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# A Population-based Study on Cervical Cancer Screening in Germany: Beyond the Baseline

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Dedicated to my late maternal grandmother Shen Su Ying† 沈素英.

Like so many others, you were taken away too early by cervical cancer.

Thank you for awakening my pursuit to do something about it.

“The world is full of what seem like intractable problems. Often we let that paralyse us. Instead, let it spur you to action.”

— Melinda French Gates

# Summary

Cervical cancer is worldwide a common yet preventable cancer. Several decades of clinical practice and research have led to practical screening tools for detecting potentially cancerous and early-stage cervical abnormalities and, more recently, the development of prophylactic vaccines against the causal agent Human Papillomavirus (HPV). These prevention tools have significantly reduced cervical cancer incidence and mortality in high-income countries (HIC) and have great potential to eliminate cervical cancer globally. The World Health Organization (WHO) has called for cervical cancer elimination by vaccinating at least 90% of girls by age 15, screening 70% of women by age 35 and treating 90% of women with cervical disease. However, issues in access and uptake of HPV vaccinations persist in many populations, including in HIC, and most of the current global adult population remains unvaccinated against HPV. Screening, therefore, remains the most critical prevention mechanism. Screening is offered in almost all HIC. Screening with cytology, however, has reached its limit in further reducing the incidence of cervical cancer due to its highly variable performance and will be challenged by increasingly vaccinated cohorts. Recent advances in HPV research have led to an increasing number of available diagnostic tools and uncertainties regarding the impact on screening and follow-up retention, requiring re-evaluation to maintain screening effectiveness. This thesis investigates two aspects of cervical screening, including optimal screening methods and patient adherence behaviours to follow-up within a HIC context.

Germany has offered longstanding and opportunistic annual cytological screenings since 1971. Despite its historical successes in reducing incidence and mortality, the incidence has since plateaued, with a substantial number of missed cancers still occurring, calling for better screening tools and improved quality assurance. Detection of HPV infection has surfaced as an effective and superior tool for screening. It has been implemented as a primary screening tool in several countries, such as the Netherlands, the United Kingdom and Australia. However, it has only recently been integrated into the German screening system as a concomitant test (co-test) to cytology. International and WHO guidelines recommend HPV testing as the preferred primary (stand-alone) screening tool, but direct evidence comparing it to co-testing is lacking. Using a population-based sample of screened women, the first study in this thesis compared screening performance by stand-alone HPV testing and co-testing as well as combinations with various tools. The results of this study demonstrated favourable harm-benefit outcomes by HPV testing stand-alone. The performance of HPV testing stand-alone was

equivalent to co-testing using liquid-based cytology and superior to co-testing with conventional cytology.

After a positive screening result, a diagnostic follow-up assessment to further determine, manage, or treat any abnormality is crucial to maintaining screening effectiveness. This forms one of the first critical assessments for patient management in the screening algorithm, designed to appropriately safeguard at-risk women and aptly discharge those at no or low risk. In cervical cancer screening, colposcopy constitutes the follow-up diagnostic assessment. Adherence to such referrals is a critical quality assurance indicator. Given the recent shift towards HPV-based screening worldwide, it was also unclear whether HPV testing would influence follow-up adherence. The second study within this thesis determined the rate of adherence to diagnostic follow-up, whether this is impacted by HPV status and which additional factors lead to attendance or lack thereof. The results revealed a substantial non-attendance rate of up to 30% and the affirmative influence of a positive HPV screening result on colposcopy attendance. Moreover, the results revealed several barriers for the patient, such as lack of time or need for childcare support.

These findings identify two areas for improvement in the current German cervical cancer screening programme. While integrating HPV testing as a co-test is an improvement for screening programmes, a better balance of benefits and harms is likely when it is used as a primary screening tool. Furthermore, adherence to follow-up after a positive screening result must be improved to maximise the effects of screening. These issues can be addressed through quality-assured modern methods, appropriate triage and education efforts of all patients and relevant stakeholders involved with screening. Further post-implementation research and evaluation of HPV-based testing strategies on a population-level are necessary to fully capture the effect of this screening tool and to assess aspects that may enhance or jeopardise the effectiveness of such a screening programme.

# Zusammenfassung

Gebärmutterhalskrebs ist weltweit eine häufige, aber vermeidbare Krebsart. Mehrere Jahrzehnte klinischer Praxis und Forschung führten zur Entwicklung wirksamer Screening-Methoden zur Früherkennung von Zervixkarzinomen und auch derer präkanzerosen Vorstufen. Ebenso führten Erkenntnisse der Karzinogenese durch bestimmte Hoch-Risiko humane Papillomaviren (HPV) zur Entwicklung prophylaktischer Impfstoffen gegen HPV. Die Einführung beider Präventionsmaßnahmen senkte die Inzidenz und Sterblichkeit von Gebärmutterhalskrebs in Ländern mit hohem Einkommen (HIC) bereits deutlich. Die Weltgesundheitsorganisation (WHO) hat dazu aufgefordert, Gebärmutterhalskrebs zu eliminieren, indem mindestens 90% der Mädchen bis zum Alter von 15 Jahren geimpft, 70% der Frauen bis zum Alter von 35 Jahren untersucht und 90% der Frauen mit Gebärmutterhalskrebs behandelt werden. Es besteht allerdings noch ein großes Potenzial bei der bevölkerungsdeckenden Umsetzung, da viele Bevölkerungsgruppen, auch in den HIC, nach wie vor Probleme mit dem Zugang und der Akzeptanz von HPV-Impfungen haben, und der größte Teil der erwachsenen Weltbevölkerung noch nicht gegen HPV geimpft ist. Daher bleibt die Früherkennung die wichtigste Präventionsmaßnahme. Screening wird in fast allen HIC angeboten. Das zytologische Screening hat jedoch seine Grenzen erreicht, um die Inzidenz von Gebärmutterhalskrebs weiter zu senken, da seine Zuverlässigkeit stark schwankt und es durch zunehmend geimpfte Kohorten in Frage gestellt wird. Die jüngsten Fortschritte in der HPV-Forschung führten zur Einführungen vieler neuer diagnostischer Instrumente, die Auswirkungen auf das Screening und die Einhaltung der Nachuntersuchungen ungewiss sind. Neue Bewertungen sind erforderlich, um die Wirksamkeit des Screenings zu erhalten. In dieser Dissertation werden zwei Aspekte von Gebärmutterhalskrebs-Screeningprogramm untersucht, darunter eine Analyse der optimalen Screening-Methoden und das Verhalten der Patientinnen bei der Nachsorge im Rahmen der HIC.

In Deutschland werden seit 1971 jährliche zytologische Screenings angeboten. Das Screening basiert auf einem opportunistischen Modell. Trotz der historischen Erfolge bei der Senkung der Inzidenz und der Gesamtmortalität hat sich die Inzidenz seither auf einem Plateau stabilisiert, wobei immer noch eine beträchtliche Anzahl von Krebserkrankungen übersehen wird, was eine verbesserte Qualitätssicherung erforderlich macht. Der Nachweis einer HPV-Infektion hat sich als wirksames und überlegenes Instrument für das Screening erwiesen und wurde in mehreren Ländern wie den Niederlanden und Australien als primäres Screening-Instrument eingeführt. Dennoch wurde dieses erst vor kurzem in das deutsche Screening-System als Kombinationstest zusammen mit der Zytologie

integriert (Co-Testing). In internationalen Leitlinien wird der HPV-Test als bevorzugtes primäres (eigenständiges) Screening-Instrument empfohlen, aber es fehlen direkte Vergleichsdaten zu Co-Testing. Anhand einer bevölkerungsbasierten Stichprobe von Frauen, die einem Screening unterzogen wurden, verglich die erste Studie in dieser Dissertation die Screening- Genauigkeit von alleinigen HPV-Tests und oder als Co-Tests sowie von Kombinationen mit verschiedenen Instrumenten. Die Ergebnisse dieser Studie zeigen ein optimales Nutzen-Schaden-Verhältnis für den HPV-Test als eigenständige Methode. Die Leistung des eigenständigen HPV-Tests war gleichwertig mit der eines Co-Tests mit flüssigkeitsbasierter Zytologie und besser als die eines Co-Tests mit konventioneller Zytologie.

Nach einem positiven Screening-Ergebnis ist eine diagnostische Nachuntersuchung zur weiteren Bestimmung, Behandlung oder Therapie von Auffälligkeiten entscheidend für die Aufrechterhaltung der Wirksamkeit des Screenings. Dies ist eine der ersten wichtigen Beurteilungen für das Patientenmanagement im Rahmen des Screening-Algorithmus, der darauf abzielt, gefährdete Frauen angemessen zu betreuen und Frauen ohne oder mit geringem Risiko bei unnötigen Nachuntersuchungen auszuschließen. Beim Gebärmutterhalskrebs-Screening stellt die Kolposkopie die diagnostische Folgeuntersuchung dar. Die Einhaltung der Nachuntersuchung ist ein wichtiger Qualitätssicherungsindikator. In Anbetracht der jüngsten Verlagerung auf HPV-basiertes Screening weltweit ist noch unklar, ob der HPV-Test die Adhärenz bei der Nachuntersuchung beeinflussen würde. In der zweiten Studie im Rahmen dieser Dissertation wurde untersucht, wie hoch die Adhärenz an der diagnostischen Nachsorge in Deutschland ist, ob diese durch den HPV-Status beeinflusst wird und welche zusätzlichen Faktoren zur Teilnahme oder Nichtteilnahme führen. Die Ergebnisse zeigten eine erhebliche Nichtteilnahmerate von bis zu 30%. Ein positives HPV-Screening-Ergebnisses erhöhte die Wahrscheinlichkeit auf die Teilnahme an einer Kolposkopie. Darüber hinaus zeigte die Studie mehrere Barrieren für Patientinnen auf, darunter Zeitmangel oder Bedarf an Unterstützung bei der Kinderbetreuung.

Diese Ergebnisse zeigen zwei Bereiche auf, in denen das derzeitige deutsche Gebärmutterhalskrebs-Screeningprogramm verbessert werden kann. Während die Integration des HPV-Tests als Co-Test eine Verbesserung für die Screening-Programme darstellt, aber ein besseres Verhältnis zwischen Nutzen und Schaden ist eher zu erwarten, wenn er als primäres Screening-Instrument eingesetzt wird. Außerdem muss die mangelnde Einhaltung der Nachsorge nach einem positiven Screening-Ergebnis verbessert werden, um die Wirkung des Screenings zu maximieren. Diese Herausforderungen können durch moderne, qualitätsgesicherte Methoden, eine angemessene Triage und Aufklärungsmaßnahmen für alle Patientinnen und all am Screening beteiligten Stakeholder angegangen werden. Weitere

Untersuchungen nach der Einführung von HPV-basierten Teststrategien sind notwendig, um die verschiedenen Aspekte, die die Wirksamkeit eines solchen Screening-Programms verbessern oder gefährden können, vollständig zu erfassen.

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# 1 Introduction

Cancers of the cervix are among the most common cancers in women worldwide. Globally in 2020, 9.2 million new cancer cases were diagnosed among women, and approximately 604,000 cases (6.5%) were due to cervical cancer, making it the fourth most common cancer in women (Ferlay et al., 2020). It is the second most common cancer in women of reproductive age 15 to 45 years, peaking at around 40 years of age (Arbyn et al., 2020). The global age-standardised rate (ASR) for incidence is 13 per 100,000 women, but this varies significantly between and within geographic regions (Singh et al., 2023). Most of the burden of cervical cancer arises from premature deaths (Global Burden of Disease Cancer Collaboration, 2017), particularly in low- and middle-income countries (LMIC) where higher rates are correlated with poorer human development index (HDI) outcomes (Huang et al., 2022).

In resource-rich and high-income countries (HIC), incidence and mortality rates were also historically high. However, until the latter half of the 20$^{th}$ century, the implementation of cervical screening practices led to significant decreases in incidence and mortalities, albeit with stagnating rates in the last three decades (Zhang et al., 2021). For example, the cervical cancer incidence rates during the 1970s in Germany and the Nordic countries were 23 to 38 per 100,000 women, decreasing to 10 per 100,000 women in the early 2000s and subsequently plateauing (Robert Koch-Institut, 2016). Despite such remarkable historical decreases in morbidity and mortality, a plateaued rate in Germany translates to roughly 4,300 cervical cancer diagnoses and 1,600 associated deaths annually, many of which were diagnosed in women that were still of working age (Robert Koch-Institut & Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V., 2021). Some of these diagnoses also occurred at advanced stages, highlighting issues within the screening system and practices. Moreover, in some HIC with long-standing screening, such as Sweden and the United Kingdom, an increase in cervical cancer incidence among women aged below 50 years has recently been observed (Huang et al., 2022; Singh

et al., 2023). Without any early intervention, the physical, economic and social burdens of cervical cancer are substantial (Ginsburg et al., 2017; Shah et al., 2020). These recent findings add impetus to address cervical cancer as a relevant and current public health issue and further investigate deficiencies in available prevention methods.

Particular breakthroughs in basic sciences research, epidemiology and public health, which range from the microscopic examination of cervical cells and the discovery of the causative agent human papillomavirus (HPV), have enabled the development of effective primary and secondary prevention measures. Implementation of these has been possible due to extensive research in public health policy (Goldie et al., 2006). Apart from cervical cancer screenings for cytological abnormalities, these measures include prophylactic vaccination against high-risk HPV (hrHPV) types associated with cervical cancer and screening for HPV infections. Screening for early disease also enables early treatment of cervical abnormalities, which could develop into invasive cervical cancer. These three measures (vaccination, screening, treatment) of the cancer care continuum form the primary pillars of the World Health Organization's (WHO) 2020 strategy towards cervical cancer elimination: to fully vaccinate 90% of girls against hrHPV by age 15 years, to screen 70% of women by a high-performance test twice between the ages 35 and 45 years, and to ensure that 90% of women identified with cervical disease receive appropriate treatment (World Health Organization, 2020). This elimination strategy aims to reduce the incidence to an ambitious global threshold of 4 new cases per 100,000 by 2030, setting a high standard even when primary and secondary prevention measures are available.

Since 2007, prophylactic vaccinations have been rolled out in many HIC, leading to some reductions in precancerous lesions and cancers (Falcaro et al., 2021; Lei et al., 2020). However, issues persist regarding the uptake and completeness of the recommended two to three doses immunisation schedule (Bruni et al., 2021). Uptake issues can be addressed by scaling up vaccine programmes, including catch-up vaccinations for older cohorts of young women (Simms et al., 2019). Recently, a single dose of the HPV vaccine demonstrated similar levels of protection compared to two and three doses across several randomised controlled trials (RCTs) within a systematic review, highlighting optimised approaches to improve coverage (Markowitz et al., 2022). This was supported by the updated WHO recommendation in December 2022, which accepts a single dose as part of the immunisation schedule (World Health Organization, 2022). The impact of increased vaccination

coverage on population-wide incidence and mortality will, however, only become apparent in many decades (Lei et al., 2020; Simms et al., 2019), and much of the target population at risk of cervical cancer (women aged 30 years and above) remains ineligible for vaccination, which is most effective before the initiation of sexual activity. The available vaccines also do not cover all hrHPV types, so HPV-vaccinated and HPV-unvaccinated cohorts remain at risk. Therefore, the second pillar of screening is vital to accelerate the prevention of avertible cases.

Screening aims to decrease incidence and mortality by swiftly detecting and treating potential (precancerous) lesions and cervical cancers. Therefore, to render the effectiveness of such screening, eligible populations must have adequate access to screening, and among those with screen-detected abnormalities, be monitored and treated where necessary within a screening algorithm. Cervical cancer screening is offered in almost nine out of ten HIC (Bruni et al., 2022). For these countries, several challenges persist, especially regarding disparities in screening coverage, retention in follow-up (the screening algorithm) and quality assurance. In HIC such as Germany, access is less of a major issue due to the availability of annual cytological screenings, which were established in 1971 (Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft et al., 2020). In a three year period, screenings were accessed by up to three-quarters of the eligible population (Klug et al., 2010), comparable to neighbouring countries with organised programmes (Bruni et al., 2022). The significant challenges beyond this 'baseline' of access to screening thus concern inaccurate screening tools, sparse information dissemination leading to poor follow-up care and lack of quality assurance (Hillemanns & Iftner, 2020).

Additionally, there are fast-evolving technologies, various screening strategies, emerging tools, and information dissemination approaches to consider within the screening algorithm (Anttila et al., 2015; Ronco et al., 2015). Shortcomings in any of these areas likely explain the plateauing incidence. Therefore, more research into the inadequacies within screening programmes is necessary in order to reach the WHO's elimination goals. This thesis, therefore, focuses on optimal screening strategies for improved detection and patient adherence behaviours within the screening algorithm. Chapter 1 aims first to give background to the cervix and its vulnerabilities and the aetiology, epidemiology and natural history of cervical dysplasia. This background highlights prevention possibilities and failures in care within a cervical cancer screening algorithm. The research gaps questions lay the groundwork for the two published studies conducted:

**Study 1:** Liang, L. A., Einzmann, T., Franzen, A., Schwarzer, K., Schauberger, G., Schriefer, D., Radde, K., Zeissig, S. R., Ikenberg, H., Meijer, C. J. L. M., Kirkpatrick, C. J., Kölbl, H., Blettner, M., & Klug, S. J. (2021). Cervical Cancer Screening: Comparison of Conventional Pap Smear Test, Liquid-Based Cytology, and Human Papillomavirus Testing as Stand-alone or Cotesting Strategies. Cancer Epidemiology, Biomarkers & Prevention, 30(3), 474-484. https://doi.org/10.1158/1055-9965.Epi-20-1003

**Study 2:** Liang, L. A., Zeissig, S. R., Schauberger, G., Merzweiler, S., Radde, K., Fischbeck, S., Ikenberg, H., Blettner, M., & Klug, S. J. (2022). Colposcopy non-attendance following an abnormal cervical cancer screening result: a prospective population-based cohort study. BMC Women's Health, 22(1), 285. https://doi.org/10.1186/s12905-022-01851-6

Chapter 2 describes the methodological approaches of these studies. The results of these investigations and the contributions are summarised in Chapter 3. Finally, these studies are discussed in detail within Chapter 4 with future implications for cervical cancer prevention measures in the 21st century.

## 1.1 Aetiology and epidemiology

### 1.1.1 The cervix: Anatomy and physiology

The anatomical term *cervix* stems from Latin roots and refers to "the neck" or "the nape" of the female reproductive organ, the uterus. The uterus is roughly pear-shaped and consists of the fundus (connecting to the fallopian tubes), the body (corpus) and the cervix. The cervix connects the uterine cavity to the vagina and consists of the inner canal (endocervix) and outer lips (ectocervix), meeting at the opening known as the external os. Due to its location and cylindrical shape, the cervix requires an intrusive and thorough investigation for disease screening and obtainment of smears for further morphological and molecular assessment (World Health Organization, 2014).

Histologically, the ectocervix is lined by a transparent squamous epithelium, while a singular layer of columnar epithelium lines the endocervix (or endocervical canal; Figure 1). Within the squamous epithelium, four layers exist: the superficial and intermediate cell layers, parabasal and basal cell layers, and meet the basement membrane lining. As for the endocervix, finger-like crypts or glands branch

out within the ridged endocervical canal, where the mucin secretion provides a protective barrier for the uterus (Figure 1). These glands extend up to 5mm deep (Prendiville & Sankaranarayana, 2017).

The point at which the squamous and columnar epithelium meet is known as the squamocolumnar junction (SCJ). This mucosal junction rotates outward in a migratory fashion (eversion) depending on age and significant physiological changes during the woman's lifetime, namely puberty and pregnancy. At menarche and during reproductive age, the columnar cells of the original SCJ gradually become replaced with a new layer of squamous epithelium, a natural process called squamous metaplasia. This replacement is made possible due to the acidic environment of the vagina whereby the exposed single-layered columnar epithelium becomes irritated, and the elongation of the endocervical canal occurs due to oestrogen secretion (Prendiville & Sankaranarayana, 2017). Consequently, a new SCJ is formed.

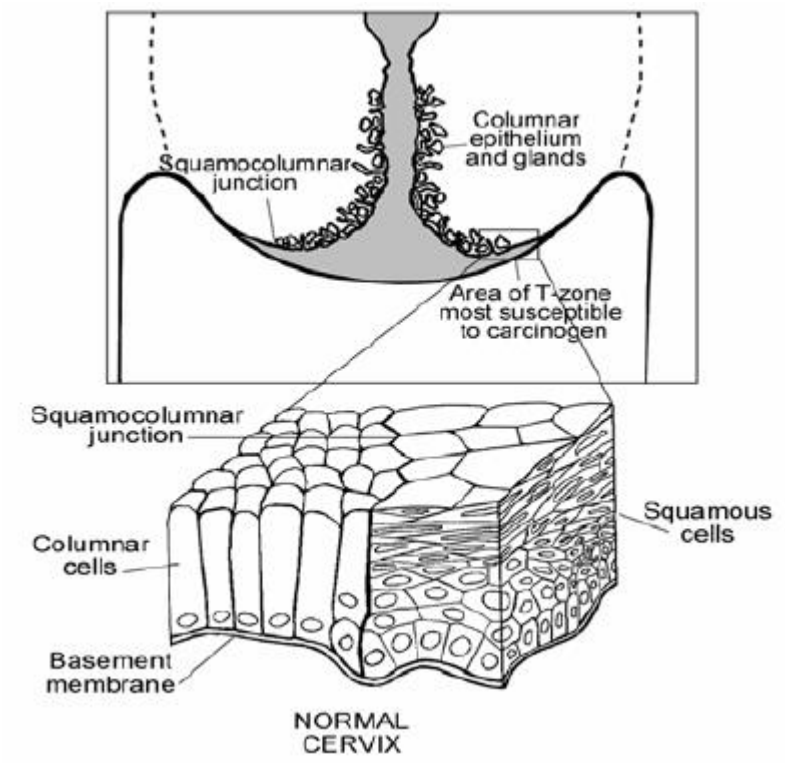



**Figure 1.** Anatomy of a normal cervix; reprinted with the permission of Blumenthal and McIntosh (2005)

The most critical area for proper assessment and, if necessary, treatment is defined as the area between the original location of the SCJ and the new SCJ, known as the transformation zone (TZ). The anatomy and physiology here are essential to note since almost all precancerous lesions and cancers of the cervix are found in this zone (Burghardt & Ostör, 1983). This area is also considered most susceptible to HPV infections (Soares et al., 2019), akin to other mucosal junctions, including the oropharynx and anus, which are also susceptible to HPV and other sexually transmitted infections (STIs) such as human immunodeficiency virus (HIV) (Doorbar & Griffin, 2019; Herfs et al., 2011). Because of these transitions and the structure of the glands within the ectocervix and endocervix, proper sampling of the area with the appropriate tools during screening, dependent on the woman's age and reproductive history, is crucial to provide an accurate picture of the disease status (World Health Organization, 2014). Furthermore, adequate visualisation and determination of the TZ by the smear-taker are essential.

The historical successes in reduced cervical cancer incidence and mortality are due to mass cytological screenings of the cervix (Meggiolaro et al., 2016; Peirson et al., 2013; Vaccarella et al., 2013). Such screenings began as early as the 1920s when novel clinical research was presented on vaginal smears taken from cancers of the uterus (Babeş, 1928; Papanicolaou, 1928). Subsequently, carcinomas in situ of the cervix were proposed to be the preceding stages of invasive cervical cancer (Reagan & Hicks, 1953). These findings devolved into a simple detection method of early asymptomatic stages of cervical cancer by obtaining cervical smears and observing morphological changes under the microscope, which spurred the beginnings of cytopathology and screening with the so-called Pap smear test (Papanicolaou, 1928, 1942; Papanicolaou & Traut, 1941).

Since the 1960s, the Pap smear and subsequent cytological evaluation have become the standard screening method for cervical cancer in many countries, predominantly in HIC (IARC Working Group on the Evaluation of Cancer-Preventive Interventions, 2022). Although this intervention was not based originally on epidemiological trials with quantitative evidence, several randomised trials and pooled observational analyses subsequently confirmed the consistent and positive impact of cytological screenings on the incidence and mortality of cervical cancer (Peirson et al., 2013; Sankaranarayanan et al., 2005; Sankaranarayanan et al., 2009). In parallel to mass cytological screenings, discovering HPV and its types were significant breakthroughs for cervical cancer prevention measures.

### 1.1.2 The role of Human Papillomavirus (HPV) and other factors

In 1838, the proposal of cell theory in both animals and humans propelled microscopic investigations of harmless skin 'tumours' (warts) into routine medical practice, emerging as the practice of tumour pathology (Hajdu, 2012). Following the discovery of viruses as infectious agents in the late 19$^{th}$ century (Beijerinck, 1898; Ivanovsky, 1892), tumour virology research on canine warts began (Ciuffo, 1907; McFadyean & Hobday, 1898), revealing the connection between papillomaviruses, the viral agent causing warts in mammals, and carcinogenic tumours (Olson & Cook, 1951; Olson et al., 1959; Rous & Beard, 1934; Shope & Hurst, 1933). Investigations into human papillomaviruses began in the 1920s due to the cosmetic rather than medical concerns of human warts (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2007). During this period, significant observations transpired with the discovery of the viral aetiology of human warts (Lutz, 1946; Strauss et al., 1949) and human papillomaviruses (Melnick, 1962; Rowson & Mahy, 1967). Consequentially, the causal role of human papillomaviruses (HPV) in squamous cell carcinomas of the skin was demonstrated in the 1970s (Jablonska et al., 1972; Orth et al., 1979; zur Hausen, 1977; zur Hausen et al., 1974).

Further investigations into genital warts (condylomata acuminata) aetiology led to the discovery of a multitude of HPV types (Gissmann & zur Hausen, 1976; Orth et al., 1977) in both condylomata acuminata (Gissmann et al., 1982; Gissmann & zur Hausen, 1980) as well as cervical cancers (Boshart et al., 1984; Dürst et al., 1983). Shortly after, it became apparent that the generation of a vaccine against HPV was possible (Zhou & Frazer, 1991; Zhou et al., 1991), and extensive epidemiological studies eventually confirmed specific HPV types as significant risk factors for cervical cancer (Bosch et al., 1995; Muñoz et al., 1992), enabling targeted approaches for prevention. With the turn of the 21$^{st}$ century, HPV was understood to be a necessary but not sufficient cause of cervical cancer (Bosch et al., 2002). Since these critical discoveries, our understanding of HPV has quickly expanded. HPV is a profoundly common sexually transmittable agent contributing to various anogenital and oropharyngeal cancers, including those of the vulva, anus and penis. However, infections with HPV are predominantly attributable to cervix cancers (de Martel et al., 2017). After Helicobacter pylori, HPV is the next most common cancer-attributable pathogen worldwide (de Martel et al., 2020).

#### 1.1.2.1 HPV structure and functions

HPV is a non-enveloped, circular, double-stranded deoxyribonucleic acid (DNA) virus consisting of approximately 8000 base pairs (zur Hausen, 2002). A protein capsid encloses the virus, and depending on the HPV type, the genome is arranged into three regions containing DNA sequences on a single DNA strand. Among these are two coding regions: the early (E) and late (L) regions.

The E region comprises several encoding proteins or oncogenes (E1, E2, E4, E5, E6 and E7). Oncogenes E1 through to E5 replicate, assemble and regulate transcription of sequences during an infectious cycle, while E6 and E7 down-regulate tumour suppressor proteins (p53 and pRB), disabling cell apoptosis and cell cycle arrest, thus allowing infected cells to proliferate. In hrHPV types, E5, E6 and E7 are key oncogenes in cervical carcinogenesis. Breakthrough studies in the late 1990s showed that E6 and E7 proteins determine the phenotype expression of cervical cancer cells, and it appears their continued expression drives cell proliferation (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012; von Knebel Doeberitz et al., 1994; von Knebel Doeberitz et al., 1992). Furthermore, E7 enables the overexpression of the tumour suppressor protein p16-INK4A, also known simply as p16 (Khleif et al., 1996; von Knebel Doeberitz et al., 1992), as well as other genes resulting from cell proliferation, Ki-67 (Wentzensen et al., 2012). E5 also appears to play a crucial and versatile role in cervical carcinogenesis. In HPV 16, E5 allows uncontrolled cell growth via stimulation of vascular endothelial growth factor (VEGF) (Straight et al., 1993) and aids in avoiding the immune clearance of infected cells (Ashrafi et al., 2005).

The L region comprises the major (L1) and minor (L2) capsid proteins necessary for virus transmission. L1 can form virus-like particles (VLP) that are gene-less capsid shells, which can induce neutralising antibody responses (Neeper et al., 1996). A third non-coding region, the upstream regulatory region or long control region, contains the necessary elements that control the expression of the E and L regions.

HPV belongs to the family of papillomaviruses, with approximately 200 HPV types classified to date (Mühr et al., 2018). There are currently 13 HPV types of importance: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The basis for oncogenic risk assessment between the types depends on the pathogenesis of the virus. The International Agency for Research on Cancer (IARC) distinguishes these

types into carcinogenic to humans (Group 1), probably carcinogenic (Group 2A), and possibly carcinogenic (Group 2B; Table 1) (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012). While virtually all cervical cancers (99.7%) are attributed to these 13 hrHPV types (IARC Group 1 and 2A) (Arbyn et al., 2014; Walboomers et al., 1999), each HPV genotype contributes varyingly to the risk of cervical cancer. For example, HPV 16 and HPV 18 account for 70% of all invasive cervical cancer (ICC) diagnoses and types 31, 33, 35, 45, 52 and 58 contribute to a further 18% of cervical cancers (de Sanjose et al., 2010; Smith et al., 2007). There are also low-risk types, but these are not classifiable as carcinogenic to humans (Group 3: HPV 6, 11) as they are associated with benign condyloma acuminata and not with malignant cervical cancer (Garland et al., 2009). These risk stratifications are essential for diagnostic and screening purposes.

**Table 1.** HPV types and their carcinogenicity for cervical cancer as classified by the IARC[a]

| **Group** | **1** | **2A (probable)** | **2B (possible)** |
|---|---|---|---|
| **HPV type(s)** | 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 | 68 | 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, 97 |

[a] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012
IARC: International Agency for Research on Cancer.

#### 1.1.2.2 HPV life cycle and the cervical dysplasia pathway

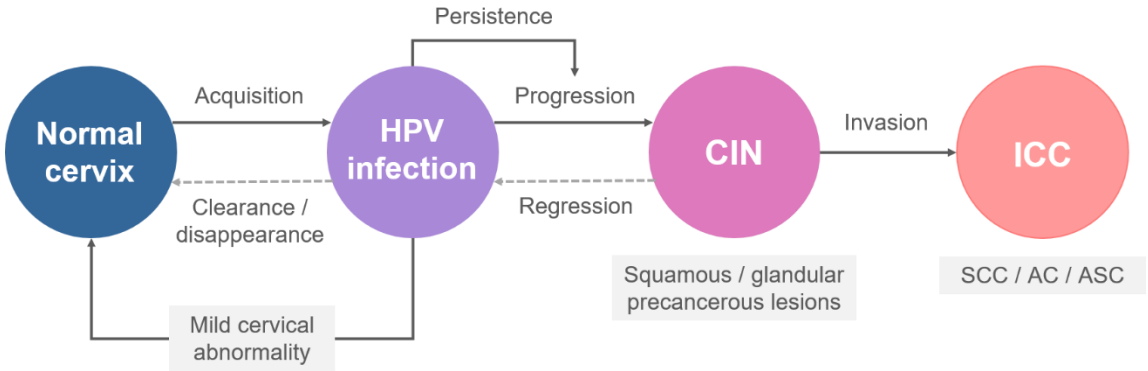
***HPV exposure***

HPV replicates exclusively in squamous epithelia. It is assumed that HPV virions penetrate the epithelium via microabrasions or tears at the basal or metaplastic epithelium of the SCJ. The underlying mechanisms for why the SCJ is most susceptible to HPV infections are unsettled (Doorbar & Griffin, 2019). It is currently proposed that the susceptibility to infection and potential resulting neoplasia are enabled via special stem cells existing below the columnar epithelium: reserve cells (Martens et al., 2009) or reserve-like cells, also known as discrete cell populations (Herfs et al., 2012). These special

cells function to remodel the cervix actively via squamous metaplasia but also appear to be bipotent, partaking in dysplastic epithelial proliferation after exposure to HPV (Doorbar & Griffin, 2019). Once HPV virions infect these special stem cells, they undergo cell division based on the expression of HPV E1 and E2 oncogenes. These infected basal cells mature, reproduce and extend within the various intraepithelial strata upwards towards the parabasal and more superficial epithelial layers with the help of early and late oncogenes, mainly E5, E6 and E7. Once virions have breached the epithelium and viral expression occurs, infected cell reproduction occurs.

***HPV infection: acquisition and viral clearance***

HPV is remarkably infectious and considered more transmissible than other STIs (Bruni et al., 2010; Burchell et al., 2006; de Sanjosé et al., 2007). It is estimated that the lifetime probability of HPV exposure is very high, from 70 to 80% (Bekkers et al., 2004) and up to 90% in some populations (Chesson et al., 2014). HPV prevalence is high in young women (below 25 years) at around 24% and declines linearly until middle age (Bruni et al., 2010). HPV acquisition generally occurs within several months following sexual debut and is also associated with multiple sexual partners, common among younger women (Castellsagué et al., 2014; Winer et al., 2003).




AC: Adenocarcinoma; ASC: Adenosquamous carcinoma; CIN: Cervical intraepithelial neoplasia; HPV: Human Papillomavirus; ICC: Invasive cervical cancer; SCC: Squamous cell carcinoma.

**Figure 2.** The natural history model of the cervical dysplasia pathway; adapted from Schiffman and Wentzensen (2013) and Gravitt and Winer (2017)

Figure 2 depicts the natural history model of the cervical dysplasia pathway. HPV may be acquired after exposure to an HPV-infected sexual partner and HPV reproduction can take weeks to months. The observed doubling time for an HPV infection in a large case-cohort study was 284 days, ranging from 188 days for hrHPV type 56 to 409 days for hrHPV type 18 (Depuydt et al., 2012). HPV acquisition does not always result in concurrent cytological abnormalities or carcinogenesis and can be detected with or without mild cervical dysplasia. One explanation for this may be due to the role of viral load in the formation of cytological abnormalities (Depuydt et al., 2009).

Within two years of acquiring HPV, over 90% of women 'clear' the infection (Muñoz et al., 2004; Woodman et al., 2001). The ability to 'clear' the infection depends on age, viral load, HPV type and sexual health and lifestyle behaviours, including smoking (Li et al., 2019; Munoz et al., 2009; Schmeink et al., 2013). It is also important to note that viral clearance or disappearance of infection may also be due to undetectable levels of HPV or latency (Gravitt & Winer, 2017). For example, in some populations, a second peak in HPV prevalence is observed in women older than 45 years (Bruni et al., 2010), possibly due to reinfection or reactivation of a past HPV infection (viral latency) (Rositch et al., 2012; Ting et al., 2015). Detection of HPV at the appropriate ages thus offers a critical window opportunity for meaningful screening and monitoring. However, infection by HPV alone is not enough to induce the development of cervical abnormalities, cervical cancer or its precursors.

***HPV persistence***

As depicted in Figure 2, the development of any subsequent cervical neoplasia after HPV acquisition appears to be driven exclusively by the persistence of a hrHPV infection (Rodríguez et al., 2010; Schiffman et al., 2011). Persistence of an HPV infection refers to the persisting or consecutive positive detection of HPV. Specific factors drive persistence, including hrHPV type, particularly HPV 16 (Castle et al., 2009; Kjaer et al., 2010; Xi et al., 2016), high viral load (Maucort-Boulch et al., 2010) and having multiple HPV infections (Castle et al., 2011). Repeated screenings for persistent HPV infections, particularly high-risk types, offer an ideal approach to distinguish women at greater or lower risk of developing cervical neoplasia and to monitor those with greater risk.

***Precursor stages: cervical intraepithelial neoplasia***

In some cases, the persistence of hrHPV-infected cells progresses towards cervical lesions (Figure 2). Growths contained only within the squamous epithelium are classified by the level of involvement of the epithelial layers, known as cervical intraepithelial neoplasia or CIN (Richart, 1973, 1990). According to this histological classification system, mild 'low-grade' abnormalities constitute CIN1, and moderate or severe 'high-grade' abnormalities are denoted respectively as CIN2, CIN3 or carcinomas in situ. CIN are squamous precursors of cervical cancer and are visible by cytology and, in advanced cases, to the naked eye. Thus, a general examination of the cervix and vagina by the smear-taker and the diagnostic swab of exfoliated cervical cells provides an ideal basis for early detection via screening measures.

Precancerous lesions can progress to invasive cancer or regress towards a normal cervix lasting months to years (Schiffman et al., 2011; Tainio et al., 2018). According to the few existing natural history studies, after two years, 50% of untreated CIN2 regressed, 32% persisted, and 18% progressed to CIN3 (Loopik et al., 2021a; Tainio et al., 2018). For CIN3, overall regression to CIN1 or less (up to 4 years later) was observed in 28% of cases, and regression towards the normal state was 18%. On the other hand, 67% of CIN3 persisted, and 2% eventually progressed to ICC (Loopik et al., 2021a; McCredie et al., 2008). In terms of duration, a progressing and persistent HPV infection is diagnosed as CIN3 after an average of 9 years (Depuydt et al., 2012). As for CIN2 or CIN3 progression towards ICC, it takes an average of 24 years, which is shortened in the presence of hrHPV type 16 (Vink et al., 2013). General CIN progression also appears to be associated with external factors, including multiparity (Muñoz et al., 2002), tobacco smoking (Nagelhout et al., 2021; Roura et al., 2014), long-term oral contraceptive use (Moreno et al., 2002; Roura et al., 2016) and coinfection with other STIs (Karim et al., 2018; Smith et al., 2004; Smith et al., 2002). However, these additional risk factors are somewhat minor compared to the persistence of hrHPV (Castellsagué & Muñoz, 2003), particularly with the same genotype (Bonde et al., 2021). These observations of progression and duration are crucial for screening (intervals, endpoints) and management practices.

On the other hand, regression of precancerous lesions is possible. Thus, monitoring for progression or regression rather than active treatment is more appropriate. Regression is significantly associated with HPV type and HPV-negative status (Loopik et al., 2021a), treatment intervention (McCredie et al., 2008)

and young age <30 years (Bekos et al., 2018; Loopik et al., 2021a). With every five years increase in age, the likelihood of regression is reduced by 21% (Bekos et al., 2018). Only a tiny proportion (~2%) of persistent HPV infections (>5 years) exist without any sign of CIN or ICC (Rodríguez et al., 2010), and almost all regress eventually (Loopik et al., 2021a). These precancerous stages of the cervical dysplasia pathway highlight the importance of ongoing screening and detection measures over a woman's lifetime to provide appropriate management where needed while balancing harms (over-treatment) and benefits (prevention).

#### 1.1.2.3 Invasive cervical cancer (ICC)

Any cluster of cells invading beyond the cervix's basal membrane is classified as ICC (Figure 2). ICCs are considered relatively rare in many HIC contexts with long-standing screening programmes, where the cumulative incidence rate of developing cervical cancer in any woman's lifetime ranges from approximately 1% among very high-HDI countries to 4.5% in countries with low HDI (Arbyn et al., 2020). ICCs are classified by their pathology and extent (staging). For pathology, ICC is sub-categorised by histology and epithelial involvement (WHO Classification of Tumours Editorial Board, 2020). Epithelial tumours include squamous cell carcinoma (SCC), adenocarcinoma (AC), neuroendocrine tumours and adenosquamous carcinoma (ASC), which are a mix of squamous and glandular cells. SCC and AC constitute most ICC, while ASC amount to between 2 to 4% of all ICC diagnoses (Castanon et al., 2016; Lei et al., 2019). Neuroendocrine tumours are rare and contribute to <2% of all ICC (de Sanjose et al., 2010; Gadducci et al., 2017).

***Squamous cell carcinoma (SCC)***

Carcinogenesis of the ectocervical epithelium is classified as SCC. Sub-types are further differentiated by HPV involvement: HPV-associated and HPV-independent tumours (WHO Classification of Tumours Editorial Board, 2020). Almost all SCCs (~95%) are HPV-associated (de Sanjose et al., 2010; Li et al., 2011; Rodríguez-Carunchio et al., 2015). HPV types 16, 31, 33, 52 and 58 are all associated with SCC, although most are due to HPV 16 (de Sanjose et al., 2010; Smith et al., 2007). SCC is the dominant sub-type of cervical cancers, accounting for over three-quarters of all invasive cancers within HIC and up to 90% in LMIC (Bray et al., 2017). In Germany, SCC accounts for 70% to 80% of all diagnoses (Robert Koch-Institut & Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V., 2021; Tanaka et

al., 2021). Smear sampling of SCC is more straightforward, particularly in younger women, as the cancerous cells are predominantly located at the TZ on the ectocervix. Screening efforts have significantly decreased SCC incidence (Adegoke et al., 2012; Bray et al., 2005b).

***Adenocarcinoma (AC) and other types***

Carcinogenesis of the glands within the endothelium is known as adenocarcinoma (AC). Like SCC, AC is sub-classified into HPV-associated and HPV-independent (WHO Classification of Tumours Editorial Board, 2020). Approximately 92% of AC are HPV-associated (Li et al., 2011). HPV genotypes 16, 18 and 45, particularly HPV 18, are commonly associated with AC, constituting 94% (Clifford et al., 2003; de Sanjose et al., 2010; Smith et al., 2007). HPV 18 is also significantly more prevalent in AC than in SCC (Li et al., 2011).

While AC occurrences (5 to 20% of ICC) are fewer than that of SCC (Bray et al., 2017), in some high-income populations, AC and adenocarcinomas in situ (AIS) have increased among young women (Bray et al., 2005a; van der Horst et al., 2017). These observations may explain increasing age-specific ICC trends in HIC with long-standing screening programmes, including Sweden, the United States and the United Kingdom (Singh et al., 2023). Explanations for such increases include the incidental findings of AC with coexisting precancerous lesions (CIN), changes in smear-taking tools, and shifting prevalence of glandular-associated HPV 18 towards younger women (Rozemeijer et al., 2015; Scherpenisse et al., 2012). Since the glands within the endocervical canal extend into finger-like crypts (Figure 1), smears must be adequately collected using tools properly designed to reach into crevices. Such tools include endocervical brushes and brooms, now the mainstay recommended tool when the TZ is not visible (Martin-Hirsch et al., 2000). Despite this vulnerability, screening practices have decreased the overall incidence of AC and screening continues to capture early-stage AC, although the extent of the impact is significantly lower than for SCC (Castanon et al., 2016; International Collaboration of Epidemiological Studies of Cervical Cancer, 2007; Sasieni et al., 2009).

As for the other types of ICC, the beneficial impact of screening has not been lost. Like its impact on SCC, albeit to a lesser extent, screening has decreased the incidence of ASC more significantly than AC (Castanon et al., 2016; Lei et al., 2019). These observations indicate an advantage in detecting the squamous component of the tumour. Undergoing at least two screenings significantly reduced the risk

of ASC and rare ICC compared to no screening (Lei et al., 2019). Almost all the ASC and rare ICC types included in the study by Lei et al. (2019) tested positive for HPV.

Fortunately, due to long-standing cytological screening practices (Meggiolaro et al., 2016; Peirson et al., 2013; Vaccarella et al., 2013), incidence rates of ICC are relatively low in HIC (Singh et al., 2023). Nonetheless, screening for cytological abnormalities and precancerous lesions does not guarantee cancer prevention. Over half of ICCs diagnosed had an inadequate screening history, and almost a third of ICCs had a false-negative cytology result prior to diagnosis (Spence et al., 2007). Therefore, advanced cervical cancers (interval cancers) can still be found even in countries with widespread screening and low incidence.

#### 1.1.2.4 Cofactors along the cervical dysplasia pathway

Several determinants have been identified as potential cofactors associated with a higher risk of precancerous lesions and cervical cancer, which may aid in further distinguishing women at greater risk. These include sexual and reproductive health as well as individual lifestyle factors. For example, many pooled and multi-centred studies have observed associations of progression to cervical cancer with the age of sexual debut (Plummer et al., 2012), a high number of sexual partners (International Collaboration of Epidemiological Studies of Cervical Cancer, 2009), high parity and long-term oral contraceptive use (International Collaboration of Epidemiological Studies of Cervical Cancer, 2007; Tekalegn et al., 2022), as well as acquisition the of other STI infections such as Chlamydia trachomatis and Herpes simplex virus-2 (Karim et al., 2018; Smith et al., 2004; Smith et al., 2002). Immune system suppression has also been suggested as an associated cofactor, particularly for older women, immunocompromised persons and persons living with HIV (Grulich et al., 2007; Kelly et al., 2018; Strickler et al., 2005). Lack of screening participation or at regular intervals is also associated with precancerous and cancerous lesions (Peirson et al., 2013). However, these studies did not capture nor adjust for HPV infection as a potential confounder.

Among studies considering HPV status (i.e. among women with HPV infection) and its progression towards ICC, cofactors such as high parity and long-term oral contraceptive use are significantly associated (Moreno et al., 2002; Munoz et al., 2009; Rositch et al., 2012). Lifestyle and health status factors such as tobacco smoking (Nagelhout et al., 2021; Roura et al., 2014) and being overweight or

obese (Clarke et al., 2018) have also been demonstrated as independent risk associations, regardless of HPV status. A recent systematic review highlighted the potential of the vaginal microbiota and its role in all stages of the HPV life cycle: HPV infection, persistence and cervical disease (Brusselaers et al., 2019). These studies point towards possible underlying hormonal and inflammatory mechanisms leading to carcinogenesis but also highlight issues of potential under-diagnosis of ICC due to sampling and visual examination difficulties and screening participation issues.

Despite the role of these cofactors, these appear to play a lesser or secondary role compared to HPV persistence and HPV type (IARC Working Group on the Evaluation of Cancer-Preventive Interventions, 2022). These findings underscore the statement that HPV is a necessary but not sufficient cause of cervical cancer (Bosch et al., 2002) and its presence or absence, in addition to known cofactors, can aid in further distinguishing subgroups of women at greater risk of developing ICC.

## 1.2 Prevention methods

### 1.2.1 Vaccination

The positive impacts of cytological screenings on the trajectory of cervical cancer incidence are indisputable. The discovery of HPV and its persistence depending on the genotype and other external factors have proved to be invaluable knowledge for additional prevention efforts. It has enabled the development of prophylactic vaccines, which prevent HPV infections and the subsequent development of cervical cancer from occurring (Koutsky et al., 2002). Since 2006, three approved vaccines have been widely distributed to target the main hrHPV types 16 and 18: Cervarix® (GlaxoSmithKline), Gardasil® and Gardasil®9 (Merck & Co., Inc.). Cervarix® is a bivalent vaccine targeting the two major hrHPV types. Gardasil® and Gardasil®9 respectively target four (quadrivalent) and nine (nonavalent) types of high and low-risk HPV responsible for ICC as well as condylomata acuminata (World Health Organization, 2022). These vaccines target the L1 proteins of HPV and their VLPs, which neutralise antibody responses before HPV is encountered (Kwak et al., 2011; Longet et al., 2011).

Prophylactic HPV vaccines have demonstrated long-term safety and prevention of HPV infection by up to 83% (Drolet et al., 2019). In the 10 to 15 years following worldwide rollout, these vaccines have

significantly reduced condylomata acuminata by up to 67% (Drolet et al., 2019), high-grade cervical lesions by up to 51% (Brotherton et al., 2015; Donken et al., 2021; Drolet et al., 2019; Pollock et al., 2014) and ICC by up to 87% in the United Kingdom and Sweden (Falcaro et al., 2021; Lei et al., 2020). Moreover, the bivalent and quadrivalent vaccines appear to offer prolonged cross-protection of other hrHPV types, such as HPV 31, which may be beneficial in further reducing cervical cancer rates (Brown et al., 2021; Kavanagh et al., 2017; Mariz et al., 2021; Tsang et al., 2020). There are, however, access and uptake issues with prophylactic HPV vaccinations (Bruni et al., 2021). Nonetheless, vaccines have provided a quick and powerful way to deter HPV infections and their subsequent diseases. Once adequately rolled out, its impact on population incidence will only be evident in several decades, and a further reduction towards elimination thresholds, even for very high HDI countries, will only appear if rapid scale-up of vaccination coverage (80-100%) using a nonavalent vaccine is adopted together with two lifetime screenings (Simms et al., 2019). This thesis focuses on the second type of prevention: screening for cervical disease.

### 1.2.2 Screening

Screening is a form of secondary prevention. Detection of HPV and its genotype and monitoring its progress is highly beneficial, given that several types are associated with carcinogenesis. Thus, many molecular assays have been designed to detect nucleic acid, for example, HPV DNA and are commercially available and validated (Arbyn et al., 2021). Even with the availability of these tools, there are challenges in detection due to the transient nature of HPV infections and the ability of HPV to survive several days without a host, adding to its high transmissibility (Roden et al., 1997; Trottier et al., 2008). The detection of HPV infection requires appropriate age thresholds and screening intervals and excellent performance, especially since HPV infections will be highly prevalent in younger women that will mostly clear within two years of detection. Furthermore, appropriate validation protocols are necessary to determine the minimum thresholds for viral load detection. Without such considerations, over-screening will occur, devoid of any benefits in preventing cases (Burger et al., 2017; Dijkstra et al., 2016).

Over-screening has economic and negative health implications, including a higher number of lifetime tests done and anxiety (Habbema et al., 2017). Over-treatment may also result if no appropriate triage mechanism is provided (Kim et al., 2018). Methods to minimise these undesired outcomes include

beginning HPV testing at a higher age threshold, extending screening intervals and using triage testing to determine the risk of progression to cervical cancer (Cuschieri et al., 2018). Additionally, active monitoring of infections via colposcopy, a specialised follow-up assessment in the screening algorithm, is crucial in safeguarding adequate yet sufficient testing and treatment (Cruickshank et al., 2015) and may even help expedite the regression of infection in some cases (Petry et al., 2018). The following section will provide an overview of screening programmes and aspects relating to this thesis.

## 1.3 Screening programme

Within screening approaches, the main pillars of cervical cancer screening programmes include screening organisation (design), screening tools, the screening algorithm and quality assurance (European Commission, 2008). In this thesis, the 'baseline' refers to the organisation of screening (the availability, invitation to or opportunity to screen) and access, underscored by the goals, endpoints, design and quality assurance principles highlighted below.

### 1.3.1 Goals and endpoints

The overarching goal of screening is to decrease the incidence and subsequent mortalities by swiftly detecting as many asymptomatic and precancerous cases in the general population as early as possible for subsequent management and treatment where necessary (IARC Working Group on the Evaluation of Cancer-Preventive Interventions, 2022). Screening for precursors of cervical cancer (CIN2 and CIN3 or worse) as a surrogate for cancer risk is appropriate and accepted (IARC Working Group on the Evaluation of Cancer-Preventive Interventions, 2022). Several reasons support using CIN2 or worse as endpoints for comparative studies rather than classical endpoints such as ICC incidence and mortality. First, cytological screenings have been in place for decades (Bruni et al., 2021) and have successfully reduced ICC incidence and mortality rates (Meggiolaro et al., 2016; Vaccarella et al., 2013). Well-screened women will rarely have an undetected ICC diagnosis and an overall low lifetime risk of developing ICC. Second, early detection of precancerous cervical lesions that may be of higher oncogenic risk is possible, and the risk of progression towards ICC can be estimated and monitored (Loopik et al., 2021a; McCredie et al., 2008). The cervical dysplasia pathway is slow-growing and regular screenings present sufficient opportunities to intervene before ICC develops. Third, when the benefits

of screening are already known and widespread, there are ethical and practical hindrances to using incidence and mortality endpoints, especially for assessing new screening tools. In contexts with high incidence and mortality rates and where resources are limited, screening for the invasive stages of the disease is a priority endpoint to reduce premature deaths. Nevertheless, the endpoints CIN2 and CIN3 or worse in screening programmes capture precancerous and cancerous lesions.

### 1.3.2 Design

In a cervical screening programme, predefined eligible women can be invited to undergo screening (via organised programmes) or are opportunistically screened. These two types of screening programmes differ in that the former (organised) invites and screens a predefined target population (by age) within a predefined screening algorithm (screening method, interval, follow-up thresholds) but also relies on rigorous quality assurance of screening service delivery and performance at every step of the screening algorithm (European Commission, 2008). Organised screening encompasses the systematic identification, invitation and appropriate follow-up or discharging of eligible and screened women. The latter programme type (opportunistic), which can be non-population based (unorganised), relies on the woman's request or the opportune offer of screening by the healthcare professional being visited (IARC Working Group on the Evaluation of Cancer-Preventive Strategies, 2005). This type of programme is only effective if a pre-existing health policy outlining the target population and screening algorithm can be utilised.

The reliance on self-referral or opportunistic screenings reveals an obvious disadvantage compared to organised programmes. Studies have robustly demonstrated more significant reductions in incidence and mortality by organised programmes than opportunistic programmes in the Nordic countries (Lăără et al., 1987; Nieminen et al., 1999), as well as in countries where opportunistic screenings have shifted to an organised programme such as the case for countries in northern, western and southern Europe (Quinn et al., 1999; Rebolj et al., 2007; Ronco et al., 2005). It has been shown that invitation to screenings significantly improves screening coverage and minimises inequalities (Staley et al., 2021). However, systematic invitations can only be carried out if a database with relevant information such as age exists and is accessible (European Commission, 2008). The systematic documentation of invitation-eligible persons also forms the denominator for screening coverage estimation (the number of screened participants invited serves as the numerator) and can serve as key indicators of quality

assurance. Furthermore, the cost-effectiveness of both programme types is vastly different, with opportunistic screening costing substantially more but with minimal gains in incidence reduction (Arbyn et al., 2009a). Finally, fewer inequalities and harmful outcomes occur within organised programmes (Miles et al., 2004), providing abundant evidence for the preference for organised programmes.

### 1.3.3 Quality assurance

Quality assurance enables the uniform provision and reduced variability of screening and services. It is an essential component of effective screening programmes and encompasses standards measured by quality indicators such as screening coverage and screening diagnostic performance, as well as monitoring and evaluation. Quality assurance should be integrated within local, national and international screening guidelines. At the local level, laboratories should commit to delivering standards that meet external validation levels through accreditation, internal validation and annual audits (Cuschieri et al., 2019). On a national level, guidelines should outline select screening tools, inter-laboratory assessments, as well as evaluation and monitoring indicators and thresholds. International guidelines should provide an overview of validated screening tools acceptable for incorporation into national guidelines and regulatory approval.

The first European Union (EU) guideline on cervical cancer screening was published in 1993 and outlined population-based screening recommendations for cytology-based smear collection and quality assurance in cytology laboratories (Coleman et al., 1993). In 2008, the second EU guideline outlined aspects of quality assurance for screening tools, procedures, reporting, evaluation, communication and qualifications (European Commission, 2008). Other supplements and recommendations to overhaul opportunistic screening programmes into organised systems were added in 2015 to integrate HPV-based testing, given the mounting evidence of its superior performance (Anttila et al., 2015; Ronco et al., 2015). These guidelines describe quality assurance indicators that focus on i) screening coverage, for example screening participation rates, ii) screening diagnostic performance, for example test specificity and iii) diagnostic follow-up and treatment, for example adherence to follow-up examination after abnormal screening results.

### 1.3.4 Access and participation

Within the cancer care continuum, one of the first failures in care that can occur in a screening programme is failure to access screening (IARC Working Group on the Evaluation of Cancer-Preventive Strategies, 2005). Barriers to access, particularly socio-cultural factors, must be considered even within an organised programme. High screening coverage (few barriers) is important for screening programme success. Screening non-participation constitutes the largest factor in screening care failures (between 40% and 54%), followed by false-negative Pap smears (29%) and poor follow-up of abnormal results (12%) (Spence et al., 2007). Among HIC, screening uptake within the previous five years was approximately 77% (121.2 million women) and lifetime screening rate was 84% (132.6 million women) (Bruni et al., 2022). This translates to approximately 36 million women who have not been screened recently or within the recommended intervals and 25 million women who have never been screened. Several novel interventions have demonstrated significant improvements in screening coverage (other than shifting to an organised programme), including instating reminders, prescheduling appointments and offering or directly sending HPV self-sampling kits (Arbyn et al., 2018; Staley et al., 2021). While screening participation regardless of design is important, adequate diagnosis, follow-up and management, and quality assurance can ensure the effectiveness of a screening programme.

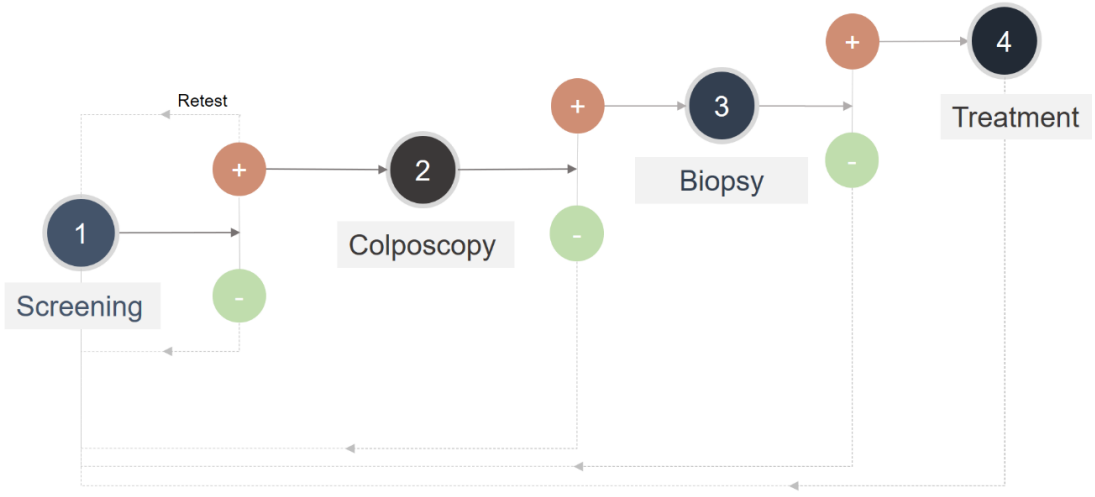
The focus of this thesis will not delve into the organisation and uptake of screening but examines failure in care beyond this screening programme baseline. The following sections provide an overview beyond the baseline of a screening programme, specifically the screening algorithm and quality assurance measures (including screening diagnostic performance, diagnostic follow-up) at each step. These are incorporated in the two studies central to this thesis.

### 1.3.5 Screening algorithm

Appropriate management and treatment of screen-positive women is crucial in an effective screening programme and forms the screening algorithm, a threshold-based decision-making aid. There are several types of screening algorithms, grouped by the WHO as a 'screen-and-treat' approach or a 'screen, triage and treat' approach (World Health Organization, 2021). In a 'screen-and-treat' approach, the decision to provide treatment is based immediately following a positive screening. They are most

appropriate in LMIC or contexts with resource constraints. In a 'screen, triage and treat' approach, treatment is appropriate only following a positive primary screening result and a positive triage assessment, with or without a histologically confirmed diagnosis. This approach is common in most HIC with screening programmes and mitigates potential over-treatment and additional harm.

The spectrum of cervical cancer control involves four critical assessments (Figure 3). The first essential assessment includes screening eligible populations who are mostly asymptomatic. In this thesis, triage assessments of the primary screening result (i.e. repeat or sequential testing) are considered part of the first assessment as they typically do not require a specialised follow-up. The second critical assessment is the follow-up evaluation of the screening result(s) by colposcopic examination (colposcopic triage). This examination includes a physical examination of the cervix and tissue biopsy sampling by an expert colposcopist, a specifically trained gynaecologist or in some cases, a specifically trained nurse. The third critical assessment is a pathologist's histological evaluation of the biopsy sample(s), which is the gold standard for disease verification. The fourth essential assessment includes treatment or determination of the best management strategies, based on the screening, colposcopy and biopsy evaluations. The following sections will delve into each assessment and describe current recommendations and quality assurance considerations.




+ indicates a positive or abnormal result; - indicates a negative or normal result.

**Figure 3.** The general algorithm of critical cervical cancer screening assessments

#### 1.3.5.1 Screening

During screening, a cervical smear is obtained for further cytological or HPV testing. In order to maximise the appointment, proficient communication with the women attending screening is necessary. Screening includes extracting a general health and medical history, an explanation of the screening test to be conducted and potential outcomes and steps after the screening result is received. A standardised reporting form should be used to detail the identity of the woman screened, report clinically relevant observations such as bleeding and provide space for the laboratory result (European Commission, 2008). Regarding cytology-based screening, reporting should be made according to a standardised national or international classification system (Coleman et al., 1993).

***Nomenclature***

For screening and cytological or histological diagnosis, several standardised classification systems exist. The general phases of cytological abnormalities typically begin with a normal state of cells and range from changes that are borderline to low-grade, high-grade, glandular or invasive (Herbert et al., 2007). The Papanicolaou classification system, a previously widely used 5-tiered system, was developed in the 1960s when cytological screenings were being rolled out (Papanicolaou, 1963). This system was adapted into the Munich Nomenclature with several modifications and used as the national classification in Germany (Cirkel et al., 2015). These modified versions include the Munich II (1990) and Munich III systems (2014). The latter is currently the standard system for cytological diagnoses in Germany. Internationally, the Bethesda classification system is regarded as the standard nomenclature. Regarding histology assessment, the Cervical Intraepithelial Neoplasia (CIN) terminology for histological lesions is widely used (Richart, 1973). The European Union (EU) quality assurance guidelines recommend using a nationally agreed nomenclature that is translatable into the Bethesda system for cytology (European Commission, 2008). The equivalent translations of the Munich (II, III) and Bethesda classification systems and the CIN terminology for histology are presented in Table 2.

**Table 2.** Comparison of the international nomenclature for cytology and histology findings

| Munich II (1990)[a] | | Munich III (2014)[b] | | Bethesda System (2014)[c] | | Histology CIN* |
|---|---|---|---|---|---|---|
| **0** | Inadequate material | **0** | Unsatisfactory specimen | **Unsatisfactory for evaluation** | | |
| **I** | Normal cell pattern | **I** | Normal / unsuspicious cell pattern | **NILM** | Negative for intraepithelial lesion / malignancy | **Normal** |
| | | **IIa** | Normal cell pattern with suspicious medical history | | | |
| **II** | Mild inflammatory, regenerative, metaplastic or degenerative changes | **-** | - | **-** | - | |
| **liw lik** | Unofficial category for "kontrollbedürftiger" or repeat smear, often used to note minimal changes and koilocytes | **II-p** | Squamous epithelium with low-grade changes of nucleus <CIN1 & with koilocystic cytoplasm / parakeratotic changes | **ASC-US** | Atypical squamous cells of undetermined significance | **Koilocytic atypia, flat condyloma, without epithelial changes; 'Low-grade' CIN** |
| | | **II-g** | Abnormal cervical glandular cells, more than reactive changes | **AGC endocervical NOS** | Atypical glandular cells, otherwise not specified | |
| | | **II-e** | Endometrial cells; Women >40 years & more than second half of the cycle | **Endometrial cells** | - | |
| **III** | Unclear findings: severely inflammatory or degenerative / poorly preserved cell material, abnormal glandular / stromal cells, dysplasia, CIS or invasive carcinoma not excluded | **-** | - | **-** | - | |
| | | **III-p** | CIN2/CIN3/SCC cannot be excluded | **ASC-H** | Atypical squamous cells of undetermined significance, cannot exclude HSIL | |
| | | **III-g** | Distinctive atypia of glandular cells, AIS / invasive AC cannot be excluded | **AGC endocervical favour neoplastic** | Atypical glandular endocervical cells favour neoplastic | |
| | | **III-e** | Abnormal endometrial cells (esp. postmenopausal) | **AGC endometrial** | Atypical glandular endometrial cells | |
| | | **III-x** | Unclear glandular cells of unknown origin | **AGC favour neoplastic** | Atypical glandular cells favour neoplastic | |
| **IIID** | Cells of mild or moderate dysplasia | **-** | - | **-** | - | |
| | | **IIID1** | Cells of mild dysplasia CIN1 | **LSIL** | Low-grade squamous intraepithelial lesion | **CIN1 'Low-grade'** |
| | | **IIID2** | High-grade | **HSIL** | High-grade squamous intraepithelial lesion | **CIN2 'High-grade'** |
| | | | | | | **CIN3** |

| Munich II (1990)[a] | | Munich III (2014)[b] | | Bethesda System (2014)[c] | | Histology CIN* |
|---|---|---|---|---|---|---|
| **IVA** | Cells of severe dysplasia or CIS | **-** | - | **-** | - | **'High-grade'** |
| | | **Iva-p** | Cells of severe dysplasia or CIS analogous with CIN3 | **HSIL** | High-grade squamous epithelial lesion | |
| | | **Iva-g** | Cells of AIS | **AIS** | Adenocarcinoma in situ – premalignant | **AIS Microinvasive lesion** |
| **IVB** | Cells of severe dysplasia or CIS; cells of invasive carcinoma not safely excluded | **Ivb-p** | CIN3 invasion cannot be excluded | **HSIL with features suspicious for invasion** | - | **CIS Microinvasive lesion** |
| | | **Ivb-g** | Cells of AIS invasion cannot be excluded | **AIS with features suspicious for invasion** | - | |
| **V** | Cells of invasive cervical carcinoma or of other malignant tumours | **-** | - | **-** | - | **Invasive carcinoma** |
| | | **V-p** | Squamous cell carcinoma | **SCC** | Squamous cell carcinoma | |
| | | **V-g** | Endocervical AC | **Endocervical AC** | - | |
| | | **V-e** | Endometrial AC | **Endometrial AC** | - | |
| | | **V-x** | Other malignant tumours, also of unclear origin | **Other malignant neoplasms** | - | |

[a] Cirkel et al., 2015
[b] Griesser et al., 2013; Griesser et al., 2015
[c] Küppers & Reich, 2016
* combined both original terminology and modified terminology (Richart, 1973, 1990);
AC: Adenocarcinoma; AIS: Adenocarcinoma in situ; CIN: Cervical intraepithelial neoplasia; CIS: Carcinoma in situ; HSIL: High-grade squamous intraepithelial lesion; LSIL: Low-grade squamous intraepithelial lesion; SCC: Squamous cell carcinoma.

### *Screening tools*

The performance of the screening tools and services used to detect precursors is crucial regarding their effectiveness. An ideal and effective test should have high sensitivity (identifying persons with disease among truly diseased persons) and high specificity (ruling out non-diseased persons among truly non-diseased persons). Otherwise, they give rise to false-negative or false-positive results, which can lead to undetected ICC or induce unnecessary fear and anxiety. If high enough, these features will effectively help the screening system correctly identify those at risk for cervical cancer and those at low or no risk of cervical cancer to be assured until the next screening round (IARC Working Group on the Evaluation of Cancer-Preventive Interventions, 2022).

Additional indicators of screening tool performance include reliability, particularly of colposcopies and among pathologists within and between clinics and laboratories. Reliability can be measured by general agreement, the proportion of total true positives and true negatives agreed upon in both evaluations (either by the same person: intra-observer or between two observers: intra-observer) among the total number of assessments. Intra-observer and inter-observer reliability are typically determined by Cohen's Kappa κ, which is the degree of agreement or disagreement beyond chance alone (Cohen, 1960; Cohen, 1968). Additionally, the costs, feasibility of implementation and potential harms must be considered (Streetly & Holland, 2009).

The WHO recommends any of the following three screening tools to be implemented for cervical cancer screening, depending on context: cytology, HPV testing or visual inspection with acetic acid (VIA) (World Health Organization, 2021). Cytology and HPV testing rely on microscopic and molecular assessment for cervical abnormalities and HPV presence. Both require adequate infrastructure and resources to function efficiently. VIA requires no magnification and is conducted by physically applying diluted acetic acid (3-5%) to the cervix, followed by identifying aceto-whitened regions for epithelial abnormalities. Despite its rapidness in detecting abnormalities and low costs, VIA is a highly subjective method. It is further hampered if the TZ is not visible and impractical if treatment is unavailable or inaccessible (World Health Organization, 2014). Due to these reasons, VIA may be a feasible screening method for low-resource settings. Recently, HPV testing was recommended as the preferred primary screening tool, although a quality-assured cytological programme may continue in the absence of operational HPV testing (World Health Organization, 2021). Both cytology and HPV-based screening have their advantages and disadvantages. Within the context of this thesis, only cytology and HPV testing screening methods will be examined, as these methods are mostly adopted or utilised in HIC settings.

### A. Cytology

A sample or smear of the cervix is taken via physical exfoliation of the TZ by a trained smear-taker for further microscopic evaluation. The smear-taker may be a general physician, a specialised physician (gynaecologist) or a nurse (World Health Organization, 2014). Importantly, adequate training is essential to obtain a good quality smear for further assessment. There are two main types of cytological assessment which vary in sample collection, preparation and functionality.

***Conventional cytology***

Historically, the mainstay of cytological screenings has been conventional cytology. The main device used to collect the cervical smear shifted from dry cotton-tipped swabs to spatulas, brushes and brooms to allow for better exfoliation of the ectocervix and endocervix (Martin-Hirsch et al., 2000). Following the smear collection, the exfoliated cells are immediately transferred to a glass slide via rolling and rotations of the device head and subsequently fixed using 95% ethyl alcohol to prevent air drying. The slides are then transferred to be microscopically evaluated by a trained cytopathologist.

The effectiveness of conventional cytology has been questioned by the significant number of false-negative results resulting in lowered sensitivity. In a Cochrane systematic review of studies, the sensitivity to detect low-grade lesions (CIN2) or worse ranged from 43% to 96% and among high-grade lesions (CIN3) or worse, it ranged between 39% to 85% (Koliopoulos et al., 2017). The pooled sensitivities were 66% and 70%, respectively. On the other hand, specificity was very high, pooled at 96% for CIN2 or worse and 97% for CIN3 or worse. False negatives are often due to sampling errors and variability in cytology assessment (Spence et al., 2007) and high false-negative results can lead to delays in cervical cancer diagnosis (Philp et al., 2018). Furthermore, in some countries, such as Germany, conventional cytology is also considered a more costly method than the more modern cytological form: liquid-based cytology (LBC), due to drawbacks in smear preparation (Armstrong & Guest, 2020).

***Liquid-based cytology (LBC)***

LBC differs from conventional cytology in the collection and preparation of the smear. Regarding smear collection, a spatula, brush or broom is used to exfoliate the cells; however, instead of fixing the cells directly to the slide, the head of the sampling device is submerged and flushed in a vial of preservative liquid, which is then transported for further preparation. Regarding smear preparation, the liquid vial is centrifuged in the laboratory. The exfoliated cells are resuspended in mucolytic and haemolytic agents, where excess substances such as blood and mucous are effectively removed. A representative sub-sample of the cells relevant for cytopathology assessment is spread into a thin layer on a glass slide for further staining and microscopy. This process allows fewer inadequate smears for cytological assessment (Beerman et al., 2009) via the concentration of relevant cells (Bernstein et al., 2001).

Despite these advantages, the sensitivity of LBC is also variable, yet marginally higher than conventional cytology, pooled at 76% for both endpoints CIN2 and CIN3 or worse (Koliopoulos et al., 2017). Although some extensive studies observed better detection capabilities of cervical abnormalities and precancerous lesions (Beerman et al., 2009; Klug et al., 2013), its sensitivity does not appear to be superior to conventional cytology in meta-analyses (Arbyn et al., 2008a; Koliopoulos et al., 2017). This difference in findings could be due to the preparation of the cells, which involves either cell enrichment via the ThinPrep® system (Hologic, United States) or cell filtration via the SurePath® (BD Diagnostics, United States) system (Medical Services Advisory Committee, 2014), or differing populations assessed (Arbyn et al., 2008a). The pooled specificities were similar yet marginally lower than conventional cytology, at 92% (CIN2 or worse) and 91% (CIN3 or worse) (Koliopoulos et al., 2017). The reliability of LBC is also variable, particularly in borderline abnormalities, with only 43% agreement between cytopathologists and blinded cytopathologist reviewers (Stoler et al., 2001). One meta-review reported improved detection of glandular lesions by LBC, probably due to computer-assisted readings (Gibb & Martens, 2011).

A further advantage of LBC includes using the residual sample for additional HPV testing. LBC shortcuts additional testing without requiring the screened women to return for a second smear. LBC can also serve as a triage method following a positive HPV test result. Additionally, modelling studies demonstrated the cost-effectiveness benefits of LBC compared to conventional cytology (Armstrong & Guest, 2020; de Bekker-Grob et al., 2012; Karnon et al., 2004). Several countries such as the United Kingdom, the Netherlands and Australia have shifted from conventional cytology to LBC screening as their primary sampling medium due to these advantages (Cancer Council Australia Cervical Cancer Screening Guidelines Working Party, 2016; Maver & Poljak, 2020).

Laboratories must comply with national nomenclature that is translatable into the Bethesda classification system, and quality assurance of cytological processes are necessary. The adequacy of educational training and expertise of cytotechnicians and cytopathologists preparing and diagnostically evaluating the smears heavily influences the quality and effectiveness of cytology-based screening (European Commission, 2008; IARC Working Group on the Evaluation of Cancer-Preventive Interventions, 2022). Diagnostic quality is also dependent on the workload. Improvements to cytological technologies also led to computer-assisted cytological assessment, whereby rapid

screening of copious slides could aid in singling out anomalies for further manual review. This technology can save resources, reduce turnaround time and improve the detection of lesions (Klug et al., 2013; Rebolj et al., 2015).

Because of the remarkably variable sensitivity and subjectivity and the historical absence of any triage mechanisms in most cytological screening programmes implemented, short and regular screening intervals of five or fewer years are necessary (Peirson et al., 2013). Some countries, such as Germany and Austria recommend cytology screening every one to two years. However, most countries with cytology screening (over 75%) recommend screening at least every three years or more (Bruni et al., 2022). Shorter intervals translate into many lifetime screening tests and associated costs (Chao et al., 2019; Petry et al., 2017). Recent longitudinal analyses from Sweden and Australia of young HPV-vaccinated cohorts showed that the positive predictive value of cytology for predicting high-grade lesions decreased as prevalence rates of hrHPV types shifted (Lei et al., 2020; Sultana et al., 2019).

Despite driving down incidence and mortality rates historically, the clear disadvantages of cytology-based screening comprise its inferior reproducibility, poorer sensitivity and thus shorter intervals leading to more lifetime screenings and costs, and declining detection ability in the face of HPV vaccination efforts. Additionally, the pitfalls in cytological screenings translate to missed cancer diagnoses, some of which are at advanced stages with poor prognoses (Spence et al., 2007). These points may explain why incidence has plateaued in HIC with HPV vaccination and cytological screening systems available and highlight the necessity for re-evaluation, as well as more accurate and balanced benefit-to-harm screening alternatives.

### B. HPV testing

Using the smear sample obtained by exfoliation of the cervix, HPV testing involves the molecular detection of free HPV virions or HPV-infected cells via nucleic acid (DNA or ribonucleic acid [RNA]). A selected group of HPV types are targeted and can include both high and low-risk types. Since the early 2000s, several large RCTs assessed the accuracy of HPV testing as a primary tool compared to cytology alone (Kitchener et al., 2009; Mayrand et al., 2007; Naucler et al., 2009; Ronco et al., 2008; Sankaranarayanan et al., 2009). They consistently found that HPV testing was superior in sensitivity to cytology, which was further reiterated in meta-analyses and real-world surveillance data (Arbyn et al.,

2012; Koliopoulos et al., 2017; Rebolj et al., 2019; Veijalainen et al., 2019). Longitudinal trial results and pooled estimates also support the superior utility of HPV testing compared to cytology (Arbyn et al., 2012; Dijkstra et al., 2016; Kitchener et al., 2011b; Leinonen et al., 2012; Murphy et al., 2012; Rijkaart et al., 2012; Ronco et al., 2014; Wright et al., 2015). HPV assays have also been investigated as triage alternatives for abnormal cytological screening results or following positive hrHPV results (Arbyn et al., 2004; Demarco et al., 2020; Polman et al., 2019), and as a test of cure following treatment of a precancerous lesion (Arbyn et al., 2012).

Until now, the United States Food and Drug Administration (FDA) has approved several HPV assays for use as an adjunct test (co-test) to cytology (Hybrid Capture® 2 [HC2], Cervista, APTIMA) and two assays for use as a primary tool stand-alone or co-test (Cobas 4800, BD Onclarity) (U.S. Food and Drug Administration (FDA), 2019). Subsequently, guidelines in several HIC with long-standing cytological screening programmes were updated to incorporate for example HPV testing either as a primary tool alone in the Netherlands (National Institute for Public Health and the Environment (RIVM), 2017; Ronco et al., 2015) or optionally as a co-test in the United States and Germany for example (Fontham et al., 2020; Gemeinsamer Bundesausschuss (G-BA), 2018b; Hillemanns et al., 2019a).

For the detection of HPV nucleic acid, several techniques exist, categorised into non-amplified and amplified methods. Non-amplified methods include direct hybridisation (dot blot, Southern blot, and filter in-situ hybridisation). These methods are no longer used in screening due to their low sensitivity, specificity, and time and resource-intensive features (Duggan et al., 1994; Schiffman & Schatzkin, 1994). Amplified methods can be either signal-amplified, in which signals are generated from probes containing the nucleic acid of HPV types, or target-amplified, where target HPV nucleic acid sequences of hrHPV types are duplicated and amplified.

***Signal amplification***

For signal-amplified assays, labelled HPV DNA or RNA probes are used to identify sequences of HPV types and bind to targeted sequences with the help of monoclonal antibodies. These bindings produce light signals based on the removal of alkaline phosphatase (dephosphorylation), which are visible under microscopy. Thresholds for these light emissions, relative light units (RLU), signal the presence or absence of HPV DNA in a sample. The most commonly used and commercially available signal

amplification assay is the HC2 (Qiagen, United States), which was the first assay approved by the FDA for use as a triage to cytology and then as a co-test (U.S. Food and Drug Administration (FDA), 2019). Samples for HC2 are collected via a liquid-based medium, including the PreservCyt® solution (Hologic, Inc., United States) used for ThinPrep or the in-house HC2 sampling kit. HC2 detects 13 hrHPV types (IARC Group 1 and 2A) using RLU thresholds. These RLU values are compared to a control threshold of 1 pg/ml (for HPV 16) to determine a viral load ratio. Thus, RLU values greater than 1.0 (equivalent to 1000-5000 HPV genomes) are classified as HPV positive.

Compared to cytology, the pooled sensitivity to detect CIN2 or worse and CIN3 or worse was substantially higher at 93% and 97%, respectively, which increased as RLU cut-offs increased (Koliopoulos et al., 2017). On the other hand, specificity is lower than for cytology, at 89% for both endpoints. In the presence of multiple infections with various HPV types, which occur more frequently in low-grade lesions than higher or borderline lesions (Schmitt et al., 2013), HC2 may be prone to misclassification bias. The HC2 system also does not contain an internal control, which could indicate the integrity of the tested sample. HPV testing via HC2,31oweverr, is the most widely evaluated and used commercial assay for HPV detection. It has been used as the standard comparator test for emerging assays (Arbyn et al., 2021; Meijer et al., 2009). As it is currently understood that the risk outcomes for specific HPV types vary, the main disadvantage of signal-amplified assays includes the inability to distinguish specific HPV types.

***Target amplification***

PCR is the main type of target amplification technique used. PCR testing begins with heating (denaturation) of the sample to break down nucleic acid. After adding a mixture of HPV DNA primers to detect nucleic acid sequences of selected HPV types or HPV groups, amplified HPV fragments (or amplicons) are repeatedly created and duplicated (annealing, extension) until they can be visualised and assessed post-PCR. The addition of HPV DNA fragments includes the use of type-specific and consensus primer systems to identify the targeted HPV types. Consensus primer systems are advantageous over individual type-specific sequences because they can target any of the L1 oncogenes of several HPV types rather than the L1 of a specific HPV type. One such consensus primer used in PCR is GP5+/6+, which amplifies a relatively short segment (150 bp fragments) of this region

(Camargo et al., 2011). When coupled with enzyme immunoassays (EIA), 14 hrHPV types (IARC Group 1, 2A and 2B) and 23 low-risk HPV types can be distinguished.

HPV testing with GP5+/6+ EIA has demonstrated superior detection compared to cytology in several randomised trials and systematic reviews, including their long-term performance (Arbyn et al., 2012; Dijkstra et al., 2016; Dillner et al., 2008; Elfström et al., 2014; Koliopoulos et al., 2017; Naucler et al., 2009; Patanwala et al., 2013; Rijkaart et al., 2012; Vesco et al., 2011). Along with HC2, GP5+/6+ EIA is used as a standard comparator for emerging HPV assays (Arbyn et al., 2016; Arbyn et al., 2021; Meijer et al., 2009). However, unlike HC2, it is not commercially available and thus not used for routine HPV testing within screening programmes. The performance of GP5+/6+ EIA has been observed to be better than other consensus primers (IARC Working Group on the Evaluation of Cancer-Preventive Strategies, 2005).

The evidence supporting HPV-based screening is persuasive, and many countries have begun to or shifted away from cytology-based screening (Maver & Poljak, 2020). Additionally, HPV testing can be carried out by the woman herself, via self-sampling, instead of having a clinician collect the smear. HPV self-sampling has demonstrated equivalent performance to the latter and is an excellent strategy to address under-screened populations or where stigma and shame may preclude screening participation (Arbyn et al., 2022a; Arbyn et al., 2018). Overall, HPV-based screening must be carefully considered to deal with false positives, which may lead to over-testing, over-referral and over-treatment. For example, the number of referrals to colposcopic examinations are typically higher for HPV-based testing than for cytological screenings (IARC Working Group on the Evaluation of Cancer-Preventive Interventions, 2022). From the patient's perspective, increases in anxiety, distress and stigma regarding HPV results may arise within an HPV-based screening programme (Bennett et al., 2021; McBride et al., 2020). A logistical challenge may also be the need for biobanking capacities to retest or audit an aliquot compared to compact cytology slides archived at room temperature (Cuschieri et al., 2019).

***Genotyping***

PCR methods via GP5+/6+ EIA do not directly reveal which HPV types are present in a sample; it requires additional genotyping processes of the duplicated amplicons produced during PCR. Visualisation of these amplicons includes sequencing or type-specific probe hybridisation, such as

reverse line blot analysis. Modern methods without post-PCR processing include real-time PCR, which emits fluorescence signals during the PCR process rather than after the procedure. The utility of HPV testing is optimised if genotyping, the detection of the specific HPV type, is considered. For example, genotyping would enable the evaluation of the long-term impact of the various HPV vaccines administered or refine the predictive outcome of a positive hrHPV screening result. There are various levels of HPV genotyping application: limited genotyping in which HPV 16, 18 and 45 are identified, extended genotyping in which additional hrHPV types are probed, or full genotyping, where all hrHPV types are separately identified (IARC Working Group on the Evaluation of Cancer-Preventive Interventions, 2022).

To date, many HPV assays have been developed for commercial application and vary in detection technique, performance and advantages (Arbyn et al., 2021). An exhaustive list of fully, partially or internally validated HPV assays with genotyping abilities is available (Arbyn et al., 2021). These assays must be analytically evaluated and clinically validated against standard comparators (HC2 and GP5+/6+ EIA) to be recommended as a screening tool. Evaluation and validation are based on important performance criteria, including sensitivity, specificity and reliability. These criteria stem from the Meijer Protocol, which serves as a benchmark for comparing and approving emerging HPV assays for CIN2 or worse lesions (Meijer et al., 2009). The VALidation of HPV GENotyping tests (VALGENT) protocol focuses on validating HPV assays with genotyping capacities and extends assessment to CIN3 or worse (Arbyn et al., 2016). Although HPV-based screening is already implemented or planned in several HIC, the strategic contribution of genotyping is still debated and mostly confined to triage alternatives for positive hrHPV primary screening results for more precise management (Demarco et al., 2020). The cost aspect and management algorithm by genotyping must also be considered in every screening programme context.

***Strategies for detection***

Currently, two main strategies are used for screening, including stand-alone or primary screening (with or without triage) and co-testing. In HIC with long-standing screening measures, screening has historically been performed with stand-alone cytology (Chrysostomou et al., 2018). According to European guidelines, cytology-based screening should be offered to young women aged from 20 to

30 years at 3 to 5-year intervals following a normal (negative) result, as HPV screening is not recommended in this age group due to the high and transient HPV prevalence (Bruni et al., 2010; European Commission, 2008). Repeat cytological testing or colposcopy examination are recommended in cases of abnormal results. In programmes with cytology-based screening where HPV testing is not yet implementable for women above 30 years, the WHO recommends 3-year (triennial) cytology screening intervals (World Health Organization, 2021).

The WHO elimination target for screening coverage aims for at least 70% of women to be screened by age 35 years by a high-performance test equivalent to or better than HPV testing (World Health Organization, 2020). Current WHO and European guidelines recommend HPV testing as the preferred primary tool for screening, with or without triage, from the age of 30 years, albeit in settings with existing organised programmes (Council of the European Union, 2022; World Health Organization, 2021). This recommendation is based on abundant evidence of its high sensitivity compared to cytology (Arbyn et al., 2012; Koliopoulos et al., 2017; Murphy et al., 2012; Ronco et al., 2014). Longitudinal results of trials (Kitchener et al., 2011b; Ogilvie et al., 2018), observational studies (Castle et al., 2018b; Dillner et al., 2008; Rebolj et al., 2019) and modelling studies (Kim et al., 2018; Lew et al., 2017) have demonstrated the long-term detection advantages of HPV testing over cytology, whereby the negative predictive value is almost 100% with screening intervals of 5 years or longer, compared to triennial intervals of cytology. It should be offered to women from age 30 years to minimise false positives and at 5 to 10-year intervals following a negative result (Council of the European Union, 2022; World Health Organization, 2021). Only commercially available and clinically validated tests with reliable and consistently high sensitivity to detect CIN2 and CIN3 or worse should be used (Arbyn et al., 2021; Ronco et al., 2015).

HPV-based screening can include HPV testing as a primary screening tool or as a co-test. Several national screening programmes, such as those in the Netherlands, Australia and the United Kingdom, align with the WHO's recent recommendation for primary HPV testing over cytology, while others, such as those in the United States and Germany, offer primary HPV testing as an alternative to primary cytology or co-testing (Bruni et al., 2022; World Health Organization, 2021). There have been ongoing debates surrounding the benefits and harms of primary HPV testing in comparison to co-testing (Castle et al., 2018a; Nayar et al., 2018; Stoler et al., 2015; Wentzensen & Arbyn, 2017). Arguments refer to the

slightly lower sensitivity and greater rate of over-referrals with primary HPV testing alone, HPV-negative ICCs and missed detection of rare and glandular cervical cancer types. Although the American Cancer Society of the United States recently updated their recommendation for primary HPV testing alone as the preferred strategy (Fontham et al., 2020), co-testing remains acceptable, specifically where access to FDA-approved HPV assays is restricted. Until 2022, European guidelines recommended primary HPV screening due to a lack of evidence for primary HPV testing versus co-testing (Ronco et al., 2015). As of December 2022, the EU updated their recommendation with HPV testing as the preferred tool (Council of the European Union, 2022).

Prior to HPV-based screening, cytological thresholds and repeat cytological testing were used to determine the need for colposcopy referral. With the shifting screening context towards HPV-based testing, triage testing mitigates the need for a direct referral to colposcopy examinations (over-referrals), particularly when it is unclear what the progression risk to precancerous lesions or cancer is. The triaging of positive primary screening results is currently implemented in several HPV-based screening programmes (Cuschieri et al., 2018). These include triage with cytology, HPV testing, other biomarkers such as p16/Ki-67, or a combination of these to determine the risk. Each varies with advantages and disadvantages in performance, costs and resources required (Arbyn et al., 2004; Cuschieri et al., 2018).

#### 1.3.5.2 Colposcopy

Colposcopy examination involves the physical and visual assessment of the cervix using a low magnification colposcope: a binocular microscope with an illumination setting. A microscope is necessary to visualise the cervix for abnormalities and carcinomas. The colposcope was developed around 1925 and comprised a lens capable of magnifying the cervix from a focal distance of 150 to 190mm (Hinselmann, 1925). After discovering that dysplastic cells and lesions do not contain glycogen, staining the cervix with iodine could help identify areas indicative of cervical abnormalities, i.e. unstained glycogen-free areas (Schiller, 1933). Iodine staining was later adopted with acetic acid staining as part of the colposcopy procedure to identify biopsy areas for further histopathology assessment (Hinselmann, 1938). As evidence emerged showing the moderate to the high accuracy of colposcopy in its ability to determine cervical lesions in women with symptoms or abnormal cytological

results (Beller & Khatamee, 1966; Hermanns et al., 1982), colposcopy was integrated chiefly as a follow-up examination following primary cytology screening. Currently, the colposcopy examination is the main procedure that screen-positive women are referred to for diagnostic investigation in a 'screen, triage and treat' approach (IARC Working Group on the Evaluation of Cancer-Preventive Interventions, 2022).

The colposcopy procedure involves the determination of the TZ and type, identification of the presence of any lesion in terms of its size and location using acetic acid and Lugol's iodine, as well as the extraction of biopsies of potentially affected areas (hence colposcopy-directed biopsy) for histopathology evaluation (Prendiville & Sankaranarayana, 2017). The type of biopsy extracted also varies by context and practice. For lesions located on the ectocervix, punch biopsies are taken, in which small round samples of the abnormality are extracted. For lesions with potential endocervical involvement, an endocervical curettage (ECC) is performed by scraping the affected cervical area with a spoon-like tool (curette). This latter method is typically conducted adjunctively to punch biopsies in the United States (European Commission, 2008). However, these sampling techniques may be insufficient in obtaining cells of microinvasive nature (Prendiville & Sankaranarayana, 2017).

Moreover, the colposcopy examination also serves as a management and communication stage, where possible treatment strategies are discussed. In some contexts, even historically in HIC, 'see-and-treat' excision approaches are performed, particularly for high-grade lesions. These include cold-knife conisation and Large Loop Excision of the Transformation Zone (LLETZ), which serve a dual purpose of removing the abnormal area as a form of treatment and also providing a biopsy sample for histological confirmation. However, these excisional methods are associated with adverse pregnancy outcomes such as preterm delivery, adverse neonatal outcomes and perinatal mortality and thus must be discussed together with the patient (Arbyn et al., 2008b; Kyrgiou et al., 2016).

In order to provide quality assurance, a certified and trained colposcopist is necessary (European Commission, 2008). Quality assurance of colposcopy includes the routine use of the International Federation for Cervical Pathology and Colposcopy (IFCPC) terminology for the identification and type classification of the TZ and lesions. Accredited training programmes and certification standards determined by national and international guidelines and the use of quality indicators to audit the

findings are also part of quality assurance measures (Bornstein et al., 2012; Luyten et al., 2015b). Based on a meta-analysis of the accuracy of colposcopy impressions (the colposcopists' judgement), the sensitivity of colposcopy impressions compared to histopathology confirmed CIN3 or worse lesions is highly variable, ranging from 29% to 100% (Mustafa et al., 2016). Among these colposcopy impressions, 464 false positives per every 1,000 colposcopies were observed. The specificity is also relatively low and ranges from 25% to 63% depending on the abnormality grade and the presence of verification bias (Mustafa et al., 2016; Underwood et al., 2012). The overall accuracy and reliability are further restricted when training and certification standards and guidelines in colposcopy vary (Mayeaux et al., 2017; Moss et al., 2015). Poor reliability correlates with fewer years of colposcopy experience and with high-grade lesion types among more experienced colposcopists (Bekkers et al., 2008; Sideri et al., 1995; Stuebs et al., 2019).

Several attempts have been made to increase the performance of colposcopy, including the incorporation of single-digitised cervical images for further blinded review (Ferris et al., 2005). However, the colposcopy impressions of these are still highly subjective and low in reliability, even among experienced colposcopists (Jeronimo et al., 2007). In a study comparing single static cervical images time series of multiple digitised images, reliability and accuracy were not improved and misclassification of high-grade lesions was observed (Perkins et al., 2022). Other methods have demonstrated improvements in the performance of colposcopy by increasing the number of biopsies taken from the cervix (Wentzensen et al., 2018; Wentzensen et al., 2015) and by taking random biopsies (Pretorius et al., 2019). It is also suggested that risk-based decision-making using a combination of screening results and colposcopy impression should be used to discern those at higher risk for precancerous lesions further, to minimise potential over-treatment and potential harm (Silver et al., 2018a).

Recent evidence shows that adherence to follow-up guidelines by providers, including timely biopsy, is suboptimal, specifically among women with discordant co-testing results (Perkins et al., 2021). Inadequate follow-up care may explain plateauing incidence rates observed in countries with long-standing screening, in addition to screening non-participation and false-negative Pap results (as highlighted in Section 1.3.4). Adherence to colposcopy referral by both the screen-positive woman and the healthcare provider substantially influences the rate of failures in care. To demonstrate, a meta-

analysis showed that 12% of ICC diagnoses are attributed to poor follow-up of abnormal screening results (Spence et al., 2007), and longer wait times for colposcopy led to fewer cancers prevented, particularly among women of lower socioeconomic status (Doubeni et al., 2018).

#### 1.3.5.3 Histopathology of the biopsy

Histopathological assessment of the colposcopy-directed biopsies is necessary to confirm the disease status of the sample and inform further management decisions by the treating physician. In practice, pathologists are not blinded to the screening and colposcopy results. Similar to cytology and colposcopy, the reliability of histological assessments is subjective, with moderate agreement (weighted κ ranges from 0.58 to 0.79) even between blinded expert pathology reviewers (with and without a major focus on cervical pathology), although this was particularly poor for CIN1 and CIN2 rather than CIN3 lesions (Carreon et al., 2007; Dalla Palma et al., 2009; Stoler et al., 2001).

Additionally, histopathological classification of disease stages is recommended, which usually follows the international CIN terminology (Table 2). However, with the shift towards HPV-based screening, a preference to use a 2-tiered terminology system has been reported in the United States and by the WHO to determine risk (Perkins et al., 2020; World Health Organization, 2021). This 2-tiered system includes the designation of HPV-infected abnormalities as low-grade squamous intraepithelial lesion (LSIL) and precancerous lesions as high-grade squamous intraepithelial lesion (HSIL), preferably with the CIN terminology. However, pathologists have not yet widely applied this terminology (Nayar et al., 2020).

Quality assurance of histopathology is also necessary to maintain uniform delivery of services. Standardised reporting, the communication of results to the treating physicians as well as to data registries should be carried out by the laboratories (European Commission, 2008). Regular training and auditing of evaluations should also be carried out. For ICC diagnoses, the WHO histological classification of tumours of the cervix should be utilised to enable comparisons (WHO Classification of Tumours Editorial Board, 2020).

#### 1.3.5.4 Management and treatment

In an organised, quality-assured screening programme, the preceding steps of the screening algorithm (screening (with or without triage), colposcopy and histopathology assessment of any biopsy) lead to few women that will require treatment. Treatment for cervical disease aims to safely and effectively remove or destroy the identified areas of the cervix indicative of early-stage invasive cancer or at a high risk of cancer progression (Prendiville & Sankaranarayana, 2017). Treatment includes removal of the lesion and the TZ, particularly if any microinvasion is diagnosed. For young women with CIN (<30 years) and potential family planning intentions, a passive observational approach to the management should take place since disease regression is more likely to occur at younger age (Bekos et al., 2018). However, this approach should be based on quality-assured colposcopy and histopathology assessments.

Two main forms of active treatment for CIN are available: excisional and ablative methods. Historically, hysterectomy (the removal of the uterus) was the mainstay form of cervical cancer treatment (Freund, 1877) prior to the discovery of the colposcope and the utility of staining with iodine and acetic acid (Hinselmann, 1925; Hinselmann, 1938; Schiller, 1933). Such colposcopy procedures led to localised treatment approaches, including the application of cold-knife conisation of the cervix, where a cone-like section of the affected cervical area is removed by a scalpel. Conisation is one type of excisional treatment method that can also be carried out by laser or electrical loops (LLETZ or otherwise known in some contexts as Loop Electrosurgical Excision Procedure, LEEP), suitable for CIN2, CIN3 and microinvasive cervical cancer (Prendiville & Sankaranarayana, 2017). Excisional treatment is preferred due to the ability to histologically confirm the disease status, especially when the preceding colposcopy impression is unsatisfactory. The major disadvantage of excisional methods involves serious adverse pregnancy outcomes (Arbyn et al., 2008b; Kyrgiou et al., 2016). Since cervical neoplasia is typically diagnosed in women of reproductive age, therefore, needs to be carefully considered before treatment. This shared decision is crucial for glandular precancers, where hysterectomy is the primary treatment approach (Prendiville & Sankaranarayana, 2017).

Ablation methods destroy tissue and include cryotherapy and thermal ablation (also known as coagulation). Laser ablation of the affected area is also possible but is less commonly used today, given

that both cryotherapy and thermal ablation methods are effective treatment methods for CIN2 or worse (Dolman et al., 2014; Randall et al., 2019). The risk of adverse pregnancy outcomes also increases after these methods but is not as high as excisional treatment (Kyrgiou et al., 2016). However, not all women are eligible for ablation treatment and eligibility largely depends on the TZ type and glandular involvement, hence the strong recommendation to assess eligibility prior to active treatment (World Health Organization, 2021). It is also impossible to confirm the destroyed tissue's histopathology or rule out actual glandular disease, making ablation a less preferable treatment approach (Prendiville & Sankaranarayana, 2017).

Not all treated and managed CIN remain risk-free. The risk of cervical cancer and other HPV-related cancers of the vagina, vulva etc., are significantly increased following both excisional and ablative treatment for CIN, albeit significantly increased by 2-fold following excisional treatment (Kalliala et al., 2020). The potential reasons for this increased risk include inadequate removal or destruction of the tissue or the genetic, immune and anogenital microbiota predisposition to persistent HPV infections and consequently cervical neoplasia (Bowden et al., 2021; Brusselaers et al., 2019). Thus, HPV testing could be used as a form of post-treatment surveillance, which is superior in determining the risk of recurrent or progressive lesions compared to post-treatment cytological testing (Clarke et al., 2020). In the event of ICC, timeliness of treatment is vital as even a four-week delay in treatment of cervical cancer may lead to an increased risk of mortality (Hanna et al., 2020). Overall, successful and effective treatment should be based on the collective results from quality-assured screening, colposcopy and histopathology assessments as well as the individual factors of the woman, including her age and family planning desires.

### 1.3.6 The German context

Cytology-based screening has been the mainstay cervical cancer screening method in Germany since 1971 (Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft et al., 2020). Since its implementation, Pap smears have been offered to all women from 20 years of age with no upper age limit at an annual interval, in contrast to international guideline recommendations at the time calling for at least 3-year intervals (IARC Working Group on the Evaluation of Cancer-Preventive Strategies, 2005). Gynaecologists and general practitioners conducted these screenings opportunistically and the

screening costs were covered by statutory health insurance. Within this opportunistic model, neither systematic invitation nor uniform quality assurance mechanisms were in place (Hillemanns, 2016). Monitoring of screening coverage or programme performance was absent nationally and state-wide (Petry et al., 2014).

Similar to many other HIC, the incidence of cervical cancer has fallen significantly since Pap smear introduction, from almost 40 per 100,000 women in the 1970s (age-standardised) to approximately 10 per 100,000 women in the early 2000s (Robert Koch-Institut, 2016). National estimates in 2017-2018 reported an age-standardised incidence of 9 per 100,000 women (Robert Koch-Institut & Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V., 2021). Relative survival rates after five and ten years are 65% and 61%, respectively, and higher for early-stage ICC (93%), decreasing linearly to 22% for late-stage ICC. These estimates indicate a plateauing effect of incidence and mortality in which cytology-based screening and an opportunistic model may no longer effectively drive down cervical cancers. Survival rates of advanced ICC indicate potential issues in timing and coverage with screenings offered opportunistically. In a recent large case-control study with population-based controls, 53% of women with ICC were screened frequently in the preceding ten years, underscoring issues with the quality of cytology-based screening (Tanaka et al., 2021). Studies based on routinely screened women in Germany have reported poor sensitivity of Pap smears, ranging from 20% to 43% (Petry et al., 2003; Schneider et al., 2000). These estimates were based on smears obtained with cotton-tipped swabs, which are no longer recommended (Martin-Hirsch et al., 2000). Furthermore, there are significant inequalities in screening participation among specific sub-populations, such as women of migrant background (Brzoska et al., 2020). However, overall screening uptake across a three-year period (74%) is comparable to other HIC with organised programmes (Bruni et al., 2022; Klug et al., 2010).

The future utility of cytology as a primary screening method will be further compromised due to the impact of vaccination against HPV. Free-of-charge HPV vaccinations were recommended for young girls from 2007 (ages 12 to 17) and expanded in 2014 to ages 9 to 14 years (Robert Koch-Institut, 2007, 2014). Rates for full or complete immunisation remain low in young girls ranging from <1% (age 9) to 44% (age 14) and up to 54% of girls by 18-years of age (Rieck et al., 2022). Despite low coverage, especially compared to other countries in the EU and other HIC (Bruni et al., 2021), HPV vaccinations have appeared to reduce the incidence of precancerous lesions (Osmani et al., 2022). These reductions

will further impact the performance of cytology and HPV testing as vaccinated girls become eligible for screening.

In 2018, the shift to an organised screening programme was announced (Gemeinsamer Bundesausschuss (G-BA), 2018a) and the subsequent rollout began in 2020 (Hillemanns & Iftner, 2020). The programme aimed to systematically inform eligible women aged 20 to 65 years via their health insurance companies of the screening programme. Despite EU recommendations to send appointment-based invitations with a call-recall system (Anttila et al., 2015), eligible women in Germany receive informational letters (without appointments) at 5-year intervals, regardless of their age and screening history (Gemeinsamer Bundesausschuss (G-BA), 2018a). As for screening strategies, women aged 20 to 34 years continue to be eligible for annual cytology-based screening rather than HPV testing due to the high prevalence of HPV and thus false positives that may arise in this age group (Bruni et al., 2010).

For women aged 35 years and above, cytology combined with HPV testing as a co-test is offered triennially. Regarding international age and interval recommendations, WHO and EU guidelines recommend HPV-based screening from 30 or 35 years, with at least 5-year intervals (Ronco et al., 2015; World Health Organization, 2021). A guideline committee in Germany first formed the expert consensus for HPV-based screening from age 30 years at either triennial or 5-yearly intervals but ultimately agreed to recommend starting age from 35 years, with the emphasis on review of the data collected within or after a 6-year transitional period (Hillemanns et al., 2019a). An evaluation of the newly organised programme is yet to be conducted and published.

The major shift from an opportunistic cytological screening model to an organised HPV-based co-testing model raises several questions. The following section gives more background to the specific aspects of the screening algorithm that are examined in this thesis.

## 1.4 Studies conducted: Research gaps and questions

This thesis includes two studies investigating two important aspects of the cervical cancer screening algorithm in Germany: optimal screening methods and patient adherence behaviours. The main research questions central to this thesis were:

- How can current and modern screening methods optimise cervical cancer screening in Germany?
- If screening detection can be optimised, what role does it play in the follow-up of abnormal results? What impacts patient adherence behaviours within the screening algorithm?

### 1.4.1 Study 1: Optimal screening methods

Several countries such as the Netherlands, the United Kingdom and Australia have opted for primary HPV screening (Bruni et al., 2022) partly due to the lack of direct evidence comparing co-testing and stand-alone HPV testing strategies head-to-head and partly due to the higher costs and harms of co-testing (Ronco et al., 2015). The United States and Germany are currently two HIC that offer co-testing. However, in the United States, primary cytology with triennial intervals and primary HPV testing at 5-year intervals are also accepted strategies (Fontham et al., 2020).

A clear and direct comparison of primary HPV testing with co-testing was lacking. It was hypothesised that the gains in superior detection predominantly stem from HPV testing (Stoler et al., 2015), and longitudinal results from prospective trials in Canada and Sweden appear to support this assumption (Elfström et al., 2014; Ogilvie et al., 2018). However, these studies were based on a randomised trial design that may not be generalisable and relied on screenings conducted within an established organised programme. Other studies have either indirectly or retrospectively compared these two strategies (Arbyn et al., 2012; Demarco et al., 2017; Schiffman et al., 2018). Additionally, stand-alone HPV testing reportedly demonstrated slightly lower in sensitivity than co-testing with cytology (Blatt et al., 2015; Gage et al., 2014). However, these results were based on retrospective cohorts of health insurance populations and laboratory samples and present major biases that must be considered.

Opponents of primary HPV testing highlight that some ICC are HPV-negative or miss non-squamous ICC and that HPV performance decreased among the older age cohorts, thus favouring co-testing strategies (Pirog et al., 2014; Stoler et al., 2015). The number of colposcopy referrals is also predicted to be higher following primary HPV screening than for co-testing, leading to higher lifetime costs (Felix et al., 2016). There is also an increase in anxiety and stigma surrounding the HPV screening result regardless of the cytological result (McBride et al., 2020; O'Connor et al., 2014). Supporters of primary HPV testing point to the longitudinal ability of HPV tests to detect CIN3 or worse lesions, where fewer high-risk cases are observed after a longer interval (Demarco et al., 2017; Dillner et al., 2008; Kitchener et al., 2011b; Ogilvie et al., 2018; Rebolj et al., 2019). This ability may translate into a significantly lower cumulative incidence (Gage et al., 2014) even after 10 years of follow-up observation (Gottschlich et al., 2021). However, a systematic review found this effect attenuated after time within trial settings (Melnikow et al., 2018). Several modelling studies also indicated that primary HPV testing was more cost-effective and required fewer screening tests than co-testing (Jin et al., 2016; Kim et al., 2018), even with an opportunistic screening model (Petry et al., 2017).

The lack of concrete evidence directly comparing the performance and related outcomes of primary HPV testing stand-alone to co-testing underscored the necessity to examine these strategies head-to-head. Moreover, there was a need to compare multiple cytology tools (conventional and LBC), multiple validated and high-performing HPV assays (HC2, GP5+/6+ PCR), and screening strategies (stand-alone, co-testing) in a population-based sample of eligible women. In order to identify an optimal screening method given the robust evidence for HPV-based screening, Study 1 (Liang et al., 2021) compared various cytology and HPV-based tools as stand-alone or co-tests. This study was based on the following research questions:

1. Which HPV-based screening strategies offer better performance in the detection of precancerous lesions?
2. Which strategy is preferable with an acceptable balance of benefits and harms?

The research results provide valuable evidence for HPV-based screening programmes and provide a baseline result to compare with the screening performance of the new German programme.

### 1.4.2 Study 2: Patient adherence behaviour

An abnormal screening result warrants further investigation and patient adherence at every step along the screening algorithm is important to measure as a key quality assurance indicator (European Commission, 2008). In a meta-analysis assessing failures in cervical cancer care, at least 12% of ICC diagnoses were due to poor or inadequate follow-up after an abnormal screening result (Spence et al., 2007). Although a significant proportion (55%) of cervical cancer care failures are due to inadequate screening histories, the screening coverage in Germany with a historically opportunistic model within a three-year period is comparable to other HIC: approximately 74% (Klug et al., 2010) and 70% (Bruni et al., 2022) respectively. This points to the possibility that a substantial proportion of preventable ICC in Germany is due to post-screening failures in care (Tanaka et al., 2021), which, if delayed, lead to poorer outcomes and survival (Doubeni et al., 2018; Hanna et al., 2020).

Regarding diagnostic follow-up assessment (colposcopy), there are currently no estimates from Germany due to the opportunistic model previously in place and current transitional period of the newly organised programme. It can be assumed that inadequate follow-up contributes to more than 12% of care failures in an opportunistic model compared to an organised one due to the significant inequalities in screening coverage (Miles et al., 2004). In countries with organised and quality-assured screening, benchmarks for the rate of diagnostic follow-up assessment should be clear and upheld. EU guidelines define this indicator as "compliance to referral for colposcopy", the proportion of women referred to follow-up that complies with the assessment (European Commission, 2008). Several national guidelines have determined the benchmark for acceptable attendance rates, including Canada, where a non-attendance rate among all referrals should be less than 15% and supported by systematic recall mechanisms (Murphy et al., 2015). The recent German screening programme reform considers this indicator vital for monitoring quality assurance but stops short of determining an acceptable threshold (Gemeinsamer Bundesausschuss (G-BA), 2018a). It is also unclear if and how recalls after non-attendance will be carried out, which within the previous opportunistic model, was left predominantly as the responsibility of the screening clinic, the screening physician and the patient.

Furthermore, given the general and recent shift towards HPV-based screening in some countries (Maver & Poljak, 2020), only one study evaluated the impact of HPV testing on colposcopy follow-up

but was limited in sample size (Buick et al., 2021). Qualitative studies have indicated the potential of positive hrHPV results to increase anxiety, stigma, concerns and shame, particularly when communication regarding the meaning of the test result is neglected (Bennett et al., 2021; McRae et al., 2014; O'Connor et al., 2014; O'Connor et al., 2018). Other studies have shown no increase in anxiety or related concerns or retreat of these initial psychological harms after 12 months (Andreassen et al., 2019; Burger et al., 2014; McBride et al., 2020). Others have found that anxiety regarding an abnormal cytological screening result drive non-attendance, particularly in smokers (Yassin et al., 2002). It is crucial, therefore, to measure the rate of non-attendance to colposcopy and investigate factors associated with non-attendance of follow-up assessments, considering HPV testing. Thus, Study 2 (Liang et al., 2022) investigated the following research questions:

1. In a population-based sample of women invited and screened with optimal tools, what proportion of women do not adhere to the screening algorithm, specifically diagnostic follow-up of abnormal screening results (colposcopy)?
2. If screening detection is optimised by HPV testing, what role does a positive HPV result have on colposcopy non-attendance?
3. What are the factors associated with follow-up or lack thereof? (Whom does it impact?)
4. What are the reasons given for non-follow-up?

This investigation contributes to the understanding of the role of HPV testing results in the diagnostic follow-up of the screening algorithm and particularly the adherence behaviours of screened women. Furthermore, the added benefits in identifying which factors are related to poor follow-up behaviour will determine potentially vulnerable sub-groups, enabling the development and testing of potential interventions to address these shortcomings.

# 2 Methods

## 2.1 The MARZY study

Despite the successes brought by opportunistic cytological screenings since 1971, the opportunistic model in Germany did not embed any systematic or centralised database for cervical screening activities or outcomes. Several federal states established cancer registries that documented invasive cancers (Arndt et al., 2020) but not for precancerous lesions. Previous German studies that assessed cytological and HPV testing performance relied on study recruitment of women who self-referred to routine screening or who were privately insured (Petry et al., 2003; Schneider et al., 2000). These limitations are important to recognise as routinely screened women within an opportunistic model may not reflect the truly eligible population. On the contrary, more representative and generalisable estimates can be obtained in a randomly selected, population-based sample of women. For example, there is a substantial variation in screening participation in Germany by many socio-economic and cultural factors, where women with migrant backgrounds or lower socio-economic status are less likely to participate in cervical cancer screening (Brzoska et al., 2020). Moreover, the calculation of test specificity from routinely screened populations is impractical since screen-negative women are not recalled for diagnostic follow-up, and thus confirmation of their negative status is missing (Arbyn et al., 2010). In order to investigate the research questions described in Chapter 1 Section 1.4, data from a population-based study were necessary.

The MARZY study (Machbarkeitsstudie für die Durchführung einer randomisierten Interventionsstudie zur Implementation eines Einladungsmodells und eines HPV Tests in die

Routinefrüherkennungsuntersuchung für das Zervixkarzinom [Feasibility study for the conduct of a randomised interventional study on the implementation of an invitation model and HPV tests within the routine cervical cancer screening system]) was conducted in the federal state of Rhineland-Palatinate, Germany, in the largest city of Mainz and the surrounding regional area of Mainz-Bingen.

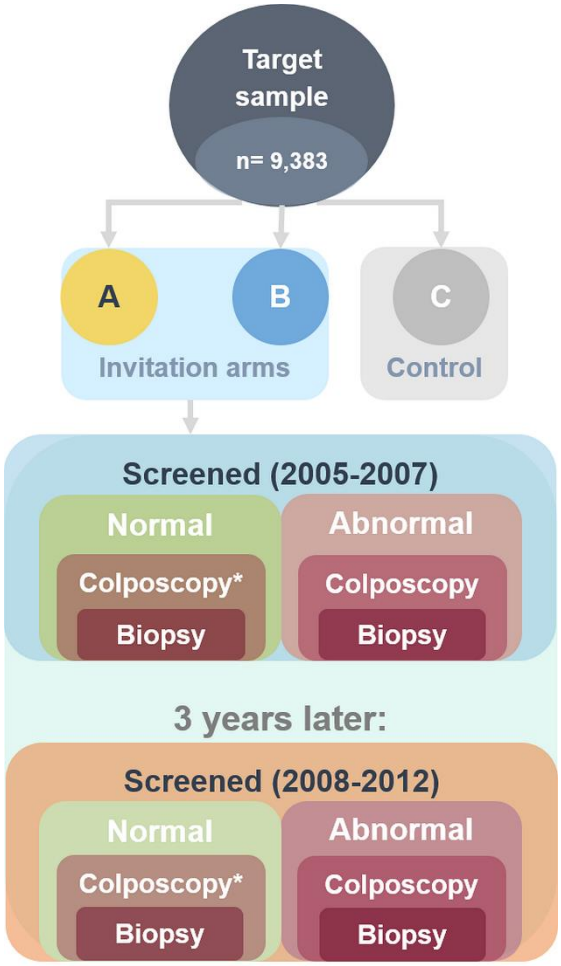
The MARZY study aimed to:

(i) Investigate the impact of an invitation letter to cervical cancer screening on screening participation mimicking an organised programme (with an invitation and call-recall),
(ii) Compare various validated HPV assays and cytology assessments as well as
(iii) Estimate the prevalence of HPV infection.

Additional goals were to examine patient adherence within the screening algorithm and to evaluate the impact of positive HPV results on the screened women and healthcare providers (Klug, 2004).

### 2.1.1 Study design

The study design of MARZY is a prospective RCT. The recruitment of women depended on specific eligibility criteria and age. The lower age limit was restricted to 30 years due to HPV testing and the upper limit was restricted to 65 years. A random selection within the target population living in the study regions city of Mainz (~195,000 inhabitants) and Mainz-Bingen (~200,000 inhabitants) were contacted via registration data provided by the local population registries of those areas. Older women (50 to 65 years) were oversampled due to unknown hysterectomy status in this age group. The inclusion criteria to participate in the screening and subsequent study modules were women who had no previous history of cervical cancer or severe lesions, had not undergone hysterectomy, were not pregnant, lived in the study region for at least six months, and women who were physically and cognitively able to participate in the study.




* Colposcopy offered to randomly selected screen-negative women to verify their status.

**Figure 4.** A general diagram of the MARZY prospective randomised trial design

As described in the published results of Radde and colleagues (2016), for the RCT module (Figure 4), 9,383 women from the target sample population were contacted and eligibility for screening based on the exclusion criteria was determined. Both invitation arms A and B were sent a basic information letter but arm B received an additional 8-page brochure containing further information regarding HPV infection and cervical cancer. Two intervention arms were incorporated to analyse the difference between the extent of information given with invitation and its impact on participation. A third arm C was assigned as the control group, to which participants did not receive any invitation to screening within MARZY but could attend routine screening within the general opportunistic model in place at the time of the study. Arm C participants were informed about the study and their screening

participation data within the opportunistic model were retrospectively collected at the end of the baseline period.

Women who were randomised to the invitation arms (A and B only) could participate in screening at any office-based gynaecologist routinely conducting screenings in the study region between 2005 and 2007 (round 1, R1). Strict recruitment monitoring and quality assurance were conducted at the practices (Zeissig et al., 2014). All gynaecologists and general practitioners performing cervical screening were informed about the study and were sent the necessary information, screening material and study material to document the recruited participants. Informed consent was collected from all the participating women in the trial component and ethical approval was granted by the German Cancer Aid (Deutsche Krebshilfe; Grant numbers: 105827, 106619, 107247, 108047 and 107159).

### 2.1.2 Screening algorithm

The screening algorithm within MARZY incorporated the same key assessments described in Chapter 1, Section 1.3.5. Invited women (arms A and B only) who attended the screening were offered their standard routine Pap screening (conventional Pap) and an additional study swab (LBC), which also enabled HPV testing in the laboratory by HC2 assay (described in Chapter 1, Section 1.3.5.1). Post hoc genotyping analyses with GP5+/6+ EIA PCR were conducted separately (Department of Pathology, Amsterdam UMC, location Vrije Universiteit Amsterdam, the Netherlands) and did not influence the screening algorithm. The instruments used for the study swab were provided by the LBC manufacturer, including a cytobrush and endocervical broom. All gynaecologists and physicians conducting the cervical screening completed a standard screening form and sent the routine Pap sample to the usual routine laboratory for processing. LBC study swabs were sent to a centralised and experienced laboratory with LBC processing and HC2 capacities to ensure quality assurance (CytoMol MVZ, Frankfurt, Germany).

The Munich Nomenclature II classified the study thresholds for abnormal screening results: Pap IIw or worse (equivalent to Bethesda: ASC-US or worse) for cytology or a positive hrHPV test result by HC2 (Table 2, Chapter 1 Section 1.3.5.1). Due to the novelty of HPV testing at the time of the MARZY study, participants with a positive hrHPV result (regardless of cytological result) were given thorough

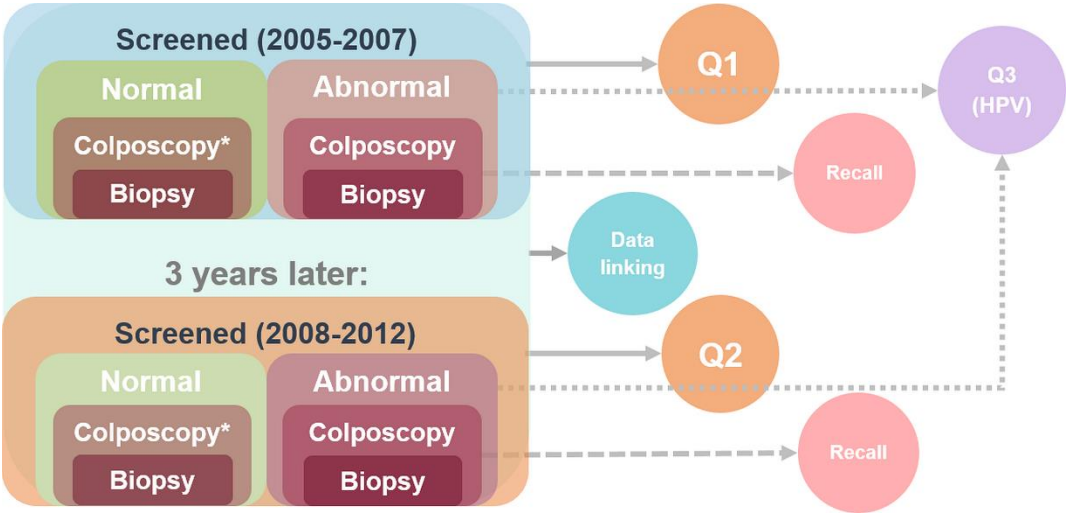
information regarding their screening result, which differed from the information received by participants who had only cytological abnormalities detected (ASC-US or worse) and those who were screen-negative upon both co-tests but randomly selected for verification. These participants were informed of their screening result and referred to diagnostic follow-up: colposcopy examination. Women with screen-negative results (both cytology and HPV negative) who were not randomly selected for colposcopy were returned to the normal screening intervals. A random sample (5%) of screen-negative women invited to colposcopy examination (Figure 4) was recruited to enable specificity to be calculated and to minimise verification bias (Arbyn et al., 2009b). These women were given an incentive to participate and all were informed about the purpose and expected procedure.

Colposcopies were conducted by expert accredited colposcopists at the Department of Obstetrics and Gynecology, Mainz University Hospital, Germany, who were not blinded to the screening results. This was to ensure maximum reliability and validity of the biopsy samples extracted from screen-positive and screen-negative women as not all screening gynaecologists are certified to conduct colposcopies (as described in Chapter 1, Section 1.3.5.2). Colposcopy examinations were conducted according to the standard routine procedure and documentation processes of the IFCPC guidelines (Walker et al., 2003). For screen-positive women, multiple punch biopsy samples were taken at the place where any lesions were identifiable to increase sampling adequacy (Wentzensen et al., 2018). An ECC was performed for women with an everted TZ. Colposcopy of screen-negative women included random punch biopsies at two quadrants of the cervix (12 and 6 o'clock).

The biopsies were sent to the centralised university pathology laboratory at the Mainz University Hospital (Mainz, Germany). Akin to real-world screening assessments, pathologists were not blinded to the screening or colposcopy results and assessed biopsy samples in accordance to the standard procedure. Two pathologists were engaged in assessing biopsy samples, and upon any disagreement in diagnosis, a third blinded pathologist was called upon to give their diagnosis (as highlighted in Chapter 1 Section 1.3.5.3). This was incorporated as the inter-rater reliability of histopathology can vary substantially (Ceballos et al., 2008; Stoler et al., 2001). The results were relayed to the treating physician for further management where necessary. Cervical disease was managed according to the recommended clinical guidelines in place at the time based on eligibility (as described in Chapter 1 Section 1.3.5.4).

For the prospective follow-up component of the MARZY study, the same screening algorithm protocol was conducted three years after the baseline screening period ended (2005-2007) from 2008 to 2010, plus additional observation time for treatment outcomes until 2012 (Figure 4). Women who participated in the baseline round and met the inclusion criteria applied at baseline were re-invited to partake in the second round of screening (R2).

### 2.1.3 Questionnaire and interview data collection




* Colposcopy offered to randomly selected screen-negative women to verify their status

**Figure 5.** Data collection sources within the MARZY study within baseline and follow-up periods

To fulfil the first MARZY study aim to investigate the impact of an invitation letter to cervical cancer screening, screening participation data were recorded in addition to various confounding factors, including sociodemographic information, screening behaviour and history, sexual reproductive health and lifestyle factors. These data were collected within the R1 baseline questionnaire (Q1) as a paper-based and self-administered survey from all women attending a screening. In the study follow-up phase R2, screening was offered again alongside another general questionnaire (Q2), collecting the same information (pertaining to the prior three years since baseline) as well as additional relevant items on sexual health such as STI history.

In concordance with the second research aim within MARZY: to compare various HPV assays and cytology assessments, only screening results from R1 were used. The screening results from R1 were

used to calculate the performance of screening tool, such as sensitivity and specificity. Parameters were calculated for each screening tool stand-alone and the combined (co-testing) strategies. The methods addressing the research questions of Study 1 are described below under Section 2.2.

Further data on screening history and results were extracted and linked between R1 and R2 since screening was offered annually to all women above age 20 in Germany. These data were extracted in parallel to the study screenings and retrospectively by study nurses who contacted the participant's gynaecology or general practitioner office. Routine screening information after R1 participation, as well as medical records from the colposcopy and pathology clinics at the hospitals, were collected and linked to study participants. All records relating to subsequent colposcopy, biopsy and disease management were extracted from these medical records. These results could provide a more comprehensive picture of the participants, especially with routine screenings still offered in the background of the study, which may have led to abnormal screening results not being captured within the study.

Given the interest in HPV-based screening and potential concerns or anxiety arising, an additional questionnaire (Q3) was administered at both screening rounds to women who were screen-positive for HPV testing. Q3 entailed several aspects of HPV awareness, knowledge, anxiety and concern about HPV links to sexual health and cancer, as well as information dissemination channels, such as how HPV results were communicated to the participants by their screening physician. At the time, no standardised and validated tool was available to capture these various HPV-related perspectives. Thus, the questionnaire incorporated several instruments, such as the Psychosocial Effects of Abnormal Pap Smears Questionnaire (PEAPS-Q) and the Cervical Dysplasia Distress Questionnaire (CDDQ) to collect this information (Bennetts et al., 1995; Shinn et al., 2004).

Further relevant data were collected from the screen-positive participants who were referred to colposcopy. Study nurses called the study participants by phone, who did not make timely appointments for colposcopy within a 3-month period. This call-recall was necessary to a) act as a reminder to attend colposcopy, b) offer reassurance and point of reference for women unsure about the meaning of the screening result and subsequent examination and c) to interview the women who

refused to make a colposcopy appointment on why they preferred not to attend. Study nurses conducted these calls at both screening rounds.

The collection of Q1, Q2, HPV-associated items and data among women referred to colposcopy could be used to explore the second aspect of this thesis, including non-participation within the screening algorithm, particularly after screen-positive results, and to evaluate the impact of positive HPV results on the screened women and provider. The methods addressing aspect 2 of this thesis are described in more detail under Section 2.3.

## 2.2 Study 1: Optimal screening methods

In order to answer the research questions on optimal screening strategies in Study 1, comparisons of diagnostic performance were conducted based on the screening and biopsy results obtained at the baseline round of the MARZY study. The study population included all women screened who provided a LBC study swab sample. The study swab also provided the residual sample for HPV testing (HC2 and GP5+/5+ PCR, hereby simply referred to as PCR). All women with at least one positive screening result (cytology-based cut-off ASC-US or worse, hrHPV-positive) were referred to colposcopy for further verification. Since clinical guidelines at the time of the study used a more stringent definition for referral compared to the study threshold, namely Pap IIID or worse (equivalent to Bethesda: LSIL or worse), this threshold was also taken into consideration for the following analyses.

### 2.2.1 Absolute accuracy indicators

An overview of absolute accuracy indicators and their calculations is provided in Table 3. These are important indicators of screening test performance (IARC Working Group on the Evaluation of Cancer-Preventive Interventions, 2022). Sensitivity was calculated as the proportion of true positives (TPs) screening results by each test conducted (conventional cytology [Pap], LBC, HC2 or PCR) as well as their combinations (Pap/HC2, Pap/PCR, LBC/HC2 and LBC/PCR) among the total histopathologically confirmed cases: CIN2 or worse and CIN3 or worse. As for specificity, the proportion of true negatives (TNs) among histopathologically confirmed 'normal' findings was calculated. A high positive predictive value (PPV; the proportion of positive test results leading to a disease diagnosis) and high negative

predictive value (NPV; the proportion of negative test results leading to no disease diagnosis) are also desirable to determine the true disease status accurately, but these depend on the base population prevalence. The PPV was calculated by the proportion of all TPs among screen-positive results. The NPV was conversely calculated by the proportion of TNs among all screen-negatives. In some cases, it is not possible to calculate a meaningful proportion when a cell in the 2x2 contingency table contains the value 0. To avoid this issue, we applied Haldane's correction by adding 0.5 to each 2x2 cell for screening test results or histopathological results that encountered this issue, namely co-testing strategies with HC2 and all PCR screening strategies stand-alone and combined (Haldane, 1956).

**Table 3.** 2x2 contingency table of the various absolute performance parameters and their respective formulae

| | | **Disease status (confirmed by gold standard)** | | |
|---|---|---|---|---|
| | | **CIN* or worse** | **Normal**** | **Calculation** |
| **Screening test** | **Positive** | True positive (TP) | False positive (FP) | $PPV = \frac{\mathrm{TP}}{\mathrm{TP} + \mathrm{FP}}$ |
| | **Negative** | False negative (FN) | True negative (TN) | $NPV = \frac{\mathrm{TN}}{\mathrm{FN} + \mathrm{TN}}$ |
| | **Calculation** | $Sensitivity = \frac{\mathrm{TP}}{\mathrm{TP} + \mathrm{FN}}$ | $Specificity = \frac{\mathrm{TN}}{\mathrm{FP} + \mathrm{TN}}$ | |

* Endpoints: CIN2 or worse, CIN3 or worse.
** Endpoints: <CIN2, <CIN3.
PPV: Positive predictive value; NPV: Negative predictive value.
Note: under co-testing, a positive screening result refers to positivity by either test included.

In order to better compare the risk of precancer or worse following a negative screening test (Wentzensen & Wacholder, 2013), the complement of the NPV, the cNPV was calculated by subtracting the proportion of TNs to screen-negative results from 1 as below:

$$cNPV = 1 - NPV = \frac{\mathrm{FN}}{\mathrm{FN} + \mathrm{TN}}$$

A low cNPV value indicates a low risk of precancer. Thus, the screening test is highly reliable in the case of a negative screening result.

A reference test or gold standard for disease verification that is 'error-free' is required to calculate the described performance parameters (Table 3) of existing and emerging screening tools (Cohen et al.,

2016). In contexts with screening services implemented, the gold standard colposcopy-directed for screen-negative women would be impractical and unethical due to its invasiveness (IARC Working Group on the Evaluation of Cancer-Preventive Interventions, 2022). However, without verification of true negative status, verification bias of the performance estimates may arise, where sensitivity is overestimated and specificity is underestimated (Begg & Greenes, 1983; de Groot et al., 2011). There are two types of verification bias. The first is classified as differential verification bias, as the disease status among screen-positives are verified, but not for the screen-negatives. Screenees referred to follow-up colposcopy may not adhere to their examination, leading to partial verification bias. Partial verification bias exists because verification of referred non-attendees is lacking. Therefore, to determine estimates with minimal verification bias, estimates can be adjusted in statistical analyses for verification bias (Arbyn et al., 2009b). Weights were assigned to the parameters to adjust for verification bias. The assignments were applied according to the three strata that received differing referral and invitation letters, which may have influenced attendance of the follow-up colposcopy examination: 1) cytological abnormality only (ASC-US or worse), 2) positive HC2 result (regardless of cytological result) and 3) co-test negative but randomly invited to colposcopy. A sampling fraction determined these weights, similar to methods carried out by Kulasingam et al (2002):

$$Adjusted\ sensitivity = \frac{\sum_{i=1}^{k} {x_i}/{f_i}}{\sum_{i=1}^{k} {n_i}/{f_i}}$$

Here $x_i$ refers to the number of TPs that attended colposcopy in any of the 3 strata (i), $n_i$ refers to the total number of histopathologically confirmed CIN2 or worse and CIN3 or worse cases and $f_i$ refers to the proportion of each stratum who attended the follow-up colposcopy examination. The weight was calculated as the inverse of this probability per stratum and applied to the accuracy estimates (Table 4).

**Table 4.** The predicted probability of attending a follow-up colposcopy appointment (denominator) for each stratum and the formula for weight allocation

| **Stratum 1: Cytology abnormality only (ASC-US or worse)** | **Stratum 2: Positive HC2 result (regardless of cytology)** | **Stratum 3: Co-test negative** |
|---|---|---|
| $= \frac{1}{0.44} = 2.27$ | $= \frac{1}{0.46} = 2.17$ | $= \frac{1}{0.04} = 0.04$ |

ASC-US: Atypical squamous cells of undetermined significance; HC2: Hybrid Capture®2.

Confidence intervals around each of these estimates were determined by the bootstrap method (number of bootstraps: b=1,000) at the lower 2.5% and upper 97.5% quantiles (Efron & Tibshirani, 1993).

### 2.2.2 Comparison of strategies

A simple comparison between two screening test strategies was tested using McNemar's paired sample statistic for the weighted absolute performance parameters. This was carried out among stratified groups: CIN or worse and 'normal' histopathology results (Kim & Lee, 2017). The disadvantage of this statistic is that one is unable to determine the direction of the association if it is detected. Therefore, a comparison directly between test strategies was carried out by determining the relative sensitivity and relative specificity. The relative sensitivity is also helpful in the absence of true negatives, for example in a real-world screening study where screen-negatives do not undergo verification of their status (Filleron, 2018; Pepe & Alonzo, 2001). Both of these parameters were calculated by the ratio of one test to the comparison test, as seen below:

$$Relative\ sensitivity = \frac{Sensitivity(Test1)}{Sensitivity(Test2)}$$

$$Relative\ specificity = \frac{Specificity(Test1)}{Specificity(Test2)}$$

These ratios were calculated for both crude accuracy parameters and verification bias-adjusted accuracy parameters to examine if any verification bias exists. To estimate the precision of the crude estimates, 95% confidence intervals were determined by standard Wald method (Fagerland et al., 2014), while 95% confidence intervals for the adjusted relative estimates were determined by bootstrapped resampling as described above.

In addition, the positive likelihood ratio (PLR) was determined, i.e. the ratio of the probability of a positive test result among those diseased (TP rate) to a positive test result in the 'normal' screened population (FP rate). Conversely, the negative likelihood ratio (NLR) was determined as the ratio of negative test results among those diseased (FN rate) to negative test results in the 'normal' screened population (TN rate). These are advantageous to PPV and NPV as they are independent of disease prevalence.

$$PLR = \frac{Sensitivity}{1 - Specificity}$$

$$NLR = \frac{1 - Sensitivity}{Specificity}$$

### 2.2.3 Potential harms

Potential harms of screening tools include false-positive and false-negative rates (FPR; FNR). These were calculated as below, based on the sensitivity and specificity determined:

$$FPR = 1 - Specificity$$

$$FNR = 1 - Sensitivity$$

Another relevant parameter for determining the potential harms of a screening strategy is the number of referrals to colposcopy necessary to detect one precancerous lesion (NNC) or the colposcopy referral

rate (European Commission, 2008). This was calculated by taking the inverse of the PPV as seen below.

$$NNC = \frac{1}{PPV} = \frac{\mathrm{TP} + \mathrm{FP}}{\mathrm{TP}}$$

The 95% confidence intervals of all potential harm parameters were obtained by the bootstrapped resampling method (b=1,000).

## 2.3 Study 2: Patient adherence behaviour

In the second study on patient adherence to colposcopy following an abnormal screening result, all screened and referred women with at least borderline cytological results (ASC-US or worse) or a hrHPV result at either R1 or R2 were included in the analyses. Participants who were invited as part of the random sample of screen-negative women (5%) were not included.

### 2.3.1 Descriptive analyses

Sociodemographics, health-related information such as smoking status, screening frequency, HPV-related information and reasons for non-attendance were described with absolute numbers and proportions among all women included in these analyses. These were stratified by HPV screening status and the cut-off for cytology results was ASC-US or worse (equivalent to Munich II Nomenclature: Pap IIw or worse). Additionally, similar to Study 1, a second cytological cut-off equivalent to low-grade cytology (LSIL) or worse was analysed in accordance with clinical guidelines in place at the time of the MARZY study.

### 2.3.2 Attendance rate

The colposcopy attendance rate is an important quality assurance indicator for cervical screening programmes. It is determined as the proportion of screened women who underwent colposcopy follow-up among the total number of women referred to colposcopy. Following an abnormal screening result, women were given up to three months of receiving their screening result to arrange a colposcopy appointment with the university clinic (Mainz, Germany). If appointments were not arranged in this timeframe, a designated study physician/nurse called the participant to encourage

arranging the appointment and to address any concerns from the patient. Additional time was given for women who eventually arranged a colposcopy appointment (regardless of whether contact by the study nurse was successful or not). Thus, the attendance rate was calculated as any colposcopy undertaken within a 4-month period. The non-attendance rate was simply determined as the complementary of the attendance rate. These rates were stratified by three relevant co-testing result groups: cytology abnormality only (ASC-US or worse), hrHPV positive only and both co-test positive.

### 2.3.3 Influence of HPV status and other factors

Due to the transient nature of HPV infections and relatively short screening intervals, it is possible that women were screen-positive and referred to colposcopy at R1 only, R2 only or at both rounds if they participated in study follow-up. The reasons for attendance or lack thereof among women referred to colposcopy at both rounds may differ between the two rounds. Thus, in this situation, to avoid overestimation of attendance-related factors, only the first referral and respective questionnaire data were included in these analyses. The outcome of interest for the analyses in Study 2 was colposcopy attendance versus non-attendance. In order to determine whether HPV status influenced colposcopy attendance and whether other potential factors were associated with attendance, binary logistic regression was applied. Binary logistic regression differs from linear regression modelling, which assumes the outcome Y is continuous and normally distributed (conditional on the independent/explanatory variables). This binary outcome can be modelled in logistic regression to predict the probability $P$ of the outcome of interest ($y_1 = 1$) or 'attendance = yes' as follows:

$$log\left(\frac{P(y_1 = 1)}{P(y_1 = 0)}\right) = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \cdots + \beta_p x_{ip}$$

Here, the log-odds of the outcome are determined by a linear predictor based on $p$ covariates $x_1, \ldots, x_p$ and a set of parameters $\beta_0, \ldots, \beta_p$. Both univariable and multivariable logistic regression were performed in order to determine the odds of colposcopy attendance, including whether the association exists beyond chance and if so, the size and statistical significance of the association. Relevant cofactors of precancerous and cancerous lesions, such as age, nationality (a proxy for migrant background), education level, HPV status etc. (as described in Chapter 1, Section 1.1.2.4) were extracted

from the study screening records, as well as from Q1, Q2 and Q3 surveys (as described in Chapter 2, Section 2.1.3). These cofactors were applied as covariates for multivariable regression modelling.

Due to the inherent loss of data from missing survey responses, available case analysis was applied in combination with imputed values to retain an adequate sample size for multivariable regression. Multiple imputation, which is the repetitive 'replacement' of missing observations based on existing observations, and their respective bootstrapped confidence intervals was carried out using the MAMI package in R (Schomaker & Heumann, 2014). This package was selected due to the capability of performing model averaging on imputed data for logistic regression and the possibility of obtaining confidence intervals based on bootstrap resampling. Bootstrapped confidence intervals were calculated based on the covariate-adjusted models instead of the likelihood ratio-based estimates, which may be over and underestimated. A total of 500 bootstrap resamples was used to determine the confidence intervals.

# 3 Studies

The following section contains the published abstracts of the two studies relevant to this thesis, as well as the respective author contributions. The full texts of the studies and their reprint permissions can be found in the appendix.

## 3.1 Study 1: Optimal screening methods

**Study 1:** **Liang, L. A.**, Einzmann, T., Franzen, A., Schwarzer, K., Schauberger, G., Schriefer, D., Radde, K., Zeissig, S. R., Ikenberg, H., Meijer, C. J. L. M., Kirkpatrick, C. J., Kölbl, H., Blettner, M., & Klug, S. J. (2021). Cervical Cancer Screening: Comparison of Conventional Pap Smear Test, Liquid-Based Cytology, and Human Papillomavirus Testing as Stand-alone or Cotesting Strategies. Cancer Epidemiology, Biomarkers & Prevention, 30(3), 474-484. https://doi.org/10.1158/1055-9965.Epi-20-1003

### 3.1.1 Abstract

**Background:** Some countries have implemented stand-alone Human Papillomavirus (HPV) testing while others consider co-testing for cervical cancer screening. We compared both strategies within a population-based study.

**Methods:** The MARZY cohort study was conducted in Germany. Randomly selected women from population registries aged ≥30 years (n=5,275) were invited to screening with Pap smear, liquid-based cytology (LBC, ThinPrep®) and HPV testing (Hybrid Capture®2, HC2). Screen positive participants

(ASC-US+ or high-risk HC2 (hrHC2)) and a random 5% sample of screen negatives were referred to colposcopy. Post hoc HPV genotyping was conducted by GP5+/6+ PCR-EIA with reverse line blotting. Sensitivity, specificity (adjusted for verification bias) and potential harms including number of colposcopies needed to detect 1 precancerous lesion (NNC) were calculated.

**Results:** In 2,627 screened women, cytological sensitivities (Pap, LBC: 47%) were lower than HC2 (95%) and PCR (79%) for CIN2+. Co-testing demonstrated higher sensitivities (HC2 co-testing: 99%; PCR co-testing: 84%), but at the cost of lower specificities (92%-95%) compared to HPV stand-alone (HC2: 95%; PCR: 94%) and cytology (97% or 99%). Co-testing versus HPV stand-alone showed equivalent relative sensitivity (HC2: 1.06, 95% CI 1.00-1.21; PCR: 1.07, 95% 1.00-1.27). Relative specificity of Pap co-testing with either HPV test was inferior to stand-alone HPV. LBC co-testing demonstrated equivalent specificity (both tests: 0.99, 95% CI 0.99-1.00). NNC was highest for Pap co-testing.

**Conclusions:** Co-testing offers no benefit in detection over stand-alone HPV testing resulting in more false-positive results and colposcopy referrals.

### 3.1.2 Contributions

Linda A. Liang is the first author of this study and contributed to the development of the methodology, data analysis and visualisation, as well as the drafting, review and editing of the published manuscript. Prof. Dr. Stefanie J. Klug conceptualised and acquired funding for the MARZY study as principal investigator and contributed to the supervision of the analyses and writing of the manuscript. Dr. Gunther Schauberger contributed to the methodology, data analysis and validation of statistical methods applied in the study. Dirk Schriefer curated and validated the data for analysis. Regarding data collection, P.D. Dr. Hans Ikenberg conducted the assessments for LBC and HC2 screening tests. Dr. Thomas Einzmann, Dr. Arno Franzen, Dr. Katja Schwarzer conducted the expert colposcopies. Prof. Dr. Dr. Heinz Kölbl was the institute director of the gynaecology department and oversaw the examinations and quality assurance of the colposcopies conducted within the study. Prof. Dr. Chris J. L. M. Meijer (and Prof. Dr. Peter J. F. Snijders†, who sadly passed away before the manuscript was published and thus could not be listed as co-author by journal guidelines) performed the post hoc GP5+/6+ PCR laboratory tests. Prof. Dr. Charles J. Kirkpatrick oversaw the pathology examinations and

quality assurance of pathology reports within the study. Kathrin Radde was the project coordinator, responsible for the administration of the study. Prof. Dr. Sylke Zeissig was the study physician. Prof. Dr. Maria Blettner contributed to the conceptualisation of the MARZY study and the subsequent acquisition of third-party funding together with Prof. Dr. Stefanie J. Klug. All co-authors of this manuscript reviewed and agreed to the final manuscript for publication.

## 3.2 Study 2: Patient adherence behaviour

**Study 2:** **Liang, L. A.**, Zeissig, S. R., Schauberger, G., Merzweiler, S., Radde, K., Fischbeck, S., Ikenberg, H., Blettner, M., & Klug, S. J. (2022). Colposcopy non-attendance following an abnormal cervical cancer screening result: a prospective population-based cohort study. BMC Women's Health, 22(1), 285. https://doi.org/10.1186/s12905-022-01851-6

### 3.2.1 Abstract

**Background:** A considerable proportion of cervical cancer diagnoses in high-income countries are due to lack of timely follow-up of an abnormal screening result. We estimated colposcopy non-attendance, examined the potential factors associated and described non-attendance reasons in a population-based screening study.

**Methods**: Data from the MARZY prospective cohort study were analysed. Co-test screen-positive women (atypical squamous cells of undetermined significance or worse [ASC-US+] or high-risk human papillomavirus [hrHPV] positive) aged 30 to 65 years were referred to colposcopy within two screening rounds (3-yr interval). Women were surveyed for sociodemographic, HPV-related and other data, and interviewed for non-attendance reasons. Logistic regression was used to examine potential associations with colposcopy attendance.

**Results:** At baseline, 2,627 women were screened (screen-positive=8.7%), and 2,093 again at follow-up (screen-positive=5.1%; median 2.7 years later). All screen-positives were referred to colposcopy, however 28.9% did not attend despite active recall. Among co-test positives (ASC-US+ and hrHPV) and only hrHPV positives, 19.6% were non-attendees. Half of only ASC-US+ screenees attended colposcopy. Middle age (adjusted odds ratio [aOR] =1.55, 95% CI 1.02, 4.96) and hrHPV positive result

(aOR=3.04, 95% CI 1.49, 7.22) were associated with attendance. Non-attendance was associated with having ≥3 children (aOR=0.32, 95% CI 0.10, 0.86). Major reasons for non-attendance were lack of time, barriers such as travel time, need for childcare arrangements and the advice against colposcopy given by the gynaecologist who conducted screening.

**Conclusions:** Follow-up rates of abnormal screening results needs improvement. A systematic recall system integrating enhanced communication and addresses follow-up barriers may improve screening effectiveness.

### 3.2.2 Contributions

Linda A. Liang is the first author of this study and contributed to the methodology, data analysis and visualisation of the study data, as well as the drafting, review and editing of the published manuscript. Prof. Dr. Stefanie J. Klug conceptualised and designed the MARZY study and acquired the project funding. Dr. Gunther Schauberger contributed to and oversaw the methodology, specifically in the data analysis. Sophie Merzweiler assisted in data checks. The screening and post-screening algorithm study was co-ordinated by Kathrin Radde. Prof. Dr. Sylke Zeissig was the study physician. Additionally, P.D. Dr. Hans Ikenberg contributed to the data collection of screening results and relaying of information to the study team. Dr. Sabine Fischbeck contributed to the data collection method by the Q3 survey. Prof. Dr. Maria Blettner contributed to the MARZY study conceptualisation and the acquisition of third-party funding together with Prof. Dr. Stefanie J. Klug. All co-authors reviewed the submitted and final versions of the published manuscript.

# 4 Discussion

Cervical cancer prevention research has evolved rapidly in the last three decades. With the increasing evidence favouring modern and accurate screening tools and the call to eliminate cervical cancer by optimising primary and secondary prevention measures by the WHO, it is essential to evaluate existing structures and provide high-quality data to guide changes and implementation. This thesis explored two critical aspects of cervical cancer screening programmes in the face of evidence of gaps, emerging screening tools and technologies and persisting failures in the cancer prevention and care continuum. In particular, the first general research question of this thesis probed the possibility of having an optimal screening strategy. The investigation determined which screening strategy could offer precise disease detection and fewer harms using emerging screening tools either stand-alone or combined as an adjunct test. Using the data from the MARZY prospective randomised trial, Study 1 was carried out to identify which screening strategy with HPV testing performed better in the detection of relevant early-stage disease and offered a better benefit-to-harm balance.

As screening for disease must include appropriate follow-up of screen-positive results, this thesis determined the adherence rate to follow-up (or lack thereof) and also examined the role of HPV status through HPV testing, given that harms such as over-referral and increased concerns of having an HPV infection can be expected. The second study used MARZY data to examine the post-screening diagnostic follow-up adherence rate after the introduction of HPV testing, specifically the adherence behaviours to colposcopy. The following sections discuss the results of both studies and the broader context of screening programmes to which they provide new evidence.

## 4.1 Optimal screening methods

The first study investigated whether screening methods, including signal-based and target-amplified HPV testing, can optimise cervical cancer screening in a German population. While there is plenty of RCT and longitudinal evidence supporting the superior detection capabilities of HPV-based screening over cytology screening as a primary tool (Arbyn et al., 2012; Dillner et al., 2008; Koliopoulos et al., 2017; Murphy et al., 2012; Mustafa et al., 2016; Ogilvie et al., 2018; Ronco et al., 2014), a direct comparison of co-testing with both cytology and HPV testing to HPV testing stand-alone outside of a trial or retrospective database setting was lacking. The results of Study 1 could demonstrate in a population-based selection of women eligible for HPV-based screening that the sensitivity of primary screening with HPV testing alone was equivalent to co-testing, and that co-testing did not add substantial benefits in the detection of relevant disease endpoints.

Although the specificity of HPV testing stand-alone was lower than cytology, a major argument to keep cytology as a primary screening tool, the specificity was significantly better than that of the co-testing strategies, and the overall specificity of stand-alone HPV testing was still high at 93-94%. When co-testing with LBC, specificity was equivalent to stand-alone HPV testing. This result indicates the favourability of LBC co-testing over conventional cytology co-testing when co-testing strategies are considered, not only due to the high accuracy but also due to the practicality of a single sample collected for dual testing purposes. A recent retrospective analysis of co-testing results from a large diagnostic laboratory in Germany also reported the increased detection capability of LBC co-testing (Xhaja et al., 2022), likely due to the use of computer-assisted imaging technologies (Klug et al., 2013). However, the potential harms of these co-testing strategies are substantial. Study 1 shows that co-testing would introduce more potential harms through a higher number of colposcopy referrals and false positives. These results align with the observations reported previously from indirect comparisons (Arbyn et al., 2012; Cox et al., 2013; Koliopoulos et al., 2017; Schiffman et al., 2018). The conclusion of Study 1 supports the favouring of primary HPV screening, when accounting for a balance of benefits and harms. It reinforces the recommendations by the WHO and EU of moving away from primary cytology-based screening for women above the age of 30 years.

## 4.2 Patient adherence behaviour

The second study examined adherence rates within the screening algorithm to colposcopy following a screen-positive result and the impact of HPV and other factors on adherence to the algorithm. Women screened within the MARZY study who had a positive HPV test result or abnormal cytology were referred to colposcopy examination. The proportion of women who did not participate in follow-up colposcopy was substantial at 29% overall and the rate varied depending on the screening result: approximately 20% for women with HPV-positive results (stand-alone and co-test positive) and half of the women with only a cytological abnormality (cytology stand-alone). High non-attendance rates (up to 45%) have been observed in settings with opportunistic screenings (Benard et al., 2005; Elit et al., 2012), in contrast to settings with organised screenings where rates are lower than 15% (Douglas et al., 2015; Jørgensen et al., 2021; Sharp et al., 2012), albeit all within cytology-based programmes. In one study piloting HPV testing within an established organised programme, non-attendance remained below 15% (Green et al., 2021).

A positive HPV result in the initial screening step significantly influenced colposcopy attendance, with women 3-fold more likely to attend colposcopy than those with cytological abnormalities. This pattern was also observed in a small pilot study from Canada, one of the first to investigate the impact of HPV testing on colposcopy attendance outside of a RCT and organised screening setting (Buick et al., 2021). Concerns regarding the positive HPV result in terms of its relation to cancer development and fertility did not significantly increase colposcopy attendance. However, the impact of having an HPV infection on sexual activity and partners was a marked topic of concern among attendees, prompting colposcopy attendance (yet not statistically significant). These concerns were likely mitigated due to the detailed information given to the referred women regarding their HPV result and its risk factors within the MARZY study, as well as communication by their screening physician and the study nurses conducting the active recall.

In addition, women aged 40-49 years were more likely than younger women to attend colposcopy, but this effect disappeared for older women ≥50 years. Having three or more children significantly reduced the likelihood of attending colposcopy. Study nurse-assisted recalls of women who had not yet arranged colposcopy appointments were helpful in improving the attendance rate. However, major

reasons for non-attendance included lack of time, barriers such as the need for childcare arrangements or travel to the clinic, and the screening physician's advice not to attend, but rather undergo retesting. These factors distinguish particular sub-groups of screen-positive women that may require more tailored interventions targeting particular age groups and prompt the provision of detailed yet simplified information regarding colposcopy (Chan et al., 2004; Eggleston et al., 2007; Ogilvie et al., 2004). Such interventions should be integrated into a systematic and uniform call-recall system, particularly if administered by the screening clinic (Dunn et al., 2013; Kristiansen et al., 2017; Oladipo et al., 2007). Study 2 highlights the need to consider appropriate and specific sub-group strategies to circumnavigate poor attendance, over-referrals and increased concerns by screened women about positive HPV results.

## 4.3 Considerations for transitioning HPV-based screening programmes

Within the context of cervical cancer prevention by screening, the two studies from the MARZY project underscore the benefits of shifting to primary HPV screening and the need to actively retain women in the screening algorithm who are positively screened. The findings from Study 1 support the preference for primary HPV screening over co-testing, even in contexts with opportunistic screening. Recent real-world evidence from the United States, which also offers screenings based on an opportunistic model, demonstrated that HPV testing contributes more to the detection of CIN2 and CIN3 or worse than cytology within co-testing strategies (Cuzick et al., 2023). Co-testing was hypothesised to add only incremental benefits to HPV testing stand-alone in terms of disease detection, and the benefits for glandular lesions would be non-existent due to the poor performance of cytology (Castle et al., 2018a; Stoler et al., 2015). These arguments are supported by the longitudinal observations of opportunistic co-testings and RCT-based HPV screenings whereby the cumulative incidence rates of CIN3 or worse were similar between co-testing and HPV testing stand-alone after multiple screening rounds (Castle et al., 2018b; Dillner et al., 2008; Gage et al., 2014). A pilot HPV-based screening project in Germany concluded similarly, despite being based on a health insurance population, prone to potential selection bias (Horn et al., 2019). The tipping point favouring primary HPV screening over co-testing however has been argued with greater cost-effectiveness indicators and fewer number of lifetime tests (Jin et al., 2016; Kim et al., 2018; Petry et al., 2017).

A frequent argument against primary HPV screening asserts the possibility of HPV-negative cancers, which can be detected instead by cytology if it were used in adjunct (Pirog et al., 2014; Tracht et al., 2017; Zhao et al., 2013). However, it is important to note the following. Even in screening programmes with co-testing, ICCs are not eliminated (Castle et al., 2017). In a large multi-centre study from the United States, the majority of women with ICC who experienced a failure in screening test had either a negative co-test (both cytology and HPV negative) or a discordant co-test (normal cytology, HPV positive) (Chao et al., 2023). Glandular lesions were also 3-fold more likely to be missed. Rare types of AC have also been associated with exposure to diethylstilbestrol (Herbst et al., 1971), a synthetic oestrogen hormone previously prescribed to prevent miscarriages, congenital disabilities and adverse pregnancy outcomes. Furthermore, cytology is known to detect glandular lesions inadequately and likely would not have detected these types (Ronnett et al., 1999). A small proportion (8%) of other glandular cervical carcinomas have been observed to be HPV-independent, likely owing to suboptimal histological preparation and less-sensitive HPV assays (Rodríguez-Carunchio et al., 2015). As for HPV-negative SCCs, they are likely to be HPV-associated but may be falsely classified due to the use of suboptimal HPV assays, suboptimal histological processing or the loss of HPV DNA in advanced tumours (Rodríguez-Carunchio et al., 2015). For instance, coincidental endometrial biopsies have also been reported as HPV-negative cervical cancers (Cuzick et al., 2023).

With many countries planning to integrate HPV testing, these observations highlight first, the importance of selecting appropriate clinically validated HPV tests with quality assurance plans and second, resource considerations within local and national guidelines (Cuschieri et al., 2019). Both HC2 and GP5+/6+ EIA PCR have limitations (no genotyping and not commercially available, respectively). In place, there are several clinically validated HPV assays with non-inferior sensitivity and specificity compared to HC2 and GP5+/6+ EIA PCR, including the signal-amplified Cervista (Hologic, Inc., United States) and target-amplified Cobas 4800 (Roche Diagnostics, Germany) and BD Onclarity (Becton, Dickinson and Company [BD], United States) (Arbyn et al., 2016; Arbyn et al., 2021). These also contain internal controls to determine whether the sample is adequate, minimising false negatives. However, only Cobas 4800 and BD Onclarity are FDA-approved for primary screening purposes. In contrast, the other assays (including HC2) are only approved for use as a co-test or for triage (U.S. Food and Drug Administration (FDA), 2019). Several assays clinically validated for triage and genotyping capacities may also soon receive regulatory approval (Arbyn et al., 2016; Arbyn et al., 2021).

Any transition from a primary cytology-based programme towards a primary HPV-based programme requires careful consideration of the resources, capacities and preparedness of the laboratories involved with diagnostics. National statistical reports from England reported difficulties in laboratory staff retention and recruitment, slower turnaround, and thus longer wait times for result delivery during the pilot period of the HPV screening programme (NHS Digital, 2020). In Germany, laboratory preparedness for HPV testing as a co-test, for which a wider variety of assays were approved, was still a major reported challenge due to a delay in communication of co-testing implementation plans (Xhaja et al., 2022). In the Australian programme, this was also a challenge for HPV self-sampling options due to hindrances in local regulatory approval (Smith et al., 2019). These logistical challenges are also impacted by the extended intervals for HPV-based screening, affecting workload and capacities up to 4-fold (Pesola et al., 2023). An attractive solution is the gradual transition of selected age cohorts to extended screening intervals rather than transitioning the entire eligible cohort immediately.

Another critical point to consider and frequently used as a counter-argument for primary HPV screening strategies is the impact on over-referrals due to the lower diagnostic specificity of HPV testing (Kim et al., 2018). The impact of integrating co-testing into the cervical screening programme in Germany is currently unknown. Until such results are published, appropriate triage and communication strategies must be a central component integrated into the screening programme to mitigate these harmful outcomes.

### 4.3.1 Triage

As a means to improve upon specificity shortcomings of primary HPV screening, triage alternatives are possible. There is, however, a lack of consensus regarding the appropriate triage strategy (Cuschieri et al., 2018). The WHO recommends primary HPV screening with or without triage in the general population (World Health Organization, 2021). There are several triage possibilities: cytology, HPV testing, genotyping, immunocytochemistry for p16/Ki-67, a combination of these, or DNA methylation and next generation sequencing (Cuschieri et al., 2018). Currently, most HIC that have implemented HPV screening or plan to, have integrated triage options and triage with cytology following a positive primary HPV screening result, a common strategy that has demonstrated a low lifetime risk of cervical cancer (Cancer Council Australia Cervical Cancer Screening Guidelines Working Party, 2016; Chao et

al., 2019; Maver & Poljak, 2020), particularly with LBC (Gustinucci et al., 2016). However, cytological triage is subject to suboptimal reproducibility and requires strict quality assurance (Ronco et al., 2016).

Triage with HPV genotyping is another viable option and demonstrates high sensitivity and specificity (Chao et al., 2019; Hashim et al., 2020) and very high NPV (>99%) (Stoler et al., 2012). Another recently FDA-approved assay, APTIMA (Hologic, Inc., United States), is based on RNA-targeted amplification, which focuses on either HPV E6 and E7 proteins or monoclonal antibodies. RNA-based detection is proposed to be a more accurate indicator of cervical neoplasia than DNA-based assays due to the ability to detect an active or transient HPV infection likely to develop into CIN (Sotlar et al., 2004). Compared to DNA-based assays, the sensitivity of APTIMA is equivalent to DNA-based assays (Strang et al., 2021), but specificity is reported to be better (Arbyn et al., 2022b). However, RNA-based assays require a high detectable level of nucleic acid in the sample, which may not be feasible large-scale (Castle et al., 2015), has inferior intra- and inter-laboratory reproducibility (Arbyn et al., 2021) and higher referral rates to follow-up assessment than DNA-based assays (Maggino et al., 2016).

Newer triage methods, including immunocytochemical staining of p16 and Ki-67, are excellent proxies for identifying productive rather than transforming HPV infections, which HPV DNA assays cannot differentiate. P16 is present in almost all CIN3 and ICC biopsies (Klaes et al., 2002; Silva et al., 2017). Ki-67 overexpression also indicates cell proliferation during the HPV cell cycle and can be coupled with p16 detection to form p16/Ki-67 dual staining. Dual staining also offers better risk stratification and mitigation of over-referrals (Cuschieri et al., 2018). The specificity of p16/Ki-67 dual staining is also superior to HPV-based triage, especially for women with borderline ASC-US abnormalities (Peeters et al., 2019). In the new German screening programme, co-testing triage is recommended by national guidelines (Gemeinsamer Bundesausschuss (G-BA), 2018b) in contrast to expert committee recommendations that specified triage by HPV or p16/Ki-67 dual staining (Hillemanns et al., 2019b).

A second delayed triage (retesting) or direct referral to a colposcopy examination may also be integrated. For example, in the United States, co-tested women can be directly referred to colposcopy based on the concurrent test result available or a second delayed triage with either HPV testing or cytology is requested (Melnikow et al., 2018). However, in such settings with multiple accepted screening strategies like in the United States (primary cytology, primary HPV testing, co-testing), triage

should be considered with particular regard to referral demand and costs. The referral rate of co-testing is high and thus requires the highest number of lifetime tests (Kim et al., 2018). Co-testing costs are notably higher than primary cytology and primary HPV testing strategies (Sawaya et al., 2019; Wright et al., 2016). Future tests such as next-generation sequencing (high throughput genomic sequencing to detect genomic variabilities) and DNA methylation assays (to detect elevated epigenetic marks or methylations) show promise as more precise and accurate indicators of cervical neoplasia (Banila et al., 2022; Cuschieri et al., 2018), but further studies on their performance and economic outcomes are necessary before these are recommended as triage solutions.

The shift from a primarily cytology-based screening programme to primary HPV screening does not only impact the screenings conducted (age and intervals) but also has a domino effect on the colposcopy rate, which increased above expected numbers following the rollout in Australia even despite genotyping and triage strategies (Machalek et al., 2019; Smith et al., 2019; Smith et al., 2022). This increase in demand for colposcopies led to longer wait times and exacerbated inequalities of colposcopy adherence. Part of the reason for the higher number of colposcopy referrals in Australia was due to inadequate adherence to recommended age groups and intervals and patient demand by healthcare providers (Smith et al., 2019). Increased colposcopy referrals were also observed in the Netherlands, England, and Finland, where HPV-based screenings were implemented (Loopik et al., 2021b; Rebolj et al., 2019; Veijalainen et al., 2019). The starting age for screening and subsequent algorithm, however, differed between these countries. Additionally, over-treatment rates did not increase, and reductions in overall referral rates are expected in subsequent screening rounds (Blatt et al., 2015; Loopik et al., 2021b; Ogilvie et al., 2018; Rebolj et al., 2019; Smith et al., 2022). Furthermore, observations from Study 1 demonstrated that the number of colposcopy referrals would be highest among all co-testing strategies, especially if only one of the two screening tests were positive.

In Study 2, women were more likely to attend colposcopy with only a hrHPV screening result than having a cytological abnormality detected. While this is a positive observation that HPV testing retains rather than repels women from further evaluation, this can also be disadvantageous from a health service capacities perspective, especially if the detected HPV infection eventually clears. Greater demand for colposcopy may be linked to concerns about the consequences of HPV infections on fertility and sexual relationships rather than the test result itself (Bennett et al., 2021; Lester & Wilson,

1999; McRae et al., 2014; O'Connor et al., 2014). Stigma and shame were highest among women who knew HPV was sexually transmitted but were unaware of the high lifetime prevalence of HPV infections (Waller et al., 2007). In populations where inadequate efforts were made to inform women of screening changes to come, negative concerns and anxiety were reported (Dodd et al., 2020b; Hendry et al., 2012). These potential harms can be minimised by educating women on the high prevalence, transient nature and available prevention methods. On the healthcare provider side, inadequate education and training of physicians and nurses eligible to conduct screenings were also associated with poor HPV knowledge and training (McSherry et al., 2018). Wide acceptance of HPV testing may only then be realised if women and healthcare providers are adequately educated and informed.

### 4.3.2 Education, information and quality assurance

#### 4.3.2.1 Women screened

While triage may help mitigate over-referrals, adequate and timely communication of information is still necessary for HPV-positive screened women, particularly women who require diagnostic follow-up. Study 2 identified a greater adherence rate when a positive HPV screening result was reported compared to a cytological abnormality. In a systematic review of women's needs in an HPV-based screening programme, having an active follow-up strategy was reportedly a critical and preferred option over observational follow-up via retesting (Frederiksen et al., 2012). Women in Study 2 were not more likely to attend colposcopy due to concerns about their HPV screening result, and adherence improved after active study nurse recall efforts. A trial from the United Kingdom comparing the psychosocial outcomes after a positive screening result demonstrated similar outcomes of having a call-recall system for follow-up (Fielding et al., 2017). Women who were assigned to the colposcopy arm reported fewer concerns regarding cervical cancer risk and worries related to their abnormal screening result than the triage (repeat cytology) arm. Several interesting reasons could explain this observation. First, this result could be due to the active consultation given to women before and during the colposcopy examination by the study physician/nurses, which may have provided reassurance. Second, some colposcopy clinics show the patient a live video of the cervix being examined, which minimises anxiety and can lead to improved post-colposcopy adherence (Galaal et al., 2011; Takacs et al., 2004; Walsh et al., 2004).

Regarding the content and delivery of the screening-related information, in Study 2, a high proportion of women, regardless of whether they attended colposcopy, were concerned with infecting their partner and the impact of a positive HPV result on their sexual relationships. A recent study from a colposcopy centre in Germany found that women referred to colposcopy within the newly implemented screening programme had very high levels of anxiety, primarily due to the procedure itself rather than the outcome (Wittenborn et al., 2022). A recent systematic review on the needs of HPV-positive women also observed that the majority of women required more information about their positive HPV result and its impact on their sexual relationships and partners (Galeshi et al., 2022). Stigma, shame and anxiety rates were lowered when participants were aware of the fact that HPV is highly transmissible yet mostly harmless virus (Waller et al., 2007). Women from Australia, Norway and the United Kingdom experienced a major shift from the biennial cytology screenings offered since the 1960s to a primary HPV screening programme with 5-year intervals. Focus groups from these populations after the rollout of primary HPV screening highlighted the necessity for timely communication of the expected changes to the screening, as well as adaptive delivery modes that are age-appropriate (Dodd et al., 2020b; Mulcahy Symmons et al., 2021). Further qualitative takeaways found poor awareness and understanding of the proposed screening test and intervals (Andreassen et al., 2019), and feelings of being disregarded in the decision-making process (Obermair et al., 2018). Integrating the needs and preferences of women eligible for screening, explicitly raising awareness, educating and doing so in a timely manner is crucial for the successful implementation of a reformed and effective screening programme.

#### 4.3.2.2 Healthcare providers

Education and information are important for the women receiving screening prevention and care and for the involved healthcare professionals to minimise potential harms of over-screening, over-referral and over-treatment. For example, in the United States, in addition to primary cytology, co-testing has been an accepted strategy with triennial intervals since 2002 and recommended with 5-yearly intervals from 2012. Despite these recommendations, co-testing screenings by physicians were not adequately adhered to compared to the long-standing cytology screenings conducted since the 1960s (Castle et al., 2022; Rendle et al., 2018; Silver et al., 2018b; Wright et al., 2021). Physicians screened women more frequently, outside the recommended age groups, and infrequently, at longer than recommended

intervals. Several reasons could explain this phenomenon, including a lack of awareness of guideline changes (Teoh et al., 2015), confusion due to discordant guidelines from various professional bodies (Wentzensen et al., 2016), lack of faith in the utility of primary HPV screening versus co-testing (Kruse et al., 2022), concerns about liability and loss of patient adherence to other examinations (Roland et al., 2013) as well as the influence of patient preference (Hawkins et al., 2013). The influence of the healthcare practice or system was also observed to influence the lack of screening guideline adherence (Tatar et al., 2020). Over time, adherence rates to screening recommendations improved, but improper adherence persists as a major challenge, compounded by the latest recommendation to primarily test with HPV (Castle et al., 2022; MacLaughlin et al., 2019).

Inadequate adherence to guidelines is not a unique problem to the United States. The rollout of Australia's HPV screening programme also demonstrated challenges to healthcare provider understanding of the screening changes (Dodd et al., 2022). Some physicians were screening outside guideline-recommended age groups and at shorter intervals, and performing co-testing despite it not being a recommended strategy. Issues in information systems integration, timely regulatory approval and shortages in laboratory equipment contributed to barriers to smooth implementation (Smith et al., 2019). These issues have also been reported by laboratories in Germany after the 2020 shift to co-testing (Marquardt & Ziemke, 2022; Xhaja et al., 2022). Implementation issues could be minimised with timely communication of the changes and the comprehensive training of clinicians involved with screening and follow-up assessments (Dodd et al., 2020a; Smith et al., 2019). The straightforwardness of the 'screen, triage and treat' algorithm with a single screening test could also improve adherence (Fontham et al., 2020; Stoler et al., 2015). A screening algorithm based on co-testing is complex due to the numerous combinations and multitude of results from both cytology and HPV testing compared to a stand-alone HPV test result (positive, negative). An overview of facilitators of proper guideline adherence among healthcare providers underscores the value of educational interventions, particularly in increasing knowledge and understanding of HPV testing and integration of electronic medical systems (Tatar et al., 2020).

#### 4.3.2.3 Quality assurance

Part of quality assurance in a screening programme involves measuring indicators such as screening coverage and colposcopy attendance. In organised screening programmes, a threshold for the latter

supports proper follow-up of women who require it. One example is given by guidelines in Canada, which has a 15% non-attendance threshold supported by systematic recall mechanisms (Murphy et al., 2015). Although the new screening programme in Germany is still in the transition phase, and preliminary data on such indicators are yet to be published, no specification of an acceptable threshold has been determined. This should be addressed as not doing so may undermine the purpose of having an organised programme.

Screening participation and timely attendance to colposcopy by the screened woman is crucial for identifying relevant cases, but quality-assured screening, colposcopy and histopathology processes minimise failures in care. For example, among women with ICC who attended colposcopy and had biopsies taken, 9% of precancers were incorrectly identified, either by the colposcopist or pathologist, particularly for glandular lesions (Chao et al., 2023). Although standardisation of the assessments by colposcopists and pathologists has been streamlined for qualifications, classification systems and procedural instructions (Arbyn et al., 2010; Bulten et al., 2011; Wentzensen et al., 2017), inadequacies in reproducibility still exist. Significant variations in quality indicators and clinical guidelines are acknowledged across national colposcopy boards (Mayeaux et al., 2017). The classification of TZ types in Germany varies between colposcopists and colposcopy clinics (Luyten et al., 2015a). A recent review of colposcopy-directed biopsies and excisional histopathological diagnoses taken within the new co-testing programme in Germany highlighted poor agreement ($\kappa$=0.35) between the two (Marquardt & Ziemke, 2022). Low to moderate reliability has also been reported in Italy and the United States (Ceballos et al., 2008; Dalla Palma et al., 2009). In Study 1, efforts were made to curtail these discrepancies. However, due to the subjective nature of these assessments, biases may still have arisen.

Additional tools may aid in determining high-risk precancers. Such tools include p16 Immunohistochemistry (IHC) staining with histopathological assessment, which has been demonstrated to be an accurate method to detect CIN3 (Galgano et al., 2010; Klaes et al., 2002; Silva et al., 2017) The use of p16-IHC staining could better differentiate CIN2 and CIN3 lesions, helping avoid unnecessary treatment or misclassification (Castle et al., 2020). A meta-analysis of studies using p16-IHC found improved inter-rater reliability of moderate to severe precancers (Reuschenbach et al., 2014). However, another systematic review reported wide variations in performance due to a lack of standardised interpretation (Tsoumpou et al., 2009). Several guidelines, including those from the

United States, Europe and Australia, consider p16-IHC an appropriate diagnostic aid for the clarification of precancerous lesions that are prone to misclassification (Cancer Council Australia Cervical Cancer Screening Guidelines Working Party, 2016; European Commission, 2008; Perkins et al., 2020).

## 4.4 Limitations

There are some limitations of the two studies in this thesis to note. First, Study 1 was based on the screening results of one screening round, i.e. cross-sectional data. Although it was possible to determine the absolute and relative performance of various screening tools and strategies accounting for verification bias, the true potential of primary HPV testing can be determined if longitudinal sensitivity and NPV are described. The MARZY study encompassed two screening rounds. However, obtaining clinically meaningful longitudinal estimates would be limited due to the total number of recruited and screened women. Study 2 incorporated data from both screening rounds regarding adherence to colposcopy follow-up. Inclusion of data from both rounds was done to provide sufficient power in the analyses, as the number of co-test positives in a predominantly routinely albeit opportunistically screened population was low. However, through the invitation intervention arms of the MARZY study, an additional 9% of women rarely or never-screened could be screened, offering a more representative picture of screen-eligible women. Additional multiple imputation analyses were carried out to address this issue.

Unfortunately, no cost-relevant data was captured in the study, which would be helpful in determining the cost-effectiveness of the screening strategies. Furthermore, there was a lack of qualitative data with respect to the preferred screening strategy (from both the woman and healthcare provider) and experiences of colposcopy among attendees. These data help inform strategies to address inadequacies in the new screening programme in Germany. In Study 2, there appeared to be improper adherence to the screening algorithm in terms of interval and triage, with some women screened earlier than three years and some being advised to undergo repeat testing instead of colposcopy as indicated by the study protocol. While quality assurance of the MARZY study was carefully carried out and feasible in one region of Germany (Zeissig et al., 2014), this emphasises the need for robust, continual and consistent upkeep of quality assurance practices across the country.

## 4.5 Strengths

Overall, the MARZY study could provide crucial population-based data to answer screening-relevant questions on screening coverage, diagnostic performance and diagnostic follow-up and treatment, forming quality assurance indications. Based on the randomised trial design, it could be demonstrated that an invitation and reminder system could significantly improve screening participation rates (Radde et al., 2016). An additional brochure detailing additional information about HPV infection, cervical dysplasia pathway and screening did not further improve participation. Central to this thesis, Study 1 could show that using an HPV-based test was superior to cytology screening in terms of the balance of benefits-to-harms. This strategy could improve failures in screening detection that contribute to the stagnant cervical cancer incidence observed. Furthermore, Study 2 highlighted a relatively high but variable non-adherence rate to colposcopy follow-up, with HPV-positive screened women three times more likely to adhere. The study identified reasons for non-attendance that are driven by time and resource barriers of the woman, but also by contradicting advice from the healthcare provider. These were observed despite the maintenance of quality assurance measures (Zeissig et al., 2014). Addressing failures in follow-up care is crucial to optimise the effects of population-wide screening.

## 4.6 Implications for future research and policy

Countries such as Germany shifting towards HPV-based screening will need to evaluate emerging tools and technologies to optimise their programme continuously. While triage following a stand-alone HPV test can mitigate harmful outcomes by identifying those at the highest risk, more precise risk stratification may be possible with the help of artificial intelligence technologies such as machine learning and deep learning. These technologies engage in automated pattern recognition of samples, and have been assessed in the context of visual inspection of the cervix for primary screening (particularly in LMICs; also referred to as automated visual evaluation [AVE]) and as colposcopy assessments (Hu et al., 2019), as an aid to cytology screening (Kitchener et al., 2011a; Klug et al., 2013; Nieminen et al., 2004; Rebolj et al., 2015), and as triage of positive HPV results (Desai et al., 2022; Wentzensen et al., 2020). Such technologies can reduce human judgement errors, i.e. subjectivity, reduce workload in contexts with human resource issues and may thus lower the costs. However, these

technologies are still evolving rapidly and require rigorous studies if they are to be integrated into cervical screening programmes.

Another likely future outlook of cervical cancer screening involves risk-stratified screening, which has the potential to improve the balance in benefits-to-harms further. One example is the integration of genotyping as the primary HPV test. In Australia, the national primary HPV screening programme relies on partial genotyping of types 16 and 18 as a form of risk stratification. Those positive for either type are directly referred to colposcopy (Cancer Council Australia Cervical Cancer Screening Guidelines Working Party, 2016). This strategy achieves maximum clinical impact in detection, however, may entail higher annual diagnostic costs for the screening programme in other contexts (Petry et al., 2017). Ethical and practical considerations also need to be resolved in contexts considering risk-stratified screening, for instance, the autonomy of the individual must be respected if hrHPV types 16, 18 or other than 16 and 18 are detected (Hall et al., 2013). Moreover, healthcare provider endorsement is crucial. Further research is required to determine the needs for healthcare providers to adequately adhere to risk-stratified screening, given that uptake of general screening recommendations is slow or even non-existent (Tatar et al., 2020).

Furthermore, the impact of HPV-vaccinated cohorts will play a prominent role in the future of screening programmes. Risk-stratified methods for accommodating vaccinated and non-vaccinated cohorts will need to be developed, perhaps with modern tools such as DNA methylation (Lehtinen et al., 2022). Data from the Australian programme indicate that a substantial number of colposcopy referrals among vaccinated cohorts will be positive for hrHPV not of types 16 and 18 and with normal or low-grade cytology, who can safely be referred for HPV retesting at 12 months to minimise over-referral (Smith et al., 2022). Alternatively, therapeutic vaccines offer a post-exposure alternative to those who missed the prophylactic vaccine prior to sexual debut. Therapeutic HPV vaccines work by inducing cell-mediated immunity against active infections via the key oncogenes E6 and E7 in hrHPV types. Several candidates are currently being trialled (Chabeda et al., 2018), and the results could potentially curb the need for intensive cervical screening.

With increasingly emerging prevention and screening tools to consider, the performance of such tools should also be evaluated longitudinally due to the progressive and regressive nature of cervical

neoplasia and specific for each context. Longitudinal outcomes can be extracted from surveillance registries or an organised programme. Of the limited longitudinal evidence available in Germany (Horn et al., 2019; Petry et al., 2017), it appears that HPV-based screening, mainly primary HPV testing, is the future. However, more rigorous evidence from the transitional period of the new screening programme will be necessary to determine whether primary HPV testing can be realised and beneficial longitudinally in Germany. Additional research efforts to optimise cervical cancer screening should include an implementation science approach with quantitative and qualitative (mixed methods) data, even for HIC (Broutet et al., 2022). Incorporation of such stakeholder-informed data can help adapt healthcare provider needs and eligible women's preferences and inform policy-makers on the most optimal and effective screening programme.

## 4.7 Conclusion

Overall, HPV-based screening translates to reliable disease detection, fewer lifetime screenings and optimal cost-effectiveness, thus significantly reducing the burden of precancerous lesions, cervical cancers and preventable mortalities. This thesis examined optimal screening strategies and found that reliance on HPV testing stand-alone would not result in more harm than a co-testing strategy, which ties a long-standing yet problematic screening tool to an objective modern one. While there are higher number of referrals and better adherence to diagnostic follow-up expected with HPV-based screening, tailored strategies are essential to improve adherence among women who should follow-up with colposcopy. To mitigate high colposcopy demand in the initial rollout of an HPV-based screening, reassurance via education and information of patients and healthcare professionals are vital.

The findings from this thesis in particular draw attention to adopting of more optimal screening strategies and highlight the need for adequate and timely communication of such major screening changes (ideally prior to implementation). Furthermore, simple information dissemination of the anticipated screening results, particularly for HPV testing and possible follow-up diagnostic steps (throughout programme rollout) needs to be implemented in a clear, transparent and standardised format, preferably within national screening guidelines. Guidelines should be informed by all stakeholders in the cervical screening programme, from the eligible women, health insurance

companies and healthcare provider to the diagnostic units involved. To improve the quality and thoroughness, a call-recall system built within a systematic organised programme with quality assurance is needed to maintain adequate screening coverage and retain women in the screening algorithm who need further management (Miles et al., 2004). These approaches ensure that women participate in screening when eligible and understand the meaning of their screening result, and adhere to follow-up where necessary. Complementary to this, greater preparatory and ongoing educational efforts and resources for healthcare providers are fundamental (Kruse et al., 2022; Tatar et al., 2020), in addition to clear and timely communication of planned changes. These mechanisms will not work unless the information technology infrastructure is ready. Such adaptions can substantially improve cervical cancer screening effectiveness and together with HPV vaccination efforts, can lead to the eventual elimination of cervical cancer.

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# Appendix

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# Cervical Cancer Screening: Comparison of Conventional Pap Smear Test, Liquid-Based Cytology, and Human Papillomavirus Testing as Stand-alone or Cotesting Strategies

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## ABSTRACT

**Background:** Some countries have implemented stand-alone human papillomavirus (HPV) testing while others consider cotesting for cervical cancer screening. We compared both strategies within a population-based study.

**Methods:** The MARZY cohort study was conducted in Germany. Randomly selected women from population registries aged ≥30 years ($n$ = 5,275) were invited to screening with Pap smear, liquid-based cytology (LBC, ThinPrep), and HPV testing (Hybrid Capture2, HC2). Screen-positive participants [ASC-US+ or high-risk HC2 (hrHC2)] and a random 5% sample of screen-negatives were referred to colposcopy. *Post hoc* HPV genotyping was conducted by GP5+/6+ PCR-EIA with reverse line blotting. Sensitivity, specificity (adjusted for verification bias), and potential harms, including number of colposcopies needed to detect 1 precancerous lesion (NNC), were calculated.

**Results:** In 2,627 screened women, cytological sensitivities (Pap, LBC: 47%) were lower than HC2 (95%) and PCR (79%) for CIN2+. Cotesting demonstrated higher sensitivities (HC2 cotesting: 99%; PCR cotesting: 84%), but at the cost of lower specificities (92%–95%) compared with HPV stand-alone (HC2: 95%; PCR: 94%) and cytology (97% or 99%). Cotesting versus HPV stand-alone showed equivalent relative sensitivity [HC2: 1.06, 95% confidence interval (CI), 1.00–1.21; PCR: 1.07, 95% CI, 1.00–1.27]. Relative specificity of Pap cotesting with either HPV test was inferior to stand-alone HPV. LBC cotesting demonstrated equivalent specificity (both tests: 0.99, 95% CI, 0.99–1.00). NNC was highest for Pap cotesting.

**Conclusions:** Cotesting offers no benefit in detection over stand-alone HPV testing, resulting in more false positive results and colposcopy referrals.

**Impact:** HPV stand-alone screening offers a better balance of benefits and harms than cotesting.

*See related commentary by Wentzensen and Clarke, p. 432*

## Introduction

With the implementation of cytologic Papanicolaou (Pap) smear as a detection method for cervical cell abnormality since the 1960s, overall cervical cancer incidence and mortality rates in high income countries have fallen drastically (1). Lately however, incidence rates have remained stagnant in many of these settings (1, 2). Despite its successes, screening with cytology is resource-intensive and prone to poor reproducibility with a widely ranging sensitivity of 43% to 96%, even in high-resource countries such as Germany (3). In addition, since the discovery of the causative agent human papillomavirus (HPV) in almost all cervical cancers, prophylactic vaccines that target HPV types attributable in up to 90% of cervical cancers have been developed (4). Consequently, as HPV-vaccinated cohorts move toward screening eligibility, accuracy of cytology will be even further compromised because of the significant reduction in precancerous and cancerous lesions (5). Therefore, more objective detection methods are needed.

Molecular testing for HPV DNA has recently appeared as an alternative screening method, offering greater reproducibility and high-throughput benefits. These advantages led to U.S. FDA approval of HPV testing as an adjunct to cytology (reflex testing) or as a concomitant test (cotest). Since then, pooled studies and meta-analyses of several randomized controlled trials and observational studies have demonstrated superior detection of HPV-based screening (both stand-alone and cotesting) in comparison with cytology (3, 6, 7). These findings coupled with results from the ATHENA trial prompted regulatory approval of HPV testing as a stand-alone screening strategy in 2014 (8). As a result, stand-alone HPV testing has become the preferred strategy over cytology in European and U.S. guidelines among others (9, 10). In the Netherlands, cytology has already been replaced by stand-alone HPV testing at 5-year intervals (11).

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**Note:** Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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There are still, however, several concerns of stand-alone HPV screening regarding lowered specificity, safety of extended screening intervals, testing in women under 30 years of age, and observations of HPV test–negative carcinomas (12, 13). These concerns have been frequently used to advocate cotesting over stand-alone HPV screening and have even bolstered cotesting as a screening modality alongside HPV testing and triennial cytology in the United States (10). While a large robust body of evidence supports HPV-based screening, there is an ongoing debate around cotesting versus stand-alone HPV testing (14). Few studies have compared accuracy of the two strategies (6, 15, 16), with some observing minor differences in detection, albeit based on retrospective analyses (17, 18). Moreover, separate comparisons between HPV testing and Pap or liquid-based cytology (LBC) are lacking, and few have compared Pap to LBC-based cotesting (19, 20). To our knowledge, no study has directly compared both Pap and LBC as cotesting strategies to stand-alone HPV testing with two standard HPV comparators. Findings from such analyses provide necessary evidence on optimal screening strategies, especially for countries considering HPV-based screening, such as Germany, which has implemented an organized screening program with cotesting only in 2020 (21). Therefore, in a large population-based sample of women within an opportunistic screening setting, we compared absolute and relative clinical test accuracy of stand-alone and cotesting strategies with conventional Pap, LBC, and two HPV tests.

## Materials and Methods

Data stem from MARZY, a randomized prospective cohort study with a population-based sample of women eligible for cervical cancer screening in Germany between 2005 and 2012. Details on recruitment and intervention have been published in detail elsewhere (22). Briefly described, a random sample of 9,383 women selected from population registries were randomized into two intervention arms (sole invitation to screening, invitation with information brochure) and a no-invitation control arm to observe differences in screening attendance. At baseline, women randomized to both intervention arms ($n = 5{,}275$; eligible = 3,759) were invited to undergo screening with a conventional Pap smear, a LBC study swab, and HPV testing (**Fig. 1**). These analyses focus on baseline-screened participants between 2005 and 2007 ($n = 2{,}627$).

### Participants

Inclusion criteria were women 30 years or older and residing within the urban and rural region of Mainz and Mainz-Bingen, Germany. Women with any previous cervical cancer diagnoses, hysterectomy, or pregnancy at baseline were excluded. To preserve real-world screening, all gynecological practices and general practitioners conducting routine cervical cancer screening within the study region or who were elected by participants outside the study region were contacted to cooperate ($n = 121$) and closely monitored for quality assurance (23). Participants provided written informed consent before undergoing screening.

### Cytology

In line with the standard practice, gynecologists first obtained a conventional Pap smear and sent the specimen fixed onto a glass slide to their routine laboratory for assessment. Diagnostic results were relayed back to the study team. A second cytologic study swab was obtained using an Ayres spatula and endocervical broom or cytobrush when the transformation zone was not visible. The cells of this specimen were directly suspended in a vial containing 20 mL of PreservCyt Liquid Solution (ThinPrep, Cytyc/Hologic) and sent to a centralized laboratory (CytoMol, Frankfurt, Germany) routinely conducting LBC assessment.

Cytologic findings at baseline were based on the Munich II Nomenclature, which was used prior to Munich III, the current classification system in Germany (24). As up to 10% of moderate cervical intraepithelial neoplasia (CIN2) and 4% of severely dysplastic CIN3 are detected in equivocal cytology (25), all women with atypical squamous cells of undetermined significance or worse (ASC-US+) were referred to colposcopy. In German nomenclature, Pap IIw is an unofficial category widely used to denote equivocal results and is considered equivalent to ASC-US (24) from the International Bethesda Classification for Cytology (2014) (26). Pap IIID is equivalent to low-grade intraepithelial lesions, LSIL, and was also assessed for comparison.

### HPV DNA testing

Remaining PreservCyt solution was directly used for HPV DNA detection by Hybrid Capture2 (HC2, Qiagen), detecting 13 high-risk HPV types (hrHPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). Detection of hrHPV was set at the manufacturer recommended cut-off ratio of 1.0 relative light units (RLU). In addition to HC2, we analyzed the accuracy of another standard HPV comparator. All available PreservCyt solution samples were processed *post hoc* using GP5+/6+ PCR with enzyme immunoassay (EIA) probes targeting 14 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and low-risk types [6, 11, 26, 30, 32, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 64, 67, 69, 70, 71, 72, 73, 81, 82 (variants mm4 and is39), 83, 84, 85, 86, 89 (formerly cp6108), 90 (formerly jc9710)]. GP5+/6+ PCR-EIA–positive samples were typed and classified by reverse line dot blot hybridization performed at the Department of Pathology, Amsterdam UMC location VU Medical Center, the Netherlands. PCR results were not used to refer women to colposcopy as these were processed *post hoc*. HrHPV types were based on IARC 2012 classifications of probably carcinogenic and cervical carcinogens thus HPV 66 was not analyzed as high-risk (27).

### Colposcopy and histology

Women were considered screen-positive if either cytology was ASC-US+ or HC2 was positive (hrHC2) and subsequently referred to colposcopy, conducted centrally by certified study colposcopists (Department of Obstetrics and Gynecology, Mainz University Hospital, Mainz, Germany). Screen-positive women who did not arrange a colposcopy appointment within 2 months were contacted and encouraged to attend. If unable or unwilling, participants were further interviewed on reasons for non-attendance. Screen-negative was defined as both cytology (negative for intraepithelial lesion or malignancy, NILM) and HC2 negative. PCR results were not considered for colposcopy referral, as this test was only conducted *post hoc*. A random sample (5%) of all screen-negative women was also invited to colposcopy (**Fig. 1**).

Colposcopic examinations were conducted in accordance to 2002 International Federation of Cervical Pathology and Colposcopy (IFCPC) guidelines (28) with 5% acetic acid application first followed by Lugol's iodine solution. Participants with macroscopically visible lesions (abnormal colposcopic findings, colposcopic features suggestive of invasive cancer) underwent punch biopsy with multiple samples obtained for multiple acetowhitened lesions. Endocervical curettage was conducted if the transformation zone was obscured. Colposcopists were additionally instructed to take two biopsies from participants without visible lesions at the 12 and 6 o' clock regions of the cervix. All biopsies were assessed centrally by an experienced histopathologist. To maintain quality assurance, all histopathologic samples were

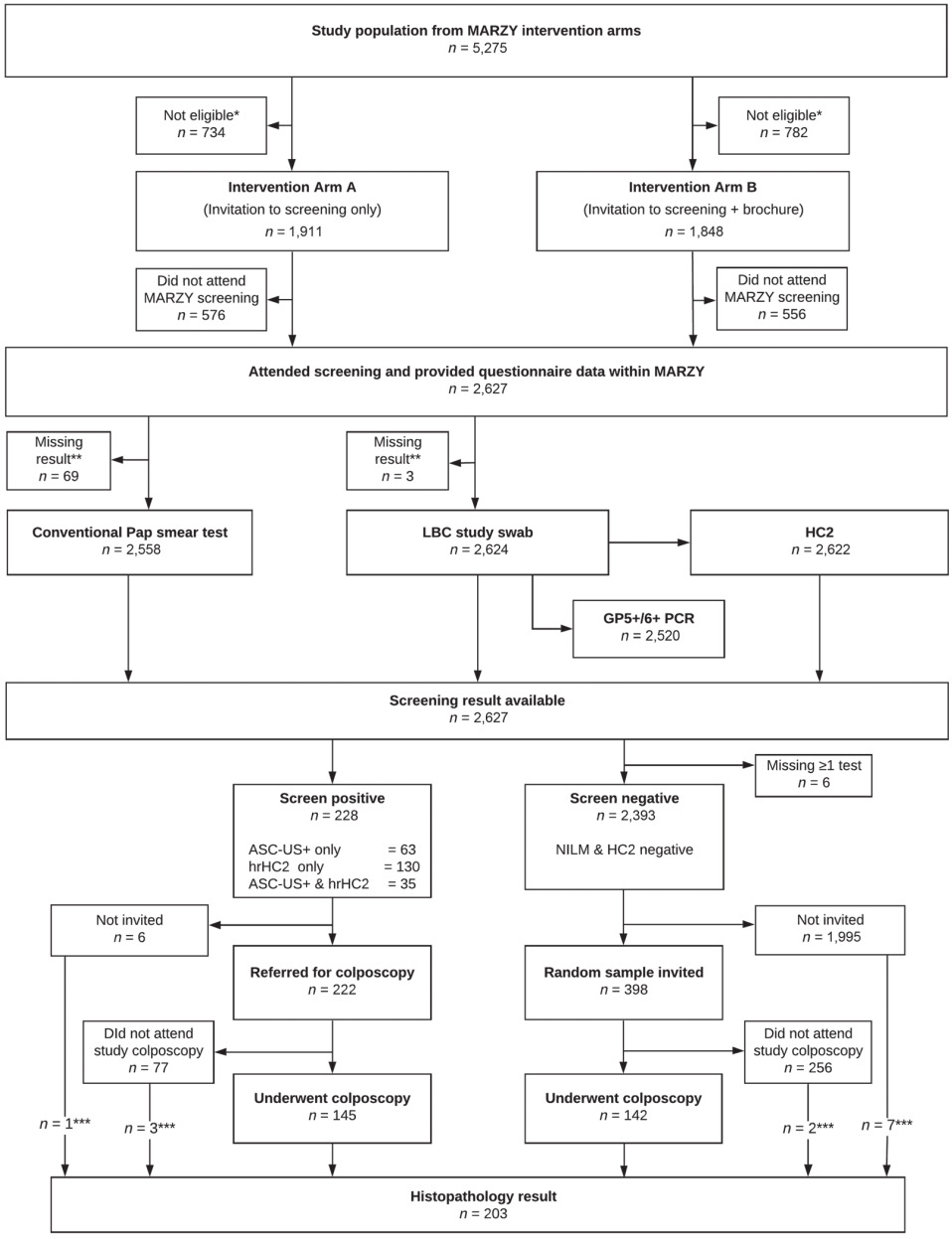


**Figure 1.**

Flow chart of study design and end results. LBC, liquid-based cytology; HC2, Hybrid Capture2 HPV test; hrHC2, high-risk HC2 type; ASC-US+, atypical squamous cells of undetermined significance or worse; NILM, negative for intraepithelial lesion or malignancy; * excluded due to hysterectomy, pregnancy, or history of cervical cancer; ** no sample obtained; *** external histopathology results reported within 1 year of study swab.

independently reviewed by a second histopathologist. A third histopathologist was called upon to settle discrepancies. The final agreed upon result was used for evaluation. In addition, external information regarding colposcopy and histopathology conducted outside the study during the study period were traced. Results reported within 1 year of the study swab were included. This active tracing of information was necessary due to the lack of centralized data registration of precancerous lesions in Germany and an opportunistic screening system. Women with suspected lesions at colposcopy or histopathologic lesions were managed as per local protocols for standard care.

## Statistical analyses

The *a priori* sample size estimation for MARZY was based on the primary outcome assuming 5% increase in participation rate

between randomized arms described elsewhere (22). The endpoints of interest for screening purposes were CIN2 or worse (CIN2+) and CIN3 or worse (CIN3+). Absolute sensitivity, specificity, and positive predictive values (PPV) were calculated. We calculated the complement of the negative predictive value (cNPV) to show the risk of CIN2+ or CIN3+ among screen-negative women (1-NPV). Although a random 5% sample of screen-negatives were invited to colposcopy, partial verification bias could still lead to an overestimation of sensitivity and an underestimation of specificity. Therefore, we adjusted all test accuracy estimates based on the probability to be followed-up for verification via the following sampling fractions (formula previously described in ref. 29: negative (0.04), cytology positive only (ASC-US+; 0.44), and hrHC2 (0.46). The inverse of these probabilities was applied as a weight to participants in their assigned strata (negative: 24.39, ASC-US+: 2.27, hrHC2: 2.17). As PCR test results did not influence test status nor strata allocation (processed *post hoc*), verification adjustment is equally appropriate for *post hoc* test results. Confidence intervals (CI) were obtained using bootstrap resampling methods ($n = 1{,}000$) at the lower 2.5% and upper 97.5% quantiles (30). To avoid problems with proportion calculations, we added 0.5 to each $2 \times 2$ contingency cell for HC2 cotesting and all PCR-based strategies (Haldane correction; ref. 31).

Comparisons of stand-alone sensitivity and specificity were conducted using McNemar's paired sample test, stratified by verified disease status. Positive and negative likelihood ratios (PLR, NLR) were calculated to compare cotesting strategies with stand-alone components. Relative sensitivity and specificity were calculated to directly compare all strategies, defined as the ratios of sensitivity and specificity between tests (no Haldane correction). CIs for crude ratios were based on Wald for paired data and adjusted ratios were based on bootstrap resampling.

For potential harms, we calculated false positive and negative rates (FPR: 1-specificity, FNR: 1-sensitivity) and the number of women needed to undergo colposcopy to detect one CIN2+ or CIN3+ case (NNC: 1/PPV) per test strategy. In sensitivity analyses, we calculated accuracy for women aged ≥35 years and of within-study collected biopsies (i.e., excluding external findings). HC2 test accuracy at higher viral load cutoffs at 2.0, 3.0, and 10.0 RLU were also conducted to determine specificity. All analyses were conducted using SAS 9.4 (SAS Institute). We complied with the STARD guidelines for reporting and followed Good Epidemiological Practice guidelines. The MARZY study was approved by the ethical committee of the state of Rhineland-Palatinate and the state government data protection office.

### Ethics approval and consent to participate

All participants provided signed informed consent to the study. The MARZY study was approved by the ethical committee of the state of Rhineland-Palatinate [Landesärztekammer Rheinland-Pfalz: 837.438.03 (4100)] and the state government data protection office. All recruitment, data collection, and analyses were performed in accordance to Good Epidemiological Practice guidelines and the Declaration of Helsinki.

### Consent for publication

Not applicable.

### Data availability statement

Anonymized data that support the findings of this study may be available from the corresponding author upon reasonable request.

## Results

Of the 5,275 women invited for screening within MARZY arms A and B, 2,627 (49.8%) were screened (**Fig. 1**). Mean age was 47.09 years (SD = 9.97; range 30–68 years). In women aged 30–39 years, 27% attended screening while only 15% of ≥60-year-old women attended. Approximately 9% of all participants either reported to have never undergone screening or did not attend screening at the recommended interval nor within a 5-year period (Supplementary Table S1 shows characteristics).

Pap and LBC detected 69 (2.7%) and 47 (1.8%) equivocal or worse cytology (ASC-US+), respectively, while HC2 and PCR detected 165 (6.3%) and 165 (6.6%) hrHPV, respectively. Among the 2,627 screened (**Fig. 1**), 228 (8.7%) were screen-positive where 63 (2.4%) were ASC-US+ only, 130 (5.0%) were hrHC2 only, and 35 (1.3%) were both ASC-US+ and hrHC2. Six women were not referred to colposcopy for reasons including planned hysterectomy elsewhere. Of 222 remaining screen-positives, despite active callback, 145 (65.3%) underwent colposcopy at the study center. Of all 2,393 screen-negatives, 142 (5.9%) attended study colposcopy (attendance rate, 142/398 = 35.7%). Colposcopies were conducted on average 4.9 months after screening (SD = 4.9), 6.0 months among screen-positives (SD = 6.4) and 3.7 months among screen-negatives (SD = 1.9).

Of the 203 histopathologic results (190 from study colposcopy: range of biopsies taken 1–5; 13 from externally conducted colposcopies), 3 squamous cell carcinomas (SCC; 1.5%), 7 high-grade (CIN3; 3.5%), 9 moderate-grade (CIN2; 4.4%), and 7 mild lesions (CIN1; 3.5%) were reported (**Fig. 1**). No adenocarcinomas or glandular lesions were detected (Supplementary Table S2). All CIN2+ were HPV positive (Supplementary Table S3).

### Absolute test accuracy

Estimates adjusted for verification bias for ASC-US+ are presented in **Table 1** (crude estimates: Supplementary Tables S4 and S5) and are based on 41 CIN2+ and 22 CIN3+ hypothetical lesions after adjustment. HC2 presented the highest sensitivities (cotesting 98.82%, stand-alone 94.56%) with HC2 stand-alone significantly more sensitive than either cytology (Pap and LBC both 47.47%; $P < 0.0001$). Specificity of HC2 stand-alone (95.12%) was significantly lower than cytology (Pap 97.48%; LBC 98.64%; $P < 0.0001$). Contrasting to stand-alone, cotesting specificity was reduced (Pap/HC2 93.09%; LBC/HC2 94.58%). For CIN3+, sensitivity of both Pap and LBC stand-alone was 70.11% and 89.67% for HC2 stand-alone. Specificities were similar to CIN2+.

With PCR, high sensitivities were also observed for CIN2+ (both cotests 84.24%, stand-alone 78.99%) and stand-alone was significantly higher than cytology ($P < 0.01$). PCR cotesting conferred the lowest specificities (Pap/PCR 92.21%; LBC/PCR 93.73%) increasing to 94.25% stand-alone, but significantly lower than cytology ($P < 0.0001$). For CIN3+, PCR presented the highest sensitivity (97.81%) but specificities were lower than cytology.

PPVs also indicated higher probability of disease by cytology, particularly with LBC, than HPV-based screening. However, for CIN2+ lesions, HC2-based strategies revealed similar PPVs to Pap. cNPVs revealed greater safety against CIN2+ among screen-negatives with HPV-based strategies, particularly HC2 cotesting (<0.1%). Safety against CIN2+ was lowest with cytology only (∼0.86%).

For LSIL+, sensitivities of cytology were lower (**Table 2**). LBC and HC2 cotesting conferred lower sensitivity than Pap and HC2 cotesting, but the former showed identical sensitivity as HC2 stand-alone. LBC and HC2 cotesting performed similarly to Pap and HC2 cotesting in terms of specificity and PPV. Sensitivity for stand-alone PCR for

**Table 1.** Sensitivity, specificity, NPV, PPV, and likelihood ratios adjusted for verification bias for ASC-US+ equivalent.

| Cut-off | Endpoint | Test | Sensitivity % (95% CI)[a] | Specificity % (95% CI)[a] | PPV % (95% CI)[a] | cNPV % (95% CI)[a] | PLR (95% CI) | NLR (95% CI) |
|---|---|---|---|---|---|---|---|---|
| **ASC-US+** | **CIN2+** | Pap | 47.47 (25.00–68.26)[b,c] | 97.48 (96.44–98.41)[b,c] | 23.25 (10.16–37.23) | 0.86 (0.37–1.38) | 18.86 (12.63–28.16) | 0.54 (0.40–0.72) |
| | | LBC | 47.47 (25.00–70.11)[b,c] | 98.64 (97.89–99.26)[b,c,d] | 35.78 (16.62–54.93) | 0.85 (0.36–1.38) | 34.79 (21.99–55.04) | 0.53 (0.40–0.71) |
| | | HC2 | 94.56 (82.09–100.0) | 95.12 (93.55–96.42) | 23.68 (14.57–33.14) | 0.09 (0.0–0.32) | 19.38 (16.10–23.33) | 0.06 (0.02–0.20) |
| | | PCR | 78.99 (58.40–94.74) | 94.25 (90.08–97.52) | 18.19 (8.33–38.26) | 0.36 (0.08–0.75) | 13.73 (10.99–17.15) | 0.22 (0.12–0.40) |
| | | Pap/HC2 | 98.82 (96.54–100.0) | 93.09 (91.09–95.07) | 19.01 (11.73–26.68) | 0.02 (0.02–0.02) | 14.31 (12.37–16.55) | 0.01 (0.00–0.20) |
| | | LBC/HC2 | 98.82 (96.54–100.0) | 94.58 (92.94–95.97) | 23.01 (13.97–31.82) | 0.02 (0.02–0.02) | 18.23 (15.47–21.49) | 0.01 (0.00–0.20) |
| | | Pap/PCR | 84.24 (66.67–100.0) | 92.21 (88.09–95.95) | 14.92 (7.52–26.46) | 0.28 (0.0–0.63) | 10.81 (8.96–13.05) | 0.17 (0.08–0.35) |
| | | LBC/PCR | 84.24 (65.10–100.0) | 93.73 (89.49–97.03) | 17.85 (8.68–34.63) | 0.27 (0.0–0.62) | 13.43 (11.00–16.40) | 0.17 (0.08–0.34) |
| **ASC-US+** | **CIN3+** | Pap | 70.11 (36.47–100.0) | 97.34 (96.24–98.27)[b,c] | 18.10 (6.78–30.54) | 0.26 (0.0–0.60) | 26.31 (18.36–37.69) | 0.31 (0.16–0.58) |
| | | LBC | 70.11 (38.12–100.0) | 98.48 (97.64–99.11)[b,c,d] | 27.86 (11.29–45.73) | 0.25 (0.0–0.57) | 46.09 (30.49–69.68) | 0.30 (0.16–0.58) |
| | | HC2 | 89.67 (65.87–100.0) | 94.41 (92.66–95.76) | 11.84 (4.66–19.62) | 0.09 (0.0–0.32) | 16.03 (12.96–19.83) | 0.11 (0.03–0.38) |
| | | PCR | 97.81 (95.71–100.0) | 93.85 (89.70–97.15) | 12.35 (4.81–26.80) | 0.02 (0.02–0.02) | 15.91 (13.52–18.72) | 0.02 (0.0–0.36) |
| | | Pap/HC2 | 97.81 (93.69–100.0) | 92.39 (90.11–94.15) | 10.13 (4.18–15.95) | 0.02 (0.02–0.02) | 12.86 (11.09–14.90) | 0.02 (0.0–0.37) |
| | | LBC/HC2 | 97.81 (93.69–100.0) | 93.87 (92.13–95.32) | 12.26 (5.02–19.38) | 0.02 (0.02–0.02) | 15.95 (13.56–18.77) | 0.02 (0.0–0.36) |
| | | Pap/PCR | 97.81 (93.69–100.0) | 91.75 (87.65–95.26) | 9.51 (3.91–17.76) | 0.02 (0.02–0.02) | 11.85 (10.27–13.67) | 0.02 (0.0–0.37) |
| | | LBC/PCR | 97.81 (93.69–100.0) | 93.25 (89.08–96.62) | 11.37 (4.64–23.26) | 0.02 (0.02–0.02) | 14.49 (12.40–16.94) | 0.02 (0.0–0.36) |

Note: cNPV = 1-NPV; complement of the NPV.
ASC-US+ = Atypical squamous cells of undetermined significance or worse.
CIN2+ = Moderate cervical intraepithelial neoplasia or worse.
CIN3+ = Severe cervical intraepithelial neoplasia (incl. carcinoma in situ) or worse.
Abbreviations: HC2, Hybrid Capture2 HPV test; LBC, liquid-based cytology; NLR, negative likelihood ratio; Pap, conventional Pap smear; PCR, GP5+/6+ HPV PCR test; PLR, positive likelihood ratio; PPV, positive predictive value.
[a]95% CI based on bootstrap resampling ($n$ = 1,000 resamples).
[b]Significant McNemar's test comparing Pap or LBC to HC2, $P < 0.05$.
[c]Significant McNemar's test comparing Pap or LBC to PCR, $P < 0.05$.
[d]Significant McNemar's test comparing LBC to Pap, $P < 0.05$.

**Table 2.** Sensitivity, specificity, NPV, PPV, and likelihood ratios adjusted for verification bias for LSIL+ equivalent.

| Cut-off | Endpoint | Test | Sensitivity % (95% CI)[a] | Specificity % (95% CI)[a] | PPV % (95% CI)[a] | cNPV % (95% CI)[a] | PLR (95% CI) | NLR (95% CI) |
|---|---|---|---|---|---|---|---|---|
| **LSIL+** | **CIN2+** | Pap | 42.22 (18.93–64.42)[b,c] | 99.40 (98.88–99.83)[b,c] | 53.06 (26.53–79.71) | 0.92 (0.46–1.49) | 70.41 (38.18–129.85) | 0.58 (0.45–0.75) |
| | | LBC | 36.77 (16.62–58.16)[b,c] | 99.07 (98.43–99.59)[b,c] | 38.73 (16.57–61.37) | 1.01 (0.49–1.60) | 39.48 (22.46–69.39) | 0.64 (0.51–0.81) |
| | | HC2 | 94.56 (82.09–100.0) | 95.12 (93.55–96.42) | 23.68 (14.57–33.14) | 0.09 (0.0–0.32) | 19.38 (16.10–23.33) | 0.06 (0.02–0.20) |
| | | PCR | 78.99 (58.40–94.74) | 94.25 (90.08–97.52) | 18.19 (8.33–38.26) | 0.36 (0.08–0.75) | 13.73 (10.99–17.15) | 0.22 (0.12–0.40) |
| | | Pap/HC2 | 98.82 (96.54–100.0) | 94.84 (93.11–96.29) | 23.90 (14.46–33.12) | 0.02 (0.02–0.02) | 19.14 (16.17–22.66) | 0.01 (0.00–0.20) |
| | | LBC/HC2 | 94.56 (82.09–100.0) | 94.95 (93.37–96.27) | 23.06 (14.25–32.39) | 0.09 (0.0–0.32) | 18.71 (15.59–22.46) | 0.06 (0.02–0.20) |
| | | Pap/PCR | 84.24 (64.92–100.0) | 93.89 (89.68–97.15) | 18.27 (9.35–35.21) | 0.27 (0.0–0.62) | 13.78 (11.27–16.86) | 0.17 (0.08–0.34) |
| | | LBC/PCR | 84.24 (65.97–100.0) | 94.07 (89.94–97.39) | 18.70 (9.48–35.80) | 0.27 (0.0–0.62) | 14.21 (11.60–17.41) | 0.17 (0.08–0.34) |
| **LSIL+** | **CIN3+** | Pap | 60.14 (25.68–90.98)[c] | 99.24 (98.67–99.70)[b,c] | 39.86 (15.19–66.48) | 0.34 (0.08–0.71) | 78.88 (45.22–137.60) | 0.40 (0.24–0.67) |
| | | LBC | 59.78 (27.63–88.89)[b,c] | 98.99 (98.34–99.50)[b,c] | 33.20 (12.39–53.20) | 0.34 (0.08–0.69) | 59.31 (35.49–99.11) | 0.41 (0.24–0.68) |
| | | HC2 | 89.67 (65.87–100.0) | 94.41 (92.66–95.76) | 11.84 (4.66–19.62) | 0.09 (0.0–0.32) | 16.03 (12.96–19.83) | 0.11 (0.03–0.38) |
| | | PCR | 97.81 (95.71–100.0) | 93.85 (89.70–97.15) | 12.35 (4.81–26.80) | 0.02 (0.02–0.02) | 15.91 (13.52–18.72) | 0.02 (0.0–0.36) |
| | | Pap/HC2 | 97.81 (93.69–100.0) | 94.12 (92.23–95.54) | 12.74 (5.28–19.87) | 0.02 (0.02–0.02) | 16.65 (14.10–19.65) | 0.02 (0.0–0.36) |
| | | LBC/HC2 | 89.67 (65.87–100.0) | 94.23 (92.47–95.61) | 11.53 (4.60–18.93) | 0.09 (0.0–0.32) | 15.55 (12.59–19.20) | 0.11 (0.03–0.38) |
| | | Pap/PCR | 97.81 (93.69–100.0) | 93.41 (89.35–96.72) | 11.64 (4.60–23.77) | 0.02 (0.02–0.02) | 14.84 (12.67–17.38) | 0.02 (0.0–0.36) |
| | | LBC/PCR | 97.81 (93.69–100.0) | 93.59 (89.52–97.01) | 11.91 (5.14–24.76) | 0.02 (0.02–0.02) | 15.26 (13.01–17.91) | 0.02 (0.0–0.36) |

Note: cNPV = 1-NPV; complement of the NPV.
LSIL+ = Low-grade squamous intraepithelial lesion or worse.
CIN2+ = Moderate cervical intraepithelial neoplasia or worse.
CIN3+ = Severe cervical intraepithelial neoplasia (incl. carcinoma *in situ*) or worse.
Abbreviations: HC2, Hybrid Capture2 HPV test; LBC, liquid-based cytology; NLR, negative likelihood ratio; Pap, conventional Pap smear; PCR, GP5+/6+ HPV PCR test; PLR, positive likelihood ratio; PPV, positive predictive value.
[a]95% CI based on bootstrap resampling ($n$ = 1,000 resamples).
[b]Significant McNemar's test comparing Pap or LBC to HC2, $P < 0.05$.
[c]Significant McNemar's test comparing Pap or LBC to PCR, $P < 0.05$.
[d]Significant McNemar's test comparing LBC to Pap, $P < 0.05$.

CIN2+ was lower than PCR cotesting sensitivities, but for CIN3+ no differences were observed.

### Relative test accuracy

In **Fig. 2**, the relative sensitivity and specificity for CIN2+ conferred similar estimates for crude and verification bias–adjusted calculations, but specificities appear under or overestimated (from unity) when potential verification bias is not accounted for. When compared with either cytology (**Fig. 2A**), HC2 stand-alone [1.99, 95% CI, 1.30–4.00] and both respective cotesting strategies detected twice as many CIN2+ lesions (Pap/HC2 2.11, 95% CI, 1.43–4.04; LBC/HC2 2.11, 95% CI, 1.39–4.01). Cotesting did not detect more CIN2+ compared with HC2 stand-alone (Pap and LBC 1.06, 95% CI, 1.00–1.21). Similar results were also observed among PCR strategies (**Fig. 2C**), however sensitivity estimates were reduced (PCR stand-alone 1.66; PCR cotesting 1.77).

Specificity of HC2 stand-alone (**Fig. 2B**) was significantly lower than cytology (Pap 0.98, 95% CI, 0.97–0.98; LBC 0.96, 95% CI, 0.96–0.97) and similar findings were observed for PCR stand-alone versus cytology (**Fig. 2D**). Pap cotesting was significantly less specific than HPV stand-alone while LBC cotesting presented no significant difference in detection compared with either HPV test stand-alone. For CIN3+, relative sensitivities were not statistically significant due to the low number of CIN3+ ($n = 10$). These relative specificities appeared similar to the CIN2+ cutoff (Supplementary Fig. S1).

### Potential harms

For CIN2+, the highest FPRs were observed with HPV testing (**Table 3**), particularly cotesting strategies (6.27%–7.79%) with the exception of HC2 cotesting (5.42%). HC2 and PCR stand-alone demonstrated moderate FPRs (4.88%–5.75%), followed by

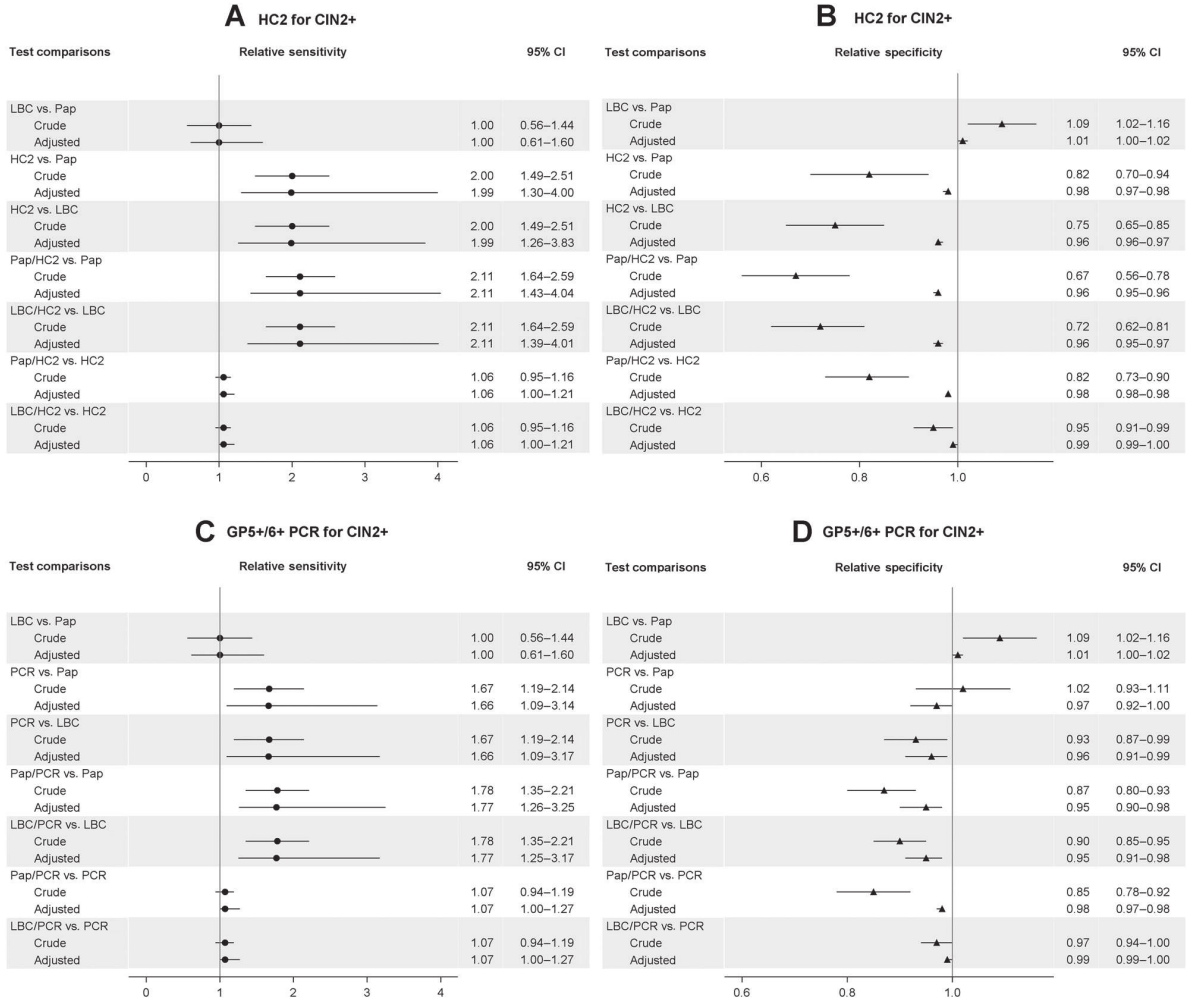


**Figure 2.**

Relative sensitivity and specificity of tests comparing both crude and adjusted estimates for CIN2 or worse at ASC-US+. **A,** Relative sensitivity for HC2. **B,** Relative specificity for HC2. **C,** Relative sensitivity for PCR. **D,** Relative specificity for PCR. Crude CIs based on Wald for paired data and adjusted CIs based on bootstrap resampling ($n = 1{,}000$); CIN2+, moderate cervical intraepithelial neoplasia or worse; Pap, conventional Pap smear; LBC, liquid-based cytology; HC2, Hybrid Capture2 HPV test; PCR, GP5+/6+ HPV PCR test.

**Table 3.** False positives, false negatives, and number needed to colposcopy at all cytology and precancerous lesion cutoffs.

| | | ASC-US+ | | LSIL+ | | |
|---|---|---|---|---|---|---|
| **Endpoint** | **Test** | **False positive rate % (95% CI)[a]** | **False negative rate % (95% CI)[a]** | **False positive rate % (95% CI)[a]** | **False negative rate % (95% CI)[a]** | **NNC 1/PPV (95% CI)[a]** |
| **CIN2+** | Pap | 2.52 (1.59–3.56) | 52.53 (31.74–75.00) | 0.60 (0.18–1.12) | 57.78 (35.58–81.07) | 4.30 (2.80–8.81) |
| | LBC | 1.36 (0.74–2.11) | 52.53 (29.89–75.00) | 0.93 (0.41–1.57) | 63.23 (41.84–83.38) | 2.79 (1.82–5.63) |
| | HC2 | 4.88 (3.58–6.45) | 5.44 (0.0–17.35) | 4.88 (3.58–6.45) | 5.44 (0.0–17.35) | 4.22 (3.00–7.10) |
| | PCR | 5.75 (2.43–9.70) | 21.01 (5.56–44.22) | 5.75 (2.43–9.70) | 21.01 (4.77–42.60) | 5.50 (2.78–10.81) |
| | Pap/HC2 | 6.91 (5.23–9.22) | 1.18 (0.84–1.89) | 5.16 (3.85–7.05) | 1.18 (0.84–1.89) | 5.30 (3.72–8.62) |
| | LBC/HC2 | 5.42 (4.03–7.06) | 1.18 (0.84–1.89) | 5.05 (3.73–6.63) | 5.44 (0.0–17.35) | 4.37 (3.07–7.10) |
| | Pap/PCR | 7.79 (4.23–11.60) | 15.76 (0.0–35.71) | 6.11 (2.58–9.94) | 15.76 (0.0–35.71) | 6.70 (3.85–12.82) |
| | LBC/PCR | 6.27 (2.99–10.25) | 15.76 (0.0–35.71) | 5.93 (2.65–9.80) | 15.76 (0.0–35.71) | 5.60 (2.97–11.04) |
| **CIN3+** | Pap | 2.66 (1.73–3.76) | 29.89 (0.0–63.04) | 0.76 (0.30–1.33) | 39.86 (12.44–83.33) | 5.52 (3.42–14.85) |
| | LBC | 1.52 (0.89–2.36) | 29.89 (0.0–62.08) | 1.01 (0.50–1.66) | 40.22 (13.91–77.87) | 3.59 (2.21–8.69) |
| | HC2 | 5.59 (4.24–7.34) | 10.33 (0.0–35.15) | 5.59 (4.24–7.34) | 10.33 (0.0–35.15) | 8.44 (5.20–20.00) |
| | PCR | 6.15 (2.88–10.05) | 2.19 (1.38–4.43) | 6.15 (2.88–10.05) | 2.19 (1.38–4.43) | 8.24 (3.91–19.59) |
| | Pap/HC2 | 7.61 (5.85–9.89) | 2.19 (1.38–4.43) | 5.88 (4.46–7.77) | 2.19 (1.38–4.43) | 10.05 (6.26–21.42) |
| | LBC/HC2 | 6.13 (4.68–7.87) | 2.19 (1.38–4.43) | 5.77 (4.39–7.53) | 10.33 (0.0–35.15) | 8.30 (5.19–17.39) |
| | Pap/PCR | 8.25 (4.72–12.28) | 2.19 (1.38–4.43) | 6.59 (3.05–10.49) | 2.19 (1.38–4.43) | 10.71 (5.76–24.67) |
| | LBC/PCR | 6.75 (3.47–10.69) | 2.19 (1.38–4.43) | 6.41 (3.10–10.32) | 2.19 (1.38–4.43) | 8.95 (4.51–20.79) |

Note: ASC-US+ = Atypical squamous cells of undetermined significance or worse.
LSIL+ = Low-grade squamous intraepithelial lesion or worse.
False positive rate = Proportion of index test positives among biopsy verified normal results (1-specificity).
False negative rate = Proportion of index test negatives among biopsy verified abnormal results i.e., CIN present (1-sensitivity).
NNC = Number of women needed to undergo colposcopy to detect 1 precancerous lesion with ASC-US+.
Abbreviations: HC2, Hybrid Capture2 HPV test; LBC, liquid-based cytology; Pap, conventional Pap smear; PCR, GP5+/6+ HPV PCR test.
[a]95% CI based on bootstrap resampling ($n$ = 1,000 resamples).

Pap (2.52%) and LBC (1.36%). For CIN3+ lesions a similar pattern was observed. Conversely, FNRs were lowest among HC2 strategies but for CIN3+, PCR-based strategies and HC2 cotesting were identical. The number of women needed to undergo colposcopy to detect one CIN2+ was highest under Pap and PCR cotesting (6.70) followed by other cotesting strategies and HPV stand-alone (HC2 4.22, PCR 5.50; **Table 3**). For CIN3+ a larger difference between Pap and LBC cotesting was observed, and had greater colposcopy referrals than HPV stand-alone and cytology.

### Sensitivity analyses

For women ≥35 years, test accuracy increased for CIN2+ (Supplementary Table S6), namely sensitivity of cytology stand-alone (up to 56.35% for Pap and LBC with ASC-US+ and 50.11% for Pap, 43.65% for LBC with LSIL+). Accuracy based on the 190 within-study histopathology results yielded similar estimates (Supplementary Table S7). After increasing the RLU cutoff of HC2 testing to 2.0, 3.0, and 10.0, further gains in specificity and PPV were observed (Supplementary Table S8). However, sensitivity was further reduced. These patterns were similar for both HC2 cotesting strategies. At all RLU cutoffs, NPV remained very similar, decreasing slightly with increasing RLU. Screening women ≥30 and ≥35 years of age revealed similar adjusted FPRs (Supplementary Figs. S2 and S3). All HPV-based strategies incurred more false positives; however, this was more pronounced among cotesting strategies.

We observed 94 discordant HPV results with genotyping information. 82 (87.2%) were HC2 negative but high-risk PCR positive and the most common detected types were HPV 16 (53.7%), 56 (12.2%), 45 (9.8%), and 18 (7.3%). All 12 PCR high-risk negative but hrHC2 positive were low-risk HPV types.

## Discussion

Few studies have compared stand-alone HPV test accuracy to cotesting strategies (6, 15–17) and to our knowledge none have directly compared the two most common cytology methods and standard HPV comparators using these strategies. On the basis of a large population-based sample of women above 30 years of age within an opportunistic screening setting and notably poor quality in cytology (3), our results demonstrated similar accuracy of stand-alone HPV testing and LBC cotesting. In particular, sensitivity of any cotesting strategy was equivalent to stand-alone HPV, and specificity of Pap cotesting was significantly lower than stand-alone HPV. Between cotesting strategies, LBC cotesting indicated some advantage over Pap cotesting where specificity was equivalent to HPV stand-alone. Furthermore, false positive test results and colposcopy referrals were highest with cotesting, particularly Pap cotesting. These results are relevant for countries that offer cotesting like Germany (32) and the United States (10), and for many other countries globally that are yet to decide on HPV-based screening.

We found neither cotesting strategies outperformed stand-alone HC2 or PCR. Between cotests, LBC cotesting was more favorable over Pap cotesting in terms of specificity and PPV. These findings correspond to meta-analysis results of five large randomized trials, although Pap and LBC-based cotesting were not assessed separately (6). In a meta-analysis of observational studies, cotesting demonstrated marginally but significantly higher sensitivity and reduced specificity over HPV testing for CIN2+; however, this was predominantly based on Pap cotesting (15). Furthermore, the higher sensitivity of cotesting could be due to the inconsistent use of the gold standard by some individual studies leading to misclassification bias (15, 33). Although these two studies indirectly compared test accuracy, that is, across study populations or varying trial arms and are thus prone to biases,

our results support the argument that cotesting, regardless of cytology method, does not outperform stand-alone HPV screening in detection.

Current arguments for cotesting are based on retrospective results from the United States, which have demonstrated marginally lower cumulative incidence of CIN3+ under triennial cotesting compared with HPV stand-alone (18). However, the translation of this marginally lower risk by cotesting into real screening practice may not be realized until many tens of thousands of women are screened (13), particularly with opportunistic screening. Cotesting arguments are also further undermined because this strategy leads to greater costs and number of lifetime tests (34, 35). Up to an additional 400 colposcopy referrals per 1,000 women could be expected when cotesting at triennial intervals (34). This evidence highlights screening algorithm complexities, greater costs, and potential harm for apparent minimal gains in detection with cotesting.

On the other hand, positivity to HPV without adequate triage may lead to an increase in colposcopies (36), which could result in overtreatment (7). In our study, colposcopies needed to detect one precancer were greatest under cotesting strategies (17). Between cotests, Pap cotesting incurred a greater degree of harms than LBC cotesting. The latter indicated similar but elevated potential harms compared with stand-alone HPV testing. It is conceivable that screening with other HPV tests detecting mRNA for example can mitigate these costs and harms (37), but these technologies may not be widely available and are not yet approved for stand-alone screening. As we observed, increasing the cutoff of viral load for HPV DNA detection might mitigate false positives, especially if using HC2 (38). In addition, compared with cotesting with triage, fewer colposcopies were needed when screening with HPV 16/18 genotyping and triage, further highlighting the benefit of stand-alone HPV testing (16).

Although observational studies with opportunistic screening (19, 29, 39) do not directly compare cotesting strategies to HPV stand-alone (40–43), our study confirms observations that HPV testing is superior to cytology in detection of precancerous lesions. We observed low accuracy of cytology, particularly for ASC-US+. However sensitivity was higher than previous reports in Germany possibly due to biopsies of nonvisible lesions, but is still low compared with other high-resource countries (3, 39, 44). This might explain why our results were higher than relative sensitivity and specificity from previous studies (3, 43). Possible reasons for poorer accuracy of Pap include the continued use of dry cotton–tipped swabs in screening and lack of standardized quality assurance with opportunistic screening (9). Fewer inadequate samples and from-the-vial testing advantages of LBC may also explain why LBC cotesting performed similarly to stand-alone HPV testing (45). Furthermore, in the same screening context, accuracy of LBC has been reported to be higher than Pap, likely due to the poor quality of the latter (46).

Our results conferred lower HC2 sensitivity than previously reported in Germany (39, 44), possibly because we recruited a random population-based sample via population registries rather than women already attending routine screening. In addition, our sample represents older women. The reduced sensitivity of HC2 for CIN3+ compared with CIN2+ is likely due to the low number of CIN3+ detected. In addition, in our study, all CIN3+ were correctly identified by HC2 cotesting and PCR-based strategies, while one woman with invasive cancer tested stand-alone HC2 negative (Supplementary Table S3). HPV test results may differ possibly due to insufficient viral load, differences in targeted regions of the HPV DNA or cross-reactivity to IARC classified group 2b types (47). Nonetheless, discordance can be avoided by stringent quality assurance and control (9). This is especially important to note as Germany rolls out cotesting of women ≥35 years within an organized screening program, but specific details on approved tests are yet to be defined (21), despite existing criteria and recommendations (48).

### Limitations

We report cross-sectional results. Longitudinal outcomes such as cumulative risk incidence among screen-negative women are needed to determine the interval of protection. Nevertheless, we were able to make direct comparisons of distinct cytologic and HPV test strategies within the same study population, which have previously not been reported. Second, despite active reminders for colposcopy, attendance was less than optimal among screen-positives (65.3%) and negatives (35.7%). Historically, follow-up colposcopies in Germany were rather uncommon and the lack of a centralized screening register complicates disease verification. There is still a need for more novel tactics to improve compliance with follow-up of positive screening results and with the roll-out of the new organized program, the latter issue of incomplete data might improve. Accordingly, we adjusted the analyses to account for verification bias and although there may be residual bias due to low sampling fractions of screen-negatives (49), our estimates aligned with previous observations (19, 29, 39, 44). Third, no masking to screening results of the colposcopist and first histopathologist was possible as we attempted to maintain real-world screening. This was addressed by independent second and third histopathology reviews. The number of severe precancerous lesions CIN3+ and cervical carcinomas was also low in our study and we included HPV-unvaccinated women.

### Conclusions

We found similar accuracy of stand-alone HPV testing and LBC cotesting, and superior accuracy of stand-alone HPV compared with Pap-based cotesting. However, adding cytology to HPV as a cotest offers nearly no benefit in detection at the cost of more false positive results and colposcopy referrals. For settings optimizing cervical cancer screening such as Germany coming from opportunistic and annual cytology-based screening, triennial cotesting in women 35 years and older is a positive first step toward HPV-based screening. Ultimately, consideration of stand-alone HPV screening once the organized program has been adequately implemented with high quality is warranted. Screening women aged ≥30 years with sole HPV-based testing should also be considered in the future to maximize early detection and to further reduce the incidence of cervical cancer toward elimination.

## Authors' Disclosures

H. Ikenberg reports co-ownership of a laboratory for cytology and molecular diagnostics. C.J.L.M. Meijer reports personal fees and other from Self-Screen, personal fees from Qiagen, other from MDxHealth, personal fees and other from SPMSD/Merck, and personal fees from GSK outside the submitted work; in addition, C.J.L.M. Meijer has a patent for HPV assay issued and licensed to self-screen and a patent for methylation markers issued and licensed to self-screen. S.J. Klug reports grants from German Cancer Aid (Deutsche Krebshilfe) during the conduct of the study. No disclosures were reported by the other authors.

## Authors' Contributions

**L.A. Liang:** Formal analysis, visualization, methodology, writing–original draft, writing–review and editing. **T. Einzmann:** Investigation. **A. Franzen:** Investigation. **K. Schwarzer:** Investigation. **G. Schauberger:** Formal analysis, validation, methodology. **D. Schriefer:** Data curation, validation. **K. Radde:** Data curation, project administration. **S.R. Zeissig:** Project administration. **H. Ikenberg:** Investigation. **C.J.L.M. Meijer:** Investigation. **C.J. Kirkpatrick:** Investigation.

**H. Kölbl:** Investigation. **M. Blettner:** Conceptualization, funding acquisition. **S.J. Klug:** Conceptualization, supervision, writing–review, funding acquisition.

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RESEARCH

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# Colposcopy non-attendance following an abnormal cervical cancer screening result: a prospective population-based cohort study

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## Abstract

**Background:** A considerable proportion of cervical cancer diagnoses in high-income countries are due to lack of timely follow-up of an abnormal screening result. We estimated colposcopy non-attendance, examined the potential factors associated and described non-attendance reasons in a population-based screening study.

**Methods:** Data from the MARZY prospective cohort study were analysed. Co-test screen-positive women (atypical squamous cells of undetermined significance or worse [ASC-US+] or high-risk human papillomavirus [hrHPV] positive) aged 30 to 65 years were referred to colposcopy within two screening rounds (3-year interval). Women were surveyed for sociodemographic, HPV-related and other data, and interviewed for non-attendance reasons. Logistic regression was used to examine potential associations with colposcopy attendance.

**Results:** At baseline, 2,627 women were screened (screen-positive = 8.7%), and 2,093 again at follow-up (screen-positive = 5.1%; median 2.7 years later). All screen-positives were referred to colposcopy, however 28.9% did not attend despite active recall. Among co-test positives (ASC-US+ and hrHPV) and only hrHPV positives, 19.6% were non-attendees. Half of only ASC-US+ screenees attended colposcopy. Middle age (adjusted odds ratio [aOR] = 1.55, 95% CI 1.02, 4.96) and hrHPV positive result (aOR = 3.04, 95% CI 1.49, 7.22) were associated with attendance. Non-attendance was associated with having ≥ 3 children (aOR = 0.32, 95% CI 0.10, 0.86). Major reasons for non-attendance were lack of time, barriers such as travel time, need for childcare arrangements and the advice against colposcopy given by the gynaecologist who conducted screening.

**Conclusions:** Follow-up rates of abnormal screening results needs improvement. A systematic recall system integrating enhanced communication and addressing follow-up barriers may improve screening effectiveness.

**Keywords:** Colposcopy, Non-attendance, Screening follow-up, Abnormal screening result, Cervical cancer screening, HPV status, HPV testing

## Background

Cervical cancer (CC) is preventable with effective primary and secondary prevention measures such as human papillomavirus (HPV) vaccination and screening. Cervical cancer screening (CCS) includes cytological assessment, viral detection of HPV or both (co-testing)

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[1]. However, following an abnormal screening result where risk of progression to CC is elevated, colposcopy is an important step to guide management [2]. Colposcopy involves magnified visual inspection of the cervix and biopsy extraction where necessary by trained and experienced colposcopists. Non-adherence to follow-up of abnormal screening results, i.e. colposcopy non-attendance, may lead to undiagnosed precancer (cervical intraepithelial neoplasia, CIN) and preventable CC [3], undermining screening effectiveness [4].

Until 2020, Germany offered free opportunistic Pap screening annually to women from age 20, but quality assurance measures were not systematically monitored [5]. Despite reasonable coverage [6] and declines in incidence, up to half of invasive CC cases were diagnosed in women screened frequently in the preceding 10 years [7]. Over two thirds of diagnoses had preceding negative screening results [8]. Failure of CCS to detect disease include sample collection issues to detect abnormal cells, but also lack of follow-up after an abnormal screening result [9]. The latter is not unique to Germany. For example in the US, 8% of CC diagnoses were attributed to colposcopy non-attendance [10] and a meta-analysis attributed 12% of CC to poor follow-up care [11]. Follow-up failures can be minimised if referrals are part of a fail-safe recall system, via systemic tracking, call-and-recall invitations and reminders [2, 12]. In 2020, HPV testing was adopted as a co-test in women 35 years of age and older in Germany [5]. Therefore, it is important to identify sub-groups likely to be non-adherent with follow-up, particularly with the addition of HPV screening.

Several studies have examined potential factors associated with colposcopy non-attendance [3, 10, 13–23]. However, most lack individual socio-demographic information [10, 13, 16, 17, 20], or are based on underserved populations such as migrants [13, 14]. The role of HPV status on follow-up attendance was explored only recently in a small pilot study [24]. Additionally, small qualitative studies have examined reasons for colposcopy non-attendance [25, 26]. We estimated colposcopy non-attendance among screen-positive women from a population-based, real-world screening study involving co-testing and examined the potential factors associated with attendance. Additionally, we described non-attendance reasons.

## Methods

### Participants and data collection

The data stem from randomly recruited participants from the general population (n = 2,627) who were screened within the randomised trial and prospective cohort MARZY study, described previously [27, 28]. Briefly, women eligible from the general population (aged 30 to 65 years, with no history of hysterectomy or CC and not pregnant) were screened by office-based gynaecologists at study baseline (R1, 2005–2007) with routine Pap smear, plus an additional MARZY study swab (liquid-based cytology, ThinPrep, Cytyc/Hologic including subsequent HPV testing, Hybrid Capture®2). HPV co-testing was investigated [27]. Participants were administered a questionnaire (Q1) relating to sociodemographic and other factors.

Positive screening results were defined as atypical squamous cells of undetermined significance or worse (ASC-US+) or high-risk HPV positive (hrHPV). Screen-positives were contacted by postal letter, which included HPV information and referred to the study colposcopy clinic (University Medical Center, Mainz; Fig. 1). These letters contained additional information on HPV infection and explained the colposcopy procedure in simple terms. Active telephone recall efforts were carried out by female study personnel to improve colposcopy attendance rates among women who did not arrange an appointment at the study clinic within 3 months of referral. Personnel also interviewed non-attendees for their reasons on non-attendance.

Screening was conducted again 3 years later (R2, 2008–2010) among women who participated in R1 and were still eligible (no hysterectomy or CC diagnoses since R1 and not pregnant). Lifestyle exposures such as smoking status were updated in a second questionnaire (Q2). Active recalls were again conducted by female study personnel if screen-positive participants had not attended colposcopy within 3 months following referral to the designated study clinic (University Medical Center, Mainz and St. Vincenz and Elisabeth Hospital, Mainz).

After R2 concluded (2010), an additional questionnaire with HPV-related questions (Q3) was administered to all hrHPV positive women, investigating perception and communication of HPV results, and HPV knowledge. As the MARZY screenings were conducted with a 3-year interval but routine Pap screenings were offered opportunistically and annually in the study region, any screenings conducted outside the study between the two MARZY rounds were retrospectively documented.

### Colposcopy -attendance

We classified colposcopy attendance status using medical records from the designated colposcopy clinics. The primary outcome was non-attendance after referral to colposcopy within a 4 month time-frame, calculated as number of non-attendees among all referrals. This definition is based on the study referral threshold (ASC-US+ or hrHPV or both positive). At the time of study conduct, the 2008 European guidelines suggested colposcopies be conducted following ASC-US+ and hrHPV

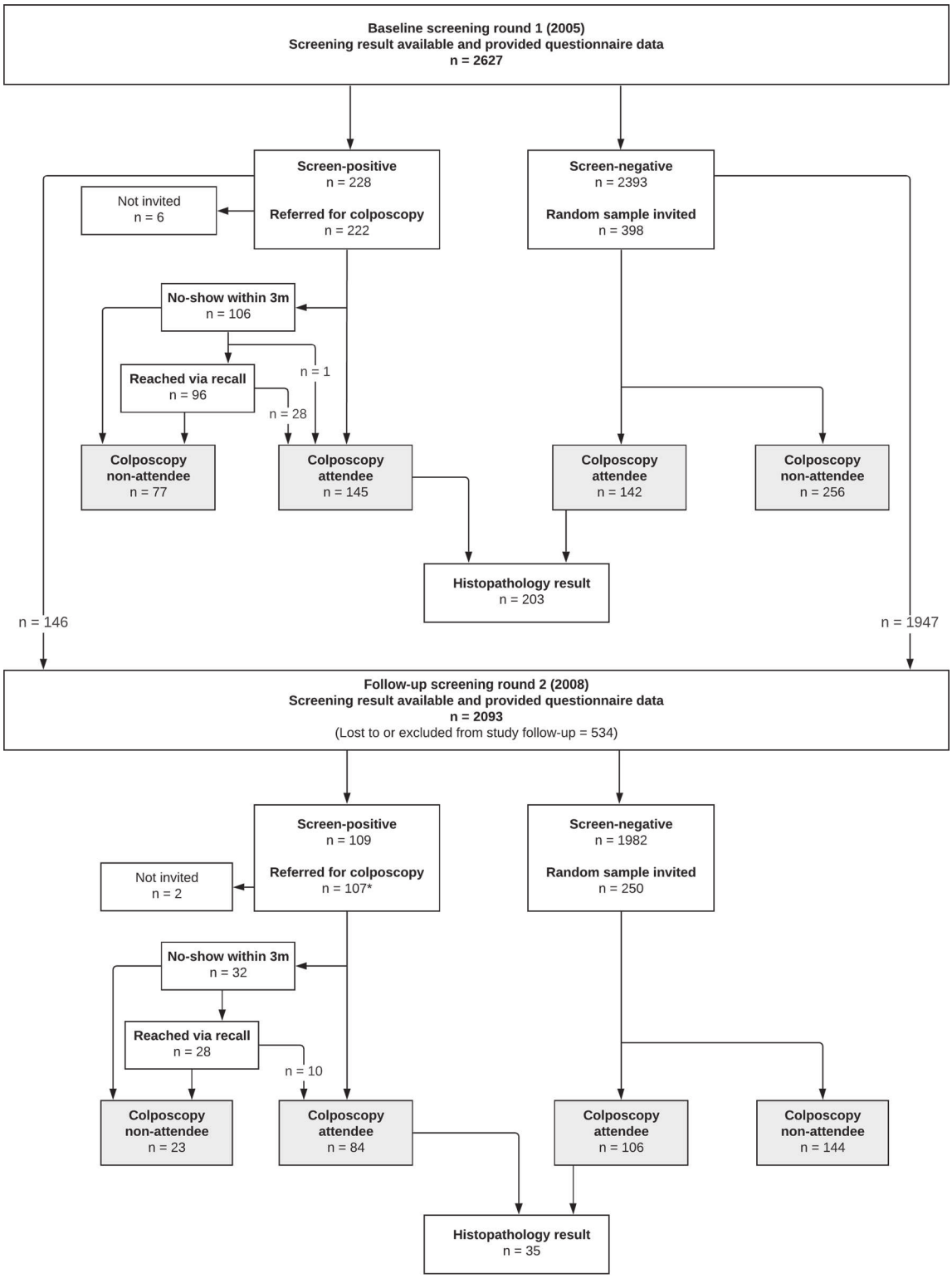


**Fig. 1** Flowchart of screening referrals and their screening results

positive results [9]. The German CCS guideline in effect at the time advised women with low-grade squamous intraepithelial lesions or worse (LSIL+) who were also hrHPV positive to undergo colposcopy [29]. Attendance was estimated for both thresholds (ASC-US+ and hrHPV positive; LSIL+ and hrHPV positive).

#### Variables of interest

Sociodemographic variables obtained included age, region of residency, nationality, highest education level attained (lower secondary; upper secondary and further), employment situation, net monthly household income (low income $\leq$ 1500€; higher income > 1500€), marital status, parity ($\leq$ 2 children; $\geq$ 3 children) and health insurance status. Smoking status, oral contraception use and hormone replacement therapy (HRT) were dichotomised (ever vs. never). Self-reported frequency of CCS attendance was grouped (regularly every 1–2 years; irregularly every 3 years or less or never). Screen-positive was defined as ASC-US+ only, hrHPV only or both ASC-US+ and hrHPV positive, and also LSIL+ only or both LSIL and hrHPV positive.

At the time of the Q3 survey, no validated HPV knowledge scale was available for use but the questionnaire items were based on extensive review of the qualitative body of evidence published. Perceived experience during and after the screening examination and concerns about infectivity or impact on sexual relationships were based on the Psychosocial Effects of Abnormal PAP Smears Questionnaire (PEAPS-Q) [30] and Cervical Dysplasia Distress Questionnaire (CDDQ) [31]. The items of interest were sub-categorised by 5-point Likert scale or binary "yes/no" answers as (i) Perception: negative screening experience (dichotomised), degree of negative reaction and understanding regarding the positive hrHPV result such as anxiety or insecurity, and (ii) HPV knowledge: as determined by the ability to identify at least 2 areas of HPV infection (virus, persistence consequences, vaccination; dichotomised), level of HPV understanding (none to good), and prior HPV knowledge to the study. Communication (iii) that occurred between the gynaecologists and participant (dichotomised), comprehensiveness of the counselling (dedicated time, provided background information and support), trust in the physician and discussion of result between the participant and friends or family members were also analysed. Concerns (iv) regarding cancer, infertility and infectivity were captured.

#### Statistical analyses

Any screen-positives leading to a referral at either round between 2005 and 2010 were included. If women were referred at both rounds, we designated questionnaire and interview data from the first referral only for regression analyses. All variables of interest were analysed using R (version 4.0.5, R Foundation for Statistical Computing, Vienna, Austria). Potential associations between attendance and individual factors were examined by univariable regression modelling and collinearity between variables were assessed. For multivariable regression, we applied multiple imputation methods to obtain model averaged estimates for missing data and computed bootstrap resampled 95% confidence intervals (CI; bootstraps = 500) using the MAMI package for R [32]. Missing data in regression models were treated as available case analyses and the adjusted odds ratios (aOR) controlled for all available confounders (age, region of residency, nationality, highest education level attained, employment situation, income, marital status, parity, smoking status, OC use, HRT use, screening frequency, screening result and insurance status), as these were previously reported to be associated with attendance [3, 10, 13–23]. Education, employment and screening result were dichotomised for regression. Non-attendee interview responses from both rounds were described together. In the case where women were non-attendees at both rounds, we designated the interview data from the first interview only. We also descriptively assessed the longitudinal outcomes (screening results, colposcopy attendance) of R1 referrals who did not attend colposcopy then but who were screened again at R2.

Informed consent was provided by all study participants prior to screening at study baseline. The MARZY study was approved by the ethical committee of the state of Rhineland-Palatinate (Landesärztekammer Rheinland-Pfalz: 837.438.03 (4100)) and the state government data protection office.

## Results

#### Colposcopy attendance status

Of 2,627 women screened at R1, 228 (8.7%) were screen-positive, 222 (8.5%) were referred to colposcopy while 6 were not invited due to pre-planned hysterectomy elsewhere (Fig. 1). Initially, 106 of these 222 screen-positive women did not attend colposcopy within 3 months following referral. With active recall efforts, 96 could be reached and 28 (29.2%) attended afterwards. One woman who was not reached by telephone eventually attended colposcopy. Finally, 145 women (65.3%) attended colposcopy within 4 months, while 77 (34.7%) did not.

At R2, 2,093 (79.7%) women were screened at a median of 2.7 years later. Of the 107 screen-positive women referred to colposcopy, 32 initially did not attend after referral and 28 were reached via active recall (Fig. 1). Ten (31.3%) women were motivated to attend. Finally, 23 (21.5%) were non-attendees and 84 attended colposcopy (78.5%; Additional file 1: Table S1).

Twenty-one women were referred at both rounds where half were referred due to only hrHPV positive results (Additional file 1: Table S2). A total of 222 women (R1) and 86 women (R2) were referred to colposcopy (n = 308) in the entire study.

Overall, among 308 total referrals, attendance was recorded in 219 (71.1%) women and non-attendance in 89 (28.9%). Mean age in both groups were similar: 45.8 years (SD = 9.1) and 45.7 years (SD = 10.1) respectively. Among both ASC-US+ and hrHPV co-test positives, 9 (19.6%) did not attend (Fig. 2A). Among LSIL+ and hrHPV positives, 6 (17.1%) did not attend colposcopy (Fig. 2B). Approximately half of only cytology-positives attended colposcopy; the majority had ASC-US (Additional file 1: Table S3). By R2, 32 women had positive routine Pap results detected between study rounds. Non-attendance rates were similar (~ 20%) after excluding these cases (Additional file 1: Figure S1).

### Sociodemographic and other factors

Compared to younger women (30–39 years), 40–49 year old women were more likely to attend colposcopy (75% vs. 69%; aOR = 1.55, 95% CI 1.02, 4.96) (Table 1). Women who resided in the urban area were less likely to attend, albeit not statistically significant (65% vs. 76%; aOR = 0.63, 95% CI 0.30, 1.00). Among women from low income households, 87% were attendees while 68% of the women from higher income households (net monthly income > 1500€) attended colposcopy. Women with higher household income or who had birthed ≥ 3 children were 67% (aOR = 0.33, 95% CI 0.11, 0.92) and 68% (aOR = 0.32, 95% CI 0.10, 0.86) less likely to attend colposcopy respectively. Smoking status, oral contraceptive use and HRT were not significantly associated with attendance. Sixty percent who attended screening irregularly (every 3 years or less) or not at all, attended colposcopy versus 73% of regular participants. A positive hrHPV screening result increased likelihood of attending by threefold (aOR = 3.04, 95% CI 1.49, 7.22; Table 1).

### Reasons for non-attendance

Overall, 83 respondents provided reasons on non-compliance (response rate R1: 68/77 (88.3%); R2: 18/23 (78.3%) (Fig. 3). Over half indicated lack of time (56%), almost half (48%) mentioned barriers such as long travel time, travel cost, childcare challenges and 29% cited lack of choice of colposcopy clinic (Fig. 3A). A fifth of the women reported to have forgotten the appointment, while 15–16% feared the procedure itself or the outcome of the examination (Fig. 3A).

Forty-four percent mentioned that their office-based gynaecologist who conducted screening advised against

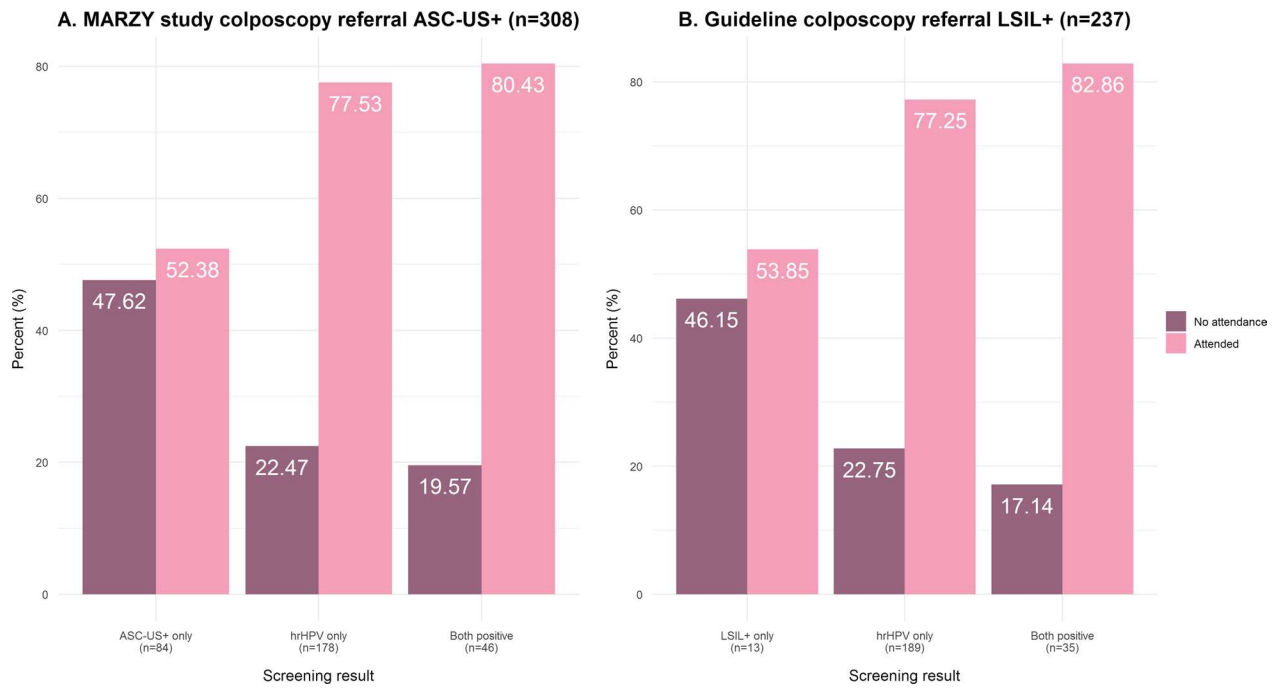


**Fig. 2** Proportion of overall referrals who attended or did not attend colposcopy by screening result at ASC-US+ threshold (**A**) and at LSIL+ (**B**). ASC-US+: Atypical squamous cells of undetermined significance or worse; hrHPV: high-risk human Papillomavirus; LSIL+: low grade squamous intraepithelial lesion or worse

**Table 1** Sociodemographic and lifestyle factors associated with colposcopy attendance among all women referred

| | Overall (n = 308) | | Logistic regression models | | | |
|---|---|---|---|---|---|---|
| | Non-attendee (n = 89) | Attendee (n = 219) | Univariable | | Multivariable[a] | |
| | n (row %) | n (row %) | OR | 95% CI* | aOR | 95% CI** |
| **Age group** | | | | | | |
| 30–39 years | 28 (31.46%) | 61 (68.54%) | Ref | | Ref | |
| 40–49 years | 29 (25.44%) | 85 (74.56%) | 1.35 | 0.73, 2.49 | 1.55 | 1.02, 4.96 |
| 50–59 years | 22 (30.14%) | 51 (69.86%) | 1.06 | 0.54, 2.09 | 1.18 | 0.63, 3.40 |
| 60+ years | 10 (31.25%) | 22 (68.75%) | 1.01 | 0.43, 2.49 | 1.07 | 0.32, 3.72 |
| Missing | 0 | 0 | | | | |
| **Nationality** | | | | | | |
| Non-German | 13 (40.62%) | 19 (59.38%) | Ref | | Ref | |
| German | 76 (27.54%) | 200 (72.46%) | 1.80 | 0.83, 3.80 | 1.58 | 0.96, 5.97 |
| Missing | 0 | 0 | | | | |
| **Study region** | | | | | | |
| Mainz-Bingen (rural) | 41 (24.12%) | 129 (75.88%) | Ref | | Ref | |
| Mainz (urban) | 48 (34.78%) | 90 (65.22%) | 0.60 | 0.36, 0.98 | 0.63 | 0.30, 1.00 |
| Missing | 0 | 0 | | | | |
| **Education** | | | | | | |
| Upper secondary or further[1] | 36 (30.77%) | 81 (69.23%) | Ref | | Ref | |
| Lower secondary[2] | 53 (27.75%) | 138 (72.25%) | 1.16 | 0.70, 1.91 | 1.01 | 0.75, 2.15 |
| Missing | 0 | 0 | | | | |
| **Employment** | | | | | | |
| Employed | 60 (27.91%) | 155 (72.09%) | Ref | | Ref | |
| Not employed[3] | 22 (32.35%) | 46 (67.65%) | 0.81 | 0.45, 1.48 | 0.97 | 0.50, 1.83 |
| Missing | 7 | 18 | | | | |
| **Net household income** | | | | | | |
| ≤ 1500€/month | 9 (13.43%) | 58 (86.57%) | Ref | | Ref | |
| > 1500€/month | 58 (31.69%) | 125 (68.31%) | 0.33 | 0.15, 0.69 | 0.33 | 0.11, 0.92 |
| Missing | 22 | 36 | | | | |
| **Marital status** | | | | | | |
| Married, divorced, widowed | 69 (27.49%) | 182 (72.51%) | Ref | | Ref | |
| Single | 17 (32.08%) | 36 (67.92%) | 0.80 | 0.43, 1.55 | 0.72 | 0.22, 1.12 |
| Missing | 3 | 1 | | | | |
| **Parity** | | | | | | |
| 0–2 | 64 (26.45%) | 178 (73.55%) | Ref | | Ref | |
| ≥ 3 | 18 (46.15%) | 21 (53.85%) | 0.42 | 0.21, 0.84 | 0.32 | 0.10, 0.86 |
| Missing | 7 | 20 | | | | |
| **Smoking status** | | | | | | |
| Never | 34 (25.76%) | 98 (74.24%) | Ref | | Ref | |
| Ever | 54 (31.03%) | 120 (68.97%) | 0.77 | 0.46, 1.27 | 0.76 | 0.32, 1.01 |
| Missing | 1 | 1 | | | | |
| **Oral contraceptive use** | | | | | | |
| Never | 17 (29.82%) | 40 (70.18%) | Ref | | Ref | |
| Ever | 72 (28.80%) | 178 (71.20%) | 1.05 | 0.55, 1.95 | 0.90 | 0.28, 1.28 |
| Missing | 0 | 1 | | | | |
| **HRT** | | | | | | |
| Never | 74 (28.24%) | 188 (71.76%) | Ref | | Ref | |
| Ever | 12 (31.58%) | 26 (68.42%) | 0.85 | 0.42, 1.83 | 0.96 | 0.29, 1.55 |
| Missing | 3 | 5 | | | | |
| **Health insurance** | | | | | | |

**Table 1** (continued)

| | Overall (n = 308) | | Logistic regression models | | | |
|---|---|---|---|---|---|---|
| | Non-attendee (n = 89) | Attendee (n = 219) | Univariable | | Multivariable[a] | |
| | n (row %) | n (row %) | OR | 95% CI* | aOR | 95% CI** |
| Statutory | 54 (28.12%) | 138 (71.88%) | Ref | | Ref | |
| Private | 9 (30.00%) | 21 (70.00%) | 0.91 | 0.40, 2.21 | 1.14 | 0.70, 4.62 |
| Missing | 26 | 60 | | | | |
| **Screening frequency** | | | | | | |
| Regular[4] | 70 (27.24%) | 187 (72.76%) | Ref | | Ref | |
| Irregular or never[5] | 19 (40.43%) | 28 (59.57%) | 0.55 | 0.29, 1.06 | 0.82 | 0.30, 1.13 |
| Missing | 0 | 4 | | | | |
| **Screening result** | | | | | | |
| ASC-US+ only | 40 (47.62%) | 44 (52.38%) | Ref | | Ref | |
| hrHPV+ only | 40 (22.47%) | 138 (77.53%) | 3.25[b] | 1.91, 5.55 | 3.04[b] | 1.49, 7.22 |
| Both positive | 9 (19.57%) | 37 (80.43%) | | | | |

OR: odds ratio; CI: confidence interval; aOR: adjusted odds ratio; Ref: reference level; HRT: hormone replacement therapy; ASC-US+: Atypical squamous cells of undetermined significance or worse; hrHPV+: high-risk Human Papillomavirus positive; both positive: ASC-US+ and hrHPV positive

[1] at least 12 years education

[2] ≤ 10 years

[3] includes other employment status such as parental leave, sick leave

[4] every 1–2 years

[5] every 3 years or less, irregular screening, rarely and no previous screening attendance

[a] Adjusted for all covariates in the model

[b] dichotomised to include hrHPV only and both co-test positive results (hrHPV and ASC-US+)

* Likelihood ratio

** Bootstrap resampled confidence intervals (n = 500)

colposcopy at the study clinic (Fig. 3B). Thirty-six percent of these women resided in the urban region, 40% were aged 40–49 years, 8% had a positive co-test at the guideline threshold LSIL+, 12% reported irregular or no screening history and 80% reported having a repeat test since the MARZY screening round (Figs. 3B.1–B.5). Among 9 co-test screen-positives (ASC-US+ and hrHPV positive) who did not attend at either round (Table 1), only one cited the advice of the screening gynaecologist as the main reason for non-attendance; the remainder reported other barriers or concerns (Additional file 1: Table S4).

#### HPV: Perception, knowledge, communication and concerns

Among women who reported negative experiences during screening, 78% attended colposcopy compared to 87% of attendees who did not report a negative screening experience and 78% who reported moderate to high levels of negative reaction to their HPV result attended compared to 84% of attendees with little to no negative reaction (Table 2). Likelihood of attending colposcopy was lowered if screening was associated with a negative experience (OR = 0.49, 95% CI 0.21, 1.09) or reaction (OR = 0.64, 95% CI 0.27, 1.41), but not statistically significant. Approximately 79% of women reporting to have HPV knowledge attended compared to 82% with no HPV knowledge. Better levels of HPV knowledge were markedly lower among attendees (75%) than those reporting poor or no HPV knowledge who also attended colposcopy (82%). Level of understanding of the HPV result was not significantly associated with attendance (OR = 1.15, 95% CI 0.42, 2.82).

For communication, 85% of attendees reported direct communication of the HPV result by their gynaecologist compared to 76% of colposcopy attendees who were not directly informed by the gynaecologist. Direct communication increased the likelihood of attending but was not statistically significant (OR = 1.34, 95% CI 0.39, 5.03). Higher proportions of attendees also reported comprehensive counselling (83% vs. 80%), and discussed their result with a friend or family member (84% vs. 74%) than those who did not report these discussions. Eighty-five percent of women who reported lack of trust in their gynaecologist went to colposcopy compared to 81% who reported trust. Approximately 77% of all hrHPV positive women who responded in Q3 were concerned about cancer.

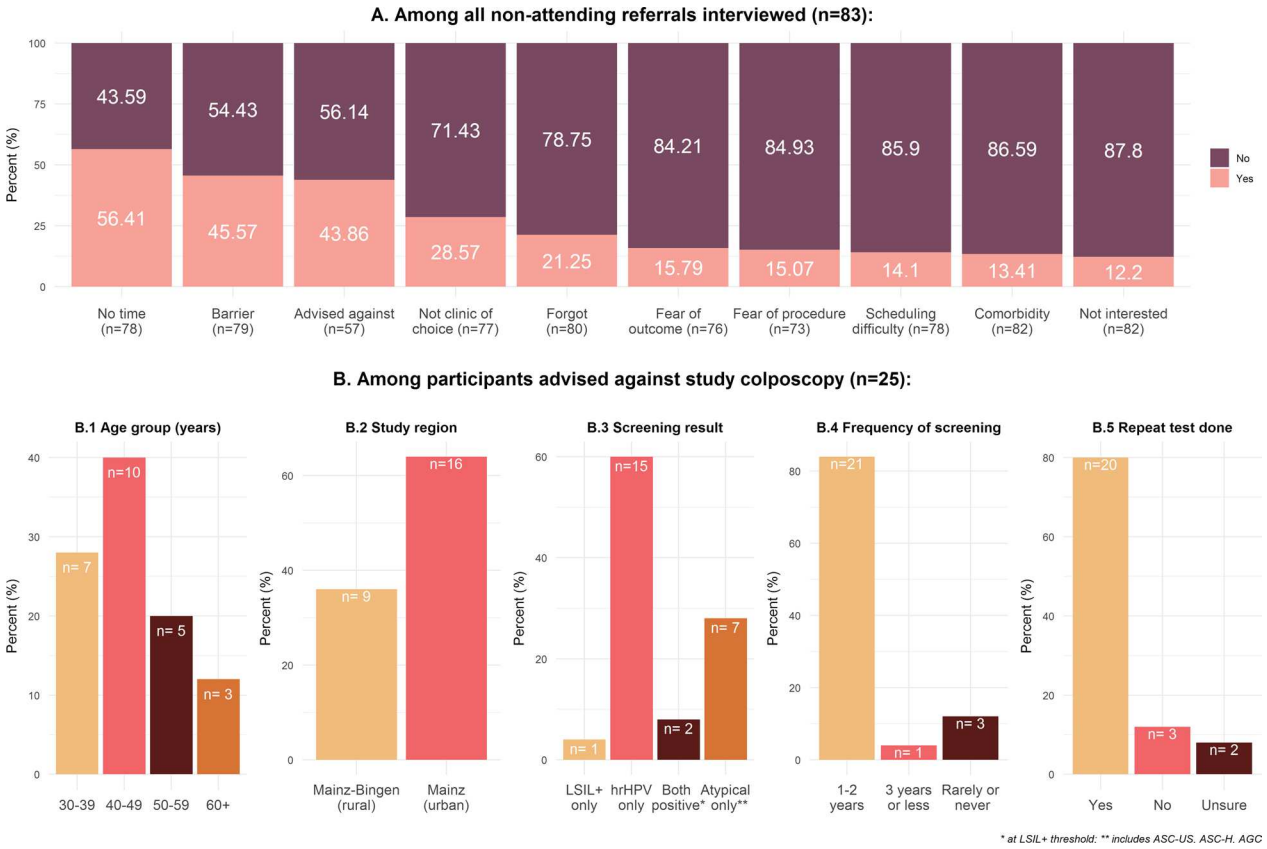


**Fig. 3** **A** Reasons for non-attendance over both rounds and **B** characteristics of the participants who were advised against attending the study colposcopy. ASC-US+: Atypical squamous cells of undetermined significance or worse; hrHPV: high-risk human Papillomavirus; LSIL+ low grade squamous intraepithelial lesion or worse

### Longitudinal outcomes

At baseline R1, 77 referrals to colposcopy at R1 did not attend. Of these, 44 were lost to follow-up and not subsequently screened at R2. Respectively, baseline data and retrospective documentation of outcomes among these women indicated that 25 women (57%) were at least hrHPV positive (hrHPV positive only or both ASC-US+ and hrHPV positive) and a total of 4 women were scheduled to later undergo hysterectomies outside of the study (Additional file 1: Table S5). Three of the 4 women who underwent hysterectomies had a positive screening result within routine screening after R1 of MARZY.

Among the 33 referrals who did not attend colposcopy at R1 and were re-screened at R2, the majority (92%) were screened routinely between study rounds with negative screening results (Table 3). Only 2 non-attendees from R1 were screen-positive upon routine screening after study baseline. At R2, 4 women (12%) were hrHPV positive only, 2 (6%) were co-test positive to both cytology and hrHPV, while 27 (82%) were screen-negative. Among the 6 women referred again to colposcopy at R2, 5 (83%) did not attend, despite all 5 having a hrHPV positive result detected at R2 screening. Two of these women also had a concurrent cytological abnormality and via retrospective data linkage, it was found that they later underwent hysterectomies due to severe cervical intraepithelial neoplasia or worse (CIN3+; Additional file 1: Table S6).

Characteristics of the non-attendees who presented again at R2 show that 67% were women aged 50 years and above and 61% resided in an urban area (Additional file 1: Table S7). Twenty-one percent had 3 or more children and 28% did not attend screening regularly. Sixty-four percent of non-attendees from R1 reported no time as a reason for non-attendance at R1 and 50% reported a barrier. Among the 5 referrals who did not attend colposcopy at either R1 or R2, common reasons were lack of time, concerns and obstacles to arranging the appointment (Additional file 1: Table S6).

**Table 2** HPV and screening-related factors of hrHPV positive women who underwent colposcopy versus hrHPV positive non-attendees

| | Overall (n = 225) | | Logistic regression model | |
|---|---|---|---|---|
| | Non-attendee (n = 49) | Attendee (n = 176) | Univariable | |
| | n (row %) | n (row %) | OR | 95% CI |
| *Perception* | | | | |
| **Negative screening experience** | | | | |
| No | 9 (13.04%) | 60 (86.96%) | Ref | |
| Yes | 26 (22.41%) | 90 (77.59%) | 0.49 | 0.21, 1.09 |
| Missing | 14 | 26 | | |
| **Level of negative reaction to HPV result**[a] | | | | |
| Little to none | 10 (15.87%) | 53 (84.13%) | Ref | |
| Moderate to high | 24 (21.82%) | 86 (78.18%) | 0.64 | 0.27, 1.41 |
| Missing | 15 | 37 | | |
| **Level of understanding regarding HPV result** | | | | |
| Little to none | 7 (22.58%) | 24 (77.42%) | Ref | |
| Most or everything | 27 (19.57%) | 111 (80.43%) | 1.15 | 0.42, 2.82 |
| Missing | 15 | 41 | | |
| *Knowledge* | | | | |
| **HPV knowledge** | | | | |
| No | 17 (18.28%) | 76 (81.72%) | Ref | |
| Yes | 18 (21.18%) | 67 (78.82%) | 0.78 | 0.37, 1.62 |
| Missing | 14 | 33 | | |
| **Level of HPV knowledge** | | | | |
| Poor to none | 5 (14.71%) | 29 (85.29%) | Ref | |
| Moderate to good | 13 (25.00%) | 39 (75.00%) | 0.64 | 0.20, 1.84 |
| Missing | 31 | 108 | | |
| **Any HPV knowledge prior to the study** | | | | |
| No | 16 (17.78%) | 74 (82.22%) | Ref | |
| Yes | 17 (19.32%) | 71 (80.68%) | 0.84 | 0.39, 1.78 |
| Missing | 16 | 31 | | |
| *Communication* | | | | |
| **Of HPV result by gynaecologist** | | | | |
| No | 8 (24.24%) | 25 (75.76%) | Ref | |
| Yes | 4 (15.38%) | 22 (84.62%) | 1.34 | 0.39, 5.03 |
| Missing | 37 | 129 | | |
| **Comprehensive explanation of HPV result by gynaecologist**[b] | | | | |
| 1 area or less | 27 (20.00%) | 108 (80.00%) | Ref | |
| At least 2 areas | 7 (16.67%) | 35 (83.33%) | 1.06 | 0.46, 2.70 |
| Missing | 15 | 33 | | |
| **Trust in gynaecologist** | | | | |
| No | 3 (15.00%) | 17 (85.00%) | Ref | |
| Yes | 23 (19.01%) | 98 (80.99%) | 0.71 | 0.16, 2.34 |
| Unsure* | 8 (27.59%) | 21 (72.41%) | | |
| Missing | 15 | 40 | | |
| **Discussed HPV result with friend or family** | | | | |
| No | 16 (26.23%) | 45 (73.77%) | Ref | |
| Yes | 18 (16.22%) | 93 (83.78%) | 1.72 | 0.80, 3.67 |
| Missing | 15 | 38 | | |

**Table 2** (continued)

| | Overall (n = 225) | | Logistic regression model | |
|---|---|---|---|---|
| | Non-attendee (n = 49) | Attendee (n = 176) | Univariable | |
| | n (row %) | n (row %) | OR | 95% CI |
| *Concerns* | | | | |
| **About cancer** | | | | |
| No | 8 (18.60%) | 35 (81.40%) | Ref | |
| Yes | 27 (19.29%) | 113 (80.71%) | 0.91 | 0.36, 2.11 |
| Missing | 14 | 28 | | |
| **About infertility** | | | | |
| No | 27 (18.88%) | 116 (81.12%) | Ref | |
| Yes | 8 (24.24%) | 25 (75.76%) | 0.76 | 0.32, 1.96 |
| Missing | 14 | 35 | | |
| **Of infecting partner** | | | | |
| No | 25 (20.83%) | 95 (79.17%) | Ref | |
| Yes | 8 (15.38%) | 44 (84.62%) | 1.52 | 0.66, 3.84 |
| Missing | 16 | 37 | | |
| **About impact on sexual intercourse** | | | | |
| No | 26 (19.85%) | 105 (80.15%) | Ref | |
| Yes | 8 (16.67%) | 40 (83.33%) | 1.30 | 0.56, 3.27 |
| Missing | 15 | 31 | | |

OR: Odds Ratio; CI: Confidence Interval; HPV: Human Papillomavirus; hrHPV: high-risk human Papillomavirus

[a] at least one of the following: anxiety, insecurity, nervousness, incomprehension, powerlessness

[b] areas include: dedicated time for explaining result, background information on HPV, answered questions or concerns from patient

* not included in logistic regression

## Discussion

In a population-based cohort study with both cytological and HPV testing (co-testing), the overall proportion of colposcopy non-attendance in screen-positive women was 29%. In referrals with ASC-US+ and hrHPV positive results, 20% did not attend despite active recall efforts. Attendance was associated with having a positive HPV status. Lack of time, barriers including childcare arrangements, travel time as well as lack of clinic choice and the advice given by the gynaecologist who conducted screening were cited as major reasons for non-attendance.

We observed higher non-attendance than in Europe (6–10%) [13, 16, 33]. In North America where CCS is offered opportunistically, non-attendance was observed in 28% of screened women [17], and up to 44% in underserved populations [34]. Low proportions of non-attendance appear to stem from organised screening contexts with active referral to colposcopy. This most likely explains the higher non-attendance rate observed in our study, since screening in Germany until 2020 was opportunistic. Historically, expert colposcopy was also not routinely performed, partly due to the annual screening interval, lack of certified dysplasia centres [35] and gynaecologists conducting repeat smears instead. This is evident in the high proportion of women in our study who were advised by their gynaecologist not to attend colposcopy and instead underwent repeat screening. Additionally, the guideline in effect at the time, when HPV screening was not offered, did not include recommendations for positive HPV or co-test results. The discrepancy between guideline and study protocol could explain this advice.

High non-adherence rates also arise from the lack of a screening registry to systematically contact non-attendees and lack of personnel to conduct recalls in non-organised programmes [36]. Randomised trials and community programs have demonstrated written reminders, preclinic calls and communication with patients significantly increase adherence to follow-up care [20, 23]. In our study, we were able to motivate a third of non-attending women to attend colposcopy by active call-recall. However, this may pose logistical challenges as the communication of results and referral is the responsibility of the screening physician, both in the previous and current screening program in Germany [5]. Management gaps between screening physicians and dysplasia centres where colposcopies are conducted also exist [35]. Enhanced patient communication conducted

**Table 3** Longitudinal outcomes of baseline round (R1) referred women who were also screened at the MARZY follow-up round (R2)

| Outcome | Non-attendee at R1 (n = 33) | Attendee at R1 (n = 109) |
|---|---|---|
| *Between MARZY study rounds* | | |
| **Screening result** | | |
| Positive | 2 (7.69%) | 19 (21.35%) |
| Negative (attended routine screening) | 24 (92.31%) | 66 (74.16%) |
| Did not undergo any screening since R1 | 0 (0.00%) | 4 (4.49%) |
| Missing | 7 | 20 |
| Total | 33 | 109 |
| *At MARZY study R2* | | |
| **Screening test result** | | |
| ASC-US+ only | 0 (0.00%) | 3 (2.75%) |
| hrHPV+ only | 4 (12.12%) | 8 (7.34%) |
| Both positive | 2 (6.06%) | 4 (3.67%) |
| Negative | 27 (81.82%) | 94 (86.24%) |
| Total | 33 | 109 |
| **Colposcopy referred and attendance status** | | |
| No attendance | 5 (83.33%) | 6 (40.00%) |
| Attended | 1 (16.67%) | 9 (60.00%) |
| Not applicable (screen-negative) | 27 | 94 |
| Total | 33 | 109 |

ASC-US+: Atypical squamous cells of undetermined significance or worse; hrHPV+: high-risk human Papillomavirus positive; both positive: ASC-US+ and hrHPV positive

by clinic staff, streamlined management between gynaecological care providers and integration within a standardised call-recall system need to be introduced to reduce anxiety and improve attendance. Similar to other countries with organised screening, a programme target of less than 15% non-attendance should also be set [12].

Almost half (48%) of referrals with cytological abnormalities did not attend colposcopy, probably due to the annual screening interval. Congruent to a recent pilot study [24], a positive hrHPV result significantly increased attendance in our study by three times. We screened participants with HPV testing in addition to cytology, which at the time was not part of routine CCS in Germany. As the majority of hrHPV referrals reported concerns about cancer in our study, additional HPV testing may have caused anxiety or concern [37], which might have led to better attendance. However, in a randomised trial to reduce anxiety by educating participants on HPV before colposcopy, knowledge significantly increased but anxiety did not decrease [38]. Balanced risk communication must be addressed in a programme that offers HPV screening, and could be differential for subgroups such as younger and older women [39]. Furthermore, attendance rates could be improved if engaging information on colposcopy and particular attention for the emotional experience are provided [25, 26]. This is important since concerns and barriers were noted as reasons for non-attendance in a small group of women that did not attend colposcopy, despite being referred in both rounds.

Women with several children were less likely to attend colposcopy. Indeed, the major reasons cited for non-attendance were lack of time and barriers including lack of childcare arrangements, transport times and general lack of clinic choice (hospitals only). Additionally, our active recall efforts may not have mitigated such barriers, rather that it was more effective among women with hesitations. Moreover, we observed better attendance among women who were communicated their positive hrHPV result by the screening gynaecologist, in alignment with previous findings [23]. In a meta-analysis, even after HPV self-sampling kits are offered as a method to address barriers, follow-up non-adherence remains around 19% [40]. These observations underscore the necessity to diversify follow-up alternatives (self-sampling) and the importance of an established relationship including trust between the patient and physician. As recall appears largely to be left to the responsibility of the provider [5], encouraging information packs, educational support for screening physicians in counselling patients backed by a systematic screening registry for call-recall should be provided [12].

### Limitations

We defined non-attendees as screen-positive to either cytology or HPV testing, rather than both cytology and HPV test positive. This may overestimate non-attendance as many who are screen-positive to one test only would normally undergo repeat Pap smear 3, 6 or 12 months later according to the guidelines in effect at the time in Germany [29]. However after restricting non-attendance to positive co-test results (ASC-US+ or LSIL+ and hrHPV positive), we found similar attendance rates. The sample size may have also restricted our analyses, particularly for the HPV-related items in Q3. However, only 18% of hrHPV positive cases were Q3 non-respondents. Additional assessment between Q3 respondents and non-respondents revealed some differences in nationality and socioeconomic status (Additional file 1: Table S8). These differences highlight potential external validity limitations of our results to un(der)screened women. Some non-attendees whom were unreachable may have sought colposcopy elsewhere, but the numbers are small.

## Conclusion

Our population-based screening study offers important insight into colposcopy non-attendance, particularly as HPV testing is being integrated into screening in many countries. We quantitatively and qualitatively described the major reasons for non-attendance, which is important to maximise screening effectiveness. A considerable proportion of women did not attend colposcopy after abnormal screening results, and this persisted even in some women who were referred twice. Certain subgroups of women could be targeted by personalised measures within a failsafe recall system, especially since HPV testing is new. Continued educational support of screening gynaecologists should also be integrated. An optimised screening management continuum can reduce loss to follow up, minimise preventable CC diagnoses and improve the overall effectiveness of cancer screening.

### Abbreviations

ASC-US: Atypical squamous cells of undetermined significance; CC: Cervical cancer; CCS: Cervical cancer screening; CIN: Cervical intraepithelial neoplasia; HC2: Hybrid Capture®2; HPV: Human papillomavirus; hrHPV: High-risk HPV; LSIL: Low-grade squamous intraepithelial lesion.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12905-022-01851-6.

**Additional file 1**. The following file contains further analyses and insight (tables S1–S8 and figure S1) to complement the main analyses of our study

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### Author contributions

Study conception and design: SJK, MB. Data collection and patient management: SRZ, KR, SF, HI. Study co-ordination: KR, SJK. Data analysis and interpretation: LAL, GS, SM, SJK. Drafting of the manuscript: LAL, SJK. Revision and final manuscript review: all co-authors. Supervision of the findings: SJK. All authors read and approved the final manuscript.

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### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

Informed consent to participate in the MARZY prospective cohort study was provided in written form by all study participants prior to screening at study baseline. The MARZY study was approved by the ethical committee of the state of Rhineland-Palatinate (Landesärztekammer Rheinland-Pfalz: 837.438.03 (4100)) and the state government data protection office. All recruitment, data collection and analyses were performed in accordance to Good Epidemiological Practice guidelines and the Declaration of Helsinki.

### Consent for publication

Not Applicable (NA).

### Competing interests

HI reports co-ownership of a laboratory for cytology and molecular diagnostics. All other authors declare no competing interests.

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