COURSE: Evidence-Based Approaches to HPV Screening implementation

Module 4B. Screening management - Visual techniques and Diagnostic tests/biopsy

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CONTENTS

INTRODUCTION AND LEARNING OBJECTIVES

Once a woman has been screened, including or not subsequent triage testing, and has obtained abnormal results that suggest a risk of CIN3+ higher than a country-specific value, a diagnosis process is required. In most countries, this process involves the visual inspection of the cervix through a colposcopy. In case of abnormal findings in the colposcopy, and according to the relevant clinical guidelines in each setting, biopsy samples are taken to histologically confirm the diagnosis.

Therefore, colposcopy examination plays a key role in the cervical precancerous lesions diagnosis and treatment of symptomatic women or those showing abnormal screening tests. Yet, the transition from a cytology to HPV-based screening program, implies two main issues to be considered:

- Women attending colposcopy will present different characteristics (HPV positive versus abnormal cytology) which might affect the colposcopy performance.
- The use of the previous and current screening history of women attending colposcopy can be used to guide the colposcopy and biopsy practice.

In some resource-constrained settings, other visual techniques are being used for the diagnosis of cervical precancerous lesions but its performance is moderate. Therefore, enhanced camera image device or artificial intelligence tools are being explored to improve the subjectivity of such visual techniques.

This module provides an overview of the accuracy and the management practice of the colposcopy in the context of an HPV-based screening program as well as describing other visual techniques mainly used in resource-constrained settings. It also introduces how artificial intelligence can improve the performance of cervical cytology or other visual inspection techniques.

It also covers the recently published WHO classification of cervical squamous intraepithelial lesions, which contains some important revisions on HPV-related lesions.

At the conclusion of this course, participants will be able to:

- Relate the terms used in colposcopy to describe the different lesional patterns
- Understand the impact that the different cervical cancer screening approaches have on the colposcopy
- Understand how colposcopy could be used according to risk-based approaches
- Recognize why training, expertise and quality control are crucial in the colposcopy performance
- Identify the benefits and the harmful effects of colposcopy
- Recognize the utility and caveats of visual techniques other than colposcopy
- Explore new technological developments in cervical cancer screening.
- Achieve a high-level understanding of how an artificial intelligence approach in cervical cancer screening could be widely implemented and sustainable
- Recognize the main differences between the recently published WHO classification of cervical squamous intraepithelial lesions compared with the previous ones
- Understand the utility of biomarkers such as p16 in the diagnosis of histological HPV-related cervical lesions

UNIT 1. VISUAL TECHNIQUES

1.1 Visual techniques

The visual examination of the cervix is required to identify potential precancerous lesions.

The colposcopy enables the inspection of the cervix in women showing abnormal screening tests, allowing the characterisation and localisation of intraepithelial lesions to guide the collection of biopsies for subsequent diagnosis confirmation. However, other visual techniques are used, mainly in resource-constrained settings where colposcopy is not always available for cervical lesions diagnosis.

An overview of these visual techniques is provided in the following units.

1.2 Colposcopy

1.2.1 Technical description of the colposcopy examination

A colposcope is a low magnification, light illuminated, stereoscopic, binocular, field microscope used for visual examination of the lower genital tract, especially the uterine cervix. Colposcopy enables the examination of the transformation zone (TZ) and the squamo-columnar junction (SCJ), where most cervical cancers arise, and characterisation and localisation of intraepithelial lesions to guide biopsies, for diagnosis confirmation, where necessary.

In 1925, Hinselmann designed the colposcope and described how to enhance the colposcopic view of the cervical epithelium to identify cervical cancer and precancer by staining the cervix with acetic acid. In 1929, Schiller showed that areas of the cervix harbouring early cervical cancer did not stain with iodine, in contrast with the dark staining of normal squamous epithelia of the ectocervix (Bappa & Yakasai, 2013; Colgan & Lickrish, 1990).

Colposcopy is a comprehensive examination providing information that is crucial to optimum clinical management. However, it must be performed by a competent and quality-assured service. Clinicians performing colposcopy must have undergone comprehensive training and the procedure must be carried out in a fully-equipped examination room (Prendiville & Sankaranarayanan, 2017).

The colposcope allows for examination of the whole lower genital tract, including the cervix, vagina and vulva as follows. First, the clinician assesses whether the examination can be performed adequately (Bornstein, Bentley, et al., 2012). If so, the cervix is then examined at low power magnification and gently cleansed with saline solution. The hormonal status and degree of inflammation are then assessed. Once adequacy has been confirmed, the TZ is examined at low power. At this point, the green filter may be used at low magnification. Then, the evaluation with acetic acid (either 3% or 5%) can be performed. A spray bottle, syringe or soaked cotton swabs should be used repeatedly to achieve complete uptake of acetic acid unprotected by mucus until the TZ type is clearly discerned. Endocervical forceps (Kogan speculum, for instance) may be necessary to achieve full visualisation of the upper limit of the TZ. It is important to perform both low- and high-powered examination of the TZ and to take note of the relevant image characteristics.

A competently performed colposcopic examination (IARC, 2022) will:

- determine the adequacy of the examination,
- evaluate the TZ site and size,
- recognize intraepithelial abnormality, if present,
- identify the most accurate biopsy site for sampling, and
- facilitate precise treatment.

1.2.2 Colposcopy terminology and lesion correlation

Different terminologies of the colposcopic findings have been used throughout the 90-year history of colposcopy (IARC, 2022).

Did you know?

The following table shows the most relevant and widely used (clinically) global colposcopic classifications and the modifications that have been introduced over time. However, the use of these terms is now obsolete and inappropriate.

Terminology (Author/ Year)	Normal findings	Abnormal findings	Other terms
Hinselmann 1933 (Mayeaux & Cox, 2013)	Thick leukoplakia	Mosaic leukoplakia	Cervico-uterine ectopy
Coppleson 1960 (Reid & Campion, 1989)	Grade I, not suspicious, white semi-transparent epithelium, flat, indistinct borders	Grade II, suspicious white Grade III, opaque epithelium with very suspicious defined borders	Transformation zone
IFCPC Graz 1975 (Stafl, 1976)	Normal colposcopy	Atypical transformation zone	Colposcopy not satisfactory Miscellaneous
Reid 1985 (Reid & Campion, 1989)	Category 1, benign, minor dysplasia	Category 2, intermediate Category 3, suspicious	4 criteria: border, colour, vessels, iodine uptake
IFCPC Rome 1990 (Stafl & Wilbanks, 1991)	Normal colposcopy Cylindrical epithelium: ectopy	Abnormal colposcopy inside/outside the transformation zone Mosaic punctuation fine/coarse	Miscellaneous not acetowhite
IFCPC Barcelona 2002 (Walker et al., 2003)	Type 1, 2, 3 transformation zone	Minor/major changes Suggestive of low/high-grade lesion	Colposcopy suggestive of invasive cancer

Worldwide, the most commonly used classification in healthcare practice is that endorsed unanimously by over 40 national societies in the triennial congress of the

International Federation for Cervical Pathology and Colposcopy (IFCPC) in Rio de Janeiro in 2011 (Bornstein et al., 2012).

International terminology for colposcopic findings (Bornstein, Bentley, et al., 2012)

General assessment		Adequate / inadequate because… (e.g. cervix obscured by inflammation, bleeding, scar) Squamo-columnar junction visibility: completely visible, partially visible, not visible Transformation zone types 1,2,3	
Normal colposcopic findings		Original squamous epithelium: Mature Atrophic Columnar epithelium Ectopy Metaplastic squamous epithelium Nabothian cysts Crypt (gland) openings Deciduosis in pregnancy	
Abnormal colposcopic findings	**General principles**	*Location of the lesion*: inside / outside the TZ and location of the lesion by clock position *Size of the lesion*: number of cervical quadrants the lesion covers and/or size of the lesion as percentage of cervix	
	Grade 1 (Minor)	Thin acetowhite epithelium Irregular, geographic border	Fine mosaic Fine punctuation
	Grade 2 (Major)	Dense acetowhite epithelium Rapid appearance of acetowhitening Cuffed crypt (gland) openings	Coarse mosaic Coarse punctuation Sharp border Inner border sign Ridge sign
	Non specific	Leukoplakia (keratosis, hyperkeratosis), erosion Lugol's staining (Schiller's test): stained/non-stained	
Suspected invasive disease		Atypical vessels Additional signs: fragile vessels, irregular surface, exophytic lesion, necrosis, ulceration (necrotic), tumour/gross neoplasm	
Miscellaneous findings		Congenital TZ, condyloma, polyp (ectocervical /endocervical), inflammation, stenosis, congenital anomaly, post-treatment consequence, endometriosis…	
Excision treatment types		Excision type 1, 2, 3	
Excision specimen dimensions		Length: distance from the distal/external margin to the proximal/internal margin	

	Thickness: distance from the stromal margin to the surface of the excised specimen Circumference (optional): the perimeter of the excised specimen

Below are a series of images (**Figures 1-3**) of the cervix obtained through colposcopy with the findings described using the 2011 ICFC accepted terminology:

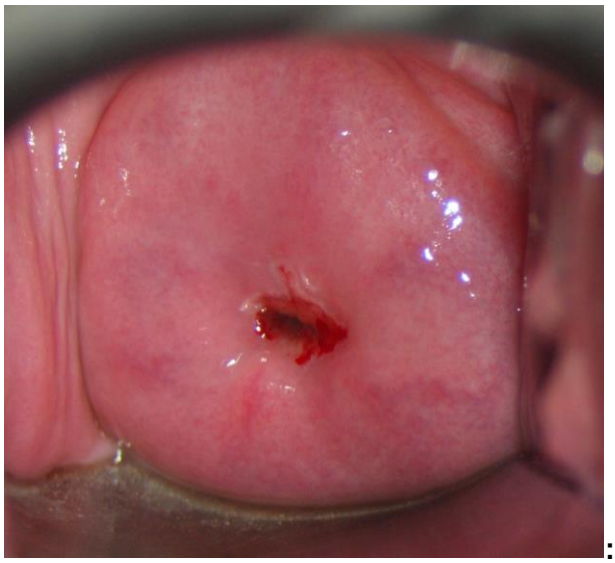

Figure 1. Colposcopy showing type 1 transformation zone (fully visible squamous columnar junction) and normal colposcopy appearance: mature squamous epithelium. Vision after application of acetic acid

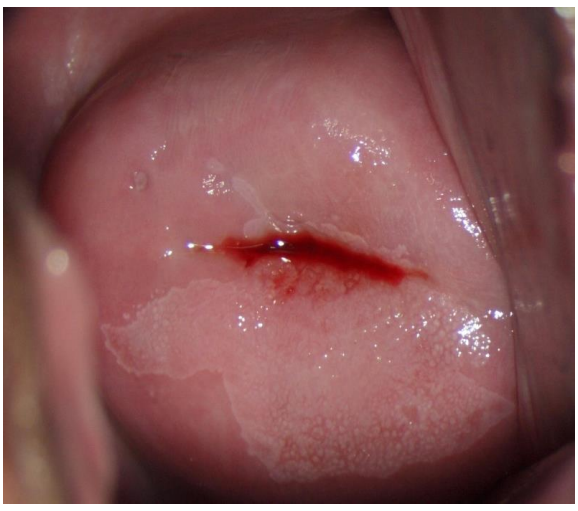

Figure 2. Colposcopy showing type 2 transformation zone (fully visible squamous columnar junction with an endocervical component) and thin acetowhite epithelium (findings grade 1) in both cervical lips. Vision after application of acetic acid

A) B)

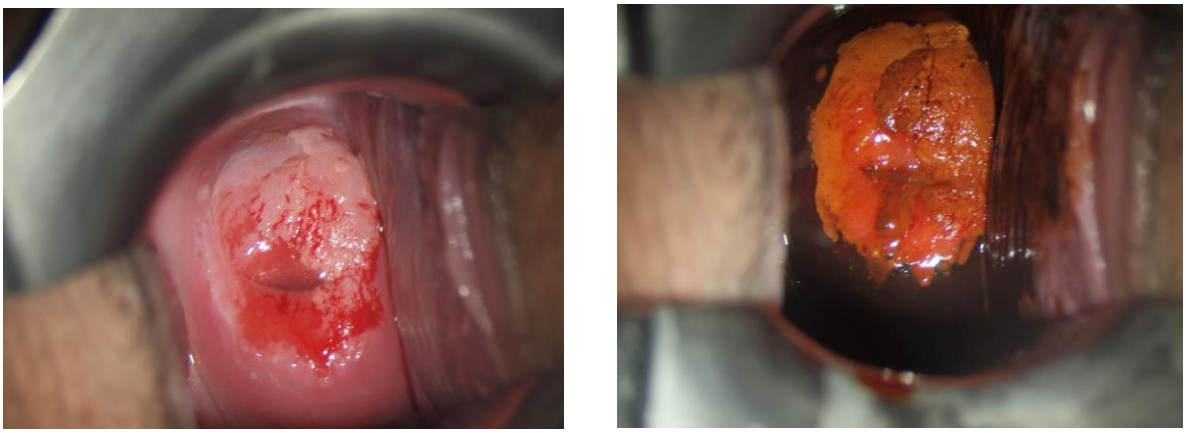

Figure 3. Colposcopy showing dense acetowhite epithelium (findings grade 2) in both cervical lips. In these images, fragile vessels and irregular surface suggesting an invasive lesion should also be noted. A) Vision with acetic acid B) Vision after Lugol

Several studies have evaluated the correlation between the categorisation of a lesion using the current IFCPC classification and the histological diagnosis. Some report a good correlation between the colposcopic impression and the final diagnosis (Petousis et al., 2018). Indeed, the identification of some specific patterns (e.g. coarse punctuation, coarse mosaic or dense acetowhitening, inner border sign and ridge sign) have demonstrated correct predictive accuracy for high-grade squamous intraepithelial lesions/cervical intraepithelial neoplasia grades 2–3 (HSIL/CIN2–3) (Li et al., 2017; Vercellino et al., 2013), with a sensitivity and specificity of colposcopic impression suggesting HSIL/CIN2+ ranging from 20% to 100% and 96% to 99%, respectively, depending on the specific pattern reported. However, other authors suggest that the grade of concordance between the colposcopic impression and the histological diagnosis mainly depends upon the training, experience and expertise of the colposcopist (Khan et al., 2017; Mayeaux & Cox, 2013) and point out the importance of quality training and assurance programmes.

Some attempts have been made to quantify the qualitative descriptions or specific patterns in scoring systems, such as the Reid Colposcopic Index (RCI) (Reid & Scalzi, 1985) or the Swede score (Alfonzo et al., 2022). It has been suggested that colposcopic findings may be best assessed using a scoring system (Alan et al., 2020; Prendiville & Sankaranarayanan, 2017; Ranga et al., 2017). However, not all reports find a better

correlation when using quantitative scores instead of colposcopy terminology. Currently, the use of these scores is not yet standard practice worldwide.

1.2.3 Colposcopy accuracy according to the cervical cancer primary screening test

Colposcopy accuracy in cytology-based screening

The performance of the colposcopy varies depending on the criteria used to take a biopsy.

In general, all studies show a high sensitivity and low specificity of colposcopy for HSIL/CIN2+ when a biopsy is taken at the cut-off of 'any colposcopic abnormality' (i.e biopsy is taken because there is thought to be SIL/CIN of any grade present) (Brown & Tidy, 2019; Mitchell et al., 1998). In contrast, at the cut-off of "high-grade impression colposcopy" (biopsy taken due to a colposcopic impression of HSIL), colposcopy has moderate sensitivity but high specificity for HSIL/CIN2+.

Did you know?

Risk estimates for HSIL/CIN3+ are much less heterogeneous than for HSIL/CIN2+. However, most available reports have evaluated colposcopy based on the risk of underlying HSIL/CIN2+ and only a limited number of studies present specific data for HSIL/CIN3+.

The use of punch or random biopsies in apparently normal tissue as the gold or reference standard to assess the sensitivity and specificity of colposcopy has been questioned, since in many clinics biopsy is only performed when disease is suspected. As a result, verification by biopsy is only performed when the outcome of colposcopy is positive and not when the outcome is negative. This form of bias results in overestimated sensitivity and underestimated specificity (Walter, 1999).

Many healthcare professionals understand a random biopsy as a biopsy taken randomly at any normal area (i.e, clockwise, at 2 and 6, for example) but the concept of random biopsy is more complex. In contrast with the term of "directed biopsy", a random biopsy is that taken in an area that does not apparently qualify as high-grade lesion.

NOTE: *The methods used to evaluate the diagnostic accuracy of colposcopy in clinical trials are subject to several types of biases such as the verification or ascertainment bias described before.*
For more information on this bias, please see ***MODULE 2****.*

To avoid this verification bias, some studies have assessed the performance of colposcopy-directed cervical punch biopsies (test positivity defined as CIN1+) to detect HSIL/CIN2+ in a subsequent excisional cervical specimen or hysterectomy (Kahramanoglu et al., 2019; Kim et al., 2020; Underwood et al., 2012). A meta-analysis of these studies found a punch biopsy sensitivity for HSIL/CIN2+ ranging from 80% to 90% and a specificity ranging from 25% to 63%. However, most studies do not specify whether the biopsies were performed for any colposcopy abnormality or because a high-grade lesion was suspected.

Did you know?

Both the sensitivity and specificity of colposcopy for CIN2+ are improved when colposcopist know the cytology result (Lalande et al., 2024 **RISCC**).

Colposcopy accuracy in HPV-based screening

Worldwide, cervical cancer screening is moving from cytology-based to HPV-based screening (Chrysostomou et al., 2018). As explained in previous modules, HPV testing detects a high proportion of infections likely to be transient or at an early stage of the carcinogenic process that may not be detectable at colposcopy. Therefore, it is

neither efficient nor viable to propose screening with referral to colposcopy for all HPV-positive women. To avoid overburdening the health system and overtreating low-risk women, a risk-based approach is needed to manage women with an HPV-positive screening test.

Moreover, though colposcopy will still play a central diagnostic role in HPV-based screening, the clinical characteristics of the referred patients will be markedly different. The accuracy of colposcopy must therefore be re-evaluated in this new scenario.

The positive predictive values (PPV) of an abnormal colposcopy for histological HSIL/CIN2+ in cytology-based screening and in an HPV-based programme are similar.

EXAMPLE

The Guanacaste cohort (Porras et al., 2012) with 7-year of follow-up after initial screening with both cytology and HPV testing, was used to assess the predictive values of colposcopy under two different screening scenarios (those referred to colposcopy due to a cytology result of atypical squamous cell of uncertain significance (ASC-US) or worse versus those with a positive HPV result):

- In women under 30 years: In comparison to women with an abnormal cytology result, a non-statistically significant reduction in the positive predictive value (PPV) of colposcopy was observed in HPV-positive women (31.8 vs 24.2%). The negative predictive value (NPV) was statistically higher in HPV positive women (85.5 vs 91.6%).
- In women 30 years or older: Both the PPV and the NPV were not statistically different in women with an abnormal cytology and those with a positive HPV result (47.8 vs 41.5% and 87% in both, respectively)

1.2.4 Risk-based colposcopy practice and the value of random biopsies

The risk of underlying cervical HSIL/CIN2+ varies greatly depending on the type of primary screening test(s) and the corresponding result(s).

Therefore, the colposcopy and biopsy practice can be modified depending on the risk of precancerous lesions (Torné et al., 2018; Wentzensen et al., 2018) estimated through the information provided by the screening test (cytology and/or molecular test results such as HPV testing and genotyping) and previous screening results as described in **Table 1.**

NOTE: *For more information on the risk stratification of women with a positive HPV result based on the primary screening test results or subsequent triage testing, please see* ***MODULE 4A****.*

Table 1. Risk of premalignant cervical lesion based on previous and current screening test results. Adapted from Egemen et al., 2020; IARC, 2022.

Clinical scenario		
HPV testing	**Cytology**	**HSIL/CIN3+ risk**
Negative (primary screening)	**Not performed**	Very low (0.15% at 5 years)
Negative (primary screening)	**ASC-US/LSIL**	Low (0.4%-2.0% at 5 years)
Negative (follow-up of previous positive low-grade result)	**Negative**	Low (0.5%-3.2% at 5 years)
Not performed	**Negative (primary screening)**	Low (0.7%-2.0% immediate risk; 2.0%-4.8% at 5 years)
Positive (primary screening)	**Negative**	Low-moderate (2.0%-4.5% immediate risk; 3.8%-7.3% at 5 years)
Positive (primary screening)	**ASC-US/LSIL**	Low-moderate (2.0%-4.5% immediate risk; 3.8%-7.3% at 5years) *
Not performed	**LSIL (primary screening)**	Moderate (10.0%-14.0% immediate risk)
Positive (follow-up of previous positive low-grade result)	**Negative/ASC-US/LSIL**	Moderate (2.6%-7.9% immediate risk; 6.6%-9.5% at 5 years)
Positive 16 and/or 18 (primary screening)	**Negative/ASC-US/LSIL**	High (5.5%-11% immediate risk; 9.0-12.0% at 5 years)
Positive (primary screening)	**HSIL/ASC-H/ACG**	High (>25% immediate risk)
Positive 16 and/or 18 (primary screening)	**HSIL/ASC-H/ACG**	High-very high (28.0%-60% immediate risk; 33.0%-64.0% at 5 years)

** The risk varies mainly depending on the availability of the results of the previous screening test. If negative, the risk of immediate HSIL/CIN3 is low. If the results are not available or unknown, the risk should be considered moderate.*

The information provided by the colposcopic impression itself can also influence the need to perform multiple biopsies, including the potential collection of non-directed (random) biopsy obtained within the TZ.

NOTE: *The value of random biopsies to improve the sensitivity of the colposcopy is currently under discussion. Some reports suggest that the collection of only colposcopy directed biopsies can miss 30–55% of the cervical HSIL/CIN2+, depending on the colposcopist (IARC, 2022). However, the benefit of collecting additional random biopsies to identify otherwise potentially missed lesions is not consistent across different studies and some authors claim that in countries in which colposcopy is part of a properly constructed, quality-assured program, normal colposcopy is associated with a very high NPV.*

Based on the screening results and the colposcopic impression, women can be stratified into the following risk levels (**Table 2**):

Table 2. Level of cervical HSIL/CIN3+ risk based on the cytology, HPV test and colposcopy results (Wentzensen et al., 2017)

Level of risk		
Low risk (<0.5% for HSIL/CIN3+)	**Intermediate risk** (0.5-28% for HSIL/CIN3+)	**High risk** (≥29% for HSIL/CIN3+)
The following 3 criteria are met: ▪ Cytology <HSIL ▪ No HPV 16/18 ▪ Normal colposcopy (no acetowhitening)	Cases not included in the other 2 groups.	At least 2 of the following criteria are met: ▪ Cytology HSIL or worse, AGC or ASC-H ▪ HPV 16 and/or 18 ▪ Colposcopy shows grade 2 findings If the three criteria above are met, the risk for HSIL/CIN3+ is >70%

Scientific societies worldwide have issued new colposcopy standards and risk-based management guidelines for the lowest and highest risk groups for HSIL/CIN3+ based on the following observations:

- For women in the lowest risk group for HSIL/CIN3+, biopsies could be avoided and a random biopsy should never be performed.
- For women in the intermediate risk group, in the case of an abnormal colposcopy that does not suggest cervical HSIL/CIN3+ (acetowhite areas compatible with metaplasia or other minor abnormalities), a cervical biopsy should be performed in women with an HSIL cytology or HPV16 and/or 18 positive tests to avoid missing histological HSIL.
- For women in the high-risk group, the benefit of taking random (non-targeted) biopsies from normal colposcopic areas within the TZ could be considered given their potential role in aiding detection of HSIL/CIN2+.

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NOTE: *For more information on national colposcopy guidelines, please see those published in the United States of America (Perkins et al., 2020) and Spain (Torné et al., 2018).*

The risk-stratification approach allows the adaptation of the colposcopy exam and the biopsy strategy to each particular case.

1.2.5 Harmful effects of colposcopy

Colposcopy is generally a well-tolerated examination with very few reported complications. The main drawbacks of colposcopy can be differentiated by its cause:

Harm caused by the procedure itself

Most of these side effects are mainly reported in women undergoing colposcopy for the first time and are minimised in subsequent examinations (O'Connor et al., 2017).

(i) *Pain/discomfort*: Some women may report mild or moderate discomfort due to prolonged placing of the speculum or application of acetic acid/iodine solution, as well as cramping or pain associated with the biopsy (Khan et al., 2017; Torné et al., 2018). However, routine pre-procedure analgesia is not recommended as clinical studies have shown that oral or topical analgesics do not consistently reduce procedural pain.

(ii) *Anxiety*: Colposcopy may have a psychological impact on women undergoing any of the procedures. Anxiety, worry and fear are the feelings most commonly described (Galaal et al., 2011; O'Connor et al., 2017). However, it is often unclear whether it is the diagnosis of an abnormal screening test or the colposcopy itself that contributes to negative feelings. Interestingly, the results of the procedure affect the course of any negative feelings. Some studies (Sharp et al., 2011, 2013) indicate that at 6 weeks post procedure, women with an abnormal TZ report significantly higher distress compared with women with a normal TZ.

(iii) *Sensitivity* or *Anaphylactic reaction to iodine*: The iodine solution used in colposcopy can produce immediate burning sensation that can be managed by washing out the vagina with normal saline solution. Isolated examples of allergic reactions have been described only very rarely (Indraccolo et al., 2009).

Harm caused by inadequate indication for colposcopy

Although colposcopy was initially used as a tool for primary screening of cervical cancer and precancer, an increased understanding of the natural history of HPV infection and its progression to cervical neoplasia has recently reduced the indications for colposcopy. Strict adherence to the colposcopy indications set out in the guidelines minimises the side-effects associated with inappropriate use of this procedure (Torné et al., 2018).

Harm caused by lack of experience or quality assurance

While colposcopy is generally a low-risk procedure, this may not necessarily be the case if it is performed without adequate training or by a service without quality assurance.

Colposcopy requires adequate training and experience to attain proficiency and ensure competence throughout the procedure. The false negative rate (missed cervical HSIL/CIN2+ lesions) of colposcopy is inversely related with the expertise of the colposcopist (Bekkers et al., 2008; Stuebs et al., 2019)

Did you know?

Colposcopy has long been the cornerstone for diagnosing cervical lesions. However, in recent years, many groups have been working on applying artificial intelligence (AI) to image interpretation with the aim to improve colposcopy accuracy and reduce inter- and intra-observer variability. While AI is not yet widely available and much work remains, it holds promise as a valuable tool for screening and diagnosis in the near future.

ACTIVITY

Read the following statements and decide if they are TRUE or FALSE.

1. The colposcope allows for examination of the whole lower genital tract, including the cervix, vagina and vulva which provides relevant information for optimum clinical management.
2. There is no correlation between the specific patterns or qualitative descriptions of a lesion and the final histologic diagnosis.
3. The performance of the colposcopy is independent on the criteria used to take a biopsy.
4. A random biopsy is a biopsy taken in an area that does not apparently qualify as high-grade lesion instead of that taken randomly at any normal area.
5. The positive predictive values (PPV) of an abnormal colposcopy for histological HSIL/CIN2+ in cytology-based screening and in an HPV-based programme are similar.

6. A risk-based approach in the colposcopy and biopsy practice can avoid overburdening the health system and overtreating women at low risk of disease.
7. Cytology and molecular test results, including HPV testing and genotyping, are used to estimate the risk of precancerous lesions.
8. The information provided by the colposcopic impression, although interesting, does not influence whether to take or not a biopsy. Therefore, random biopsies are always recommended in the event of colposcopy referral.
9. The harmful effects of colposcopy are those associated with the procedure itself, an inadequate indication or the lack of experience or quality assurance.
10. The false negative rate (missed cervical HSIL/CIN2+ lesions) of colposcopy is independent from the expertise of the colposcopist.

The correct answers are:

1 True, 2 False, 3 False, 4 True, 5 True, 6 True, 7 True, 8 False, 9 True, 10 False.

1.3 Other visual methods for resource-constrained settings

When colposcopy is not available, such as in some resource-constrained settings, other visual methods are used. The visual techniques for screening and management of cervical lesions and cancer can be divided into two categories based on the level of information collected:

- **Naked eye examination with acetic acid (VIA) or Lugol's iodine (VILI)** which has been widely used to screen women for cervical precancerous lesions in low- and middle-income countries (LMIC).
- **Camera enhanced image capture** which has been more recently used to improve the performance of VIA

Naked eye examination (VIA, VILI)

Naked eye examination of the cervix with acetic acid and/or iodine as a means of detecting cervical precancerous lesions arose because of the poor performance of the screening methods used in high income countries (cytology followed by colposcopy) when applied to LMIC.

The results of a VIA examination are categorised as negative, positive or suspicious of cancer (Sankaranarayanan & Wealey, 2003; World Health Organization Regional Office for South-East Asia, 2017). VIA positivity rates vary considerably (5-35%) compared with other screening tests, which suggests that VIA performance is highly subjective and provider-dependent, with little reproducibility and varying thresholds used for test positivity in different studies (Almonte et al., 2015; Huchko et al., 2015; Shastri et al., 2014). The advantage of naked eye examination is that where the TZ is fully visible, VIA-positive women may be treated immediately during the same session with cryotherapy/thermal ablation using a single visit screen-and-treat approach (World Health Organization, 2021; World Health Organization, Geneva, Switerland, 2019). However, these women can also be referred for triage with colposcopy and treatment in the conventional manner.

VIA sensitivity for detecting HSIL/CIN2+ ranges from 22.0% to 91.0% and specificity varies from 47.0% to 99.0% (World Health Organization, 2021).

VIA should not be performed in post-menopausal women, given the TZ cannot be correctly assessed.

Lugol's iodine (5%), for staining the cervix, has historically been used as a screening test for cervical precancer and cancer. However, it is relatively expensive. It can be prepared locally and should be discarded after 3 to 6 months.

Camera enhanced visual inspection

There are several emerging devices such as digital cervicography, smartphone attachments, intravaginal endoscopes or portable monoscopic devices for either improving or assisting VIA. As yet, there have been no large randomised controlled trials that would enable an objective assessment of their clinical value.

For more information on these, please see Goldstein et al., 2020 and Parham et al., 2015.

ACTIVITY

Read the following statements and decide if they are TRUE or FALSE.

1. In resource-constrained settings, the only visual technique possible is the use of naked eye examination with acetic acid (VIA).
2. VIA positivity rates and performance vary considerably between studies because of its subjectivity.
3. VIA allows for a screen-and-treat strategy so that VIA positive women can undergo cryotherapy or thermal ablation in a single visit.

The correct answers are:

1 False, 2 True, 3 True.

1.4 Deep Learning / Artificial Intelligence

1.4.1 Introduction to Deep Learning / Artificial Intelligence

Automated approaches to image classification (Gulshan et al., 2016) have the potential to:

- improve efficiency, reproducibility and coverage of screening programmes
- reduce barriers to access
- improve patient outcomes by facilitating early detection and treatment.

Following this rationale, artificial intelligence (AI) has been explored in several medical fields including screening for diseases such as retinopathy in the diabetic population, or evaluation of X-rays or mammograms in cancer screening. In cervical cancer prevention, AI has been applied to the automated visual evaluation of cervical images, cervical cytology and biomarker-enhanced cervical cytology (Parham et al., 2023; Schiffman et al., 2017; Wentzensen et al., 2020).

Artificial intelligence (AI) refers to **computer systems** which are able to perform tasks that normally require human intelligence, such as visual perception, speech recognition, decision-making and language translation.

AI is a broad category that encompasses different analytical approaches (**Figure 4**): machine learning, neural networks and deep learning.

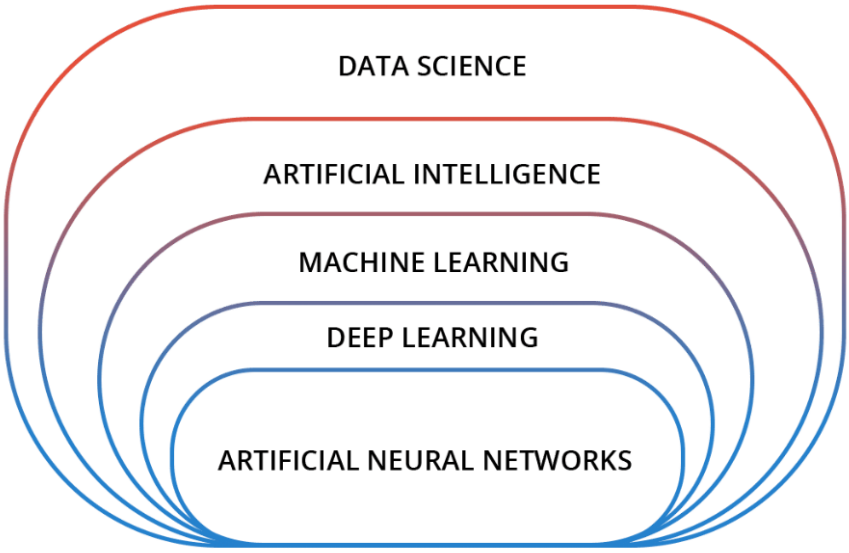

Figure 4. The broad category of artificial intelligence Adapted from Choi et al., 2020.

What is machine learning?

Machine learning is a type of AI that uses machines (computers) to detect data patterns that can be used to predict **outcomes** with a high degree of accuracy.

What is deep learning?

Deep learning is inspired by the network of neurons in the human brain. It is a specific type of machine learning method that uses many layers of arithmetic operations (Choi et al., 2020; Liu et al., 2019). It is an AI technology that can identify and classify objects, people, writing, and even actions within still or moving images. Typically driven by deep neural networks, image recognition is used for fingerprint ID systems, mobile check deposit apps, video and medical image analysis, self-driving cars, and much more.

In a deep learning model for image analysis, each individual pixel in an image provides information gathered through different layers on various characteristics such as texture, edges, curves, etc. Using big data and advanced computational resources, these elements are analysed to provide an accurate diagnosis (outcome).

A computer is more likely to provide an accurate diagnosis, as human classification of images remains subjective, even when performed by experts. Instead of shades of acetowhitening, a computer 'sees' a matrix of numbers representing pixels (**Figure 5**).

Computer vision typically involves computing the presence of numerical patterns in this matrix, then applying machine learning algorithms to distinguish images on the basis of these patterns.

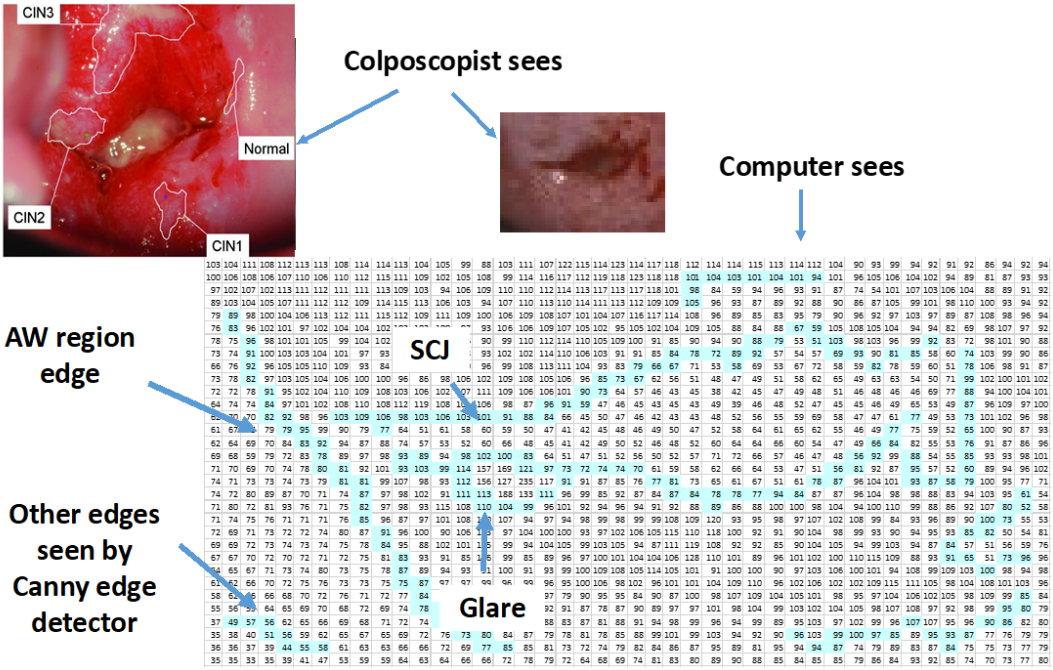

Figure 5 . Human versus computer vision of a cervical image. (Chartrand et al., 2017)

In this section, we will briefly explore some examples as well as key elements in building an automated approach that could aid cervix visualisation in cervical cancer screening and reduce the subjectivity inherent in the interpretation of cervical cytology or visual inspection.

1.4.2 Developing a machine learning algorithm

Developing a machine learning algorithm is an iterative experimental process whereby the models (or algorithms) must be trained (the model is taught to identify the outcome of interest based on the reference standard used, such as a histological diagnosis) and tuned to the context and data in question. Once trained, the algorithm must be tested on previously unseen data by the model to ensure that the original data were not biased.

Care must be taken to avoid models too specific for a given data set – overfitting – as this would impact the model's generalisability (Doshi-Velez & Perlis, 2019; Nevala, 2017).

1.4.3 Practical example of machine learning in cervical image diagnostics

Several studies have been published or are ongoing to evaluate the use of cervical images after acetic acid application using a deep learning technique called automated visual evaluation (AVE). AVE has garnered attention for its potential to enhance screening strategies in low- and middle-income countries (de Sanjosé et al., 2024), however its intended use is not limited to low resource settings as application of AI to colposcopes is also under development (Yang et al., 2023).

Figure 6 outlines the training process. In summary, the model (Ahmed et al., 2023; Hu et al., 2019; Parham et al., 2023) is generally trained using a certain proportion of the study images, with the histological evaluation of each case serving as the ground truth. Once trained, the algorithm is tested internally, and to ensure that the model is fully validated, it must be tested with a new dataset that the algorithm has not previously encountered.

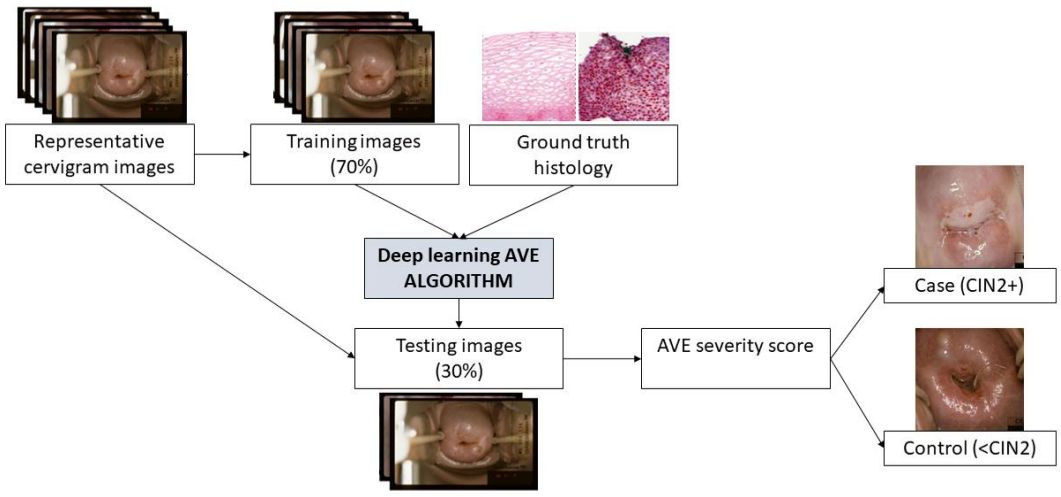

Figure 6 Training a supervised deep learning model. Original artwork by Kanan Desai

Studies reporting very high performance, such as those by Hu et al. (2019, 2024), need to confirm that results hold after external validation to guarantee that the models were not overfitting the data (Desai et al., 2020; Xue et al., 2020). AVE shows promise as an assistive technology when performing screen-triage-and-treat strategies (Pal et al., 2021; Parham et al., 2023; Xue et al., 2020). However, AVE cannot be applied "out of the box" to new patient populations or used with different image capture devices than those used to train the original algorithm. Unless setting-specific images are provided to retrain the algorithm, it will fail to distinguish precancer/cancer at a rate much higher than chance alone (Desai et al., 2020).

Did you know?

Ascertainment of the SCJ/TZ type is crucial to manage women safely and not miss occult disease. At this time, not fully validated AI systems to assess SCJ/TZ have been published. AI systems are rapidly evolving and likely work as an assisted technology to human evaluation (Wu et al., 2024).

1.4.4 Practical considerations in the application of AI to cervical image diagnostics

Despite the promise of AI, it is important to assess any study reporting on machine learning in cervical cancer screening before the use of the reported algorithm for lesion management in clinical practice (**Box 1**).

Box 1. Checklist for evaluating studies on AI-based diagnostic algorithms for cervical cancer screening (Desai et al., 2020)

Accuracy of the predictions.

Does the algorithm discriminate well between normal and precancerous lesions? Has the algorithm demonstrated that its use improves clinical outcome management? Has the clinical indication for the algorithm been clearly specified?

An algorithm achieving excellent results in a screening clinic may not be capable of improving clinical outcomes in the colposcopy population, since the latter may not include enough 'normal' cervices (i.e. the controls tend to look similar to actual cases, making discrimination more difficult).

Reference standard. What reference standard for precancer has been used to train the algorithm? *Histology is superior to expert review or cytology. Algorithms trained using visual impressions as ground truth labels will be limited by the inherent performance of VIA or colposcopic impression.*
Validation set. Has the model been validated in large and heterogeneous populations? Was the study based on the HIV population? Did the study include populations with a high prevalence of cervicitis and schistosomiasis? *An algorithm based on non-HIV women may not be automatically transferrable to HIV-positive women, since HIV-positive women have more severe lesions. The presence of cervicitis and schistosomiasis on the cervix may confuse the algorithm.*
Algorithm evaluation. How was the algorithm evaluated? How does it perform when compared to other methods? *Results should ideally be tested in randomised clinical trials in actual clinical practice. The algorithm should also be at least non-inferior to the comparative screening method (e.g. VIA).*
Image source. Has the algorithm been assessed using images captured with a specific camera device? *An algorithm trained on one type of digital camera image (e.g. cervigrams) may not be transferrable to other type of cervical image (e.g. smartphone).*
Algorithm purpose. Was the algorithm evaluated for use as a stand-alone tool or as an assistive technology for the healthcare provider?
Image quality. Is there a well-established approach for assessing image quality? Does the algorithm accept blurry, unfocussed images?

To conclude this extremely brief introduction to AI, the health professional exploring the use of an AI-based intervention should consider whether there is sufficient evidence that the approach provides a reasonable prediction comparable to that made by the best expert in the field.

ACTIVITY

Read the following statements and decide if they are TRUE or FALSE.

1. In cervical cancer screening, artificial intelligence has only been applied to the automated visual evaluation of cervical images from low-resource settings.
2. A computer is more likely to provide an accurate diagnosis, as human classification of images remains subjective, even when performed by experts.
3. Any study reporting on the performance of computer-aided diagnosis needs to be carefully assessed before its use for lesion management in clinical practice.

The correct answers are:

1 False, 2 True, 3 True.

UNIT 2. DIAGNOSTIC TESTS / BIOPSY

The classification of HPV-related tumours has evolved over the past decade in light of new and often key molecular discoveries that have influenced the categorisation of a number of these neoplasms.

NOTE: *The World Health Organization (WHO) recently published the fifth edition of Female Genital Tumours (IARC, 2020), containing some important revisions on HPV-related lesions.*

The evolution of the current morphological classification towards an integrated morphological-molecular classification will have an impact on diagnosis and treatment. In fact. HPV detection has dramatically changed the approach to epithelial tumours of the lower female genital tract.

Adenocarcinomas of the uterine cervix are now divided into HPV-associated and HPV-independent adenocarcinomas, with subsequent implications for prognosis.

Squamous tumours of the lower female genital tract are also divided into HPV-associated and HPV-independent, given important differences in their prognosis (Nicolás et al., 2019; Rodríguez-Carunchio et al., 2015). In this new classification, squamous precancerous lesions are primarily classified according to the two-tiered 'Lower Anogenital Squamous Terminology (LAST) Standardization project (Darragh et al., 2012), though the three-tiered intraepithelial neoplasia classification (CIN1/2/3) can still be used (Mills et al., 2020).:

According to the LAST classification, **LSIL** and **HSIL** are the preferred terms for both tissue and cytology specimens from the cervix on account of the improved reproducibility and enhanced biological relevance of the two-tiered LSIL/HSIL system versus the previous three-tiered CIN1/2/3 system, and the clarity of tissue-agnostic terminology given the shared pathogenesis of HPV-driven SIL across anogenital anatomical sites. HSIL may be subdivided into HSIL/CIN2 and HSIL/CIN3 because the first shows significantly higher regression rates, particularly in young women (aged <30) (Tainio et al., 2018).

Use of p16 immunostaining in squamous lesions classification:

Recent studies have put forward a reproducible histological surrogate for HPV infection (p16 immunostaining), allowing for classification of histological samples without HPV testing.

The tumour suppressor protein p16 is overexpressed in nearly all HSIL and some LSIL as a result of compensatory upregulation related to interference of the high-risk HPV oncoprotein E7 with pRb (Steenbergen et al., 2014). Application of p16 immunostaining is recommended by the LAST Standardization project in three specific contexts (Darragh et al., 2012):

- to aid in distinguishing HSIL from mimics of precancer (immature metaplasia, atrophy, reparative changes or tangential sectioning);
- to supplement morphological assessment for biopsy specimens interpreted as ≤LSIL which are at high risk for missed high-grade disease based on previous tests such as a Pap smear or HPV test; and
- to inform the diagnosis of HSIL (CIN2) versus LSIL (CIN1) in morphologically equivocal cases where the tissue positivity would support HSIL (CIN2).

Overapplication of p16 is discouraged in biopsy specimens with a clear HSIL (CIN3) or LSIL diagnosis.

A significant proportion of morphologically unequivocal LSIL show positive p16 staining which should not be reclassified as HSIL (CIN2) as it does not imply a significantly increased risk (Mills et al., 2015; Sagasta et al., 2016).

p16 has shown little value in differential diagnosis of LSIL versus morphological changes with no significance in the pathway of carcinogenesis. In such instances, HPV RNA in situ hybridisation can prevent overdiagnosis of LSIL in morphologically equivocal cases, though its use should be restricted to morphologically challenging lesions to avoid unnecessary costs (Mills et al., 2017).

NOTE: *Below you can find the essential and desirable criteria for the diagnosis of precancerous lesions.*

Essential criteria for SIL (CIN) diagnosis (Mills et al., 2020)

HSIL (CIN2)
Full-thickness atypia characterised by basaloid cells and mitotic activity extending into the upper half to upper two thirds of the epithelium, but with retained koilocytic change on the surface.
HSIL (CIN3)
Full-thickness atypia wherein the base of the lesion is often indistinguishable from the surface; mitotic activity may be identified throughout the epithelium; the upper portions of the epithelium show a significantly higher NC ratio than in LSIL and HSIL (CIN2).

An improved classification /diagnosis of HSIL to identify those that are more prone to progress can lead to better inform clinicians on the need for treatment of CIN2/3 lesions.

Did you know?

HPV E4 is a marker of a productive infection whereas p16, Ki-67 and host-cell DNA methylation are markers of transforming infections. Combinations of these markers are being explored to identify lesions requiring treatment (Leeman et al., 2020 RISCC; Vink et al., 2021 **RISCC**, 2023 **RISCC**).

ACTIVITY

Read the following statements and decide if they are TRUE or FALSE.

1. The current WHO guidelines recommend the classification of genital tumors in HPV-associated or HPV-independent.
2. p16 immunostaining may assist to elucidate the HPV involvement in precancerous lesions without needing separate HPV testing.
3. Use of p16 staining is encouraged also in clearly classified lesions.

The correct answers are:

1 True, 2 True, 3 False.

SUMMARY

- When performing a colposcopy examination, it is important to conduct both low- and high-powered examination of the TZ taking notes of the relevant image characteristics.
- Specific colposcopy patterns such as coarse punctuation, coarse mosaic or dense acetowhitening and inner border sign and ridge sign have demonstrated correct predictive accuracy for high-grade squamous intraepithelial lesions/cervical intraepithelial neoplasia grades 2–3 (HSIL/CIN2–3).
- The risk of underlying histological HSIL/CIN 2-3 can be estimated by assessing the information provided by the screening test (cytology and/or molecular test results such as HPV testing and genotyping) together with the colposcopy impression.
- Colposcopy requires adequate training and experience to attain proficiency and ensure competence throughout the procedure.
- Other visual techniques such as naked eye examination with acetic acid (VIA) or Lugol's iodine (VILI); and camera enhanced visual inspection are being used, mainly in resource-constrained settings, for the diagnosis and treatment of cervical lesions.
- AVE shows promise as an assistive technology for cervical image evaluation in the context of cervical cancer screening
- WHO guidelines classify cervical tumours as HPV-associated that include around 95% of the tumors or HPV-independent tumours (5%). p16, an indirect surrogate for HPV infections, can assist in dubious cases.

BIBLIOGRAPHY

Ahmed, S. R., Befano, B., Lemay, A., Egemen, D., Rodriguez, A. C., Angara, S., Desai, K., Jeronimo, J., Antani, S., Campos, N., Inturrisi, F., Perkins, R., Kreimer, A., Wentzensen, N., Herrero, R., Del Pino, M., Quint, W., de Sanjose, S., Schiffman, M., & Kalpathy-Cramer, J. (2023). Reproducible and clinically translatable deep neural networks for cervical screening. *Scientific Reports*, *13*(1), 21772. https://doi.org/10.1038/s41598-023-48721-1

Alan, M., Gunyeli, I., Gultekin, M., Sancl, M., & Yuce, K. (2020). Correlation of Swede score colposcopy scoring system and histopathological results in patients with high-risk HPV infection other than HPV16 and 18. *International Journal of Gynecological Cancer*, *30*(1), 35–50. https://doi.org/10.1136/ijgc-2019-000932

Alfonzo, E., Holmberg, E., Milsom, I., & Strander, B. (2022). Colposcopic assessment by Swedescore, evaluation of effectiveness in the Swedish screening programme: A cross-sectional study. *BJOG: An International Journal of Obstetrics and Gynaecology*, *129*(8), 1261–1267. https://doi.org/10.1111/1471-0528.17054

Almonte, M., Ferreccio, C., Luciani, S., Gonzales, M., Delgado, J. M., Santos, C., Alvarez, M., Cuzick, J., & Sasieni, P. (2015). Visual Inspection after Acetic Acid (VIA) is highly heterogeneous in primary cervical screening in Amazonian Peru. *PLoS ONE*, *10*(1). https://doi.org/10.1371/journal.pone.0115355

Bappa, L. A., & Yakasai, I. A. (2013). Colposcopy: The scientific basis. *Annals of African Medicine*, *12*(2), 86–89. https://doi.org/10.4103/1596-3519.112396

Bekkers, R. L., Nieuwenhof, H. P. van de, Neesham, D. E., Hendriks, J. H., Tan, J., & Quinn, M. A. (2008). Does experience in colposcopy improve identification of high grade abnormalities? *European Journal of Obstetrics and Gynecology and Reproductive Biology*. https://doi.org/10.1016/j.ejogrb.2008.07.007

Bornstein, J., Bentley, J., Bösze, P., Girardi, F., Haefner, H., Menton, M., Perrotta, M., Prendiville, W., Russell, P., Sideri, M., Strander, B., Tatti, S., Torne, A., & Walker, P. (2012). 2011 colposcopic terminology of the international federation for cervical pathology and colposcopy. *Obstetrics and Gynecology*, *120*, 66–172. https://doi.org/10.1097/AOG.0b013e318254f90c

Bornstein, J., Sideri, M., Tatti, S., Walker, P., Prendiville, W., & Haefner, H. K. (2012). 2011 terminology of the vulva of the international federation for cervical pathology and colposcopy. *Journal of Lower Genital Tract Disease*, *16*(3), 290–295. https://doi.org/10.1097/LGT.0b013e31825934c7

Brown, B. H., & Tidy, J. A. (2019). The diagnostic accuracy of colposcopy – A review of research methodology and impact on the outcomes of quality assurance. *European Journal of Obstetrics and Gynecology and Reproductive Biology*, *240*, 182–186. https://doi.org/10.1016/j.ejogrb.2019.07.003

Chartrand, G., Cheng, P. M., Vorontsov, E., Drozdzal, M., Turcotte, S., Pal, C. J., Kadoury, S., & Tang, A. (2017). Deep learning: A primer for radiologists. *Radiographics*, *37*(7), 2113–2131. https://doi.org/10.1148/rg.2017170077

Choi, R. Y., Coyner, A. S., Kalpathy-Cramer, J., Chiang, M. F., & Campbell, J. P. (2020). Introduction to Machine Learning, Neural Networks, and Deep Learning. *Translational Vision Science & Technology*, *9*(2), 14. https://doi.org/10.1167/tvst.9.2.14

Chrysostomou, A. C., Stylianou, D. C., Constantinidou, A., & Kostrikis, L. G. (2018). Cervical cancer screening programs in Europe: The transition towards HPV vaccination and population-based HPV testing. In *Viruses*. https://doi.org/10.3390/v10120729

Colgan, T. J., & Lickrish, G. M. (1990). The topography and invasive potential of cervical adenocarcinoma in situ, with and without associated squamous dysplasia. *Gynecologic Oncology*. https://doi.org/10.1016/0090-8258(90)90182-K

Darragh, T. M., Colgan, T. J., Cox, J. T., Heller, D. S., Henry, M. R., Luff, R. D., McCalmont, T., Nayar, R., Palefsky, J. M., Stoler, M. H., Wilkinson, E. J., Zaino, R. J., Wilbur, D. C., Wilbur, D. C., Darragh, T. M., Henry, M. R., McCalmont, T., Luff, R. D., Wilkinson, E. J., … Spires, S. (2012). The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: Background and Consensus Recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Archives of Pathology & Laboratory Medicine*, *136*(10), 1266–1297. https://doi.org/10.5858/arpa.LGT200570

de Sanjosé, S., Perkins, R. B., Campos, N., Inturrisi, F., Egemen, D., Befano, B., Rodriguez, A. C., Jerónimo, J., Cheung, L. C., Desai, K., Han, P., Novetsky, A. P., Ukwuani, A., Marcus, J., Ahmed, S. R., Wentzensen, N., Kalpathy-Cramer, J., Schiffman, M., & PAVE Study

Group. (2024). Design of the HPV-automated visual evaluation (PAVE) study: Validating a novel cervical screening strategy. *eLife*, *12*, RP91469. https://doi.org/10.7554/eLife.91469

Desai, K. T., Ajenifuja, K. O., Banjo, A., Adepiti, A. C., Novetsky, A. P., Sebag, C., Einstein, M. H., Oyinloye, T., Litwin, T., Horning, M. P., Olanrewaju, F., Oripelaye, M., Afolabi, E., Odujoko, O., Castle, P. E., Antani, S. K., Wilson, B., Hu, L., Mehanian, C., … Schiffman, M. (2020). Design and feasibility of a novel program of cervical screening in Nigeria: Self-sampled HPV testing paired with visual triage. *Infectious Agents and Cancer (Submitted)*.

Doshi-Velez, F., & Perlis, R. H. (2019). Evaluating Machine Learning Articles. In *JAMA - Journal of the American Medical Association* (Vol. 322, Issue 18, pp. 1777–1779). American Medical Association. https://doi.org/10.1001/jama.2019.17304

Egemen, D., Cheung, L. C., Chen, X., Demarco, M., Perkins, R. B., Kinney, W., Poitras, N., Befano, B., Locke, A., Guido, R. S., Wiser, A. L., Gage, J. C., Katki, H. A., Wentzensen, N., Castle, P. E., Schiffman, M., & Lorey, T. S. (2020). *Risk Estimates Supporting the 2019 ASCCP Risk-Based Management Consensus Guidelines*. *24*(2), 132–143. https://doi.org/10.1097/LGT.0000000000000529

Galaal, K., Bryant, A., Deane, K. H. O., Al-Khaduri, M., & Lopes, A. D. (2011). Interventions for reducing anxiety in women undergoing colposcopy. In *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.CD006013.pub3

Goldstein, A., Goldstein, L. S., Lipson, R., Bedell, S., Wang, J., Stamper, S. A., Brenner, G., Goldstein, G. R., O'Keefe, K. D., O'Keefe, S. C., O'Keefe, M., O'Keefe, T., Goldstein, A. R., & Zhao, A. (2020). Assessing the feasibility of a rapid, high-volume cervical cancer screening programme using HPV self-sampling and digital colposcopy in rural regions of Yunnan, China. *BMJ Open*, *10*(3). https://doi.org/10.1136/bmjopen-2019-035153

Gulshan, V., Peng, L., Coram, M., Stumpe, M. C., Wu, D., Narayanaswamy, A., Venugopalan, S., Widner, K., Madams, T., Cuadros, J., Kim, R., Raman, R., Nelson, P. C., Mega, J. L., & Webster, D. R. (2016). Development and validation of a deep learning algorithm for detection of diabetic retinopathy in retinal fundus photographs. *JAMA - Journal of the American Medical Association*, *316*(22), 2402–2410. https://doi.org/10.1001/jama.2016.17216

Hu, L., Bell, D., Antani, S., Xue, Z., Yu, K., Horning, M. P., Gachuhi, N., Wilson, B., Jaiswal, M. S., Befano, B., Long, L. R., Herrero, R., Einstein, M. H., Burk, R. D., Demarco, M., Gage, J. C., Rodriguez, A. C., Wentzensen, N., & Schiffman, M. (2019). An Observational Study of Deep Learning and Automated Evaluation of Cervical Images for Cancer Screening. *JNCI: Journal of the National Cancer Institute*, *111*(9), 923–932. https://doi.org/10.1093/jnci/djy225

Hu, L., Mwanahamuntu, M. H., Sahasrabuddhe, V. V., Barrett, C., Horning, M. P., Shah, I., Laverriere, Z., Banik, D., Ji, Y., Shibemba, A. L., Chisele, S., Munalula, M. K., Kaunga, F., Musonda, F., Malyangu, E., Hariharan, K. M., & Parham, G. P. (2024). Internal validation of Automated Visual Evaluation (AVE) on smartphone images for cervical cancer screening in a prospective study in Zambia. *Cancer Medicine*, *13*(11), e7355. https://doi.org/10.1002/cam4.7355

Huchko, M. J., Sneden, J., Sawaya, G., Smith-Mccune, K., Maloba, M., Abdulrahim, N., Bukusi, E. A., & Cohen, C. R. (2015). Accuracy of visual inspection with acetic acid to detect cervical cancer precursors among HIV-infected women in Kenya. *International Journal of Cancer*, *136*(2), 392–398. https://doi.org/10.1002/ijc.28996

IARC. (2022). *Cervical Cancer Screening* (Vol. 18). IARC Press. http://www.iarc.fr/en/publications/pdfs-online/prev/handbook10/

Indraccolo, U., Palombino, K., & Greco, P. (2009). Anaphylactic-like reaction to lugol solution during colposcopy. *Southern Medical Journal*. https://doi.org/10.1097/SMJ.0b013e31818894f4

International Agency for Research of Cancer (IARC) (Ed.). (2020). *WHO Classification of Tumours Editorial Board. Female Genital Tumours* (5th ed.).

Kahramanoglu, I., Demirkiran, F., Turan, H., Bese, T., Yilmaz, N., Ilvan, S., & Arvas, M. (2019). The use of colposcopic punch biopsy in the management of abnormal cervical cytology: A 5-year retrospective audit. *Journal of Obstetrics and Gynaecology: The Journal of the Institute of Obstetrics and Gynaecology*, *39*(1), 110–114. https://doi.org/10.1080/01443615.2018.1468740

Khan, M. J., Werner, C. L., Darragh, T. M., Guido, R. S., Mathews, C., Moscicki, A. B., Mitchell, M. M., Schiffman, M., Wentzensen, N., Massad, L. S., Mayeaux, E. J., Waxman, A. G., Conageski, C., Einstein, M. H., & Huh, W. K. (2017). ASCCP Colposcopy Standards:

Role of Colposcopy, Benefits, Potential Harms, and Terminology for Colposcopic Practice. *Journal of Lower Genital Tract Disease*, *21*(4), 223–229. https://doi.org/10.1097/LGT.0000000000000338

Kim, S. I., Kim, S. J., Suh, D. H., Kim, K., No, J. H., & Kim, Y. B. (2020). Pathologic discrepancies between colposcopy-directed biopsy and loop electrosurgical excision procedure of the uterine cervix in women with cytologic high-grade squamous intraepithelial lesions. *Journal of Gynecologic Oncology*, *31*(2), e13. https://doi.org/10.3802/jgo.2020.31.e13

Lalande, E., Clarke, H., Undurraga, M., Nguyen, V. Q. H., Jaksic, C., Goffin, F., Arbyn, M., Jeronimo, J., Tille, J.-C., Saiji, E., Vassilakos, P., & Petignat, P. (2024). Knowledge of cytology results affects the performance of colposcopy: A crossover study. *BMC Women's Health*, *24*(1), 189. https://doi.org/10.1186/s12905-024-03025-y

Leeman, A., Jenkins, D., Del Pino, M., Ordi, J., Torné, A., Doorbar, J., Meijer, C. J. L. M., Van Kemenade, F. J., & Quint, W. G. V. (2020). Expression of p16 and HPV E4 on biopsy samples and methylation of FAM19A4 and miR124-2 on cervical cytology samples in the classification of cervical squamous intraepithelial lesions. *Cancer Medicine*, *9*(7), 2454–2461. https://doi.org/10.1002/cam4.2855

Li, Y., Duan, X., Sui, L., Xu, F., Xu, S., Zhang, H., & Xu, C. (2017). Closer to a Uniform Language in Colposcopy: Study on the Potential Application of 2011 International Federation for Cervical Pathology and Colposcopy Terminology in Clinical Practice. *BioMed Research International*, *2017*. https://doi.org/10.1155/2017/8984516

Liu, Y., Chen, P. H. C., Krause, J., & Peng, L. (2019). How to Read Articles That Use Machine Learning: Users' Guides to the Medical Literature. In *JAMA - Journal of the American Medical Association* (Vol. 322, Issue 18, pp. 1806–1816). American Medical Association. https://doi.org/10.1001/jama.2019.16489

Mayeaux, E., & Cox, J. (2013). *Colposcopia. Texto y atlas* (W. Kluwer and L.W. Wilkins, Ed.; 3rd editio).

Mills, A. M., Carrilho, C., & Focci, G. (2020). Squamous intraepithelial lesions of the uterine cervix. In *Female Genital Tumours.* (5th ed., Vol. 4). International Agency for Research on Cancer. https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/Female-Genital-Tumours-2020

Mills, A. M., Dirks, D. C., Poulter, M. D., Mills, S. E., & Stoler, M. H. (2017). HR-HPV E6/E7 mRNA in situ hybridization. Validation against PCR, DNA in situ hybridization, and p16 immunohistochemistry in 102 samples of cervical, vulvar, anal, and head and neck neoplasia. *American Journal of Surgical Pathology*, *41*(5), 607–615. https://doi.org/10.1097/PAS.0000000000000800

Mills, A. M., Paquette, C., Castle, P. E., & Stoler, M. H. (2015). Risk stratification by p16 immunostaining of CIN1 biopsies: A retrospective study of patients from the quadrivalent HPV vaccine trials. *American Journal of Surgical Pathology*, *39*(5), 611–617. https://doi.org/10.1097/PAS.0000000000000374

Mitchell, M. F., Schottenfeld, D., Tortolero-Luna, G., Cantor, S. B., & Richards-Kortum, R. (1998). Colposcopy for the diagnosis of squamous intraepithelial lesions: A meta-analysis. *Obstetrics and Gynecology*, *91*(4), 626–631. https://doi.org/10.1016/S0029-7844(98)00006-4

Nevala, K. (2017). *The LEARNING MACHINE Primer* (SAS Best Practices). SAS Institute Inc.

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

O'Connor, M., O'Brien, K., Waller, J., Gallagher, P., D'Arcy, T., Flannelly, G., Martin, C. M., McRae, J., Prendiville, W., Ruttle, C., White, C., Pilkington, L., O'Leary, J. J., & Sharp, L. (2017). Physical after-effects of colposcopy and related procedures, and their inter-relationship with psychological distress: A longitudinal survey. *BJOG: An International Journal of Obstetrics and Gynaecology*. https://doi.org/10.1111/1471-0528.14671

Pal, A., Xue, Z., Befano, B., Rodriguez, A. C., Long, L. R., Schiffman, M., & Antani, S. (2021). Deep Metric Learning for Cervical Image Classification. *IEEE Access: Practical Innovations, Open Solutions*, *9*, 53266–53275. https://doi.org/10.1109/access.2021.3069346

Parham, G. P., Egemen, D., Befano, B., Mwanahamuntu, M. H., Rodriguez, A. C., Antani, S., Chisele, S., Munalula, M. K., Kaunga, F., Musonda, F., Malyangu, E., Shibemba, A. L., de Sanjose, S., Schiffman, M., & Sahasrabuddhe, V. V. (2023). Validation in Zambia of a cervical screening strategy including HPV genotyping and artificial

intelligence (AI)-based automated visual evaluation. *Infectious Agents and Cancer*, *18*(1), 61. https://doi.org/10.1186/s13027-023-00536-5

Parham, G. P., Mwanahamuntu, M. H., Kapambwe, S., Muwonge, R., Bateman, A. C., Blevins, M., Chibwesha, C. J., Pfaendler, K. S., Mudenda, V., Shibemba, A. L., Chisele, S., Mkumba, G., Vwalika, B., Hicks, M. L., Vermund, S. H., Chi, B. H., Stringer, J. S. A., Sankaranarayanan, R., & Sahasrabuddhe, V. V. (2015). Population-level scale-up of cervical cancer prevention services in a low-resource setting: Development, implementation, and evaluation of the cervical cancer prevention program in Zambia. *PLoS ONE*, *10*(4). https://doi.org/10.1371/journal.pone.0122169

Perkins, R. B., Guido, R. S., Castle, P. E., Chelmow, D., Einstein, M. H., Garcia, F., Huh, W. K., Kim, J. J., Moscicki, A., Nayar, R., Saraiya, M., & George, F. (2020). *2019 ASCCP Risk-Based Management Consensus Guidelines for Abnormal Cervical Cancer Screening Tests and Cancer Precursors*. *24*(2), 102–131. https://doi.org/10.1097/LGT.0000000000000525

Petousis, S., Christidis, P., Margioula-Siarkou, C., Sparangis, N., Athanasiadis, A., & Kalogiannidis, I. (2018). Discrepancy between colposcopy, punch biopsy and final histology of cone specimen: A prospective study. *Archives of Gynecology and Obstetrics*, *297*(5), 1271–1275. https://doi.org/10.1007/s00404-018-4714-8

Porras, C., Wentzensen, N., Rodríguez, A. C., Morales, J., Burk, R. D., Alfaro, M., Hutchinson, M., Herrero, R., Hildesheim, A., Sherman, M. E., Wacholder, S., Solomon, D., & Schiffman, M. (2012). Switch from Cytology-based to HPV-based Cervical Screening: Implications for Colposcopy. *International Journal of Cancer. Journal International Du Cancer*, *130*(8), 1879–1887. https://doi.org/10.1002/ijc.26194

Prendiville, W., & Sankaranarayanan, R. (2017). Colposcopy and Treatment of Cervical Precancer. In *IARC Technical Report* (Vol. 45, Issue 45). IARC Technical Publications; 45.

Ranga, R., Rai, S., Kumari, A., Mathur, S., Kriplani, A., Mahey, R., Agarwal, N., Kachhawa, G., Vanamail, P., & Bhatla, N. (2017). A Comparison of the Strength of Association of Reid Colposcopic Index and Swede Score with Cervical Histology. *Journal of Lower Genital Tract Disease*, *21*(1), 55–58. https://doi.org/10.1097/LGT.0000000000000278

Reid, R., & Campion, M. (1989). Lesiones del cuello uterino relacionadas con papilomavirus humano: Biología y características colposcópica. *Clin Obstet Ginecol*, *1*, 151–173.

Reid, R., & Scalzi, P. (1985). Genital warts and cervical cancer: VII. An improved colposcopic index for differentiating benign papillomaviral infections from high-grade cervical intraepithelial neoplasia. *American Journal of Obstetrics and Gynecology*, *153*(6), 611–618. https://doi.org/10.1016/S0002-9378(85)80244-1

Rodríguez-Carunchio, L., Soveral, I., Steenbergen, R. D. M., Torné, A., Martinez, S., Fusté, P., Pahisa, J., Marimon, L., Ordi, J., & Del Pino, M. (2015). HPV-negative carcinoma of the uterine cervix: A distinct type of cervical cancer with poor prognosis. *BJOG: An International Journal of Obstetrics and Gynaecology*, *122*(1), 119–127. https://doi.org/10.1111/1471-0528.13071

Sagasta, A., Castillo, P., Saco, A., Torné, A., Esteve, R., Marimon, L., Ordi, J., & del Pino, M. (2016). P16 staining has limited value in predicting the outcome of histological low-grade squamous intraepithelial lesions of the cervix. *Modern Pathology*, *29*(1), 51–59. https://doi.org/10.1038/modpathol.2015.126

Sankaranarayanan, R., & Wealey, R. (2003). A Practical Manual on Visual Screening for Cervical Neoplasia. *IARC Technical Publication No. 41*, *41*.

Schiffman, M., Yu, K., Zuna, R., Terence Dunn, S., Zhang, H., Walker, J., Gold, M., Hyun, N., Rydzak, G., Katki, H. A., & Wentzensen, N. (2017). Proof-of-principle study of a novel cervical screening and triage strategy: Computer-analyzed cytology to decide which HPV-positive women are likely to have ≥CIN2. *International Journal of Cancer*, *140*(3), 718–725. https://doi.org/10.1002/ijc.30456

Sharp, L., Cotton, S., Carsin, A. E., Gray, N., Thornton, A., Cruickshank, M., & Little, J. (2013). Factors associated with psychological distress following colposcopy among women with low-grade abnormal cervical cytology: A prospective study within the Trial of Management of Borderline and Other Low-grade Abnormal smears (TOMBOLA). *Psycho-Oncology*, *22*(2), 368–380. https://doi.org/10.1002/pon.2097

Sharp, L., Cotton, S., Gray, N., Avis, M., Russell, I., Walker, L., Waugh, N., Whynes, D., Woolley, C., Thornton, A., Smart, L., Cruickshank, M., & Little, J. (2011). Long-term psychosocial impact of alternative management policies in women with low-

grade abnormal cervical cytology referred for colposcopy: A randomised controlled trial. *British Journal of Cancer*, *104*(2), 255–264. https://doi.org/10.1038/sj.bjc.6606042

Shastri, S. S., Mittra, I., Mishra, G. A., Gupta, S., Dikshit, R., Singh, S., & Badwe, R. A. (2014). Effect of VIA screening by primary health workers: Randomized controlled study in Mumbai, India. *Journal of the National Cancer Institute*, *106*(3). https://doi.org/10.1093/jnci/dju009

Stafl, A. (1976). New nomenclature for colposcopy.R eportof the committee on terminology. *Obstetrics & Gynecology*, *48*(1), 123-124.

Stafl, A., & Wilbanks, G. (1991). An international terminology of colposcopy: Report of the Nomenclature Committee of the International Federation of Cervical Pathology and Colposcopy. *Obstetrics & Gynecology*, *77*(2), 313–314.

Steenbergen, R. D. M., Snijders, P. J. F., Heideman, D. a M., & Meijer, C. J. L. M. (2014). Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. *Nature Reviews. Cancer*, *14*(6), 395–405. https://doi.org/10.1038/nrc3728

Stuebs, F. A., Schulmeyer, C. E., Mehlhorn, G., Gass, P., Kehl, S., Renner, S. K., Renner, S. P., Geppert, C., Adler, W., Hartmann, A., Beckmann, M. W., & Koch, M. C. (2019). Accuracy of colposcopy-directed biopsy in detecting early cervical neoplasia: A retrospective study. *Archives of Gynecology and Obstetrics*, *299*(2), 525–532. https://doi.org/10.1007/s00404-018-4953-8

Tainio, K., Athanasiou, A., Tikkinen, K. A. O., Aaltonen, R., Cárdenas, J., Hernándes, Glazer-Livson, S., Jakobsson, M., Joronen, K., Kiviharju, M., Louvanto, K., Oksjoki, S., Tähtinen, R., Virtanen, S., Nieminen, P., Kyrgiou, M., & Kalliala, I. (2018). Clinical course of untreated cervical intraepithelial neoplasia grade 2 under active surveillance: Systematic review and meta-analysis. In *BMJ (Online)* (Vol. 360). https://doi.org/10.1136/bmj.k499

Torné, A., del Pino, M., Andía, D., Castro, M., de la Fuente, J., Hernández, J., López, J., Martínez, J., Medina, N., Quílez, J., Ramírez, M., & Ramón y Cajal, J. (2018). *Colposcopy guidelines. Standards of quality* (A. Torné & M. del Pino, Eds.). Publicaciones AEPCC. 2018.

Underwood, M., Arbyn, M., Parry-Smith, W., De Bellis-Ayres, S., Todd, R., Redman, C. W. E., & Moss, E. L. (2012). Accuracy of colposcopy-directed punch biopsies: A systematic review and meta-analysis. In *BJOG: An International Journal of Obstetrics and Gynaecology*. https://doi.org/10.1111/j.1471-0528.2012.03444.x

Vercellino, G. F., Erdemoglu, E., Chiantera, V., Vasiljeva, K., Drechsler, I., Cichon, G., Schneider, A., & Böhmer, G. (2013). Validity of the colposcopic criteria inner border sign, ridge sign, and rag sign for detection of high-grade cervical intraepithelial neoplasia. *Obstetrics and Gynecology*, *121*(3), 624–631. https://doi.org/10.1097/AOG.0b013e3182835831

Vink, F. J., Dick, S., Heideman, D. A. M., De Strooper, L. M. A., Steenbergen, R. D. M., Lissenberg-Witte, B. L. W., DNTP group, Floore, A., Bonde, J. H., Valenčak, A. O., Poljak, M., Petry, K. U., Hillemanns, P., van Trommel, N. E., Berkhof, J., Bleeker, M. C. G., & Meijer, C. J. L. M. (2021). Classification of high-grade CIN by p16ink4a , Ki-67, HPV E4 and FAM19A4/miR124-2 methylation status demonstrates considerable heterogeneity with potential consequences for management. *International Journal of Cancer*, *149*(3), 707–716. https://doi.org/10.1002/ijc.33566

Vink, F. J., Meijer, C. J. L. M., Hesselink, A. T., Floore, A. N., Lissenberg-Witte, B. I., Bonde, J. H., Pedersen, H., Cuschieri, K., Bhatia, R., Poljak, M., Oštrbenk Valenčak, A., Hillemanns, P., Quint, W. G. V., Del Pino, M., Kenter, G. G., Steenbergen, R. D. M., Heideman, D. A. M., & Bleeker, M. C. G. (2023). *FAM19A4/miR124-2* Methylation Testing and Human Papillomavirus (HPV) 16/18 Genotyping in HPV-Positive Women Under the Age of 30 Years. *Clinical Infectious Diseases*, *76*(3), e827–e834. https://doi.org/10.1093/cid/ciac433

Walker, P., Dexeus, S., Palo, G. De, Barrasso, R., Campion, M., Girardi, F., Jakob, C., & Roy, M. (2003). International Terminology of Colposcopy: An Updated Report From the International Federation for Cervical Pathology and Colposcopy. *Obstetrics & Gynecology*, *101*(1), 175–177. https://doi.org/10.1097/00006250-200301000-00032

Walter, S. D. (1999). Estimation of test sensitivity and specificity when disease confirmation is limited to positive results. *Epidemiology*. https://doi.org/10.1097/00001648-199901000-00012

Wentzensen, N., Lahrmann, B., Clarke, M. A., Kinney, W., Tokugawa, D., Poitras, N., Locke, A., Bartels, L., Krauthoff, A., Walker, J., Zuna, R., Grewal, K. K., Goldhoff, P. E., Kingery,

J. D., Castle, P. E., Schiffman, M., Lorey, T. S., & Grabe, N. (2020). Accuracy and Efficiency of Deep-Learning–Based Automation of Dual Stain Cytology in Cervical Cancer Screening. *JNCI: Journal of the National Cancer Institute*, *113*(November 2019), 1–8. https://doi.org/10.1093/jnci/djaa066

Wentzensen, N., Schiffman, M., Silver, M. I., Khan, M. J., Perkins, R. B., Smith, K. M., Gage, J. C., Gold, M. A., Conageski, C., Einstein, M. H., Mayeaux, E. J., Waxman, A. G., Huh, W. K., & Massad, L. S. (2017). ASCCP Colposcopy Standards: Risk-Based Colposcopy Practice. *Journal of Lower Genital Tract Disease*, *21*(4), 230–234. https://doi.org/10.1097/LGT.0000000000000334

Wentzensen, N., Walker, J., Smith, K., Gold, M. A., Zuna, R., Massad, L. S., Liu, A., Silver, M. I., Dunn, S. T., & Schiffman, M. (2018). A prospective study of risk-based colposcopy demonstrates improved detection of cervical precancers. *American Journal of Obstetrics and Gynecology*, *218*(6), 604.e1-604.e8. https://doi.org/10.1016/j.ajog.2018.02.009

World Health Organization. (2021). *WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention, second edition: Use of mRNA tests for human papillomavirus (HPV)*. World Health Organization. https://apps.who.int/iris/handle/10665/350652

World Health Organization, Geneva, Switerland. (2019). *WHO guidelines for the use of thermal ablation for cervical pre-cancer lesions*. World Health Organization. http://www.who.int/reproductivehealth/publications/thermal-ablation-for-cervical-pre-cancer-lesions/en/

World Health Organization Regional Office for South-East Asia. (2017). *Training of health staff in VIA, HPV detection test and cryotherapy- Trainees' handbook*. World Health Organization. Regional Office for South-East Asia. https://iris.who.int/handle/10665/259001

Wu, T., Lucas, E., Zhao, F., Basu, P., & Qiao, Y. (2024). Artificial intelligence strengthenes cervical cancer screening – present and future. *Cancer Biology & Medicine*, 1–16. https://doi.org/10.20892/j.issn.2095-3941.2024.0198

Xue, Z., Novetsky, A. P., Einstein, M. H., Marcus, J. Z., Befano, B., Guo, P., Demarco, M., Wentzensen, N., Long, L. R., Schiffman, M., & Antani, S. (2020). A demonstration of automated visual evaluation (AVE) of cervical images taken with a smartphone

camera. *International Journal of Cancer*, ijc.33029. https://doi.org/10.1002/ijc.33029

Yang, F., Zamzmi, G., Angara, S., Rajaraman, S., Aquilina, A., Xue, Z., Jaeger, S., Papagiannakis, E., & Antani, S. K. (2023). Assessing Inter-Annotator Agreement for Medical Image Segmentation. *IEEE Access*: *Practical Innovations, Open Solutions*, *11*, 21300–21312. https://doi.org/10.1109/access.2023.3249759