22-31.

90. Wentzensen, N., Arbyn, M., Berkhof, J., Bower, M., Canfell, K., Einstein, M., Farley, C., Monsonego, J., and Franceschi, S., *Eurogin 2016 Roadmap: how HPV knowledge is changing screening practice.* Int J Cancer, 2017. **140**(10): p. 2192-2200.

91. NordScreen. *Cancer Screening Fact Sheet - Sweden – Cervix – 2017*. 2017; Available from: http://nordscreen.org/wp-content/uploads/2017/05/Cervix-Fact-Sheet-Sweden-2017.pdf.

92. Socialstyrelsen. *Livmoderhalscancer – screening med HPV-test*. 2022; Available from: https://www.socialstyrelsen.se/kunskapsstod-och-regler/regler-och-riktlinjer/nationella-screeningprogram/slutliga-rekommendationer/livmoderhalscancer/#:~:text=Alla%20regioner%20ska%20erbjuda%20avgiftsfri,och%20varf%C3%B6r%20den%20%C3%A4r%20motiverad.

93. Altobelli, E., Rapacchietta, L., Profeta, V.F., and Fagnano, R., *HPV-vaccination and cancer cervical screening in 53 WHO European Countries: An update on prevention programs according to income level.* Cancer Med, 2019. **8**(5): p. 2524-2534.

94. Sorbye, S., *Forebygging av livmorhalskreft i Norge, Sverige og Danmark.* BestPractice, 2017.

95. The Finnish Medical Society Duodecim. *Kohdunkaulan, emättimen ja ulkosynnytinten solumuutokset*. 2021; Available from: https://www.kaypahoito.fi/hoi50049#readmore.

96. NordScreen. *Cancer Screening Fact Sheet - Finland - 2016*. 2016; Available from: https://nordscreen.org/wp-content/uploads/2017/04/Cervix-Fact-Sheet-Finland-2016.pdf.

97. National Institute for Public Health and the Environment Ministry of Health Welfare and Sport. *Cervical cancer screening programme*. 2022; Available from: https://www.rivm.nl/en/cervical-cancer-screening-programme.

98. Rebolj, M., Rimmer, J., Denton, K., Tidy, J., Mathews, C., Ellis, K., Smith, J., Evans, C., Giles, T., Frew, V., Tyler, X., Sargent, A., Parker, J., Holbrook, M., Hunt, K., Tidbury, P., Levine, T., Smith, D., Patnick, J., Stubbs, R., Moss, S., and Kitchener, H., *Primary cervical screening with high risk human papillomavirus testing: observational study.* BMJ, 2019. **364**: p. l240.

99. Public Health England. *NHS Cervical Screening Programme-Cervical screening and human papillomavirus (HPV) testing*. 2019; Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/820430/HPV_primary_screening_leaflet.pdf.

100. Public Health England. *HPV primary screening pilot protocol algorithm*. 2016; Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/529496/HPVPSFlowchart-Version3_Jan16.CURRENTppt.pdf.

101. Cuschieri, K., Ronco, G., Lorincz, A., Smith, L., Ogilvie, G., Mirabello, L., Carozzi, F., Cubie, H., Wentzensen, N., Snijders, P., Arbyn, M., Monsonego, J., and Franceschi, S., *Eurogin roadmap 2017: Triage strategies for the management of HPV-positive women in cervical screening programs.* Int J Cancer, 2018. **143**(4): p. 735-745.

102. Turkey Public Health Institution. *Turkey Cancer Control Programme*. 2016; Available from: https://www.iccp-portal.org/system/files/plans/Turkiye_Kanser_Kontrol_Program_English.pdf.

103. Huh, W.K., Ault, K.A., Chelmow, D., Davey, D.D., Goulart, R.A., Garcia, F.A., Kinney, W.K., Massad, L.S., Mayeaux, E.J., Saslow, D., Schiffman, M., Wentzensen, N., Lawson, H.W., and Einstein, M.H., *Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance.* Obstet Gynecol, 2015. **125**(2): p. 330-7.

104. Cancer Council Australia. *National Cervical Screening Program: Guidelines for the management of screen-detected abnormalities, screening in specific populations and investigation of abnormal vaginal bleeding*. 2017; Available from: https://wiki.cancer.org.au/australiawiki/images/a/ad/National_Cervical_Screening_Program_guidelines_long-form_PDF.pdf.

105. Monie, A., Hung, C.F., Roden, R., and Wu, T.C., *Cervarix: a vaccine for the prevention of HPV 16, 18-associated cervical cancer.* Biologics, 2008. **2**(1): p. 97-105.

106. Harper, D.M. and DeMars, L.R., *HPV vaccines - A review of the first decade.* Gynecol Oncol, 2017. **146**(1): p. 196-204.

107. Lehtinen, M., Lagheden, C., Luostarinen, T., Eriksson, T., Apter, D., Harjula, K., Kuortti, M., Natunen, K., Palmroth, J., Petaja, T., Pukkala, E., Siitari-Mattila, M., Struyf, F., Nieminen, P., Paavonen, J., Dubin, G., and Dillner, J., *Ten-year follow-up of human papillomavirus vaccine efficacy against the most stringent cervical neoplasia end-point-registry-based follow-up of three cohorts from randomized trials.* BMJ Open, 2017. **7**(8): p. e015867.

108. Harper, D.M., Franco, E.L., Wheeler, C.M., Moscicki, A.B., Romanowski, B., Roteli-Martins, C.M., Jenkins, D., Schuind, A., Costa Clemens, S.A., Dubin, G., and group, H.P.V.V.S., *Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial.* Lancet, 2006. **367**(9518): p. 1247-55.

109. Cancer Registry of Norway. *Cervical Cancer Screening Programme*. 2023; Available from: https://www.kreftregisteret.no/en/screening/cervix/org/#:~:text=Norwegian%20health%20authorities%20recommend%20all,30%2B%20year%2Dolds).

110. Cancer Registry of Norway. *Masseundersøkelsen mot livmorhalskreft*. 2008; Available from: www.kreftregisteret.no/globalassets/publikasjoner-og-rapporter/livmorhalskreft/rapport_screeningintervall.pdf.

111. Kreftregisteret, *Kvalitetsmanual: Masseundersøkelsen mot livmorhalskreft*. 2005: Oslo, Norway.

112. Norwegian Health Directorate, *HPV-TEST I PRIMÆRSCREENING MOT LIVMORHALSKREFT: Kontrollert implementering og evaluering av forbedret helsetjeneste*. 2013.

113. Engesæter, B., Skare, G.B., Groeneveld, L., and Tropé, A., *Årsrapport 2019 - Screeningaktivitet og resultater fra Livmorhalsprogrammet*. 2021.

114. Engesæter, B., Groeneveld, L., Skare, G.B., and Tropé, A., *Årsrapport 2021 - Screeningaktivitet og resultater fra Livmorhalsprogrammet*. 2021.

115. Cancer Registry of Norway. *Primær HPV-test - nå også for kvinner under 34 år*. 2022; Available from: https://www.kreftregisteret.no/screening/livmorhalsprogrammet/hpv/.

116. Margrethe Greve-Isdahl. *HPV-vaksine (Humant papillomavirus) - veileder for helsepersonell*. 2022; Available from: https://www.fhi.no/nettpub/vaksinasjonsveilederen-for-helsepersonell/vaksiner-mot-de-enkelte-sykdommene/hpv-vaksinasjon-humant-papillomavir/.

117. Norwegian Institute of Public Health. *National surveillance of HPV vaccination programme*. 2018; Available from: https://www.fhi.no/en/studies/hpv-follow-up/national-surveillance-of-hpv-vaccination-programme/.

118. Norwegian Institute of Public Health. *National surveillance of the HPV vaccination programme (project description) - project description*. 2018 2022; Available from: https://www.fhi.no/en/projects/nasjonal-oppfolging-av-hpv-vaksinasjonsprogrammet-prosjektbeskrivelse/.

119. Norwegian Institute of Public Health. *Vaccination for Human Papillomavirus (HPV) (Indicator 22)*. 2022; Available from: https://www.fhi.no/en/op/Indicators-for-NCD/vaccine-and-screening/hpv-vaccine-indicator-22/.

120. van den Brule, A.J., Pol, R., Fransen-Daalmeijer, N., Schouls, L.M., Meijer, C.J., and Snijders, P.J., *GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes.* J Clin Microbiol, 2002. **40**(3): p. 779-87.

121. Snijders, P.J., van den Brule, A.J., Jacobs, M.V., Pol, R.P., and Meijer, C.J., *HPV DNA detection and typing in cervical scrapes.* Methods Mol Med, 2005. **119**: p. 101-14.

122. dos Santos, S.I., *Cancer Epidemiology: Principles and Methods*. 1999: International Agency for Research on Cancer and World Health Organization.

123. Izquierdo, J.N. and Schoenbach, V.J., *The potential and limitations of data from population-based state cancer registries.* American journal of public health, 2000. **90**(5): p. 695-698.

124. Thygesen, L.C. and Ersbøll, A.K., *When the entire population is the sample: strengths and limitations in register-based epidemiology.* Eur J Epidemiol, 2014. **29**(8): p. 551-8.

125. Ranstam, J., *Methodological note: accuracy, precision, and validity.* 2008. **49**(1): p. 105-106.

126. Bhopal, R.S., *Concepts of Epidemiology : Integrating the Ideas, Theories, Principles, and Methods of Epidemiology*. 2016, Oxford, UNITED KINGDOM: Oxford University Press, Incorporated.

127. Bankhead CR, S.E., Nunan D., *Catalogue of bias collaboration.*, in *Information bias*. 2019, Sackett Catalogue Of Biases.

128. Pham, A., Cummings, M., Lindeman, C., Drummond, N., and Williamson, T., *Recognizing misclassification bias in research and medical practice.* Family Practice, 2019. **36**(6): p. 804-807.

129. Miquel Porta, *A Dictionary of Epidemiology*. 6th ed. 2014, New York: Oxford University Press.

130. Sorbye, S.W., Suhrke, P., Reva, B.W., Berland, J., Maurseth, R.J., and Al-Shibli, K., *Accuracy of cervical cytology: comparison of diagnoses of 100 Pap smears read by four pathologists at three hospitals in Norway.* BMC Clin Pathol, 2017. **17**: p. 18.

131. Sornapudi, S., Stanley, R.J., Stoecker, W.V., Long, R., Xue, Z., Zuna, R., Frazier, S.R., and Antani, S., *DeepCIN: Attention-Based Cervical histology Image Classification with Sequential Feature Modeling for Pathologist-Level Accuracy.* J Pathol Inform, 2020. **11**: p. 40.

132. Reynolds L A, T.E.M. *History of cervical cancer and the role of human papillomavirus, 1960–2000*. in *Wellcome Witnesses to Twentieth Century Medicine*. 2009.

133. Institute for Quality and Efficiency in Health Care, *What is overdiagnosis?* 2006-, Cologne, Germany: InformedHealth.org [Internet].

134. Armstrong, N., *Overdiagnosis and overtreatment as a quality problem: insights from healthcare improvement research.* BMJ Qual Saf, 2018. **27**(7): p. 571-575.

135. Landy, R., Birke, H., Castanon, A., and Sasieni, P., *Benefits and harms of cervical screening from age 20 years compared with screening from age 25 years.* Br J Cancer, 2014. **110**(7): p. 1841-6.

136. Molden, T., Kraus, I., Karlsen, F., Skomedal, H., and Hagmar, B., *Human papillomavirus E6/E7 mRNA expression in women younger than 30 years of age.* Gynecol Oncol, 2006. **100**(1): p. 95-100.

137. Moscicki, A.B., Ma, Y., Wibbelsman, C., Powers, A., Darragh, T.M., Farhat, S., Shaber, R., and Shiboski, S., *Risks for cervical intraepithelial neoplasia 3 among adolescents and young women with abnormal cytology.* Obstet Gynecol, 2008. **112**(6): p. 1335-42.

138. Moscicki, A.B., Ma, Y., Wibbelsman, C., Darragh, T.M., Powers, A., Farhat, S., and Shiboski, S., *Rate of and risks for regression of cervical intraepithelial neoplasia 2 in adolescents and young women.* Obstet Gynecol, 2010. **116**(6): p. 1373-80.

139. Boardman, L.A. and Robison, K., *Screening adolescents and young women.* Obstetrics and gynecology clinics of North America, 2013. **40**(2): p. 257-268.

140. Public Health England. *Cervical screening: programme overview*. 2023; Available from: https://www.gov.uk/guidance/cervical-screening-programme-overview.

141. The Cancer Registry of Norway. *Primary HPV test - now also for women under 34*. 2023; Available from: https://www.kreftregisteret.no/screening/livmorhalsprogrammet/hpv/.

142. Polman, N.J., Snijders, P.J.F., Kenter, G.G., Berkhof, J., and Meijer, C., *HPV-based cervical screening: Rationale, expectations and future perspectives of the new Dutch screening programme.* Prev Med, 2019. **119**: p. 108-117.

143. Gruppo Italiano Screening del Cervicocarcinoma, *Protocollo operativo per lo screening del tumore della cervice uterina con test HPV primario.* 2012.

144. Stefani, C., Liverani, C.A., Bianco, V., Penna, C., Guarnieri, T., Comparetto, C., Monti, E., Valente, I., Pieralli, A.L., Fiaschi, C., and Origoni, M., *Spontaneous regression of low-grade cervical intraepithelial lesions is positively improved by topical bovine colostrum preparations (GINEDIE®). A multicentre, observational, italian pilot study.* Eur Rev Med Pharmacol Sci, 2014. **18**(5): p. 728-33.

145. St-Martin, G., Thamsborg, L.H., Andersen, B., Christensen, J., Ejersbo, D., Jochumsen, K., Johansen, T., Larsen, L.G., Waldstrøm, M., and Lynge, E., *Management of low-grade cervical cytology in young women. Cohort study from Denmark.* Acta Oncologica, 2021. **60**(4): p. 444-451.

146. Clifford, G.M., Smith, J.S., Plummer, M., Munoz, N., and Franceschi, S., *Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis.* Br J Cancer, 2003. **88**(1): p. 63-73.

147. Smith, J.S., Lindsay, L., Hoots, B., Keys, J., Franceschi, S., Winer, R., and Clifford, G.M., *Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update.* Int J Cancer, 2007. **121**(3): p. 621-32.

148. Li, N., Franceschi, S., Howell-Jones, R., Snijders, P.J., and Clifford, G.M., *Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication.* Int J Cancer, 2011. **128**(4): p. 927-35.

149. Guan, P., Howell-Jones, R., Li, N., Bruni, L., de Sanjosé, S., Franceschi, S., and Clifford, G.M., *Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer.* Int J Cancer, 2012. **131**(10): p. 2349-59.

150. Denny, L., Adewole, I., Anorlu, R., Dreyer, G., Moodley, M., Smith, T., Snyman, L., Wiredu, E., Molijn, A., Quint, W., Ramakrishnan, G., and Schmidt, J., *Human papillomavirus prevalence and type distribution in invasive cervical cancer in sub-Saharan Africa.* Int J Cancer, 2014. **134**(6): p. 1389-98.

151. 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., De Souza, S.C., Jenkins, D., Holl, K., and Group, H.S.S., *Differences in human papillomavirus type distribution in high-grade cervical intraepithelial neoplasia and invasive cervical cancer in Europe.* Int J Cancer, 2013. **132**(4): p. 854-67.

152. Ogembo, R.K., Gona, P.N., Seymour, A.J., Park, H.S., Bain, P.A., Maranda, L., and Ogembo, J.G., *Prevalence of human papillomavirus genotypes among African women with normal cervical cytology and neoplasia: a systematic review and meta-analysis.* PLoS ONE, 2015. **10**(4): p. e0122488.

153. Quek, S.C., Lim, B.K., Domingo, E., Soon, R., Park, J.S., Vu, T.N., Tay, E.H., Le, Q.T., Kim, Y.T., Vu, B.Q., Cao, N.T., Limson, G., Pham, V.T., Molijn, A., Ramakrishnan, G., and Chen, J., *Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical intraepithelial neoplasia across 5 countries in Asia.* International Journal of Gynecological Cancer, 2013. **23**(1): p. 148-56.

154. Suthipintawong, C., Siriaunkgul, S., Tungsinmunkong, K., Pientong, C., Ekalaksananan, T., Karalak, A., Kleebkaow, P., Vinyuvat, S., Triratanachat, S., Khunamornpong, S., and Chongsuwanich, T., *Human papilloma virus prevalence, genotype distribution, and pattern of infection in Thai women.* Asian Pac J Cancer Prev, 2011. **12**(4): p. 853-6.

155. Basu, P., Roychowdhury, S., Bafna, U.D., Chaudhury, S., Kothari, S., Sekhon, R., Saranath, D., Biswas, S., Gronn, P., Silva, I., Siddiqi, M., and Ratnam, S., *Human Papillomavirus Genotype Distribution in Cervical Cancer in India: Results from a Multi-center Study.* Asian Pacific Journal of Cancer Prevention, 2009. **10**(1): p. 27-34.

156. Kraus, I., Molden, T., Holm, R., Lie, A.K., Karlsen, F., Kristensen, G.B., and Skomedal, H., *Presence of E6 and E7 mRNA from human papillomavirus types 16, 18, 31, 33, and 45 in the majority of cervical carcinomas.* Journal of Clinical Microbiology, 2006. **44**(4): p. 1310-1317.

157. Sorbye, S.W., Fismen, S., Gutteberg, T.J., Mortensen, E.S., and Skjeldestad, F.E., *Primary cervical cancer screening with an HPV mRNA test: a prospective cohort study.* BMJ Open, 2016. **6**(8): p. e011981.

158. Westre, B., Giske, A., Guttormsen, H., Sorbye, S.W., and Skjeldestad, F.E., *5-type HPV mRNA versus 14-type HPV DNA test: test performance, over-diagnosis and overtreatment in triage of women with minor cervical lesions.* BMC Clin Pathol, 2016. **16**: p. 9.

159. Polman, N.J., Veldhuijzen, N.J., Heideman, D.A.M., Snijders, P.J.F., Meijer, C., and Berkhof, J., *HPV-positive women with normal cytology remain at increased risk of CIN3 after a negative repeat HPV test.* Br J Cancer, 2017. **117**(10): p. 1557-1561.

160. Katki, H.A., Kinney, W.K., Fetterman, B., Lorey, T., Poitras, N.E., Cheung, L., Demuth, F., Schiffman, M., Wacholder, S., and 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**(7): p. 663-72.

161. Wright, T.C., Stoler, M.H., Behrens, C.M., Sharma, A., Zhang, G., and 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.* Gynecologic Oncology, 2015. **136**(2): p. 189-197.

162. Ronco, G., Dillner, J., Elfstrom, K.M., Tunesi, S., Snijders, P.J., Arbyn, M., Kitchener, H., Segnan, N., Gilham, C., Giorgi-Rossi, P., Berkhof, J., Peto, J., Meijer, C.J., and International, H.P.V.s.w.g., *Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials.* Lancet, 2014. **383**(9916): p. 524-32.

163. Rad, A., Sørbye, S.W., Tiwari, S., Løchen, M.-L., and Skjeldestad, F.E., *Risk of Intraepithelial Neoplasia Grade 3 or Worse (CIN3+) among Women Examined by a 5-Type HPV mRNA Test during 2003 and 2004, Followed through 2015.* Cancers, 2023. **15**(12): p. 3106.

164. Riibe, M.O., Sorbye, S.W., Simonsen, G.S., Sundsfjord, A., Ekgren, J., and Maltau, J.M., *Risk of cervical intraepithelial neoplasia grade 3 or higher (CIN3+) among women with HPV-test in 1990-1992, a 30-year follow-up study.* Infect Agent Cancer, 2021. **16**(1): p. 46.

165. Khan, M.J., Castle, P.E., Lorincz, A.T., Wacholder, S., Sherman, M., Scott, D.R., Rush, B.B., Glass, A.G., and Shiffman, M., *The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice.* Journal of the National Cancer Institute, 2005. **97**(14): p. 1072-1079.

166. Ramakrishnan, S., Partricia, S., and Mathan, G., *Overview of high-risk HPV's 16 and 18 infected cervical cancer: pathogenesis to prevention.* Biomed Pharmacother, 2015. **70**: p. 103-10.

167. Powell, N.G., Hibbitts, S.J., Boyde, A.M., Newcombe, R.G., Tristram, A.J., and Fiander, A.N., *The risk of cervical cancer associated with specific types of human papillomavirus: a case-control study in a UK population.* Int J Cancer, 2011. **128**(7): p. 1676-82.

168. Moberg, M., Gustavsson, I., Wilander, E., and Gyllensten, U., *High viral loads of human papillomavirus predict risk of invasive cervical carcinoma.* Br J Cancer, 2005. **92**(5): p. 891-4.

169. International Agency for Research on Cancer, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Human Papillomaviruses*. 2007: France, Lyon. p. 1-636.

170. Castle, P.E., Glass, A.G., Rush, B.B., Scott, D.R., Wentzensen, N., Gage, J.C., Buckland, J., Rydzak, G., Lorincz, A.T., and Wacholder, S., *Clinical human papillomavirus detection forecasts cervical cancer risk in women over 18 years of follow-up.* J Clin Oncol, 2012. **30**(25): p. 3044-50.

171. Naucler, P., Ryd, W., Tornberg, S., Strand, A., Wadell, G., Hansson, B.G., Rylander, E., and Dillner, J., *HPV type-specific risks of high-grade CIN during 4 years of follow-*

up: A population-based prospective study. British Journal of Cancer, 2007. **97**(1): p. 129-132.

172. Arbyn, M., Tommasino, M., Depuydt, C., and Dillner, J., *Are 20 human papillomavirus types causing cervical cancer?* J Pathol, 2014. **234**(4): p. 431-5.

173. Sundstrom, K. and Dillner, J., *How many Human Papillomavirus types do we need to screen for?* J Infect Dis, 2020.

174. Nygard, M., Hansen, B.T., Kjaer, S.K., Hortlund, M., Tryggvadottir, L., Munk, C., Lagheden, C., Sigurdardottir, L.G., Campbell, S., Liaw, K.L., and Dillner, J., *Human papillomavirus genotype-specific risks for cervical intraepithelial lesions.* Hum Vaccin Immunother, 2021. **17**(4): p. 972-981.

175. Bruni, L., Diaz, M., Castellsague, X., Ferrer, E., Bosch, F.X., and de Sanjose, S., *Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings.* J Infect Dis, 2010. **202**(12): p. 1789-99.

176. World Health Organization. *Cervical Cancer Elimination Initiative*. 2021; Available from: https://www.who.int/initiatives/cervical-cancer-elimination-initiative.

177. Arbyn, M., Roelens, J., Simoens, C., Buntinx, F., Paraskevaidis, E., Martin-Hirsch, P.P., and Prendiville, W.J., *Human papillomavirus testing versus repeat cytology for triage of minor cytological cervical lesions.* Cochrane Database Syst Rev, 2013(3): p. CD008054.

178. The ASCUS-LSIL Traige Study (ALTS) Group, *A randomized trial on the management of low-grade squamous intraepithelial lesion cytology interpretations.* Am J Obstet Gynecol, 2003. **188**(6): p. 1393-400.

179. Sorbye, S.W., Fismen, S., Gutteberg, T.J., Mortensen, E.S., and Skjeldestad, F.E., *HPV mRNA is more specific than HPV DNA in triage of women with minor cervical lesions.* PLoS ONE, 2014. **9**(11): p. e112934.

180. Kendall, B.S., Bush, A.C., Olsen, C.H., and Zahn, C.M., *Reflex high-risk human papillomavirus testing for women with atypical squamous cells of undetermined significance in cytologic smears: effects since implementation in a large clinical practice.* Am J Clin Pathol, 2005. **123**(4): p. 524-8.

181. Bergeron, C., Cas, F., Fagnani, F., Contrepas, A., Wadier, R., and Poveda, J.D., *[Assessment of human papillomavirus testing on liquid-based Cyto-screen system for women with atypical squamous cells of undetermined significance. Effect of age].* Gynecol Obstet Fertil, 2006. **34**(4): p. 312-6.

182. Selvaggi, S.M., *ASC-US and high-risk HPV testing: performance in daily clinical practice.* Diagn Cytopathol, 2006. **34**(11): p. 731-3.

183. Ronco, G., Cuzick, J., Segnan, N., Brezzi, S., Carozzi, F., Folicaldi, S., Dalla Palma, P., Del Mistro, A., Gillio-Tos, A., Giubilato, P., Naldoni, C., Polla, E., Iossa, A., Zorzi, M., Confortini, M., Giorgi-Rossi, P., and group, N.w., *HPV triage for low grade (L-SIL) cytology is appropriate for women over 35 in mass cervical cancer screening using liquid based cytology.* Eur J Cancer, 2007. **43**(3): p. 476-80.

184. Arbyn, M., Martin-Hirsch, P., Buntinx, F., Van Ranst, M., Paraskevaidis, E., and Dillner, J., *Triage of women with equivocal or low-grade cervical cytology results: a meta-analysis of the HPV test positivity rate.* J Cell Mol Med, 2009. **13**(4): p. 648-59.

185. Lie, A.K., Trope, A., Skare, G.B., Bjorge, T., Jonassen, C.M., Brusegard, K., and Lonnberg, S., *HPV genotype profile in a Norwegian cohort with ASC-US and LSIL cytology with three year cumulative risk of high grade cervical neoplasia.* Gynecol Oncol, 2018. **148**(1): p. 111-117.

186. Trope, A., Sjoborg, K.D., Nygard, M., Roysland, K., Campbell, S., Alfsen, G.C., and Jonassen, C.M., *Cytology and human papillomavirus testing 6 to 12 months after ASCUS or LSIL cytology in organized screening to predict high-grade cervical neoplasia between screening rounds.* J Clin Microbiol, 2012. **50**(6): p. 1927-35.

187. Thomsen, L.T., Dehlendorff, C., Junge, J., Waldstrom, M., Schledermann, D., Frederiksen, K., and Kjaer, S.K., *Human papillomavirus mRNA and DNA testing in women with atypical squamous cells of undetermined significance: A prospective cohort study.* Int J Cancer, 2016. **139**(8): p. 1839-50.

188. Verdoodt, F., Szarewski, A., Halfon, P., Cuschieri, K., and Arbyn, M., *Triage of women with minor abnormal cervical cytology: meta-analysis of the accuracy of an assay targeting messenger ribonucleic acid of 5 high-risk human papillomavirus types.* Cancer Cytopathol, 2013. **121**(12): p. 675-87.

189. Benevolo, M., Vocaturo, A., Caraceni, D., French, D., Rosini, S., Zappacosta, R., Terrenato, I., Ciccocioppo, L., Frega, A., and Giorgi Rossi, P., *Sensitivity, specificity, and clinical value of human papillomavirus (HPV) E6/E7 mRNA assay as a triage test for cervical cytology and HPV DNA test.* J Clin Microbiol, 2011. **49**(7): p. 2643-50.

190. Pedersen, K., Sorbye, S.W., Kristiansen, I.S., and Burger, E.A., *Using novel biomarkers to triage young adult women with minor cervical lesions: a cost-effectiveness analysis.* BJOG, 2017. **124**(3): p. 474-484.

191. Reinholdt K., J.K.E., Dehlendorff C.,Munk C., Kjær S.K., Thomsen L.T., *Triage of low-grade squamous intraepithelial lesions using human papillomavirus messenger ribonucleic acid tests—A prospective population-based register study.* Acta Obstetricia et Gynecologica Scandinavica, 2019. **00**: p. 1–9.

192. Haldorsen, T., Skare, G.B., Ursin, G., and Bjorge, T., *Results of delayed triage by HPV testing and cytology in the Norwegian Cervical Cancer Screening Programme.* Acta Oncol, 2015. **54**(2): p. 200-9.

193. Sorbye, S.W., Arbyn, M., Fismen, S., Gutteberg, T.J., and Mortensen, E.S., *HPV E6/E7 mRNA testing is more specific than cytology in post-colposcopy follow-up of women with negative cervical biopsy.* PLoS ONE, 2011. **6**(10): p. e26022.

194. Nygard, M., Roysland, K., Campbell, S., and Dillner, J., *Comparative effectiveness study on human papillomavirus detection methods used in the cervical cancer screening programme.* BMJ Open, 2014. **4**(1): p. e003460.

195. Sorbye, S.W., Fismen, S., Gutteberg, T.J., Mortensen, E.S., and Skjeldestad, F.E., *HPV mRNA testing in triage of women with ASC-US cytology may reduce the time for CIN2+diagnosis compared with repeat cytology.* Curr Pharm Des, 2013. **19**(8): p. 1401-5.

196. Ibanez, R., Moreno-Crespi, J., Sarda, M., Autonell, J., Fibla, M., Gutierrez, C., Lloveras, B., Alejo, M., Catala, I., Alameda, F., Casas, M., Bosch, F.X., and de Sanjose, S., *Prediction of cervical intraepithelial neoplasia grade 2+ (CIN2+) using HPV DNA testing after a diagnosis of atypical squamous cell of undetermined significance (ASC-US) in Catalonia, Spain.* BMC Infect Dis, 2012. **12**: p. 25.

197. Sorbye, S.W., Arbyn, M., Fismen, S., Gutteberg, T.J., and Mortensen, E.S., *Triage of women with low-grade cervical lesions--HPV mRNA testing versus repeat cytology.* PLoS ONE, 2011. **6**(8): p. e24083.

198. Kelly, R.S., Patnick, J., Kitchener, H.C., Moss, S.M., and Group, N.H.S.I., *HPV testing as a triage for borderline or mild dyskaryosis on cervical cytology: results from the Sentinel Sites study.* Br J Cancer, 2011. **105**(7): p. 983-8.

199. Sorbye, S.W., Fismen, S., Gutteberg, T.J., and Mortensen, E.S., *HPV mRNA test in women with minor cervical lesions: experience of the University Hospital of North Norway.* J Virol Methods, 2010. **169**(1): p. 219-22.

200. Molden, T., Nygard, J.F., Kraus, I., Karlsen, F., Nygard, M., Skare, G.B., Skomedal, H., Thoresen, S.O., and Hagmar, B., *Predicting CIN2+ when detecting HPV mRNA and DNA by PreTect HPV-proofer and consensus PCR: A 2-year follow-up of women with ASCUS or LSIL Pap smear.* Int J Cancer, 2005. **114**(6): p. 973-6.

201. Sherman, M.E., Schiffman, M., Cox, J.T., and Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study, G., *Effects of age and human papilloma viral load on colposcopy triage: data from the randomized Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS).* J Natl Cancer Inst, 2002. **94**(2): p. 102-7.

202. Rad, A., Sørbye, S.W., Brenn, T., Tiwari, S., Løchen, M.-L., and Skjeldestad, F.E., *13-Type HPV DNA Test versus 5-Type HPV mRNA Test in Triage of Women Aged 25–33 Years with Minor Cytological Abnormalities–6 Years of Follow-Up.* International Journal of Environmental Research and Public Health, 2023. **20**(5): p. 4119.

203. Koliopoulos, G., Chrelias, C., Pappas, A., Makridima, S., Kountouris, E., Alepaki, M., Spathis, A., Stathopoulou, V., Panayiotides, I., Panagopoulos, P., Karakitsos, P., and Kassanos, D., *The diagnostic accuracy of two methods for E6&7 mRNA detection in women with minor cytological abnormalities.* Acta Obstet Gynecol Scand, 2012. **91**(7): p. 794-801.

204. Paolino, M., Gago, J., Pera, A.L., Cinto, O., Thouyaret, L., and Arrossi, S., *Adherence to triage among women with HPV-positive self-collection: a study in a middle-low income population in Argentina.* Ecancermedicalscience, 2020. **14**: p. 1138.

205. Gago, J., Paolino, M., and Arrossi, S., *Factors associated with low adherence to cervical cancer follow-up retest among HPV+/ cytology negative women: a study in programmatic context in a low-income population in Argentina.* BMC Cancer, 2019. **19**(1): p. 367.

Paper I

Observational Study

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HPV types in cervical cancer tissue in South Africa

A head-to-head comparison by mRNA and DNA tests

Amir Rad, MSc[a,*], Sveinung Wergeland Sørbye, MD, PhD[b], Greta Dreyer, MD, PhD[c], Siri Hovland, MSc[d], Bente Marie Falang, BSc[d], Melanie Louw, MD[e], Finn Egil Skjeldestad, MD, PhD[a]

Abstract

Accurate identification of human papillomavirus (HPV)-types in cervical cancer tissue may be important for tailoring tests for primary screening and types to be included in a vaccine. The aim of this study was to compare test-performance of a 45-type HPV deoxyribonucleic acid (DNA)-test with a 9-type HPV messenger ribonucleic acid (mRNA)-test in cervical cancer tissues.

In a case-series design 188 women with diagnosed cervical cancer during the period January 2008 to July 1, 2011 at the Gynaecological Oncology Unit, University of Pretoria, South Africa were recruited to the study. After cases with negative internal controls for DNA/mRNA detection (n = 18) and unconfirmed histology (n = 3) of cervical cancer were excluded, 167 women remained eligible for analysis. We compared 45 DNA-types detected through general primer (GP)$5^+/6^+$ polymerase chain reaction (PCR) and reverse line blot (RLB) genotyping with a modified version of the mRNA test PreTect HPV-Proofer detecting 9 genotypes (16, 18, 31, 33, 35, 45, 51, 52, 58).

Histological types were 92.2% squamous cell carcinoma, 4.8% adenocarcinoma, and 3.0% adenosquamous carcinoma. Overall, HPV was detected in 95.2% (159/167) of specimens. The DNA- and mRNA tests each rendered 153/167 (91.6%) HPV positive results. When restricting the analysis to the 9 high-risk HPV-types included in the mRNA test, 91.6% (153/167) and 88.0% (147/167) were positive by the mRNA- and DNA-tests ($P = .28$), respectively. After hierarchical categorization of 9 comparable types, we found concordance in 66 of 67 specimens for HPV16, 25 of 27 specimens for HPV18, 19 of 21 specimens for HPV45, and only in 33 of 45 for HPV31, 33, 35, 51, 52, 58. The positivity rate for the HPV types 16, 18, and 45 and the positivity rate for HPV 16, 18, 45, 33 and 35 by both tests was 66% to 68% and 80% to 83%, respectively.

Overall and when considering established high-risk types, the mRNA test has at least as high detection rate as the DNA test. The mRNA test can be an appropriate research tool to describe causative HPV-types in cervical cancer tissue for health care planning purposes.

Abbreviations: bp = base pairs, CIN3 = cervical intraepithelial neoplasia grade 3, DNA = deoxyribonucleic acid, dsDNA = double strand DNA, EIA = enzyme immunoassay, GP = general primer, HIV = human immunodeficiency virus, HPV = human papillomavirus, IARC = international agency for research on cancer, ICC = invasive cervical cancer, ISC = intrinsic sample control, ISH = in situ hybridization, ISM = Department of Community Medicine (Norwegian: *Institutt for samfunnsmedisin*), mRNA = messenger ribonucleic acid, NASBA = nucleic acid sequence based amplification, ORF = open reading frame, PCR = polymerase chain reaction, pRb = protein retinoblastoma, RLB = reverse line blot, SCC = squamous cell carcinoma, SPF = short PCR fragment, SPSS = Statistical Package for the Social Sciences.

Keywords: cervical cancer, DNA diagnostics, HPV-type distribution, mRNA diagnostics, prevalence

1. Introduction

Cervical cancer ranks in women as the fourth most common cancer worldwide.[1] In South Africa, cervical cancer is the most frequent cancer in women aged 15 to 44 years and the second among women of all ages.[2]

Human papillomavirus (HPV) infection is the necessary, but not sufficient, cause for cervical cancer.[3–5] Persistent HPV infection is the most important risk factor for cervical cancer.[6] HPV-targeted screening programs and HPV vaccination are implemented in many countries to reduce cervical cancer incidence, morbidity, and mortality.[7] Knowledge of HPV type distribution in cervical precancerous and cancer histology is important to prioritize HPV types in future HPV prophylactic vaccines and HPV-based screening tests.[8]

Evaluation of the carcinogenic properties of HPV lacks longitudinal studies with cervical cancer as endpoint.[9,10] Meta-analysis on prevalence of HPV types detected in cervical cancer reutilize the same studies,[5,8,11,12] which lack consistency in HPV detection methods applied, number of HPV types targeted, and often validation of histological diagnoses are missing. In addition, most studies report all HPV types identified

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[a] Department of Community Medicine, Faculty of Health Sciences, UiT The Arctic University of Norway, [b] Department of Clinical Pathology, University Hospital of North Norway, Tromsø, Norway, [c] Department of Obstetrics and Gynaecology, University of Pretoria, Pretoria, South Africa, [d] PreTect AS, Klokkarstua, Norway, [e] Department of Anatomical Pathology, University of Pretoria, Pretoria, South Africa.

[*] Correspondence: Amir Rad, Department of Community Medicine, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Troms 9037, Norway (e-mail: amir.rad@uit.no).

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without a hierarchical approach to the types that are major drivers in the oncogenesis. This may overestimate the role of the low prevalent types, which often are appearing as coinfections in cervical cancer tissue. Knowledge on the biological mechanisms of HPV carcinogenicity is limited to basic research applied mostly to HPV16- and less to HPV18-infected cell lines,[13–15] while evidence from basic research on the carcinogenic properties of other HPV types is lacking.

HPV tests differ in their clinical performance, sensitivity, and specificity.[16–20] The characteristics of HPV tests are different in targeted nucleic acid (deoxyribonucleic acid [DNA] or ribonucleic acid [RNA]), targeted genes in HPV genome, and the ability of separate genotyping.[21] The general primer (GP)5+/6+ polymerase chain reaction (PCR)-enzyme immunoassay (EIA) (polymerase chain reaction-*reverse* line blot) assay targets the L1 region of HPV DNA with no ability to report genotypes separately, while PreTect HPV-Proofer targets E6/E7 regions of HPV messenger ribonucleic acid (mRNA) and can detect types 16, 18, 31, 33, and 45, separately.[21]

DNA-based HPV tests detect the presence of HPV at DNA level and not necessarily the transcriptional and translational activity of the HPV DNA. The oncogenic activity of HPV type 16 is known to be through the expression of viral genes E6 and E7, following inactivation of cell tumor suppressor proteins p53 and protein retinoblastoma (pRB).[22] The E6 and E7 gene expression from HPV types 16, 18, 31, 33, and 45 has been confirmed in the majority of cervical carcinomas.[23] The mRNA-based HPV test detects the E6 and E7 oncogenic expression of HPV and it is based on the real-time multiplex nucleic acid sequence-based amplification (NASBA) assay called PreTect HPV-Proofer.[24] On the other hand, there are several methods to test the presence of HPV DNA including PCR, reverse line blot (RLB) sequencing, in situ hybridization (ISH) and EIA, with PCR being the most commonly applied method for HPV DNA analysis. The PCR-based tests are using either consensus PCR primers that can cover a range of DNA types or type-specific PCR primers that work for specific genotypes. Depending on PCR primers, the size of the PCR-amplified fragment differs; for instance, the amplified fragment for MY09/11 is about 450 base pairs (bp) while the GP5/6 fragment size is approximately 140 bp.[25] The short PCR fragment primers (SPF10), which were developed for universal detection of HPV, target only 65 bp of the L1 open reading frame (ORF) in at least 43 HPV genotypes.[26,27] The SPF10 primers are more sensitive than other primers, especially when multiple HPV genotypes are present.[27,28] It is noticeable that as the applied primers in HPV DNA test have fewer base pairs, the ability of the test to detect the presence of HPV DNA in tumor tissues and, consequently the test sensitivity, increases. Conversely, the specificity of these tests drops and it becomes less informative on the oncogenic properties. The DNA-based HPV tests detect the HPV viral DNA presence, which might be in transient phase and not active oncogenes while mRNA test positivity implies continuous expression of the viral E6 and E7 oncogenes.

The aim of this study was to compare the test-performance of a 45-type HPV DNA-test with a nine-type HPV mRNA-test in cervical cancer tissues.

2. Materials and methods

This study was performed in collaboration between the Institute of Community Medicine (ISM) and Department of Clinical Pathology, Faculty of Health Sciences, University of Tromso, Norway; PreTect AS, Klokkarstua, Norway; and the Gynecologic Oncology Unit, Departments of Obstetrics and Gynaecology and Anatomical Pathology, University of Pretoria, South Africa. The Research Ethics Committee of the Faculty of Health Sciences of the University of Pretoria reviewed and approved the study protocol (27/2008, 108/2008, 189/2012). All the participants gave written informed consent. At the time of presentation for the evaluation and staging of disease, tissue biopsies were taken for histological confirmation of the diagnosis of invasive epithelial cervical cancer and HPV analysis. Two adjacent punch biopsies were taken at the 3 o'clock and 9 o'clock positions. One biopsy from each position was preserved in formalin and sent to the Department of Anatomical Pathology at the University of Pretoria for histological diagnosis. Two pathologists reviewed all histological diagnosis until consensus was reached.

The second biopsy from each position was preserved in a standard commercially available methanol-buffer solution, PreTect TM (PreTect AS) and shipped to Norway for HPV DNA and mRNA analyses. These biopsies were cut in small pieces on a cold metal block using a scalpel and transferred to a microcentrifuge tube prior to addition of 1 mL lysis buffer (NucliSens, BioMerieux, France), followed by homogenization for 30 seconds using a pellet pestle and incubation at 37°C for 30 minutes. Total nucleic acids (DNA/RNA) were extracted using NucliSENS miniMAG (BioMerieux, 200297, Boxtel, The Netherlands) according to the manufacturer's instructions and kept at −70°C prior to DNA/mRNA testing performed on the same extracts. All laboratory testing was performed blindly.

Human papillomavirus DNA analysis, testing for 39 individual types (HPV 6, 11, 16,18, 26, 30, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54,55, 56, 57, 58, 59, 61, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82/MM4, 82/IS39, and CP6108) and 6 rare HPV types (HPV32, 83, 84, 85, 86, and JC9710) as a pool, was performed on GP5+/6+ polymerase chain reaction products using RLB assay.[29,30] Polymerase chain reaction toward the B-globin gene was included as DNA control for all HPV-negative samples.

Human papillomavirus mRNA E6/E7 analysis, testing for 9 individual HPV types (HPV 16, 18, 31, 33, 45, 35, 51, 52, 58) was performed using an extended version of PreTect HPV-Proofer, a diagnostic kit for the qualitative detection and direct typing of E6/E7 mRNA from 5 HPV types[16,18,31–33] plus 4 additional HPV types (35,51,52,58). The kit is based on real-time NASBA technology combining nucleic acid amplification and simultaneous detection with specific Molecular Beacon probes. NASBA is an enzymatic 1-step amplification process that is able to specifically amplify RNA in a double-strand DNA (dsDNA) background under isothermal conditions (41°C). By using an RNA T7-polymerase promoter to generate multiple RNA products at 41°C, double-stranded DNA is not denatured and consequently not amplified, hence the presence of genomic dsDNA will not cause false positives.[31]

Intrinsic sample control (ISC) directed against mRNA from a human housekeeping gene is included in the kit to assess specimen quality and reveal possible factors that may inhibit the amplification, hereby monitoring the entire test process. Standardized artificial oligonucleotides corresponding to the respective viral sequences were used as positive controls for each of the HPV types and water as negative control. The PreTect Analysis Software (PAS, PreTect AS) performed all assay validation, where all controls and specimen ISC results have to be valid to report an HPV result.

A total of 188 patients with invasive cervical cancer referred to the gynaecologic oncology unit at the University of Pretoria during the period January 1, 2008 to July 31, 2011 were recruited

Table 1

Study population characteristics, HIV status, and histology by age (%).

Characteristics	25–39 y N = 38	40–89 y N = 129	25–89 y N = 167
HIV status			
Unknown	0	0.8	0.6
Negative	18.4	69.8	58.1
Positive	81.6	29.5	41.3
Histology			
Squamous carcinoma	100	89.9	92.2
Adenocarcinoma	0	6.2	4.8
Adenosquamous carcinoma	0	3.9	3.0

HIV = human immunodeficiency virus.

to the study. We excluded women without validated histological diagnosis of cervical cancer (n = 3) and samples with low quality of genomic material by using ISC (n = 18). The final study population comprised 167 patients.

All data analyses were done in SPSS, version 24.0. We applied a 2-tailed 2-proportion Z-test to compare positivity rates between the tests with significance level $P < .05$.

We applied a hierarchical approach where one type is counted only once by decreasing prevalence order of HPV types in our own data.

3. Results

The majority of patients (77.2%) were older than 40 years (range 25–89 years), and 41.3% (69/167) were HIV positive. Histology results showed 92.2% (154/167) cases of squamous cell carcinoma (SCC), 4.8% adenocarcinoma, and 3.0% adenosquamous carcinoma. Among women less than 40 years, 81.6% (31/38) were HIV-positive. All women in this age group had SCC. Among women older than 40 years, 29.5% (38/129) were HIV-positive, and 90% (116/129) were diagnosed with SCC (Table 1).

Considering both the DNA- and mRNA test results, 95.2% (159/167) of the specimens were HPV positive in at least 1 test, while the DNA- and the mRNA-tests each rendered 91.6% (153/167) HPV positive results. In 7.8% (13/167) specimens HPV were detected only by the DNA test and similarly in 7.8% (13/167) specimens, HPV were detected only by mRNA test. There were 11 double infections detected by mRNA analysis and 4 double infections detected by DNA analysis.

When analyzing the 9 most prevalent HPV types hierarchically (16>18>45>35>33>52>31>58>51), 91.6% (153/167) were positive by the mRNA test and 88.0% (147/167) by the DNA-test ($P = .28$). In total, 83.8% (140/167) were positive for the same HPV type by both tests, while there were 26 discordant results. We found concordance in 66 of 67 cases of HPV16, 25 of 27 cases of HPV18, 19 of 21 cases of HPV45, 15 of 18 cases of HPV35, and 18 of 27 cases of HPV types 33, 52, 31, 58, and 51 collectively. HPV types 30, 56, 69, 73, 82 were not included in the mRNA test, which added 6 more positive cases by the DNA test (Table 2, lower panel).

Among the HPV-negative cases, 8 biopsies were negative in both the tests. In addition, 6 biopsies were negative only by the DNA test and 6 biopsies were negative only by the mRNA test.

The positivity rate for the HPV types 16, 18, and 45 and the positivity rate for HPV 16, 18, 45, 33, and 35 by both tests summarized to 66% to 68% and 80% to 83%, respectively. Thirty-eight women were less than 40 years of age, among whom 31 women were HIV-positive. Twenty-nine of these 31 HIV infected women tested positive for HPV by both tests; 29 and 26 out of 31 HIV positive women were positive for the 9 high risk types by the mRNA- and the DNA-test, respectively. Table 3 displays a more complete comparison of the concordance and discordance in DNA/mRNA analyses among HIV negative and positive women. Positivity rates of type-specific HPV by DNA- and mRNA-tests did not differ in any of the comparisons (data not shown).

4. Discussion

In this case-series of 167 women diagnosed with cervical cancer, 95.2% (159/167) were HPV positive in at least 1 test. There were no differences in overall comparison with types detected by the 45 types DNA test (91.6%) and the 9 types mRNA test (91.6%).

4.1. Overall positivity rate

In most prevalence studies of HPV detection in cervical cancer tissue, 2 to 5 different methods are used to diagnose the virus. A

Table 2

Concordant and discordant pairs in DNA/mRNA analysis of type-specific HPV detection.

	HPV-type	Total numbers positive (N)	mRNA-positive only (N)	Both mRNA and DNA positive (N)	DNA-positive only (N)
Types present in both tests	16	67	0	66	1
	18	27	2	25	0
	45	21	2	19	0
	35	18	1	15	2
	33	9	3	6	0
	52	7	2	5	0
	31	4	0	3	1
	58	4	1	0	3
	51	3	2	1	0
Types present in the DNA-test, only	30	1	NA		1
	56	1	NA		1
	69	1	NA		1
	73	1	NA		1
	82	2	NA		2
	Total		13	140	13

DNA = deoxyribonucleic acid, HIV = human immunodeficiency virus, HPV = human papillomavirus, mRNA = messenger ribonucleic acid.

Table 3

Comparison in DNA/mRNA analysis of type-specific HPV detection in HIV negative/positive women stratified by age.

		HIV negative N = 7		HIV positive N = 31	
Age	**HPV type**	**mRNA**	**DNA**	**mRNA**	**DNA**
25–39 y	Negative	0	0	2	2
	16	4	4	6	6
	18	1	1	10	9
	45	1	1	6	5
	35	1	1	3	3
	33	0	0	0	0
	52	0	0	1	0
	31	0	0	1	2
	58	0	0	0	0
	51	0	0	2	1
	30	NA	0	NA	0
	56	NA	0	NA	0
	69	NA	0	NA	1
	73	NA	0	NA	1
	82	NA	0	NA	1
		HIV negative N = 90		**HIV positive N = 38**	
	HPV type	**mRNA**	**DNA**	**mRNA**	**DNA**
40–89 y	Negative	10	10	2	2
	16	39	39	16	17
	18	12	12	4	3
	45	6	5	8	8
	35	11	11	1	2
	33	5	5	4	1
	52	4	3	2	2
	31	2	2	0	0
	58	1	1	0	2
	51	0	0	1	0
	30	NA	1	NA	0
	56	NA	1	NA	0
	69	NA	0	NA	0
	73	NA	0	NA	0
	82	NA	0	NA	1

DNA = deoxyribonucleic acid, HIV = human immunodeficiency virus, HPV = human papillomavirus, mRNA = messenger ribonucleic acid.

valid positive test result is based on at least 1 test being positive. Our overall 95.2% HPV positivity rate was higher than in another African study (Ghana, Nigeria, and South Africa (90.4%))[34] and similar to what has been reported from a European study that tested for HPV DNA in cervical cancer tissue.[32] Another study from Norway found an overall 97% HPV positivity rate in tissue from squamous cell carcinoma,[23] while the type-specific PCR primers, consensus Gp5+/6+ PCR primers for HPV DNA, and 8-types E6/E7 mRNA test had equal 92% positivity rates.[23] Similarly, a study from India reported an overall 91.7% positivity rate of HPV in cervical cancer specimens with no difference in positivity rate between MY09/11 L1 consensus PCR applied HPV DNA test and the PreTect HPV-Proofer (5 types).[35] A meta-analysis summarizing results from case-series of HPV prevalence in cervical cancer tissue, regardless of method and number of methods used, demonstrated overall 87% HPV DNA positivity, reaching 94% in cervical cancer specimens from Africa.[8]

4.2. HPV type distribution

Our analyses are based on a hierarchical approach where each type is counted only once by order of decreasing prevalence. We found the same order of prevalence as summarized by Smith et al[8] in 5 studies from Africa, except for HPV 33 and HPV 52. Smith et al[8] counted each type more than once if they occurred as double/tripled infections. However, we found a lower prevalence of HPV 16, similar prevalence of HPV 18, and higher prevalence for HPV 45, 33, and 52 than displayed by Smith et al[8] and in another study from Ghana, Nigeria, and South Africa.[34] Compared with a European study on prevalence of HPV types in cervical cancer tissue, we again found a lower prevalence of HPV16, and relatively higher prevalence of HPV 45, 35, and 52.[32]

4.3. Prevalence, persistency, and progressive ability

Evaluation of the carcinogenic properties of HPV suffers from the lack of long-term prospective studies with cervical cancer as endpoint.[9,10] Most of the reviews and meta-analysis considered prevalence from case-control or case-series studies.[5,8,11,12] Although HPV types may differ by order of magnitude in risk for cervical cancer,[36] the International Agency for Research on Cancer (IARC) did not rank the HPV types according to this risk, except for types 16 and 18. They simply concluded that HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 are carcinogenic in the human cervix.[36,37]

In cervical carcinogenesis, genotype-specific HPV persistence is associated with higher risk of cervical cancer than transient HPV

infection.[10,38–40] Longitudinal prospective studies showed that some of the HPV types that are classified by the IARC and other above-mentioned studies as high risk or carcinogenic, had no or little potentiality for progression to high-grade cervical lesions and cancer.[10,41–45]

HPV types 16, 18, and 45 have been detected more frequently in invasive cervical cancer (ICC) than cervical intraepithelial neoplasia grade 3 (CIN3) cases, suggesting differences in type-specific risks for progression and the necessity of treatment for cervical lesions related to these types.[8,32,33] This, together with the narrow median age differences between CIN3 and SCC in women diagnosed with HPV types 16, 18, and 45, indicates the progressive nature of HPV types 16, 18, and 45.[32]

4.4. HPV types distribution among HIV positive/negative women

In this study, similar to other studies from Mozambique,[46] Kenya,[47] and South Africa,[47] we did not detect significant differences in positivity rates of HPV types by HIV status. Since cell-mediated immunity is crucial in clearing HPV infection and for regression of cervical lesions,[48] we expected a different HPV-type distribution. Among immunocompromised women "low risk types" could become more "oncogenic," but our results did not confirm such a theory. Lack of knowledge on the time of HIV acquisition is another difficulty in determining the oncogenic potential of HPV types by HIV status.[47] In case the HIV infection took place after HPV infection and, especially, in the last years before cervical cancer development, the HIV-associated immune impairments would not affect the responsible HPV type.[47] Moreover, it is supposed that micronutrient deficiency and chronic infections in African countries may also suppress the immune system and, consequently, fade the association between type-specific HPV infection and HIV status.[49]

4.5. Strengths

We consider the application of NASBA technology as a strength in mRNA detection method. This technology amplifies RNA under isothermal conditions, which avoids denaturing and, in turn, amplification of double stranded DNA. Therefore, the false positives from the presence of genomic dsDNA in the background of mRNA may be prevented.[31] The usage of 2 different methods for HPV detection, together with the high concordance (84%) in type detection between methods, is considered another strength.

In addition, we consider the hierarchical approach in performing analyses for multiple infection cases as a strength. The hierarchical analysis of single infections avoids overestimation of the less prevalent types in cervical cancer specimen. The results from hierarchical studies provided more accurate information on the role of HPV16/18 compared with other oncogenic HPV types for the risk of CIN3 and cancer.[43,45]

4.6. Limitations

In a global perspective, our sample size may be considered a limitation,[8,32] however, from a regional or national perspective, our sample size is above average of published studies. Some HPV types that tested positive using the DNA test were not covered by the mRNA test and thus could not be confirmed as carcinogenic. This could be a limitation for the mRNA detection and also type specific comparisons. However, these HPV-types are considered to have low oncogenic properties.

5. Conclusion

Overall and when considering established high-risk types, the mRNA test has at least as high a detection rate as the DNA test. The mRNA test can be an appropriate research tool to describe causative HPV-types in cervical cancer tissue for health care planning purposes.

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References

[1] Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–86.
[2] Bruni L, Barrionuevo-Rosas L, Albero G, et al. Human Papillomavirus and Related Diseases in the World. Summary Report 2016.
[3] Walboomers J, Jacobs M, Manos M, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 189:12–9.
[4] Matsukura T, Sugase M. Pitfalls in the epidemiologic classification of human papillomavirus types associated with cervical cancer using polymerase chain reaction: driver and passenger. Int J Gynecol Cancer 2008;18:1388.
[5] Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518–27.
[6] zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002;2:342–50.
[7] Bosch FX, Robles C, Diaz M, et al. HPV-FASTER: broadening the scope for prevention of HPV-related cancer. Nat Rev Clin Oncol 2016;13: 119–32.
[8] Smith JS, Lindsay L, Hoots B, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007;121:621–32.
[9] Castle PE, Rodriguez AC, Burk RD, et al. Short term persistence of human papillomavirus and risk of cervical precancer and cancer: population based cohort study. Brit Med J 2009;339:b2569.
[10] Kjaer SK, Frederiksen K, Munk C, et al. Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. J Natl Cancer Inst 2010; 102:1478–88.
[11] Clifford GM, Smith JS, Plummer M, et al. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer 2003;88:63–73.
[12] Clifford GM, Smith JS, Aguado T, et al. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. Br J Cancer 2003;89:101–5.
[13] Scheffner M, Munger K, Byrne JC, et al. The State of the P53 and Retinoblastoma Genes in Human Cervical-Carcinoma Cell-Lines. Proc Natl Acad Sci USA 1991;88:5523–7.
[14] Li F, Cui J. Human telomerase reverse transcriptase regulates vascular endothelial growth factor expression via human papillomavirus oncogene E7 in HPV-18-positive cervical cancer cells. Med Oncol 2015;32:199.
[15] Shen-Gunther J, Wang CM, Poage GM, et al. Molecular Pap smear: HPV genotype and DNA methylation of ADCY8, CDH8, and ZNF582 as an integrated biomarker for high-grade cervical cytology. Clin Epigenetics 2016;8:96.
[16] Szarewski A, Ambroisine L, Cadman L, et al. Comparison of predictors for high-grade cervical intraepithelial neoplasia in women with abnormal smears. Cancer Epidemiol Biomarkers Prev 2008;17:3033–42.
[17] Ovestad IT, Vennestrom U, Andersen L, et al. Comparison of different commercial methods for HPV detection in follow-up cytology after ASCUS/LSIL, prediction of CIN2-3 in follow up biopsies and spontaneous regression of CIN2-3. Gynecol Oncol 2011;123:278–83.
[18] Szarewski A, Mesher D, Cadman L, et al. Comparison of seven tests for high-grade cervical intraepithelial neoplasia in women with abnormal smears: the Predictors 2 study. J Clin Microbiol 2012;50: 1867–73.

[19] Cuzick J, Cadman L, Mesher D, et al. Comparing the performance of six human papillomavirus tests in a screening population. Br J Cancer 2013;108:908–13.
[20] Cuzick J, Ahmad AS, Austin J, et al. A comparison of different human papillomavirus tests in PreservCyt versus SurePath in a referral population-PREDICTORS 4. J Clin Virol 2016;82:145–51.
[21] Arbyn M, Snijders PJF, Meijer CJLM, et al. Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? Clin Microbiol Infect 2015;21:817–26.
[22] Villa LL. Biology of genital human papillomaviruses. Int J Gynecol Obstet 2006;94:S3–7.
[23] Kraus I, Molden T, Holm R, et al. Presence of E6 and E7 mRNA from human papillomavirus types 16, 18, 31, 33, and 45 in the majority of cervical carcinomas. J Clin Microbiol 2006;44:1310–7.
[24] Molden T, Kraus I, Skomedal H, et al. PreTect (TM) HPV-Proofer: real-time detection and typing of E6/E7 mRNA from carcinogenic human papillomaviruses. J Virol Methods 2007;142:204–12.
[25] Husnjak K, Grce M, Magdic L, et al. Comparison of five different polymerase chain reaction methods for detection of human papillomavirus in cervical cell specimens. J Virol Methods 2000;88:125–34.
[26] Kleter B, van Doorn LJ, ter Schegget J, et al. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. Am J Pathol 1998;153:1731–9.
[27] Kleter B, van Doorn LJ, Schrauwen L, et al. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Clin Microbiol 1999;37:2508–17.
[28] Micalessi MI, Boulet GA, Vorsters A, et al. A real-time PCR approach based on SPF10 primers and the INNO-LiPA HPV Genotyping Extra assay for the detection and typing of human papillomavirus. J Virol Methods 2013;187:166–71.
[29] van den Brule AJ, Pol R, Fransen-Daalmeijer N, et al. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol 2002;40:779–87.
[30] Snijders PJ, van den Brule AJ, Jacobs MV, et al. HPV DNA detection and typing in cervical scrapes. Methods Mol Med 2005;119:101–14.
[31] Heim A, Grumbach IM, Zeuke S, et al. Highly sensitive detection of gene expression of an intronless gene: amplification of mRNA, but not genomic DNA by nucleic acid sequence based amplification (NASBA). Nucleic Acids Res 1998;26:2250–1.
[32] Tjalma WA, Fiander A, Reich O, et al. Differences in human papillomavirus type distribution in high-grade cervical intraepithelial neoplasia and invasive cervical cancer in Europe. Int J Cancer 2013; 132:854–67.
[33] de Sanjose S, Quint WGV, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010;11:1048–56.
[34] Denny L, Adewole I, Anorlu R, et al. Human papillomavirus prevalence and type distribution in invasive cervical cancer in sub-Saharan Africa. Int J Cancer 2014;134:1389–98.
[35] Basu P, Roychowdhury S, Bafna UD, et al. Human papillomavirus genotype distribution in cervical cancer in India: results from a multi-center study. Asian Pac J Cancer P 2009;10:27–34.
[36] IARC Working Group on the Evaluation of Carcinogenic Risks to HumansHuman papillomaviruses. IARC Monogr Eval Carcinog Risks Hum 2007;90:1–636.
[37] IARC Working Group on the Evaluation of Carcinogenic Risks to HumansBiological Agents. A review of human carcinogens. IARC Monogr Eval Carcinog Risks Hum 2012;100(Pt B):1–441.
[38] Koshiol J, Lindsay L, Pimenta JM, et al. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol 2008;168:123–37.
[39] Schiffman M, Castle PE, Jeronimo J, et al. Human papillomavirus and cervical cancer. Lancet 2007;370:890–907.
[40] Kjaer S, Hogdall E, Frederiksen K, et al. The absolute risk of cervical abnormalities in high-risk human papillomavirus-positive, cytologically normal women over a 10-year period. Cancer Res 2006;66:10630–6.
[41] Schiffman M, Herrero R, Desalle R, et al. The carcinogenicity of human papillomavirus types reflects viral evolution. Virology 2005;337:76–84.
[42] Naucler P, Ryd W, Tornberg S, et al. HPV type-specific risks of high-grade CIN during 4 years of follow-up: a population-based prospective study. Brit J Cancer 2007;97:129–32.
[43] Khan MJ, Castle PE, Lorincz AT, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst 2005;97:1072–9.
[44] Castle PE, Glass AG, Rush BB, et al. Clinical human papillomavirus detection forecasts cervical cancer risk in women over 18 years of follow-up. J Clin Oncol 2012;30:3044–50.
[45] Sorbye SW, Fismen S, Gutteberg TJ, et al. Primary cervical cancer screening with an HPV mRNA test: a prospective cohort study. BMJ Open 2016;6:e011981.
[46] Naucler P, Mabota da Costa F, da Costa JL, et al. Human papillomavirus type-specific risk of cervical cancer in a population with high human immunodeficiency virus prevalence: case-control study. J Gen Virol 2011;92:2784–91.
[47] De Vuyst H, Ndirangu G, Moodley M, et al. Prevalence of human papillomavirus in women with invasive cervical carcinoma by HIV status in Kenya and South Africa. Int J Cancer 2012;131:949–55.
[48] Williamson AL, Marais D, Passmore JA, et al. Human papillomavirus (HPV) infection in Southern Africa: prevalence, immunity, and vaccine prospects. IUBMB Life 2002;53:253–8.
[49] Strickler HD, Palefsky JM, Burk RD. HPV types present in invasive cervical cancers of HIV-seropositive women. Int J Cancer 2008;123: 1224–5.

Paper II

cancers

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Article

Risk of Intraepithelial Neoplasia Grade 3 or Worse (CIN3+) among Women Examined by a 5-Type HPV mRNA Test during 2003 and 2004, Followed through 2015

Amir Rad [1,2,*], Sveinung Wergeland Sørbye [3], Sweta Tiwari [1], Maja-Lisa Løchen [1,2] and Finn Egil Skjeldestad [1]

[1] Department of Community Medicine, UiT The Arctic University of Norway, 9037 Tromsø, Norway; sweta.tiwari@uit.no (S.T.); maja-lisa.lochen@uit.no (M.-L.L.); finn.e.skjeldestad@uit.no (F.E.S.)
[2] Department of Clinical Medicine, UiT The Arctic University of Norway, 9037 Tromsø, Norway
[3] Department of Pathology, University Hospital of North Norway, 9019 Tromsø, Norway; sveinung.wergeland.sorbye@unn.no
* Correspondence: amir.rad@uit.no

Simple Summary: Cervical cancer is the fourth most common cancer in women worldwide. Persistent high-risk human papillomavirus (HPV) can cause invasive cervical cancer through a series of precancerous lesions. Screening women with either cytology examinations or a test detecting HPV infection can prevent cervical cancer. HPV tests are substituting cytology examinations in cervical cancer screening, but more knowledge is needed about the screening performance of HPV tests, especially among younger women. We aimed to determine the long-term performance of a five-type HPV mRNA test to predict CIN3+. These results contribute to the knowledge of the reliability of HPV mRNA testing in cervical cancer screening. Our findings suggest that women with a negative result may extend the screening interval up to 10 years.

Abstract: Background: The study's purpose was to evaluate the performance of a five-type HPV mRNA test to predict cervical intraepithelial neoplasia grade 3 or worse (CIN3+) during up to 12 years of follow-up. Methods: Overall, 19,153 women were recruited by gynecologists and general practitioners in different parts of Norway between 2003 and 2004. The study population comprised 9582 women of these women, aged 25–69 years with normal cytology and a valid five-type HPV mRNA test at baseline. Follow-up for CIN3+ through 2015 was conducted in the Norwegian Cervical Cancer Screening Programme. Results: The cumulative incidence of CIN3+ by baseline status for HPV mRNA-positive and mRNA-negative women were 20.8% and 1.1%, respectively ($p < 0.001$). Age did not affect the long-term ability of the HPV mRNA test to predict CIN3+ during follow-up. Conclusion: The low long-term risk of CIN3+ among HPV mRNA-negative women and the high long-term risk among HPV mRNA-positive women strengthen the evidence that the five-type HPV mRNA test is an appropriate screening test for women of all ages. Our findings suggest that women with a negative result may extend the screening interval up to 10 years.

Keywords: cervical cancer screening; screening; HPV mRNA test; CIN3+

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1. Introduction

According to the last global ranking by the World Health Organization (WHO) in 2020, cervical cancer was ranked as the fourth most common cancer in women [1]. High-risk human papillomavirus (HPV) infection is a necessary cause of cervical cancer, which develops slowly over the course of several years through a series of precancerous lesions [2]. By cervical cancer screening of women in the target age group, followed by treatment of detected precancerous lesions and the development of invasive cervical cancer can be prevented. Several randomized trials have shown that cervical cancer screening by HPV

testing is more effective than cytology-based screening [3] and several HPV tests have been validated for this purpose. The main differences between HPV tests are which nucleic acid they detect—that of targeted HPV genes (DNA) or that of the transcription of the HPV genome (mRNA)—and the ability of the test to detect and distinguish among HPV types [4].

Cervical cancer is not caused by the HPV infection per se but by the continuous over-expression of the E6 and E7 oncogenes of high-risk HPV types [5]. Expression of the E6 and E7 oncogenes of HPV16, 18, 31, 33 and 45 have been detected in the majority of cervical carcinomas [6]. HPV mRNA tests detect the presence of HPV at the transcriptional level, which indicates continuous expression of the E6 and E7 oncogenes [7]. While both HPV DNA and HPV mRNA tests show high sensitivity to predict cervical intraepithelial neoplasia grade 3 or worse (CIN3+), HPV mRNA tests have a higher specificity than HPV DNA tests [8–10]. The lower specificity of HPV DNA tests is more pronounced in younger women [11].

Most longitudinal studies have evaluated the risk of high-grade cervical lesions in women screened with HPV DNA tests. A 10-year cohort study showed cumulative incidence rates for CIN3+ of 17.2% and 13.6%, respectively, in women who were either HPV16+ or HPV18+ at the screening time [12]. However, the corresponding cumulative incidence rate in women positive for all other HPV types was only 3.0%, which was still higher than the rate among women who were HPV negative at the screening time (0.8%) [12]. Another longitudinal study reported 12-year cumulative risks of CIN3+ among women positive for HPV16, 18, 31 and 33 at the screening time of 26.7%, 19.1%, 14.3% and 14.9%, respectively [13].

In Norway, HPV DNA testing started to be implemented for cervical cancer screening in women aged 34–69 years in 2015 but women 25–33 years are still screened with cytology [14]. Since 2023, HPV DNA testing started to be applied every five years for all women aged 25–69 years in Norway [15]. HPV mRNA tests have been reported to be more specific than HPV DNA tests in the triage of women with minor cervical lesions at screening [9,16,17], but there is a gap in the literature and few published studies on the long-term performance of HPV mRNA tests in screening. This study aimed to evaluate the ability of a five-type HPV mRNA test to predict high-grade cervical lesions during approximately 12 years of follow-up. This study is an update of our previous publication on the performance of a five-type HPV mRNA test (PreTect HPV-Proofer, PreTect AS, Klokkarstua, Norway) in screening with histologically confirmed CIN3+ [18] with extended follow-up time. In this update, the "normal" and "unsatisfactory" cytology status were distinguished in our data source.

2. Materials and Methods

2.1. Data Source and Study Sample

This study received and used data from the Norwegian Cervical Cancer Screening Programme (NCCSP), a division of the Cancer Registry of Norway (CRN), which records the cytology results, HPV results and biopsy results generated in all Norwegian laboratories. Cytology results are classified according to the Bethesda system [19], and histology results are reported applying CIN nomenclature [20]. We applied the 11-digit personal identification number appointed to all Norwegian citizens or immigrants to merge lifetime data on cervical cytology and histology from four national registries administered by the CRN.

Overall, 19,153 women, 13–87 years of age were recruited by gynecologists and general practitioners in different parts of Norway between 1 May 2003 and 31 December 2004. This study was conducted by the departments of pathology and microbiology, University Hospital of Northern Norway, Tromsø. We then excluded women outside the target screening age (younger than 25 and older than 69 years old), women with previous atypical squamous cells of undetermined significance or worse (ASC-US+) cytology, women with a previous histology diagnosis of CIN1+, women with unsatisfactory cytology or no cytology sample collected at baseline and those with no follow-up information. This resulted in a

final analytical sample of 9582 women (Table 1). We then followed these women for CIN3+ through 2015.

Table 1. Selection of study sample from 19,153 eligible women. Characteristics at baseline. ASC-US+: atypical squamous cells of undetermined significance or worse, CIN1+: cervical intraepithelial neoplasia grade 1 or worse.

Eligible for Study Participation		**19,153**
Exclusion Criteria	***n***	
Age < 25 years	2020	
Age > 69 years	223	
Previous diagnosis of CIN1+	883	
Previous ASC-US+ cytology	4756	
Unsatisfactory cytology	501	
No cytology sample collected	627	
No follow-up information	561	
Total exclusions	9571	
Final study sample		**9582**

2.2. Screening Guidelines

During the screening period, Norwegian health authorities recommended all women aged 25–69 years be screened with cytology every 3 years [21]. There were no recommendations for HPV testing. The follow-up recommendations during the baseline period were precisely clarified on the website of the CRN [21]. Women with high-grade cytology (atypical squamous cells cannot rule out high-grade lesions/high-grade squamous intraepithelial lesions) were immediately referred to colposcopy and biopsy. Women with ASC-US or low-grade squamous intraepithelial lesions were recommended repeated cytology after 6 months. Women with twice repeated ASC-US/LSIL received triage by repeated cytology after an additional 12 months. Women with 3 times ASC-US/LSIL within 18 months were referred to colposcopy and biopsy. In 2005, delayed HPV triage was implemented in Norwegian screening guidelines. Women with ASC-US/LSIL were referred to repeat cytology and HPV testing after 6–12 months. Women with a positive HPV test and abnormal cytology were recommended for colposcopy and biopsy. Women with a positive HPV test and normal cytology were referred to repeat HPV testing after 12 months. Women with two positive HPV tests in row were referred for colposcopy and biopsy.

2.3. Human Papillomavirus mRNA Testing

All HPV mRNA testing conducted during the baseline period took place at the same laboratory. The HPV mRNA test was performed only one time for each woman in the study at the screening time. Among women screened by conventional cytology, an extra specimen was collected and placed in a methanol-containing transport medium (PreTect TM, PreTect AS, Klokkarstua, Norway) for the purpose of HPV mRNA testing. For women screened by liquid-based cytology (LBC), the residual LBC sample preserved in ThinPrep-solution (Hologic Inc., Marlborough, MA, USA) was used for HPV mRNA analysis. From the cervical material, RNA was isolated and preserved in PreTect TM or Thin-Prep medium. HPV mRNA analysis was conducted for all samples by PreTect HPV-Proofer (PreTect AS, Klokkarstua, Norway) according to manufacturer's instructions. The test detects E6/E7 mRNA transcripts from HPV types 16, 18, 31, 33 and 45 with simultaneous genotype-specific identification including a sample integrity control ensuing sample adequacy. The HPV mRNA testing for women took place between 1 May 2003 and 31 December 2004.

2.4. Outcome

The women in the study sample were followed through 2015 for histologically confirmed CIN3+. Only cervical cancer cases that were validated by the CRN against hospital

pathology reports were considered true cases of cancer. CIN2, CIN1 and no CIN were considered as absence of disease.

2.5. Statistical Analyses

We applied the Pearson Chi-square test for comparisons, Kaplan–Meier survival analyses to show the cumulative status of considered variables during follow-up, and the Wilcoxon (Gehan) statistical method to make pairwise comparisons of categories. 1-survival curves were used to display the cumulative incidence of CIN3+ by HPV status, type and age group.

HPV status was assessed as positive or negative by type: HPV16, HPV18 and HPV31/33/45 (the prevalence of these three types was low, so they were combined). Categorization by HPV type was conducted in hierarchical fashion, i.e., women with multiple infections were assigned to a single category in the following order: HPV16, HPV18 and HPV31/33/45 to reflect the oncogenicity of these HPV types. Age was categorized as 25–33 years and 34–69 years. We had a total of 11 years and 8 months follow-up (mean of 99.8 months), but data are shown for 10 years (120 months), due to the small number of women that remained in the analyses thereafter. All analyses were performed in SPSS version 29.0, and p-values < 0.05 were considered significant.

3. Results

3.1. Human Papillomavirus Status at Baseline

Of the 9582 women included in the present analysis, 3.2% (n = 303) were HPV mRNA-positive at baseline; 1.5% (n = 140) for HPV16, 0.5% (n = 44) for HPV18 and 1.2% (n = 119) for HPV31/33/45. Among HPV mRNA-positive women, 46.2% were positive for HPV16, 14.6% for HPV18 and 39.3% for HPV31/33/45. HPV 16 is the most prevalent HPV type, which is followed by HPV45, HPV18, HPV33 and HPV31. At the screening time, 27.2% of women were aged 25–33 years and 72.8% were aged 34–69 years. The HPV positivity rate was 2.6 times higher in the younger than the older age group (5.7% vs. 2.2%; $p < 0.001$; Table 2). Similar age differences were observed for type-specific HPV positivity ($p < 0.001$; Table 2).

Table 2. Human papillomavirus (HPV) status at baseline by age. Positivity rates were significantly different in each row ($p < 0.001$).

HPV Status *	25–33 Years n = 2610 (%)	34–69 Years n = 6972 (%)	Total n = 9582 (%)
HPV Negative	94.3	97.8	96.8
HPV Positive	5.7 *	2.2 *	3.2
HPV16	2.8 *	1.0 *	1.5
HPV18	0.8 *	0.3 *	0.5
HPV31/33/45	2.1 *	0.9 *	1.2

* Categorization by HPV type was conducted in a hierarchical approach, i.e., women with multiple infections were assigned to a single category in the following order: HPV16, HPV18 and HPV31/33/45, to reflect the oncogenicity of these HPV types. Paired comparisons in each row of the table were significant according to Pearson X^2 test results ($p < 0.001$).

3.2. Cumulative Incidence of Cervical Intraepithelial Neoplasia Grade 3 or Worse

Of the 303 women who were HPV-positive at the screening time, the cumulative incidence of CIN3+ during follow-up was 20.8% (n = 63). Among the 9279 women who were HPV-negative, this incidence was 1.1% (n = 104) ($p < 0.001$).

3.3. Cervical Intraepithelial Neoplasia Grade 3 or Worse by Human Papillomavirus Type

The cumulative incidence of CIN3+ was alike among HPV16+ and HPV18+ women (22.9% and 22.7%, respectively; p = 0.836) (Figure 1). The cumulative incidence of CIN3+ was higher among HPV16+ or HPV18+ women than HPV31/33/45-positive women, but

this difference was not statistically significant (15.1%; $p = 0.118$). Among HPV-negative women, the cumulative incidence of CIN3+ was 1.1% (Figure 1).

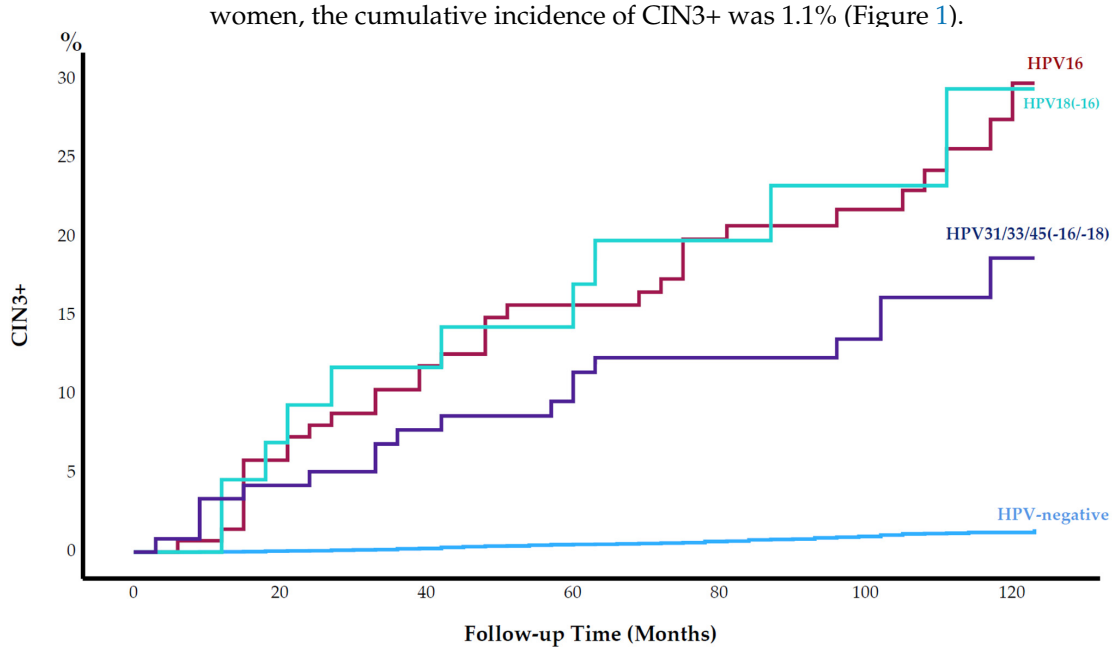

Figure 1. Cumulative incidence of cervical intraepithelial neoplasia grade 3 or worse (CIN3+) (%) by human papillomavirus (HPV) type from baseline throughout 120 months of follow-up.

3.4. Cervical Intraepithelial Neoplasia Grade 3 or Worse by Age

The cumulative incidence of CIN3+ among women aged 25–33 years was significantly higher than that among women aged 34–69 years during follow-up (2.2% vs. 1.6%, respectively; $p = 0.028$). After stratifying for HPV status, we observed no statistically significant differences in the cumulative incidence of CIN3+ between the age groups (Figure 2). During the follow-up period, the difference between the cumulative incidence of CIN3+ among women aged 25–33 years and women aged 34–69 years remained insignificant.

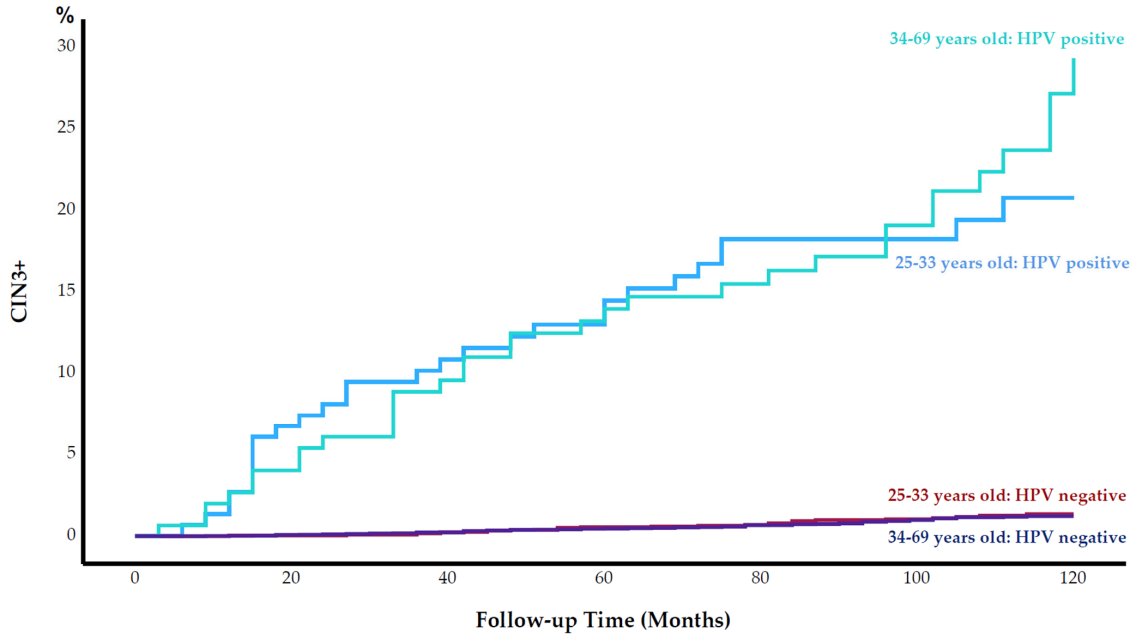

Figure 2. Cumulative incidence of cervical intraepithelial neoplasia grade 3 or worse (CIN3+) (%) by human papillomavirus (HPV) status and by age group during 120 months of follow-up.

3.5. Cancer Cases

Five cervical cancers were diagnosed during follow-up; all in women aged older than 38 years. Two were HPV-negative at baseline, one was positive for HPV16, one for HPV18 and one for HPV45. The overall cervical cancer incidence rate was 5 per 100,000 woman years. Cervical cancer incidence rates for HPV-positive and HPV-negative women were 99 (CI: 79.5–118.5) and 2 (CI: 0.8–4.8) per 100,000 woman years, respectively. Three women were diagnosed within the NCCSP, after being referred due to abnormal cytology, whereas two cases were diagnosed during delayed screening or after referral due to the appearance of symptoms. Four cases were in cancer stage I. One case was in cancer stage 2B. Table 3 displays the characteristics of the five cervical cancer cases over the study years.

Table 3. Characteristics of cervical cancer cases.

Case No.	At Study Start				At Diagnosis			
	Age (yrs.)	HPV Type	Screening History Prior to Study Start	Time to Last Smear Prior to Study Start (Months)	Diagnosed in	Time from Study Start (Months)	Histological Type	Stage
1	38	45	1 normal smear	32	Regular screening	93	SCC	1
2	39	16	8 normal smears	23	Regular screening	38	SCC	1
3	41	18	3 normal smears	34	Delayed screening	58	ADC	2B
4	48	Neg.	4 normal smears	33	Regular screening	28	SCC	1A
5	51	Neg.	6 normal smears	27	Delayed screening	65	SCC	1

SCC stands for squamous cell carcinoma. ADC stands for adenocarcinoma. yrs. stand for years. Neg. stands for negative.

4. Discussion

4.1. Cervical Intraepithelial Neoplasia Grade 3 or Worse among Human Papillomavirus-Positive Women

The 10-year cumulative incidence of CIN3+ among the HPV mRNA-positive women was 20.8% (CI: 16.2–25.4). The ability of the five-type HPV mRNA-positive test to predict CIN3+ in our study was higher than the 12-year cumulative risks of CIN3+ among 13-type HPV DNA-positive women in a Danish study (14%) [13] and the 10-year cumulative risks of CIN3+ among 13-type HPV DNA-positive women in a US study (11.3%) [12].

4.2. Cervical Intraepithelial Neoplasia Grade 3 or Worse among Human Papillomavirus-Negative Women

The cumulative incidence of CIN3+ within 6 years of follow-up was 0.62% in our previous study [18] while the cumulative incidence of CIN3+ within 10 years of follow-up increased slightly to 1.1% in this study, which can be compared to the 10-year cumulative risk of CIN3+ in 13-type HPV DNA-negative women aged over 16 years [12]. The HPV mRNA test showed equally high sensitivity to predict CIN3+ compared to HPV DNA tests, and it showed a higher specificity than HPV DNA tests [8–10]. Considering these findings, it is rational to argue that women with a negative HPV mRNA test result could wait up to 10 years for their next screening.

This is consistent with the 2021 WHO guideline for screening and treatment of cervical pre-cancer lesions, which recommends that the screening interval be extended to 10 years for women with an HPV DNA-negative test [22].

Therefore, our data support the effectiveness of the five-type HPV mRNA test as an appropriate screening test for women of all ages. However, further research is required to confirm the long-term effectiveness of the HPV mRNA test and to determine the optimal screening interval.

4.3. Age-Independent Ability to Predict Cervical Intraepithelial Neoplasia Grade 3 or Worse

Although the HPV prevalence was higher in the younger than the older age group, the cumulative incidence of CIN3+ was similar among HPV-positive women and HPV-negative women (Figure 2). In a 30 year follow-up study of women screening by HPV DNA test, the highest prevalence of HPV was observed among women aged 25–33 years; however, the risk of cervical cancer was low among women in this age group [23]. Moreover, due to the high HPV DNA positivity rate and low specificity of the HPV DNA test, screening of women younger than 35 years old by HPV DNA test was not recommended [3]. This age-related difference in performance between the HPV mRNA test and an HPV DNA test might be due to what the tests detect on a molecular level. While detection at the DNA level might be infections in a transient phase, with a higher probability of regression, detection at the mRNA level implies an integration phase of the virus into the host genome with more likelihood to progress to a cervical lesion.

4.4. Human Papillomavirus Positivity Rate

The specificity of a screening test is an essential characteristic for an effective screening program, as it accurately identifies individuals who will not develop the disease, minimizing false-positive results. A highly specific screening test with a low positivity rate results in a low follow-up rate, which is a critical aspect of screening programs. In this study, we applied a five-type HPV mRNA test in screening, which has been demonstrated to have higher specificity and lower positivity rates than 14-type HPV DNA tests [8,9,24,25]. The overall HPV mRNA positivity rate was 3.2% at screening time, with higher rates observed in women aged 25–33 years (5.7%) compared to those aged 34–69 years (2.2%). This positivity rate was lower than the HPV DNA positivity rates reported in other studies [12,13] such as an American study reporting a positivity rate of 5.1% in women aged 30 years and older [26]. In a European meta-analysis, the overall HPV DNA positivity rate was 9.4% in women aged 20–64 years with variations observed between different countries [27]. The lower positivity rate of the HPV mRNA test in this study is consistent with its higher specificity, suggesting that it may cause fewer false-positive results and a lower referral rate. Although we were unable to determine the sensitivity and specificity of the HPV mRNA test in this study due to the lack of confirmed histological results for all women, its low positivity rate and the observed low long-term risk of CIN3+ among HPV mRNA-negative women support its effectiveness as an appropriate screening test for women of all ages.

4.5. Human Papillomavirus Types Included in the mRNA Test

The question of which HPV types should be included in an HPV test used for screening is a matter of discussion. The risk of CIN3+ is strongly associated with persistent infection with HPV16, 18, 31, 33 and 45 [12,13,28–34]. The HPV mRNA test in our study detects these five high-risk HPV types. A previous report showed that 60.6% of invasive cervical cancers were attributable to HPV16 infection alone, and 70.8% were attributable to HPV16 and/or HPV18 infections [35]. The proportion increases to 84.3% of invasive cervical cancers when expanding the number of high-risk HPV types to five (HPV16, 18, 31, 33 and 45) [35]. A Swedish study examined 808 screen-detected invasive cervical cancers and found six HPV types (16, 18, 31, 33, 45 or 52) in 85.3% of the cases [36]. HPV35, 39, 51, 56, 58, 59, 66 and 68 were detected in only 12 cases (1.5%, for all eight types combined). Therefore, limiting screening to the types included in the five-type HPV mRNA test could greatly improve the specificity of screening programs [36]. The nine-valent HPV vaccine (Gardasil 9) includes HPV6, 11, 16, 18, 31, 33, 45, 52 and 58. Thus, for HPV-vaccinated women, screening tests should be limited to the seven high-risk HPV types (16, 18, 31, 33, 45, 52 and 58), as screening for all 14 HPV types might result in a suboptimal balance of harms and benefits [37]. The results from our study indicate that five HPV types may be sufficient for use in cervical cancer screening.

4.6. Cancer Cases

The incidence rates of cervical cancer in HPV mRNA-positive and -negative women were 99 (CI: 79.5–118.5) and 2 (CI: 0.8–4.8) per 100,000 woman per year. The incidence of cervical cancer in women with a negative five-type HPV mRNA test in this study is comparable to that among women with a negative 13-type HPV DNA test in other studies [26,27]. In a meta-analysis of four European countries, the cumulative incidence rate of cancer in women with a negative HPV DNA test at the screening was 2 per 100,000 woman per year [27] at 6.5 years of follow-up. The cumulative cancer rates among HPV DNA-negative women in Italy, the Netherlands, Sweden and England were 0.5, 0.3, 2.9 and 2.6 per 100,000 woman per year, respectively [27]. In an American study including 315,061 women, the incidence rate of cervical cancer was 3.8 per 100,000 woman per year in HPV DNA-negative women [26]. The low incidence rates of cervical cancer after a negative HPV test are in line with the World Health Organization's strategy and goals to reach and maintain a cervical cancer incidence rate below 4 per 100,000 woman per year [38]. Another point regarding the results of cancer cases in our study is that three to eight years had passed from the HPV mRNA test positive results at the screening time until cervical cancer cases were diagnosed (Table 3). If the women with a positive HPV mRNA test were followed up more closely and received treatment for the precancerous stages before the development of cancer, more cancer cases could be prevented.

4.7. Strengths

The NCCSP at the CRN is a nationwide, register-based platform starting in 1995. Compulsory reporting from cytology and pathology departments to the CRN is unique and allowed us to obtain improved information on cytology and histology some years prior to the study's start, at baseline and during follow-up. Other advantages of this study include the relatively large study sample of women with normal cytology and the long follow-up time.

The HPV laboratory worked independently of cytology laboratories. They were, thus, blinded to cytology results at baseline; cytology laboratories were similarly blinded to HPV mRNA results at baseline.

4.8. Limitation

Due to the lack of HPV tests before and/or after the HPV mRNA test at screening, it was impossible for us to know about the persistence of HPV infections before and/or after screening. Another limitation was incomplete screening histories and treatment of CIN before 1995. Determining the sensitivity and specificity of the HPV mRNA test would be desirable; however, the study did not comprise confirmed histological results for all women.

5. Conclusions

The low long-term risk of CIN3+ among HPV mRNA-negative women, and the high long-term risk among HPV mRNA-positive women strengthens the evidence that the five-type HPV mRNA test is an appropriate screening test for women of all ages. Our findings suggest that women with a negative HPV mRNA result may extend the screening interval up to 10 years.

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Informed Consent Statement: National health registries are exempted from informed consent at the same time as participants were informed about their rights to withhold information on request. As researchers, we worked with anonymous data with no access to laboratory identification nor any personal data except for age.

Data Availability Statement: The data presented in this study are available in this article.

Conflicts of Interest: The authors A.R. and S.T. 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. The author M.-L.L. has received lecture fees from Sanofi, Bayer and BMS/Pfizer with no relation to this study. S.W.S. has received travel grants from PreTect. F.E.S. received reimbursement for travel expenses for Eurogin 2022 from PreTect.

References

1. The Global Cancer Observatory. World-Source: Globocan 2020. 2021. Available online: http://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf (accessed on 1 April 2023).
2. Cuschieri, K.S.; Cubie, H.A.; Whitley, M.W.; Gilkison, G.; Arends, M.J.; Graham, C.; McGoogan, E. Persistent high risk HPV infection associated with development of cervical neoplasia in a prospective population study. *J. Clin. Pathol.* **2005**, *58*, 946–950. [CrossRef]
3. 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.; 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] [PubMed]
4. Arbyn, M.; Snijders, P.J.F.; Meijer, C.J.L.M.; Berkhof, J.; Cuschieri, K.; Kocjan, B.J.; Poljak, M. Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? *Clin. Microbiol. Infect.* **2015**, *21*, 817–826. [CrossRef] [PubMed]
5. Woodman, C.B.; Collins, S.I.; Young, L.S. The natural history of cervical HPV infection: Unresolved issues. *Nat. Rev. Cancer* **2007**, *7*, 11–22. [CrossRef] [PubMed]
6. Kraus, I.; Molden, T.; Holm, R.; Lie, A.K.; Karlsen, F.; Kristensen, G.B.; Skomedal, H. Presence of E6 and E7 mRNA from human papillomavirus types 16, 18, 31, 33, and 45 in the majority of cervical carcinomas. *J. Clin. Microbiol.* **2006**, *44*, 1310–1317. [CrossRef]
7. Rad, A.; Sorbye, S.W.; Dreyer, G.; Hovland, S.; Falang, B.M.; Louw, M.; Skjeldestad, F.E. HPV types in cervical cancer tissue in South Africa: A head-to-head comparison by mRNA and DNA tests. *Medicine* **2017**, *96*, e8752. [CrossRef]
8. Westre, B.; Giske, A.; Guttormsen, H.; Sorbye, S.W.; Skjeldestad, F.E. 5-type HPV mRNA versus 14-type HPV DNA test: Test performance, over-diagnosis and overtreatment in triage of women with minor cervical lesions. *BMC Clin. Pathol.* **2016**, *16*, 9. [CrossRef]
9. Sorbye, S.W.; Fismen, S.; Gutteberg, T.J.; Mortensen, E.S.; Skjeldestad, F.E. HPV mRNA is more specific than HPV DNA in triage of women with minor cervical lesions. *PLoS ONE* **2014**, *9*, e112934. [CrossRef]
10. Ratnam, S.; Coutlee, F.; Fontaine, D.; Bentley, J.; Escott, N.; Ghatage, P.; Gadag, V.; Holloway, G.; Bartellas, E.; Kum, N.; et al. Aptima HPV E6/E7 mRNA test is as sensitive as Hybrid Capture 2 Assay but more specific at detecting cervical precancer and cancer. *J. Clin. Microbiol.* **2011**, *49*, 557–564. [CrossRef] [PubMed]
11. Reinholdt, K.; Juul, K.E.; Dehlendorff, C.; Munk, C.; Kjær, S.K.; Thomsen, L.T. Triage of low-grade squamous intraepithelial lesions using human papillomavirus messenger ribonucleic acid tests—A prospective population-based register study. *Acta Obstet. Gynecol. Scand.* **2020**, *99*, 204–212. [CrossRef]
12. Khan, M.J.; Castle, P.E.; Lorincz, A.T.; Wacholder, S.; Sherman, M.; Scott, D.R.; Rush, B.B.; Glass, A.G.; Shiffman, M. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J. Natl. Cancer Inst.* **2005**, *97*, 1072–1079. [CrossRef] [PubMed]
13. Kjaer, S.K.; Frederiksen, K.; Munk, C.; Iftner, T. Long-term Absolute Risk of Cervical Intraepithelial Neoplasia Grade 3 or Worse Following Human Papillomavirus Infection: Role of Persistence. *J. Natl. Cancer Inst.* **2010**, *102*, 1478–1488. [CrossRef] [PubMed]
14. Cancer Registry of Norway. HPV in Primary Screening. 2018. Available online: https://www.kreftregisteret.no/screening/livmorhalsprogrammet/Helsepersonell/screeningstrategi-og-nasjonale-retningslinjer/HPV-i-primarscreening/ (accessed on 1 April 2023).
15. Cancer Registry of Norway. Primary HPV Test—Now Also for Women under 34. 2023. Available online: https://www.kreftregisteret.no/screening/livmorhalsprogrammet/hpv/ (accessed on 1 April 2023).
16. Szarewski, A.; Mesher, D.; Cadman, L.; Austin, J.; Ashdown-Barr, L.; Ho, L.; Terry, G.; Liddle, S.; Young, M.; Stoler, M.; et al. Comparison of seven tests for high-grade cervical intraepithelial neoplasia in women with abnormal smears: The Predictors 2 study. *J. Clin. Microbiol.* **2012**, *50*, 1867–1873. [CrossRef]

17. Arbyn, M.; Roelens, J.; Cuschieri, K.; Cuzick, J.; Szarewski, A.; Ratnam, S.; Reuschenbach, M.; Belinson, S.; Belinson, J.L.; Monsonego, J. The APTIMA HPV assay versus the Hybrid Capture 2 test in triage of women with ASC-US or LSIL cervical cytology: A meta-analysis of the diagnostic accuracy. *Int. J. Cancer* **2013**, *132*, 101–108. [CrossRef] [PubMed]
18. Sorbye, S.W.; Fismen, S.; Gutteberg, T.J.; Mortensen, E.S.; Skjeldestad, F.E. Primary cervical cancer screening with an HPV mRNA test: A prospective cohort study. *BMJ Open* **2016**, *6*, e011981. [CrossRef]
19. International Agency for Research on Cancer (IARC). Cytopathology of the Uterine Cervix—Digital Atlas (The Bethesda System). 2021. Available online: https://screening.iarc.fr/atlasclassifbethesda.php (accessed on 1 April 2023).
20. World Health Organization (WHO). Comprehensive Cervical Cancer Control: A guide to essential practice. In *Comprehensive Cervical Cancer Control: A Guide to Essential Practice*; WHO: Geneva, Switzerland, 2006.
21. Cancer Registry of Norway. Masseundersøkelsen mot Livmorhalskreft. 2008. Available online: www.kreftregisteret.no/globalassets/publikasjoner-og-rapporter/livmorhalskreft/rapport_screeningintervall.pdf (accessed on 1 April 2023).
22. World Health Organization (WHO). *WHO Guideline for Screening and Treatment of Cervical Pre-Cancer Lesions for Cervical Cancer Prevention*, 2nd ed.; WHO: Geneva, Switzerland, 2021.
23. Riibe, M.O.; Sorbye, S.W.; Simonsen, G.S.; Sundsfjord, A.; Ekgren, J.; Maltau, J.M. Risk of cervical intraepithelial neoplasia grade 3 or higher (CIN3+) among women with HPV-test in 1990–1992, a 30-year follow-up study. *Infect. Agents Cancer* **2021**, *16*, 46. [CrossRef]
24. Rad, A.; Sørbye, S.W.; Brenn, T.; Tiwari, S.; Løchen, M.-L.; Skjeldestad, F.E. 13-Type HPV DNA Test versus 5-Type HPV mRNA Test in Triage of Women Aged 25–33 Years with Minor Cytological Abnormalities–6 Years of Follow-Up. *Int. J. Environ. Res. Public Health* **2023**, *20*, 4119. [CrossRef]
25. Cuzick, J.; Cadman, L.; Mesher, D.; Austin, J.; Ashdown-Barr, L.; Ho, L.; Terry, G.; Liddle, S.; Wright, C.; Lyons, D.; et al. Comparing the performance of six human papillomavirus tests in a screening population. *Br. J. Cancer* **2013**, *108*, 908–913. [CrossRef]
26. 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] [PubMed]
27. Ronco, G.; Dillner, J.; Elfstrom, K.M.; Tunesi, S.; Snijders, P.J.; Arbyn, M.; Kitchener, H.; Segnan, N.; Gilham, C.; Giorgi-Rossi, P.; et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: Follow-Up of four European randomised controlled trials. *Lancet* **2014**, *383*, 524–532. [CrossRef]
28. Ramakrishnan, S.; Partricia, S.; Mathan, G. Overview of high-risk HPV's 16 and 18 infected cervical cancer: Pathogenesis to prevention. *Biomed. Pharmacother.* **2015**, *70*, 103–110. [CrossRef] [PubMed]
29. Powell, N.G.; Hibbitts, S.J.; Boyde, A.M.; Newcombe, R.G.; Tristram, A.J.; Fiander, A.N. The risk of cervical cancer associated with specific types of human papillomavirus: A case-control study in a UK population. *Int. J. Cancer* **2011**, *128*, 1676–1682. [CrossRef] [PubMed]
30. Moberg, M.; Gustavsson, I.; Wilander, E.; Gyllensten, U. High viral loads of human papillomavirus predict risk of invasive cervical carcinoma. *Br. J. Cancer* **2005**, *92*, 891–894. [CrossRef] [PubMed]
31. International Agency for Research on Cancer. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Human Papillomaviruses*; International Agency for Research on Cancer: Lyon, France, 2007; pp. 1–636.
32. Castle, P.E.; Rodriguez, A.C.; Burk, R.D.; Herrero, R.; Wacholder, S.; Alfaro, M.; Morales, J.; Guillen, D.; Sherman, M.E.; Solomon, D.; et al. Short term persistence of human papillomavirus and risk of cervical precancer and cancer: Population based cohort study. *Br. Med. J.* **2009**, *339*, b2569. [CrossRef] [PubMed]
33. Castle, P.E.; Glass, A.G.; Rush, B.B.; Scott, D.R.; Wentzensen, N.; Gage, J.C.; Buckland, J.; Rydzak, G.; Lorincz, A.T.; Wacholder, S. Clinical human papillomavirus detection forecasts cervical cancer risk in women over 18 years of follow-up. *J. Clin. Oncol.* **2012**, *30*, 3044–3050. [CrossRef] [PubMed]
34. Naucler, P.; Ryd, W.; Tornberg, S.; Strand, A.; Wadell, G.; Hansson, B.G.; Rylander, E.; Dillner, J. HPV type-specific risks of high-grade CIN during 4 years of follow-up: A population-based prospective study. *Br. J. Cancer* **2007**, *97*, 129–132. [CrossRef]
35. Arbyn, M.; Tommasino, M.; Depuydt, C.; Dillner, J. Are 20 human papillomavirus types causing cervical cancer? *J. Pathol.* **2014**, *234*, 431–435. [CrossRef]
36. Sundstrom, K.; Dillner, J. How many Human Papillomavirus types do we need to screen for? *J. Infect. Dis.* **2021**, *223*, 1510–1511. [CrossRef]
37. Nygard, M.; Hansen, B.T.; Kjaer, S.K.; Hortlund, M.; Tryggvadottir, L.; Munk, C.; Lagheden, C.; Sigurdardottir, L.G.; Campbell, S.; Liaw, K.L.; et al. Human papillomavirus genotype-specific risks for cervical intraepithelial lesions. *Hum. Vaccin. Immunother.* **2021**, *17*, 972–981. [CrossRef]
38. World Health Organization. Cervical Cancer Elimination Initiative. 2021. Available online: https://www.who.int/initiatives/cervical-cancer-elimination-initiative (accessed on 1 April 2023).

Paper III

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MDPI

Article

13-Type HPV DNA Test versus 5-Type HPV mRNA Test in Triage of Women Aged 25–33 Years with Minor Cytological Abnormalities–6 Years of Follow-Up

Amir Rad [1,*], Sveinung Wergeland Sørbye [2], Tormod Brenn [3], Sweta Tiwari [3], Maja-Lisa Løchen [1] and Finn Egil Skjeldestad [3]

[1] Department of Community Medicine & Department of Clinical Medicine, UiT The Arctic University of Norway, 9037 Tromsø, Norway
[2] Department of Pathology, University Hospital of North Norway, 9019 Tromsø, Norway
[3] Department of Community Medicine, UiT The Arctic University of Norway, 9037 Tromsø, Norway
* Correspondence: amir.rad@uit.no

Abstract: Background: A specific, cost-effective triage test for minor cytological abnormalities is essential for cervical cancer screening among younger women to reduce overmanagement and unnecessary healthcare utilization. We compared the triage performance of one 13-type human papillomavirus (HPV) DNA test and one 5-type HPV mRNA test. Methods: We included 4115 women aged 25–33 years with a screening result of atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesions (LSIL) recorded in the Norwegian Cancer Registry during 2005–2010. According to Norwegian guidelines, these women went to triage (HPV testing and repeat cytology: 2556 were tested with the Hybrid Capture 2 HPV DNA test, which detects the HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68; and 1559 were tested with the PreTect HPV-Proofer HPV mRNA test, which detects HPV types 16, 18, 31, 33, and 45). Women were followed through December 2013. Results: HPV positivity rates at triage were 52.8% and 23.3% among DNA- and mRNA-tested women ($p < 0.001$), respectively. Referral rates for colposcopy and biopsy and repeat testing (HPV + cytology) after triage were significantly higher among DNA-tested (24.9% and 27.9%) compared to mRNA-tested women (18.3% and 5.1%), as were cervical intraepithelial neoplasia grade 3 or worse (CIN3+) detection rates (13.1% vs. 8.3%; $p < 0.001$). Ten cancer cases were diagnosed during follow-up; eight were in DNA-tested women. Conclusion: We observed significantly higher referral rates and CIN3+ detection rates in young women with ASC-US/LSIL when the HPV DNA test was used at triage. The mRNA test was as functional in cancer prevention, with considerably less healthcare utilization.

Keywords: cervical cancer screening; triage; HPV DNA test; HPV mRNA test; CIN3+

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1. Introduction

Cytology-based screening has reduced the incidence and mortality of cervical cancer in countries with organized screening programs [1]. However, because cytology-based screening has low sensitivity in detecting high-grade lesions (cervical intraepithelial neoplasia grade 3 or worse, CIN3+) [2], several countries have replaced it with human papillomavirus (HPV) test-based screening [3–5]. Organized cervical screening programs aim to obtain the best possible balance between reducing the risk of cervical cancer and limiting over-management [6]. European guidelines recommend HPV test-based screening at 5-year intervals for women aged 30–60 years [7], and several countries follow these guidelines [3,8], though some have targeted screening populations of different ages [4,9]. In 2015, Norway implemented HPV DNA testing at 5-year intervals in selected counties to screen women aged 34–69 years [10] but continued to use cytology at 3-year intervals to

screen women aged 25–33 years [10]. Since 2023, HPV testing is also applied for women under 34 years [11].

Indeed, HPV test-based screening for women aged 30 years or younger is not cost-effective, given the high prevalence of HPV infection [12] and low incidence of high-grade cervical lesions in this age group [13,14]. The global HPV prevalence in women aged 25–34 years was reported to be 13.9% [12], compared to 32% in women younger than 30 years in Norway [15]. Moreover, most young women clear their HPV infections within 1 (70%) or 2 years (91%) [16], so cytology-based rather than HPV test-based screening is considered better for young women [17]. Still, the proportion of women with abnormal cytology results in this age group is quite high, making good management strategies essential [18]. Compared to women with normal cytology, women with minor cytological abnormalities have a higher risk of high-grade dysplasia [19]. However, as most minor cytological abnormalities regress spontaneously [20], careful triage is crucial to avoid unnecessary referrals and healthcare utilization. The referral of all women with cytology results of atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesions (LSIL) would result in overmanagement and overtreatment [21].

In a previous study, the reported 5-year risks of CIN3+ for women with screening results of ASC-US and LSIL were 2.6% and 5.2%, respectively [22]. Moreover, the progression rates from normal cytology and ASC-US/LSIL to CIN3 vary by HPV type, being faster for HPV16 than for HPV18, 31, 33, 45, and other oncogenic types [23]. This may make younger women particularly prone to the overtreatment of CIN [21], as a positive HPV test in this age group could trigger a referral and treatment process for infections that would otherwise have cleared spontaneously [20]. The degree of overtreatment will depend upon the number of HPV types targeted by the applied HPV tests, as well as other test properties [23].

It has been shown that a 5-type HPV mRNA test has a higher clinical specificity and positive predictive value than a 14-type HPV DNA test in the triage of women with minor cytological abnormalities at screening [24]. In the present study, we compared the performance of two triage tests—one 13-type HPV DNA test and one 5-type HPV mRNA test—among women aged 25–33 years with screening results of ASC-US or LSIL.

2. Materials and Methods

2.1. Data Source and Study Population

Data were obtained from the Norwegian Cervical Cancer Screening Programme (NCCSP), a division of the Cancer Registry of Norway (CRN). All cytology and pathology departments in Norway must report their results to the CRN, where results are classified according to the Bethesda System [25] and the World Health Organization dysplasia nomenclature [26], respectively. There were 47,705 women with ASCUS/LSIL registered in the NCCSP database during the time of the study. The 11-digit personal identification number assigned to all persons in Norway at birth or immigration was used to merge NCCSP data with that from other databases of the CRN.

2.2. Study Sample and Screening Algorithm

In the present analysis, we aimed to assess the performance of HPV DNA testing (using Hybrid Capture II) and HPV mRNA testing (using PreTect HPV-Proofer) in a specific subpopulation of women in Norway. Our study sample consisted of women aged 25–33 years who underwent cervical cancer screening between 2005–2010 and had a recorded screening result of ASC-US or LSIL in the NCCSP. Inclusion in the study sample required the absence of a previous diagnosis of CIN1+ or HSIL. Pragmatically, we identified 4115 women who met these criteria, thus forming our final study sample. Subsequently, we followed their medical records until 31 December 2013. This population was of interest as, unlike women aged 34–69 years and older who have been screened using HPV testing since 2015, women aged 25–33 years underwent cytological screening, and in cases with minor cytological abnormalities, HPV testing was used for triage. At the end of the post-

screening follow-up period, we defined women with indication for colposcopy and biopsy or repeat testing (HPV and cytology) without it being performed as women with incomplete follow-up.

The screening algorithm that was in effect in Norway during the study period recommended that women with screening results of ASC-US/LSIL attend triage within 6–12 months thereafter; triage consisted of both HPV testing and repeat cytology. Depending on the triage results, the screening algorithm recommended either further follow-up or a return to the 3-year screening interval without further follow-up. To reflect best practices, we categorized the post-triage study sample into three screening algorithm-recommended (SAR) groups: SAR referral for colposcopy and biopsy, SAR referral for repeat testing (HPV and cytology), or SAR return to the 3-year screening interval (i.e., return to routine screening, Figure 1). However, actual clinical practice may deviate from the recommended practice. To increase study power, we expanded the window for triage to 3–18 months after screening results.

2.3. HPV Testing

Data on the type of HPV test used for triage in different laboratories were taken from the NCCSP database. The type of HPV test was not randomized; laboratories in each geographical region made their own decisions about which commercial HPV test to use. When women with minor cytological abnormalities were triaged using conventional or liquid-based cytology (LBC), extra specimens were collected and placed in preservation and transport media for DNA (digene Specimen Transport Medium [27]) and mRNA tests (PreTect TM [28]). We confined our analyses to data reported from laboratories that used either Hybrid Capture 2 (HC2, Qiagen), an HPV DNA test that detects 13 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) [29], or PreTect HPV-Proofer (PreTect AS, Klokkarstua, Norway), an mRNA test that genotypes the E6/E7 full-length mRNA transcripts of five HPV types (16, 18, 31, 33, and 45) [30]. The qualitative assays were based on real-time, nucleic acid sequence-based amplification (NASBA) technology, targeting full-length E6/E7 transcripts, and included an intrinsic sample control to ensure sample adequacy [31]. Positive and negative assay controls corresponding to the viral mRNA for all targets validate the results reported by PreTect Analysis Software (https://www.pretect.no/pretecthpvprooferorg, available online: 22 February 2023). Total nucleic acids were isolated from 1 mL of the leftover LBC material preserved in ThinPrep using PreTect X (PreTect AS, Klokkarstua, Norway) and subsequently analyzed for mRNA expression according to the manufacturer's instructions. The HPV DNA status of the specimens was detected by the hc2 HPV kit following the manufacturer's protocols [29]. The HC2 reports only positive/negative results based on positivity for at least one included HPV type. Consequently, we report positive/negative HPV results for both DNA and mRNA tests.

2.4. Outcomes and Follow-Up

The primary study outcomes were HPV positivity rates at triage, SAR referral rates for colposcopy and biopsy or repeat testing after triage, and CIN3+ detection rates from triage to the end of follow-up (31 December 2013). We also explored CIN3+ detection rates at 42 (one screening interval: 36 months + 6 months) and 78 months (two screening intervals: 72 + 6 months) post-screening. We compared these outcomes between women who received HPV DNA and HPV mRNA testing at triage.

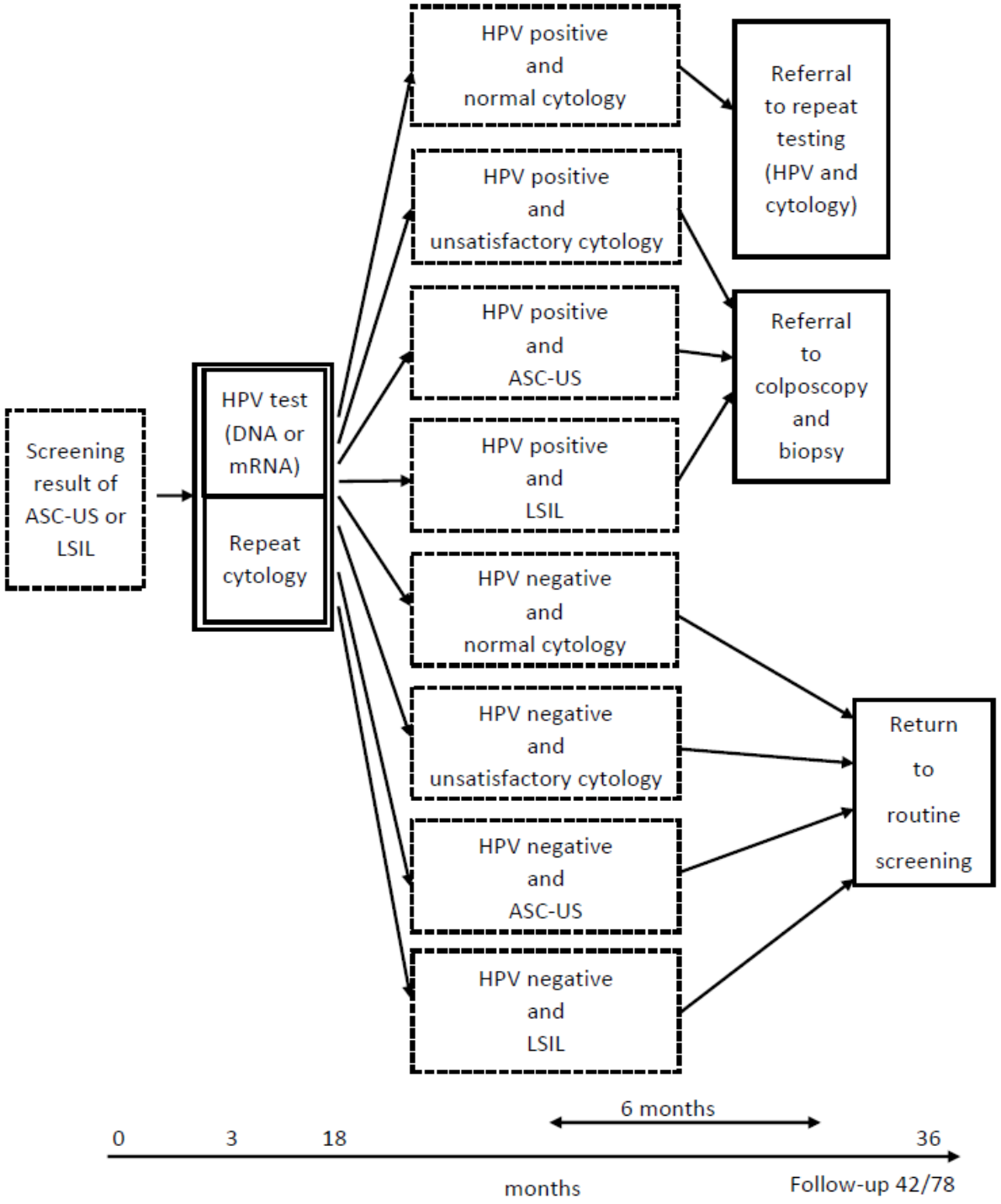

Figure 1. Screening algorithm in Norway during the study period. HPV, human papillomavirus; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesions; return to routine screening, return to the 3-year screening interval without further follow-up.

2.5. Statistical Analyses

All analyses were conducted in SPSS 29.0. Chi-square tests were performed for categorical variables, with a p-value < 0.05 as the significance level. In order to compare the age distributions among HPV DNA-tested and HPV mRNA-tested women, we divided women into age groups of 25–29 and 30–33 years old.

3. Results

Of the 4115 included women, 62.1% (2556/4115) received HPV DNA testing and 37.9% (1559/4115) received HPV mRNA testing at triage. The distributions for age, screening result (ASC-US or LSIL), and the most recent cytology result before screening are reported for DNA-tested and mRNA-tested women in Table 1.

Table 1. Comparison of characteristics among HPV DNA-tested and HPV mRNA-tested women.

		HPV DNA n = 2556 (%)	HPV mRNA n = 1559 (%)	p-Value
Age (years)	25–29	60.4	59.9	0.771
	30–33	39.6	40.1	
Screening result	ASC-US	65.9	68.8	0.06
	LSIL	34.1	31.2	
Most recent cytology result before screening	No Previous Test	35.5	36.0	0.603
	Unsatisfactory	3.0	3.5	
	Normal	61.5	60.5	

Abbreviations: HPV, human papillomavirus; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion.

The HPV positivity rate at triage among DNA-tested women was more than twice that of mRNA-tested women (52.8% vs. 23.3%; $p < 0.001$) (Table 2). Moreover, the SAR referral rate for colposcopy and biopsy after triage was significantly higher among DNA-tested women compared to mRNA-tested women (24.9% vs. 18.3%; $p < 0.01$), a pattern that was also observed for the SAR referral rate for repeat testing after triage (27.9% vs. 5.1%; $p < 0.001$). Consequently, according to the screening algorithm, more mRNA-tested than DNA-tested women should have returned to routine screening (76.7% vs. 47.2%; $p < 0.01$) (Table 2).

Table 2. HPV and cytology results at triage among HPV DNA-tested and HPV mRNA-tested women.

Triage Results		HPV DNA n = 2556 (%)	HPV mRNA n = 1559 (%)
HPV	**Cytology**		
Positive	Normal	713 (27.9%)	79 (5.1%)
	Unsatisfactory	32 (1.3%)	3 (0.2%)
	ASC-US	285 (11.2%)	131 (8.4%)
	LSIL	320 (12.5%)	151 (9.7%)
Total		1350 (52.8%)*	364 (23.3%) *
Negative	Normal	1042 (40.8%)	852 (54.7%)
	Unsatisfactory	43 (1.7%)	26 (1.7%)
	ASC-US	93 (3.6%)	206 (13.2%)
	LSIL	28 (1.1%)	111 (7.1%)
Total		1206 (47.2%) *	1195 (76.7%) *

* $p < 0.001$. Abbreviations: HPV, human papillomavirus; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion.

The CIN3+ detection rate at 42 months post-screening among women with SAR referrals for colposcopy and biopsy after triage was similar in DNA-tested and mRNA-tested women (31.7% vs. 30.5%; $p = 0.72$) (Table 3). This similarity remained when looking

at the CIN3+ detection rates at 78 months post-screening (33.4% vs. 32.3%; $p = 0.73$), as there were few cases of CIN3+ diagnosed at 42 months (Table 3).

Table 3. Status and CIN3+ detection rates at 42 and 78 months post-screening by the screening algorithm-recommended (SAR) group after triage among HPV DNA-tested and HPV mRNA-tested women.

SAR Group after Triage	Status	42 Months Post-Screening		*p*-Value	78 Months Post-Screening		*p*-Value
		HPV DNA	HPV mRNA		HPV DNA	HPV mRNA	
SAR Referral for Colposcopy and Biopsy		*n* = 637 (%)	*n* = 285 (%)		*n* = 637 (%)	*n* = 285 (%)	
	Did not attend	7 (1.1)	1 (0.4)		7 (1.1)	1 (0.4)	
	Incomplete follow-up	292 (45.8)	113 (39.6)		135 (21.2)	45 (15.8)	
	Returned to routine screening	86 (13.5)	38 (13.3)		229 (35.9)	99 (34.7)	
	CIN2	50 (7.8)	46 (16.1)		53 (8.3)	48 (16.8)	
	CIN3+	202 (31.7)	87 (30.5)	0.72	213 (33.4)	92 (32.3)	0.73
SAR Referral for Repeat Testing (HPV and Cytology)		*n* = 713 (%)	*n* = 79 (%)		*n* = 713 (%)	*n* = 79 (%)	
	Did not attend	37 (5.2)	3 (3.8)		37 (5.2)	3 (3.8)	
	Incomplete follow-up	376 (52.7)	45 (57.0)		181 (25.4)	17 (21.5)	
	Returned to routine screening	139 (19.5)	8 (10.1)		322 (45.2)	34 (43.0)	
	CIN2	29 (4.1)	3 (3.8)		31 (4.3)	3 (3.8)	
	CIN3+	132 (18.5)	20 (25.3)	0.145	142 (19.9)	22 (27.8)	0.099
SAR Return to Screening		*n* = 1206 (%)	*n* = 1195 (%)		*n* = 1206 (%)	*n* = 1195 (%)	
	Did not attend	308 (25.5)	241 (20.2)		308 (25.5)	241 (20.2)	
	Incomplete follow-up	799 (66.3)	819 (68.5)		560 (46.4)	511 (42.8)	
	Returned to routine screening	87 (7.2)	103 (8.6)		319 (26.5)	398 (33.3)	
	CIN2	1 (0.1)	10 (0.8)		2 (0.2)	12 (1.0)	
	CIN3+	11 (0.9)	22 (1.8)	0.05	17 (1.4)	33 (2.8)	0.02

Abbreviations: HPV, human papillomavirus; CIN2, cervical intraepithelial neoplasia grade 2; CIN3+, cervical intraepithelial neoplasia grade 3 or worse; return routine to screening, return to the 3-year screening interval without further follow-up.

Eight cases of cervical cancer (5 squamous cell carcinomas and 3 adenocarcinomas) had been diagnosed at 78 months post-screening among DNA-tested women, while only two squamous cell carcinomas had been diagnosed among mRNA-tested women. Two of the DNA-tested women and one mRNA-tested woman most likely had false-negative HPV results at triage (data not shown).

Although there were only marginal differences in the detection rate of CIN3+ at 42 and 78 months post-screening (Table 3), different HPV positivity rates at triage led to large differences in SAR referral rates for colposcopy and biopsy and repeat testing after triage, resulting in an overall CIN3+ detection rate that was significantly higher among DNA-tested (13.1%; 345/2556) compared to mRNA-tested women (8.3%; 129/1559) at 42 months post-screening ($p < 0.001$). The corresponding values at 78 months post-screening were 14.6% (372/2556) and 9.4% (147/1559) ($p < 0.001$). The proportion of both DNA-tested and mRNA-tested women with incomplete follow-up decreased from 42 to 78 months post-screening; accordingly, the proportion of women that returned to routine screening increased among all SAR groups after triage (Table 3). Our findings showed a 6-year CIN3+ risk of 2.8% among women with triage results of ASC-US/LSIL and a negative HPV mRNA test, versus 1.4% among women with triage results of ASC-US/LSIL and a negative HPV DNA test (Table 3).

4. Discussion

In this study, we evaluated the background characteristics of women who underwent HPV DNA testing (using Hybrid Capture II) and those who underwent HPV mRNA testing (using PreTect HPV-Proofer). We assessed factors that could impact the outcomes of the tests, including the age distribution, the distribution of ASC-US and LSIL results in the screening process, and the most recent cytology results before screening occurred. Our analysis indicated a high level of homogeneity between the two groups, thus reducing the risk of selection bias. The HPV DNA test detects 13 HPV types, whereas the HPV mRNA test only detects 5 types, leading to a higher HPV positivity rate among the DNA-tested women. In accordance with the Norwegian screening algorithm, this higher positivity rate resulted in a higher referral rate for colposcopy/biopsy and repeat testing for the DNA-tested women, leading to a higher overall rate of CIN3+ detection. However, this came at a cost of significantly more health resources, as measured by the number of follow-up visits and colposcopy/biopsy procedures. Our findings reveal that despite the higher resource utilization and CIN3+ detection rate in the DNA-tested women, the HPV DNA test was not more effective in preventing cervical cancer, which was the primary goal of the screening.

4.1. HPV Positivity Rates at Triage

In our low-risk population of women aged 25–33 years with ASC-US/LSIL, the HPV positivity rate at triage among DNA-tested women was lower (52.8%) than the global rate observed among women of all ages with ASC-US/LSIL who received this triage test (59.4%) [32]. The HPV positivity rate observed when a 5-type HPV mRNA test was used in the triage of Danish women aged 23–39 years with LSIL (34.7%) [33] was higher than the rate we recorded among our mRNA-tested women (23.3%). This could be because only women with LSIL were included in the Danish study, as HPV prevalence is generally higher in women with LSIL than those with ASC-US [32]. Another Danish study of women under 30 years of age with ASC-US/LSIL reported HPV positivity rates at triage for DNA- (any assay), 14-type mRNA-, and 5-type mRNA-tested women of 82.5%, 73.5%, and 40%, respectively [21]. However, HPV prevalence tends to decrease with age [12]; therefore, the higher positivity rate in the Danish study compared to ours might be due to the inclusion of women who were younger than those in our study population.

4.2. Referral Rates for Colposcopy and Biopsy and Repeat Testing after Triage

According to Norwegian guidelines, significantly more DNA-tested women in our study sample were to be referred for colposcopy and biopsy and repeat testing. In the Danish study, which included women under 30 years of age with ASC-US/LSIL, biopsy rates in DNA- (67%), 14-type mRNA- (77%), and 5-type mRNA-tested women (58%) [21] were generally higher than the SAR referral rates for colposcopy and biopsy we observed in our study. However, the Danish referral rate lessened as the HPV types included in the assay decreased.

4.3. CIN3+ Detection Rate

As a consequence of the higher SAR referral rates for colposcopy and biopsy and repeat testing, DNA-tested women had a significantly higher overall CIN3+ rate compared to mRNA-tested women at 42 months post-screening. Our results support the general conclusions that, in the triage of women with minor cytological abnormalities, an HPV mRNA test has lower referral rates than an HPV DNA test [24,34,35]. However, compared to the 14-type HPV DNA test, the reliability of the 5-type HPV mRNA test to rule out CIN3+ among women who test negative has been considered too low to be used as a basis for the determination to return to routine screening. Considering the 6-year CIN3+ risk of 2.8% among women with triage results of ASC-US/LSIL and a negative HPV mRNA test versus 1.4% among women with triage results of ASC-US/LSIL and a negative HPV DNA test in our study—and based on the principle of "equal management for equal risk", which guides patient management in screening—our results suggest that women who are HPV-negative

at triage require a further 1-year surveillance period, in order to respect the accepted 5-year CIN3+ risk threshold (>0.55 <5.0%), as the 5-year CIN3+ risk rate is 2.6% for ASC-US alone [22,36,37]. In accordance with a model-based economic evaluation analysis of the triage of young adult women with minor cervical lesions [38], SAR referral for colposcopy and biopsy was twice as high among our DNA-tested women as our mRNA-tested women. Thus, compared with the mRNA test, the usage of the DNA test at triage increased the workload of gynecologists and laboratories by more than double.

4.4. HPV Types Included in the Test

The number of HPV types included in the test may influence positivity, referral, and CIN3+ detection rates. A previous report showed that only 5–6 HPV types (16, 18, 31, 33, 45, or 52) were present in 85% of invasive cervical cancer cases, while the other eight HPV types included in 13–14 type HPV DNA tests were detected just in 1.5% of invasive cervical cancer cases [39]. Thus, the 5-fold higher SAR referral rate for repeat testing that we observed among DNA-tested women compared to mRNA-tested women may be attributed to the higher number of oncogenic HPV types included in the DNA test. This observation was also made in a Danish study, in which a 14-type HPV mRNA test yielded higher positivity rates than a 5-type HPV mRNA test [33]. The influence of the number of HPV types included in the mRNA test on both positivity rates and referral rates was also observed in the other Danish study of women under 30 years of age with ASC-US/LSIL [21]; when the 14-type and 5-type HPV mRNA tests were compared, the positivity rate decreased from 73.5% to 40% and the biopsy rate decreased from 77% to 58% [21]. Moreover, generally, when more types are included in the HPV test, the sensitivity for CIN2+/CIN3+ increases, but the specificity for CIN2+/CIN3+ decreases. A 14-type HPV DNA test (Cobas) showed higher sensitivity to detect CIN2+ than a 5-type HPV mRNA test (Proofer) in the triage of women with ASC-US/LSIL (100% vs. 79%), while its specificity was lower (84% vs. 91%) [34]. This reduction in specificity with an increasing number of HPV types is more visible with the decreasing age of the study population. A 14-type HPV mRNA test (Aptima) showed higher sensitivity to detect CIN2+ in the triage of women with LSIL aged 23–65 years compared to a 5-type HPV mRNA test (Proofer) (94% vs. 77%), while its specificity was considerably lower (34% vs. 69%) [33]. Although the gap in test sensitivity (93% vs. 80%) did not change substantially, the gap in specificity (19% vs. 64%) widened among women aged 23–29 years [33].

4.5. Overmanagement and Overtreatment

The Norwegian health care system has allocated limited resources for colposcopy and biopsy examinations [40]; therefore, the risk of overmanagement and overtreatment is of great concern in the NCCSP. The number of cervical cancers has increased from 311 in 2012 to 340 in 2021 in Norway, and the incidence rate showed an increasing trend (from $12.4/10^5$ in 2010 to $14.5/10^5$ in 2019) before decreasing slightly to $12.6/10^5$ in 2021 [18].

The higher overall CIN3+ detection rate among our DNA-tested women compared to mRNA-tested women was attributable to significantly higher healthcare utilization in the former group, as measured by the number of SAR referrals for colposcopy and biopsy or repeat testing. Overall, 345 DNA-tested women (13.1%) had been diagnosed with CIN3+ at 42 months post-screening, with 17 more diagnosed at 78 months post-screening. Among mRNA-tested women, 129 (8.3%) had been diagnosed with CIN3+ at 42 months, and 33 more at 78 months, post-screening. This revealed the burden of overmanagement among DNA-tested women, as 216 ($345 - 129 = 216$) more DNA-tested women with CIN3+ received treatment to avoid 16 ($33 - 17 = 16$) more women having CIN3+ results within 36 months, i.e., before the next screening round.

The number of treatments using the loop electrical excision procedure (LEEP) in Norway has increased from 3743 in 2010 to 7354 in 2021 without any reduction in the number of women with cervical cancer [18]. A low SAR referral rate for colposcopy and biopsy will reduce healthcare costs, as well as the use of LEEP treatment and its negative impacts

(e.g., unfavorable pregnancy outcomes, such as preterm birth, spontaneous abortion, and psychological stress [41]).

Not all CIN3 cases, and only a minority of CIN2 cases, will progress to cancer [42–44]. Research has shown that only 30% of large CIN3+ lesions will progress to cervical cancer during 30 years if left untreated [43], meaning that a substantial proportion of women with these conditions will undergo biopsy, and potentially LEEP treatment, unnecessarily. The purpose of the NSSCP is not to find as many CIN3+ as possible but to prevent as many cancer cases as possible and simultaneously balance benefits and harms. Even though more DNA-tested women in our study received LEEP treatment, eight cases of cervical cancer appeared among them during the study period, while only two cancer cases were diagnosed among mRNA-tested women. This illustrated the possibility of preventing more cancer cases and avoiding overtreatment by using a more specific triage test and targeting women with the highest cancer risk [34].

4.6. Strengths

The importance of this study lies in the long-term comparison of the predictive and performative value of HPV DNA and HPV mRNA tests in the triage of a large number of 25–33-year-old women with minor cytological abnormalities at screening. We used the NCCSP database, a nationwide, register-based platform embedded within the CRN, which allowed us to identify women with minor cytological abnormalities in Norway. Moreover, we used the 11-digit personal identification number assigned to all persons at the time of birth or immigration in Norway to merge data on cytology results, histological results, cancer cases, etc.

Although ours is not a randomized study, we consider the similarity in the characteristics of our DNA- and mRNA-tested women (Table 1) as an advantage, because it mimics the condition of randomized selection. Another unique advantage of our study is that we followed all women for up to 6 years after triage.

4.7. Limitations

Our analysis assumed that women, general practitioners, and gynecologists consistently followed the screening algorithm perfectly; however, in real clinical practice, there are probably more referrals for cytology/biopsy and repeat testing. Therefore, we also investigated the CIN3+ detection rate among women who were returned to screening (Table 3), to reflect the reality that some women are referred for colposcopy and biopsy even when such a referral is not recommended in the screening guidelines. The proportions of women with incomplete follow-up decreased during the study period. However, the proportions of women who did not attend and women with incomplete follow-up remained considerably high, especially among women with SAR referral for routine screening (63–71.9% at 78 months post-screening). This research was conducted in Norway, a country with the highest human development index between 2005–2010 and free public access to essential healthcare services. With a generally high socio-economic status and high coverage for vaccination and screening, it should also be expected that women would have a high rate of adherence to follow-up.

5. Conclusions

In this study, we evaluated the performance of HPV DNA testing (using Hybrid Capture II) and HPV mRNA testing (using PreTect HPV-Proofer) in a population of young women with ASC-US/LSIL. We found that when the HPV DNA test was used for triage, there was a significantly higher rate of referral for colposcopy and biopsy, repeat testing, and CIN3+ detection. However, this did not result in improved cancer prevention compared to the mRNA test. The mRNA test demonstrated similar efficacy in cancer prevention while requiring significantly less healthcare utilization. Based on these findings, healthcare authorities considering a triage test for minor cytological abnormalities among younger

women may prefer the mRNA test to minimize overmanagement and reduce unnecessary healthcare utilization.

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Informed Consent Statement: National health registries are exempted from informed consent; at the same time, participants were informed about their rights to withhold information upon request. As researchers, we worked with anonymous data with no access to laboratory identification or any personal data except for age.

Data Availability Statement: Not applicable.

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References

1. Jansen, E.E.L.; Zielonke, N.; Gini, A.; Anttila, A.; Segnan, N.; Vokó, Z.; Ivanuš, U.; McKee, M.; de Koning, H.J.; de Kok, I.M.C.M.; et al. Effect of organised cervical cancer screening on cervical cancer mortality in Europe: A systematic review. *Eur. J. Cancer* **2020**, *127*, 207–223. [CrossRef] [PubMed]
2. Koliopoulos, G.; Nyaga, V.N.; Santesso, N.; Bryant, A.; Martin-Hirsch, P.P.; Mustafa, R.A.; Schünemann, H.; Paraskevaidis, E.; Arbyn, M. Cytology versus HPV testing for cervical cancer screening in the general population. *Cochrane Database Syst. Rev.* **2017**, *8*, CD008587. [CrossRef] [PubMed]
3. Polman, N.; Snijders, P.; Kenter, G.; Berkhof, J.; Meijer, C. HPV-based cervical screening: Rationale, expectations and future perspectives of the new Dutch screening programme. *Prev. Med.* **2018**, *119*, 108–117. [CrossRef] [PubMed]
4. England, P.H. Cervical Screening: Programme Overview. 2021. Available online: https://www.gov.uk/guidance/cervical-screening-programme-overview (accessed on 5 December 2022).
5. National Cervical Screening Program: Guidelines for the Management of Screen-Detected Abnormalities, Screening in Specific Populations and Investigation of Abnormal Vaginal Bleeding. Available online: https://wiki.cancer.org.au/australiawiki/images/a/ad/National_Cervical_Screening_Program_guidelines_long-form_PDF.pdf (accessed on 5 December 2022).
6. WHO. *WHO Guideline for Screening and Treatment of Cervical Pre-Cancer Lesions for Cervical Cancer Prevention*, 2nd ed.; WHO: Geneva, Switzerland, 2021.
7. von Karsa, L.; Arbyn, M.; De Vuyst, H.; Dillner, J.; Dillner, L.; Franceschi, S.; Patnick, J.; Ronco, G.; Segnan, N.; Suonio, E.; et al. European guidelines for quality assurance in cervical cancer screening. Summary of the supplements on HPV screening and vaccination. *Papillomavirus Res.* **2015**, *1*, 22–31. [CrossRef]
8. Institution, T.P.H. Turkey Cancer Control Programme. 2016. Available online: https://www.iccp-portal.org/system/files/plans/Turkiye_Kanser_Kontrol_Program_English.pdf (accessed on 5 December 2022).
9. Socialstyrelsen. Senaste Versionen av HSLF-FS 2019:14 Socialstyrelsens Föreskrifter Och Allmänna Råd om Villkor för Avgiftsfri Screening. 2021. Available online: https://www.socialstyrelsen.se/kunskapsstod-och-regler/regler-och-riktlinjer/foreskrifter-och-allmanna-rad/konsoliderade-foreskrifter/201914-om-villkor-for-avgiftsfri-screening/ (accessed on 5 December 2022).
10. Norway, C.R.O. HPV I Primærscreening. Cancer Registry of Norway. Available online: https://www.kreftregisteret.no/en/screening/cervix/Helsepersonell/screeningstrategi-og-nasjonale-retningslinjer/hpv-i-primarscreening/ (accessed on 5 December 2022).
11. Norway, T.C.R.O. Primary HPV Test—Now also for Women Under 34. 2023. Available online: https://www.kreftregisteret.no/screening/livmorhalsprogrammet/hpv/ (accessed on 5 December 2022).
12. Bruni, L.; Diaz, M.; Castellsagué, X.; Ferrer, E.; Bosch, F.X.; de Sanjosé, S. Cervical Human Papillomavirus Prevalence in 5 Continents: Meta-Analysis of 1 Million Women with Normal Cytological Findings. *J. Infect. Dis.* **2010**, *202*, 1789–1799. [CrossRef]

13. Moscicki, A.-B.; Ma, Y.; Wibbelsman, C.; Powers, A.; Darragh, T.M.; Farhat, S.; Shaber, R.; Shiboski, S. Risks for Cervical Intraepithelial Neoplasia 3 Among Adolescents and Young Women with Abnormal Cytology. *Obstet. Gynecol.* **2008**, *112*, 1335–1342. [CrossRef]
14. Moscicki, A.-B.; Ma, Y.; Wibbelsman, C.J.; Darragh, T.M.; Powers, A.; Farhat, S.; Shiboski, S. Rate of and Risks for Regression of Cervical Intraepithelial Neoplasia 2 in Adolescents and Young Women. *Obstet. Gynecol.* **2010**, *116*, 1373–1380. [CrossRef]
15. Molden, T.; Kraus, I.; Karlsen, F.; Skomedal, H.; Hagmar, B. Human papillomavirus E6/E7 mRNA expression in women younger than 30 years of age. *Gynecol. Oncol.* **2006**, *100*, 95–100. [CrossRef]
16. Ho, G.Y.; Bierman, R.; Beardsley, L.; Chang, C.J.; Burk, R.D. Natural History of Cervicovaginal Papillomavirus Infection in Young Women. *N. Engl. J. Med.* **1998**, *338*, 423–428. [CrossRef]
17. Boardman, L.A.; Robison, K. Screening adolescents and young women. In *Obstetrics and Gynecology Clinics of North America*; W.B. Saunders Ltd.: Philadelphia, PE, USA, 2013; pp. 257–268.
18. Engesæter, B.L.G.; Skare, G.B.; Tropé, A. *Årsrapport 2021—Screeningaktivitet og Resultater fra Livmorhalsprogrammet*; Cancer Registry of Norway: Oslo, Norway, 2021.
19. Baldauf, J.J.; Ritter, J. Comparison of the risks of cytologic surveillance of women with atypical cells or low-grade abnormalities on cervical smear: Review of the literature. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **1998**, *76*, 193–199. [CrossRef]
20. Stefani, C.; A Liverani, C.; Bianco, V.; Penna, C.; Guarnieri, T.; Comparetto, C.; Monti, E.; Valente, I.; Pieralli, A.L.; Fiaschi, C.; et al. Spontaneous regression of low-grade cervical intraepithelial lesions is positively improved by topical bovine colostrum preparations (GINEDIE®). A multicentre, observational, Italian pilot study. *Eur. Rev. Med. Pharmacol. Sci.* **2014**, *18*, 728–733. [PubMed]
21. St-Martin, G.; Thamsborg, L.H.; Andersen, B.; Christensen, J.; Ejersbo, D.; Jochumsen, K.; Johansen, T.; Larsen, L.G.; Waldstrøm, M.; Lynge, E. Management of low-grade cervical cytology in young women. Cohort study from Denmark. *Acta Oncol.* **2020**, *60*, 444–451. [CrossRef] [PubMed]
22. Katki, H.A.; Schiffman, M.; Castle, P.E.; Fetterman, B.; Poitras, N.E.; Lorey, T.; Cheung, L.; Raine-Bennett, T.R.; Gage, J.C.; Kinney, W.K. Benchmarking CIN 3+ Risk as the Basis for Incorporating HPV and Pap Cotesting into Cervical Screening and Management Guidelines. *J. Low. Genit. Tract Dis.* **2013**, *17*, S28–S35. [CrossRef] [PubMed]
23. Tjalma, W.A.; Fiander, A.; Reich, O.; Powell, N.; Nowakowski, A.; Kirschner, B.; Koiss, R.; O'Leary, J.; Joura, E.A.; Rosenlund, M.; et al. Differences in human papillomavirus type distribution in high-grade cervical intraepithelial neoplasia and invasive cervical cancer in Europe. *Int. J. Cancer* **2012**, *132*, 854–867. [CrossRef]
24. Sørbye, S.W.; Fismen, S.; Gutteberg, T.J.; Mortensen, E.S.; Skjeldestad, F.E. HPV mRNA Is More Specific than HPV DNA in Triage of Women with Minor Cervical Lesions. *PLoS ONE* **2014**, *9*, e112934. [CrossRef] [PubMed]
25. Nayar, R.; Wilbur, D.C. The Pap Test and Bethesda 2014. "The reports of my demise have been greatly exaggerated". (after a quotation from Mark Twain). *Acta. Cytol.* **2015**, *59*, 121–132. [CrossRef]
26. WHO. Comprehensive Cervical Cancer Control: A guide to essential practice. In *Comprehensive Cervical Cancer Control: A Guide to Essential Practice*; WHO: Geneva, Swrizterland, 2006.
27. QIAGEN. Digene Specimen Transport Medium. 2023. Available online: https://www.qiagen.com/de/products/diagnostics-and-clinical-research/sexual-reproductive-health/cervical-cancer-screening/digene-specimen-transport-medium (accessed on 5 December 2022).
28. PreTect, T.M. 2023. Available online: https://www.pretect.no/products/pretecttm (accessed on 5 December 2022).
29. QIAGEN. Digene HC2 HPV DNA Test. 2023. Available online: https://www.qiagen.com/us/products/diagnostics-and-clinical-research/sexual-reproductive-health/cervical-cancer-screening/digene-hc2-hpv-dna-test (accessed on 5 December 2022).
30. PreTect. PRETECT HPV-PROOFER. 2023. Available online: https://www.pretect.no/products/pretecthpvproofermoreinformation (accessed on 5 December 2022).
31. PreTect. NASBA—NUCLEIC ACID SEQUENCE BASED AMPLIFICATION. 2023. Available online: https://www.pretect.no/nasba (accessed on 5 December 2022).
32. Arbyn, M.; Martin-Hirsch, P.; Buntinx, F.; Van Ranst, M.; Paraskevaidis, E.; Dillner, J. Triage of women with equivocal or low-grade cervical cytology results: A meta-analysis of the HPV test positivity rate. *J. Cell Mol. Med.* **2009**, *13*, 648–659. [CrossRef]
33. Reinholdt, K.J.K.E.; Dehlendorff, C.; Munk, C.; Kjær, S.K.; Thomsen, L.T. Triage of low-grade squamous intraepithelial lesions using human papillomavirus messenger ribonucleic acid tests—A prospective population-based register study. *Acta Obstet. Gynecol.Scand.* **2019**, *17*, e0275858. [CrossRef]
34. Westre, B.; Giske, A.; Guttormsen, H.; Sørbye, S.; Skjeldestad, F.E. 5-type HPV mRNA versus 14-type HPV DNA test: Test performance, over-diagnosis and overtreatment in triage of women with minor cervical lesions. *BMC Clin. Pathol.* **2016**, *16*, 9. [CrossRef]
35. Thomsen, L.T.; Dehlendorff, C.; Junge, J.; Waldstrom, M.; Schledermann, D.; Frederiksen, K.; Kjaer, S.K. Human papillomavirus mRNA and DNA testing in women with atypical squamous cells of undetermined significance: A prospective cohort study. *Int. J. Cancer* **2016**, *139*, 1839–1850. [CrossRef]
36. Katki, H.A.; Schiffman, M.; Castle, P.; Fetterman, B.; Poitras, N.; Lorey, T.; Cheung, L.; Raine-Bennett, T.; Gage, J.; Kinney, W. Five-year risks of CIN 3^+ and cervical cancer among women who test Pap-negative but are HPV-positive. *J. Low Genit. Tract. Dis.* **2013**, *17* (Suppl. S1), S56–S63. [CrossRef] [PubMed]

37. Egemen, D.; Cheung, L.; Chen, X.; Demarco, M.; Perkins, R.; Kinney, W.; Poitras, N.; Befano, B.; Locke, A.; Guido, R.; et al. Risk Estimates Supporting the 2019 ASCCP Risk-Based Management Consensus Guidelines. *J. Low Genit. Tract. Dis.* **2020**, *24*, 132–143. [CrossRef] [PubMed]
38. Pedersen, K.; Sorbye, S.; Kristiansen, I.; Burger, E.A. Using novel biomarkers to triage young adult women with minor cervical lesions: A cost-effectiveness analysis. *BJOG Int. J. Obstet. Gynaecol.* **2017**, *124*, 474–484. [CrossRef] [PubMed]
39. Sundstrom, K.; Dillner, J. How many Human Papillomavirus types do we need to screen for? *J. Infect. Dis.* **2020**, *22*, 1510–1511. [CrossRef]
40. Norway, C.R.O. Cervical Cancer Screening Programme. 2021. Available online: https://www.kreftregisteret.no/en/screening/cervix/org/ (accessed on 5 December 2022).
41. Bjørge, T.; Skare, G.; Bjørge, L.; Tropé, A.; Lönnberg, S. Adverse Pregnancy Outcomes After Treatment for Cervical Intraepithelial Neoplasia. *Obstet. Gynecol.* **2016**, *128*, 1265–1273. [CrossRef]
42. Castle, P.E.; Schiffman, M.; Wheeler, C.; Solomon, D. Evidence for frequent regression of cervical intraepithelial neoplasia-grade 2. *Obstet. Gynecol.* **2009**, *113*, 18–25. [CrossRef]
43. McCredie, M.R.; Sharples, K.; Paul, C.; Baranyai, J.; Medley, G.; Jones, R.; Skegg, D. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: A retrospective cohort study. *Lancet Oncol.* **2008**, *9*, 425–434. [CrossRef]
44. Ronco, G.; Giorgi-Rossi, P.; Carozzi, F.; Confortini, M.; Palma, P.D.; Del Mistro, A.; Ghiringhello, B.; Girlando, S.; Gillio-Tos, A.; De Marco, L.; et al. New Technologies for Cervical Cancer screening Working. 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]