



Risk stratification of HPV-positive results using extended genotyping and cytology: Data from the baseline phase of the Onclarity trial

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HIGHLIGHTS

- Triage of HPV-positive women is critical for optimal patient management.
- For primary HPV screening, in addition to cytology, approved triage options include extended genotyping and dual-stain cytology.
- Extended genotyping with or without cytology can stratify risk and provide a range of thresholds to guide patient management.
- Extended genotyping resulted in a good balance of disease detection and risk stratification.
- p16/Ki-67 dual-stain cytology performs similarly to extended genotyping, especially when combined with HPV16/18 genotyping.

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ABSTRACT

Background. Optimizing the balance between colposcopy referrals and the detection of high-grade cervical intraepithelial neoplasia (CIN) during cervical cancer screening requires robust triage strategies. We evaluated the performance of extended HPV genotyping (xGT), in combination with cytology triage, and compared it to previously published performance data for high-grade CIN detection by HPV16/18 primary screening in combination with p16/Ki-67 dual staining (DS).

Methods and materials. The baseline phase of the Onclarity trial enrolled 33,858 individuals, yielding 2978 HPV-positive participants. Risk values for $\geq$ CIN3 were determined for Onclarity result groupings corresponding to HPV16, not HPV16 but HPV18 or 31, not HPV16/18/31 but HPV33/58 or 52, not HPV16/18/31/33/58/52 but HPV35/39/68 or 45 or 51 or 56/59/66 across all cytology categories. Published data from the IMPACT trial for HPV16/18 plus DS was utilized as a comparator during ROC analyses.

Results. There were 163 $\geq$ CIN3 cases detected. The $\geq$ CIN3 risk stratum hierarchy (% risk of $\geq$ CIN3) that resulted from this analysis included: $>$ LSIL (39.4%); HPV16, $\leq$ LSIL (13.3%); HPV18/31, $\leq$ LSIL (5.9%); HPV33/58/52/45, ASC-US/LSIL (2.4%); HPV33/58/52, NILM (2.1%); HPV35/39/68/51/56/59/66, ASC-US/LSIL (0.9%); and HPV45/35/39/68/51/56/59/66, NILM (0.6%). For $\geq$ CIN3 ROC analysis, the optimal cutoff for sensitivity versus specificity was approximated between not HPV16 but HPV18 or 31, any cytology ($\geq$ CIN3 sensitivity = 85.9% and colposcopy-to- $\geq$ CIN3 = 7.4) and not HPV16/18/31 but HPV33/58/52, NILM ($\geq$ CIN3 sensitivity = 94.5% and colposcopy-to- $\geq$ CIN3 = 10.8). HPV16/18 with DS triage showed a sensitivity of 94.3%, with a colposcopy-to- $\geq$ CIN3 ratio of 11.4.

Conclusions. xGT performed similarly compared to HPV primary screening plus DS for detection of high-grade CIN. xGT provides results that stratify risk in a flexible and reliable manner for colposcopy risk thresholds set by different guidelines or organizations.

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1. Introduction

Almost all cases of cervical cancer are associated with persistent infection by essentially one of 13–14 high-risk human papillomavirus (HPV) genotypes [1,2]. Cervical cancer screening via primary HPV testing continues to gain consensus in many countries as the preferred

approach [3]. HPV testing offers better sensitivity for the detection of cervical intraepithelial neoplasia grades 2 or 3 and greater ($\geq$ CIN2 and $\geq$ CIN3, respectively) compared to cytology-based screening alone or with HPV-based triage for ASC-US (atypical squamous cells-undetermined significance) [4–7]. Importantly, a negative HPV test offers much better assurance that high-grade cervical disease is not present, allowing for a five-year return for routine screening instead of a three-year return, which is currently suggested for cytology-based screening, alone [8–11]. A few countries continue to employ cotesting (some combination of cytology and HPV testing) and have not transitioned solely to HPV primary screening [12]. However, cotesting leads to a relatively high number of screening tests and colposcopy referrals, and a higher cost, [4,8,13] compared to HPV primary screening, with no significant increase in assurance, over HPV primary screening, that a negative screening result reflects the absence of disease [4,10,14].

Although the large majority of $\geq$ CIN2 and $\geq$ CIN3 cases are associated with an HPV infection, [15,16] most HPV infections are transient and clear without leading to high-grade cervical disease [17,18]. Therefore, it is not clinically beneficial, or feasible, to send all HPV-positive women to immediate colposcopy and HPV primary screening requires effective triage to better identify those at greatest risk for developing high-grade cervical disease [3]. Due to its availability in most countries and relatively high specificity, [11] cytology-based testing was the first accepted triage approach for minimizing the number of colposcopies required for the detection of high-grade cervical disease [19].

In order to provide both high diagnostic accuracy and effective patient management, while reducing complexity for both health care providers and patients, the ASCCP revised their US cervical cancer screening guidelines in 2019. The new guidelines outline decision making following screening results that are based on risk for high-grade cervical disease with an underlying concept of “equal management for equal risk.” They also employed clinical action thresholds, based on the risk for $\geq$ CIN3, to help determine whether a woman should go to immediate colposcopy and possibly clinical treatment, or return for follow-up testing at some time interval (one, three, or five years) [20,21].

Individual identification of HPV16 and 18, the two genotypes associated with 60 to 70% of all cervical cancer, [16] can improve risk stratification for high-grade cervical disease and justify an immediate colposcopy without an inflated colposcopy-to-disease detected ratio [19]. A positive HPV result for any of the “other 12” HPV genotypes (HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), has traditionally been managed based on cytology triage [19,22]. However, new clinically validated triage approaches are now available to improve risk stratification for primary HPV results that encompass any of the genotypes within the “other 12” grouping. One approach, extended genotyping, involves identification of individual HPV genotypes, or groups of HPV genotypes, beyond HPV16 and 18 [3,23–25]. This results in $\geq$ CIN2/3 risk discrimination among the 12, non-HPV16/18 high-risk genotypes in order to more accurately determine the best course of clinical action during and following initial screening. For example, results from the baseline phase of the Onclarity trial demonstrated that HPV31 carries a high enough immediate risk for $\geq$ CIN3 to justify a colposcopy without additional testing [26]. Similar conclusions can be drawn for other genotypes, including HPV33, based on large US and non-US cervical cancer screening trials [24]. Alternatively, work by Wright et al. (2019) from the baseline phase of the Onclarity trial, and from other studies, demonstrates that some “high-risk” genotypes, including HPV35, 39, 51, 56, 59, 66, and 69, actually have a low enough $\geq$ CIN3 risk when associated with normal or equivocal cytology results, to warrant a return to follow-up testing in lieu of an immediate colposcopy [27]. Thus, extended genotyping can provide information that can justifiably include or exclude women from immediate colposcopy, depending on their specific HPV genotype, and achieve patient management that is optimized to cytology-based testing or cotesting.

Another promising triage approach involves the utilization of immunocytochemistry to detect overexpression of the tumor suppressor, p16 and the cell proliferation marker, Ki-67, on liquid-based cytology (LBC) specimens [3,28–30]. Dual staining (DS) for coexpression of these protein markers in the same cell is strongly associated with transforming HPV infections that cause cell cycle dysregulation and cervical neoplasia [31]. Previously, DS testing has demonstrated improved sensitivity (with no sacrifice in specificity) compared to cytology in cross-sectional analysis for the detection of $\geq$ CIN3 in HPV-positive women [28]. Other approaches are being investigated for effective triage, including methylation assays, which would not require a speculum exam for triage, and could be performed in a self-collection setting [32,33].

This study utilized data from the baseline phase of the Onclarity trial to assess performance of extended genotyping, combined with cytology, for detection of $\geq$ CIN2 and $\geq$ CIN3 in women undergoing routine cervical cancer screening. The objective was to establish actionable $\geq$ CIN2/3 risk bands associated with combined extended genotyping and cytology results and determine the corresponding clinical performance characteristics (e.g., sensitivity, ratio of colposcopies-to-disease detected) associated with the relevant risk bands. A secondary objective of this study was to evaluate the diagnostic performance of extended genotyping with combined cytology versus primary HPV16/18 utilizing DS as a triage for the other 12 genotypes. In order to achieve this second goal, we utilized published data from the Improving Primary screening And Colposcopy Triage (IMPACT) cervical cancer screening trial, which previously evaluated the clinical performance of DS as a triage approach during routine cervical cancer screening [28,34]. The similarity of the clinical trial design, demographics, HPV test positivity and cytology and histology results from both the Onclarity and IMPACT trials facilitated this analysis.

2. Methods and materials

2.1. LBC and HPV testing

LBC for this study was conducted as described previously [15]. Briefly, cervical samples were collected using an endocervical broom or brush/spatula, according to manufacturer's instructions, and collected in a BD SurePath™ vial (“SurePath;” Becton, Dickinson and Company, BD Life Sciences – Integrated Diagnostic Solutions, Burlington, NC). Additional processing utilized the BD PrepMate™ and BD PrepStain™ systems (Becton, Dickinson and Company, BD Life Sciences – Integrated Diagnostic Solutions, Burlington, NC); cytological evaluation and results were reported according to the 2001 Bethesda System.

HPV testing was performed as described, previously, [15] using the BD Onclarity™ assay (“Onclarity;” Becton, Dickinson and Company, BD Life Sciences – Integrated Diagnostic Solutions, Sparks, MD) from a SurePath specimen aliquot. Onclarity is a qualitative PCR-based assay that uses fluorescent probe technology to detect E6 and E7 DNA regions of the HPV genome. Onclarity simultaneously detects 14 high-risk HPV types with individual genotyping for the E6/7 coding regions of HPV16, 18, 31, 45, 51, 52, 33/58 (pooled result), 35/39/68 (pooled result), and 56/59/66 (pooled result).

2.2. Histology and disease ascertainment

For Onclarity results, histological methods and assessment were performed as described previously [15]. For this study, $\geq$ CIN2 lesion detection (CIN2, CIN3, adenocarcinoma in situ, and invasive cervical cancer), following biopsy, was the primary study endpoint. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions (LAST) guidelines for adjudication of CIN2 cases was also utilized; p16^{INK4A} (p16)-assisted immunohistochemistry was used to supplement hematoxylin and eosin (H&E) histopathology.

Here, LAST terminology incorporates Bethesda terminology and is simplified to the CIN terminology; thus, CIN1 indicates low-grade squamous intraepithelial lesion (LSIL), CIN2 here indicates high-grade squamous intraepithelial lesion (HSIL) (CIN2), and CIN3 indicates HSIL (CIN3) [35]. All H&E-stained biopsies and endocervical curettage samples were reviewed, initially, by two pathologists who had access to the subject's age, but were otherwise masked to all other information. When two of three pathologists provided concordant diagnoses, using eight disease criteria, consensus was reached: unsatisfactory, negative (including no significant pathological findings, reactive or inflammatory processes, atypical squamous cell glandular changes, or squamous metaplasia), CIN1, CIN2, CIN3, adenocarcinoma in situ, squamous cell carcinoma (SCC), and adenocarcinoma and adenosquamous carcinoma; the latter two were compiled into one category. Discordance between (all three) diagnoses for a specimen prompted a review by all three pathologists, together, to achieve a consensus diagnosis.

2.3. Data analysis

HPV-positive subjects were split into four groups by genotype, according to prior published risk data: [26] HPV16, not HPV16 but HPV18 or 31, not HPV16/18/31 but HPV33/58 or 52, not HPV16/18/31/33/58/52 but HPV35/39/68 or 45 or 51 or 56/59/66. In addition, cytology results were categorized into three groups, from high to low risk of CIN disease: >LSIL, ASC-US/LSIL, and NILM (negative for intraepithelial lesions or malignancies). A total of seven separate strata, ordered from high to low risk, were obtained at the end, by combining several of the 12 genotype and cytology combinations: >LSIL combined into one group regardless of genotype; HPV16 combined into one group regardless of cytology; HPV18/31 combined into one group regardless of cytology. Baseline risk of disease was estimated with 95% confidence intervals by the Wilson score method. For strategy evaluation, HPV45 ASC-US/LSIL subjects were upgraded to the same group as HPV33/58/52 ASC-US/LSIL due to the previously published importance of HPV45 in cervical cancer cases [36–38]. The two studies, IMPACT and Onclarity, had sufficient ≥CIN3 cases to allow for sensitivity estimation with a margin of error <3.5%.

This report is aligned with STARD reporting standards. The study protocol received approval from institutional review boards at each study site; written, informed consent was obtained prior to any trial-related activities. The principles set forth by the Declaration of Helsinki and Good Clinical Practice were observed during the conduct of this study [15].

3. Results

3.1. Participant reconciliation and inclusion for the Onclarity and IMPACT trials

The population of enrolled participants at the baseline phase of the Onclarity trial included 33,858 individuals; the demographic information and prevalence of cytology, HPV results, and cervical disease has been described, previously [15]. Here, only individuals ≥25 years of age were included. There were 29,513 individuals in that age group with evaluable cytology and valid HPV results. Overall, there were 3748 HPV-positive women with evaluable cytology (Table S1), and 2976 HPV-positive individuals had evaluable cytology and satisfactory histology (Table S2 and Fig. S1).

Below, data are utilized from previously published results from the IMPACT cervical cancer screening trial, which had a study population and design that is similar to the Onclarity trial (Table 1 and Supplemental Material). The IMPACT trial initially enrolled 35,263 individuals; of those, 29,664 women had HPV-negative or invalid HPV results, leaving 5250. After 564 women were excluded due to an incomplete colposcopy-to-biopsy visit, 4934 women remained. From that group, 3911 women had valid HPV and DS results, and a satisfactory histology result [28].

Table 1
Characteristics of Onclarity and IMPACT Trials.

Demographics	Onclarity trial [15]	IMPACT trial [34]
Initial enrollment	33,858	35,263
Primary screening population (≥25 years of age)	29,833	35,263
Age; years (median)	≥25 (39)	≥25 (39)
Vaccinated Participants; N _e (%)	1637 (5.5)	4297 (12.3)
≥25 years of age with evaluable cytology and valid HPV; N _e	29,513	34,807
HPV Prevalence; N _e (%)	3748 ^a (12.7)	5250 ^b (15.1)
Colposcopic Biopsies; N _e (%)	5534 ^a (18.8)	6349 ^b (18.2)
Negative; N _e (%)	4692 (84.8)	5277 (83.1)
CIN1; N _e (%)	490 (8.9)	477 (7.5)
CIN2; N _e (%)	179 (3.2)	353 (5.6)
CIN3; N _e (%)	160 (2.9)	222 (3.5)
Cancer; N _e (%)	13 ^c (0.2)	20 (0.3)

Abbreviations: IMPACT; IMproving Primary screening And Colposcopy Triage; N_e, number; HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia.

^a Denominator = 29,513; based on participants, ≥25 years, with evaluable cytology and valid HPV results.

^b Denominator = 34,807; based on participants, ≥25 years, with evaluable cytology and valid HPV results.

^c Includes 9 adenocarcinoma-in-situ, 3 squamous cell carcinomas, and 1 adenosquamous carcinoma.

3.2. Clinical performance and risk stratification through extended genotyping with cytology triage

Within the HPV-positive subjects, 490 were HPV16 positive, 172 were HPV18 positive, and 314 were HPV31 positive. Of the remaining HPV-positive subjects, 780 were 33/58/52/45 positive and 1220 were 35/39/68/51/56/59/66 HPV-positive (Table S2). Overall, there were 163 cases of ≥CIN3 and 153 CIN2 cases diagnosed at baseline; there were 2660 ≤CIN1 results at baseline. By Onclarity result, HPV16 accounted for approximately one-quarter of the CIN2 cases and almost half of the ≥CIN3 cases. HPV31 was the second most prevalent Onclarity result for both the CIN2 and ≥CIN3 categories. The prevalence of the additional Onclarity results varied, but ranged from 0.6% to 6.9% within ≥CIN3 and from 2.2% to 7.8% within ≥CIN2 (Table S2).

Immediate ≥CIN3 and ≥CIN2 risk point estimates (with accompanying 95% confidence intervals) were determined for HPV-positive individuals; results are shown in Fig. 1 and are stratified by cytology category (either >LSIL, ASC-US/LSIL, or NILM). Within each cytology category, Onclarity results were grouped as follows: (a) HPV16; (b) HPV18 and 31; (c) HPV33/58 and 52; and (d) HPV35/39/68, 45, 51, and 56/59/66. A consistent, stepwise reduction in risk was observed from left (HPV16) to right (35/39/45/51/56/59/66/68) of the x-axes in Fig. 1, regardless of cytology category, for both ≥CIN3 and ≥CIN2 disease.

Based on the results shown in Fig. 1, hierarchical screening strata were constructed to delineate risk groupings based on combinations of extended genotyping and cytology results. The hierarchical order of the strata (established by rank ordering from the highest to lowest CIN risk using a combination of HPV genotype and cytology category, excluding the prior HPV genotype group from each subsequent group) was as follows: (1) >LSIL, (2) HPV16 (any cytology), (3) not HPV16 but HPV18 [or] 31 (any cytology), (4) not HPV16/18/31 (any cytology) but HPV33/58 [or] 52 [or] 45 (ASC-US or LSIL cytology), (5) not HPV16/18/31 (any cytology) or HPV33/58/52/45 (ASC-US or LSIL cytology) but HPV33/58 [or] 52 (NILM cytology), (6) not HPV16/18/31 (any cytology) or HPV33/58/52/45 (ASC-US or LSIL cytology) or HPV33/58/52 (NILM cytology) but HPV35/39/68 [or] 51 [or] 55/59/66 (ASC-US or LSIL cytology), (7) not HPV16/18/31 (any cytology) or HPV33/58/52/45 (ASC-US or LSIL cytology) or HPV33/58/52 (NILM cytology) or HPV35/39/68/51/55/59/66 (ASC-US or LSIL cytology) but HPV45 [or] 35/39/68 [or] 51 [or] 55/59/66 (NILM cytology). Table 2 also includes cumulative risk values, which become progressively lower as each new stratum is built onto the previous one. Colposcopies-to-disease detected, a measure of diagnostic

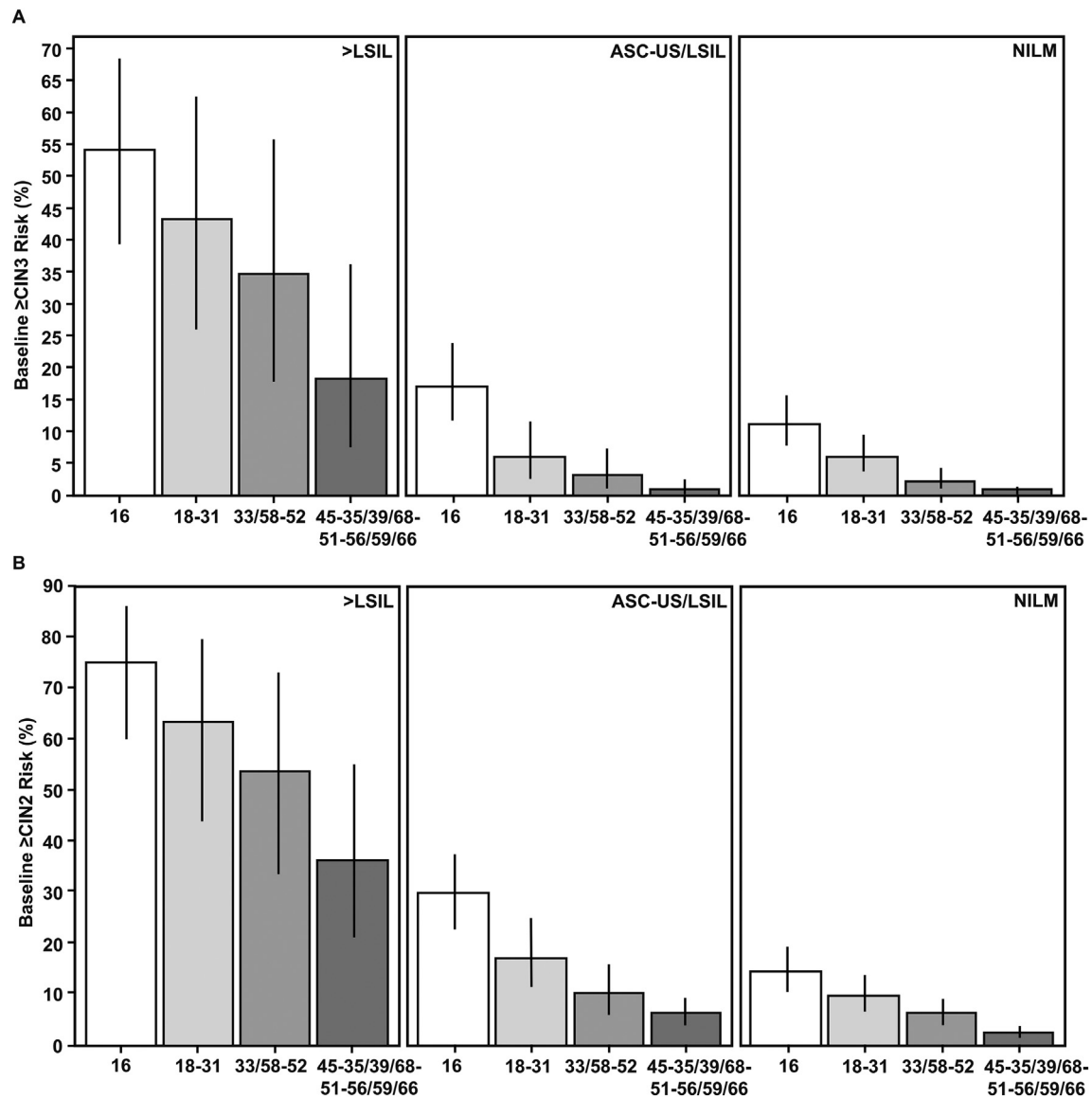


Fig. 1. Baseline (immediate) $\geq$ CIN3 (A) and $\geq$ CIN2 risk (B) is shown for Onclarity result groupings HPV16; HPV18/31; HPV33/58, 52, 45; and HPV35/39/68, 51, 56/59/66 across >LSIL, ASC-US/LSIL, and NILM cytology results. The line originating from the point estimate indicates upper- and lower-bound 95% confidence intervals. Abbreviations: CIN, cervical intraepithelial neoplasia; LSIL, low-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells-undetermined significance; NILM, negative for intraepithelial lesions or malignancies.

efficiency, worsened by referring women from additional strata, with decreasing disease risk.

Performance values for detection of $\geq$ CIN3 are also listed in Table 2 for extended genotyping alone and DS alone, or for HPV16/18 primary screening (using the cobas HPV assay) utilizing DS as a triage for non-HPV16/18, HPV-positive individuals. As expected, DS alone provided better $\geq$ CIN3 risk discrimination but lower sensitivity for detection than DS when used as a triage approach for HPV16/18 primary screening. HPV16/18 primary screening with DS provided performance characteristics that were similar to extended genotyping with cytology triage at stratum 5. Extended genotyping alone produced similar results to that of DS but combining extended genotyping results with cytology categories improved sensitivity and colposcopic efficiency (Table 2).

3.3. Receiver-operator curve (ROC) analysis

Fig. 2 shows a ROC for $\geq$ CIN3 detection that includes all baseline participants within the HPV primary screening population with a positive HPV result. The screening strata from Table 2 are plotted along the

curve. Along the curve, strata 3–5 occupy areas that represent a reasonable balance between disease detected and number of colposcopy referrals. A ROC for $\geq$ CIN2 detection was also generated and showed a less steep rise in sensitivity per change in specificity (Fig. S2). Superimposed on the plot in Fig. 2 are DS results, both alone and when used as a triage following HPV16/18 partial genotyping. For $\geq$ CIN3, the optimal cutoff for sensitivity versus specificity was situated between the not HPV16 but HPV18 or 31 (any cytology) group ($\geq$ CIN3 sensitivity = 85.9%; colposcopy-to- $\geq$ CIN3 ratio = 7.4) and the not HPV16/18/31 (any cytology) but HPV33/58 or 52 (NILM cytology) group ($\geq$ CIN3 sensitivity = 94.5%; colposcopy-to- $\geq$ CIN3 ratio = 10.8). DS alone showed a sensitivity between these points of 89.5% with a colposcopy-to- $\geq$ CIN3 ratio of 9.2, while HPV16/18 with DS triage showed a sensitivity of 94.3% and a colposcopy-to- $\geq$ CIN3 ratio of 11.4.

4. Discussion

Here we demonstrate the clinical utility of extended genotyping combined with cytology for stratifying immediate $\geq$ CIN3 risk within

Table 2Performance for detection of $\geq$ CIN3 through risk-based screening, utilizing extended genotyping or partial genotyping with DS triage.

Trial	Baseline $\geq$ CIN3	Stratum n (N = 2976)	Individual Stratum		Accumulating Strata ^a		Specificity	Sensitivity	Colposcopy-to- $\geq$ CIN3
	Triage Strategy		Stratum Disease Cases	Cumulative Disease Cases	Stratum Risk	Cumulative Risk			
Onclarity xGT with cytology	>LSIL	137	54	54	39.4%	39.4%	97.0%	33.1%	2.5
	HPV16	442	59	113	13.3%	19.5%	83.4%	69.3%	5.1
	(any cytology)								
	HPV18/31 (any cytology)	456	27	140	5.9%	13.5%	68.2%	85.9%	7.4
	HPV33/58/52/45	205	5	145	2.4%	11.7%	61.1%	89.0%	8.6
	(ASC-US/LSIL)								
	HPV33/58/52 (NILM)	419	9	154	2.1%	9.3%	46.5%	94.5%	10.8
Onclarity xGT only	HPV35/39/68/51/56/59/66	352	3	157	0.9%	7.8%	34.1%	96.3%	12.8
	(ASC-US/LSIL)								
	HPV45/35/39/68/51/35/39/66	965	6	163	0.6%	5.5%	0.0%	100%	18.3
	(NILM)								
	HPV16	490	85	85	17.3%	17.3%	85.6%	52.1%	5.8
	HPV18/31	486	40	125	8.2%	12.8%	69.7%	76.7%	7.8
	HPV33/58/52/45	780	26	151	3.3%	8.6%	42.9%	92.6%	11.6
IMPACT	HPV35/39/68/51/56/59/66	1220	12	163	1.0%	5.5%	0.0%	100%	18.3
	Dual Stain	1887	n/a	205	n/a	10.9%	54.0%	89.5%	9.2
	HPV16/18 (Dual Stain)	2461	n/a	216	n/a	8.8%	38.6%	94.3%	11.4

Abbreviations: CIN, cervical intraepithelial neoplasia; DS, dual staining; xGT, extended genotyping; LSIL, low-grade squamous intraepithelial lesion; HPV, human papillomavirus; ASC-US, atypical squamous cells-undetermined significance; NILM, negative for intraepithelial lesions or malignancies.

^a Accumulating strata indicates a stepwise addition of positive strata results from the previous strata while moving down the column (e.g., the HPV18/31, any cytology row includes the >LSIL and the HPV16, any cytology positive results).

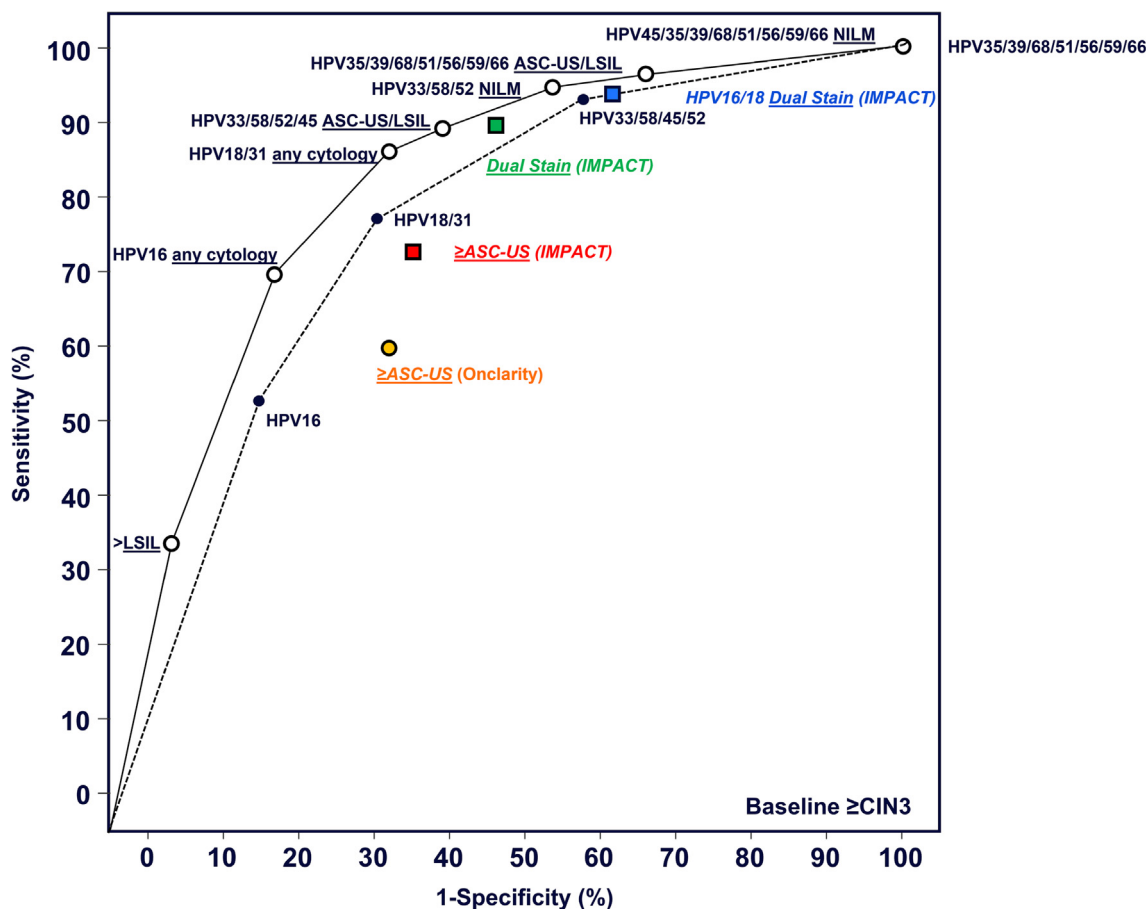


Fig. 2. Receiver-operator curve with sensitivity on the y-axis and 1-specificity along the x-axis for detection of $\geq$ CIN3. Combinations of Onclarity result groupings, either alone (hashed line), or in combination with cytology findings (solid line), within the HPV-positive population, from the baseline phase of the Onclarity trial, are plotted in hierarchical fashion so that changes in specificity are captured as sensitivity increases in an incremental fashion. Also plotted in the figure are sensitivity/specificity relationships for diagnostic performance of ASC-US cytology only (from the Onclarity and IMPACT trials), DS-positive only (from the IMPACT trial), and HPV16/18 limited genotyping with DS triage (from the IMPACT trial). Abbreviations: LSIL, low-grade squamous intraepithelial lesion; HPV, human papillomavirus; ASC-US, atypical squamous cells-undetermined significance; NILM, negative for intraepithelial lesions or malignancies; CIN, cervical intraepithelial neoplasia.

the baseline screening population of the Onclarity cervical cancer screening trial. New ASCCP guidelines promote the concept of “equal management for equal risk” as the central tenet for clinical decision-making regarding colposcopy referral and time intervals associated with a return for repeat testing. We analyzed all HPV-positive women that constituted the HPV primary screening population (≥ 25 years of age) from the Onclarity trial. We established risk strata using a combination of extended genotyping and cytology results and demonstrate the versatility of extended genotyping for devising efficient triage strategies following HPV primary screening. This is important because the risk threshold for a colposcopy referral, for example, differs in many countries, based on policy decision making. Some policies are more conservative and employ lower risk thresholds for a colposcopy referral in an attempt to detect all pre-cancer, which can result in colposcopies for women who are not at high risk for developing cancer. Other countries employ high immediate colposcopy thresholds in order to reduce false positive results and instead utilize return visits at appropriate time intervals to detect high-grade cervical disease [11,24,39,40]. Regardless of the actual risk threshold value that has been established, extended genotyping with cytology triage provides utilitarian results that identify values along the $\geq$ CIN3 risk continuum and, therefore, should be applicable in any risk-based screening program.

The actual composition of the different risk strata reported here are consistent with the literature regarding individual risk for high-grade cervical disease associated with specific genotypes, or groups of genotypes. As expected, HPV16 carried the highest risk, second only to $>$ LSIL cytology in combination with an HPV-positive result; HPV16 was also the most prevalent genotype within $\geq$ CIN3 and $\geq$ CIN2 disease. This is consistent with many other prospective screening trials, real-world evaluations, and retrospective studies involving individual genotype risk [24,41]. Although HPV18 carries a lower risk for high-grade cervical disease compared to HPV16, it is still clinically relevant due to its prevalence within SCC and adenocarcinoma, the latter of which can be difficult to detect through cytology-based screening [9,38]. Although HPV16/18 primary screening (with cytology triage for an HPV result of “other 12”) is FDA-approved and supported by the ASCCP as a screening strategy, [19] the concept of extended genotyping or other approaches for risk-based screening is under consideration by the ASCCP, based on recent FDA approvals [20]. Previous studies have demonstrated that extended genotyping stratifies risk in a manner that is clinically useful for patient management in different cytology categories. For individuals with NILM cytology, risk-based approaches would include genotype results in addition to HPV16 or 18 for immediate colposcopy, including HPV31 or HPV33. Conversely, some genotypes carry a low enough risk for high-grade cervical disease to exclude immediate colposcopy, even in the presence of an equivocal or low-grade cytology result [27,42].

During HPV16/18 primary screening, the application of DS can also achieve effective risk stratification for individuals positive for one of the “other 12” high-risk genotypes such that a positive DS result lifts the risk above the threshold for immediate colposcopy while a negative DS result could warrant a return for repeat testing after some interval of time [3,28–30]. Results from two trials (ATHENA and IMPACT) and from the Kaiser Permanente Northern California (KPNC) Pap Cohort have demonstrated the clinical utility for DS, either alone or in combination with some form of primary HPV screening, for risk stratification and optimized patient management during cervical cancer screening. Analysis of results from the ATHENA trial supports the potential for DS as a superior triage approach when compared to cytology-based triage that can also incorporate limited genotyping through HPV16/18 detection [29]. Similar conclusions came from the KPNC Cervical Cancer Screening registry, which showed that DS demonstrates better risk stratification over a five-year period when compared to a cytology-based triage approach [30]. The similar risk profiles and demographics of the two large national registration trials allowed us to compare the respective performance of the two triage approaches. Performance of DS alone or DS in

combination with HPV16/18 triage aligned very closely with extended genotyping plus cytology triage. Extended genotyping, combined with cytology, offers the advantage of multiple points on the ROC graph, which allows the application of different risk thresholds in different geographic regions, where risk tolerances and clinical practices may be different. DS triage, by contrast, results in a single point, which solely determines colposcopy referral or a return to a specific screening interval. Extended genotyping also enables effective triage of HPV-positive patients without the added cost of an additional cytology slide and staining, and allows for test interpretation by leveraging pre-existing cytology data (cotesting) or the results from a routine and readily available reflex cytology test (primary HPV screening). Finally, unlike DS, extended genotyping can be performed on self-collected specimens, which can extend coverage to under-screened women [43,44]. The results of this study suggest that extended genotyping alone can perform similarly to DS, enabling the potential triage of HPV-positive women directly from the self-sampling result, without the use of a clinician-collected specimen. Where the Pap result is available, combining with extended genotyping improves both sensitivity and specificity.

4.1. Strengths and limitations

Strengths of this analysis include those that have been previously noted for both the Onclarity and IMPACT trials [28,34]. As described in the results, both prospective trials were relatively large, were characterized by similar participant demographics, and included similar prevalence values for cytology, HPV, and cervical pathology. In addition, both trials were designed to maximize disease ascertainment from screening populations. All participants with any abnormal screening result, as well as a subset of women with a negative screening result, were referred to colposcopy. Colposcopy and biopsy procedures were standardized for all participants in each, respective, trial, and biopsy specimens received a blinded consensus pathology review by gynecologic pathologists, which utilized p16 immunohistochemistry to facilitate adjudication of CIN2 interpretations.

Although the Onclarity and IMPACT trials were similar in design and performance parameters, this study did not include a direct comparison between DS and extended genotyping/cytology triage using the same pool of participants. Thus, the analyses are potentially subject to confounding factors that might be difficult to reconcile without proper controls. On the other hand, the overall data pertaining to DS and extended genotyping, here, demonstrate results that are consistent with risk stratification previously shown for both screening approaches. On balance, this analysis provides a reasonable approach to compare the two screening strategies.

While the two compared studies are large and focus on the important $\geq$ CIN3 endpoint, additional studies and/or analyses may be beneficial to better understand how extended genotyping and dual stain triage strategies perform.

5. Conclusion

Extended genotyping with reflex to cytology for selected HPV genotypes achieves similar $\geq$ CIN3 sensitivity (94.5% vs 94.3%), specificity (46.5% vs 38.6%), and colposcopies-per-case detected (10.8 vs 11.4) as primary HPV screening with 16/18 genotyping and DS; fewer tests would be needed for cytology vs DS, as HPV31/33/58/52 would not need to be triaged. For guideline committees looking to reduce the number of colposcopy referrals and maximize colposcopy efficiency, HPV33/58/52 could also be triaged by cytology. If ASC-US/LSIL is used as the cutoff for referral, such a strategy would be similar to DS, while if $>$ LSIL is used as the cutoff for referral, the strategy would have higher specificity for a small decrease in sensitivity. Extended genotyping triage alone with colposcopy referral for HPV16/18/31/33/58/52/45 performs similarly to primary HPV screening with 16/18 genotyping and DS, with no additional tests required; such a strategy is only slightly

inferior to extended genotyping with reflex to cytology for the lowest-risk HPV genotypes and could prove beneficial in settings where cytology is not easily available, such as testing under self-collection programs. In general, extended genotyping with or without reflex to cytology offers a unique ability to tailor clinical action thresholds to align with the risk preferences of different national screening programs, while simultaneously improving colposcopy efficiency.

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Potential conflicts of interest

MHS serves as a consultant in clinical trial design and as an expert pathologist for HPV diagnostic and/or vaccine trials for Becton, Dickinson and Company, BD Life Sciences – Integrated Diagnostic Solutions, Roche, Abbott, Inovio Pharmaceuticals and Merck, and as a speaker for Roche, Merck and Becton, Dickinson and Company, BD Life Sciences.

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All authors contributed to the interpretation of the data, critically revised the manuscript for important intellectual content, and approved the final version to be published. Becton, Dickinson and Company employees that are also authors, played the following roles during the study and development of the paper: VP facilitated data analyses and revision of the manuscript; KY facilitated conception and design of the study, data acquisition and interpretation, and drafting and revision of the manuscript; JA and LV facilitated study conception and design, and manuscript revision. All authors provided final approval of the manuscript and agree to be accountable for the accuracy and integrity of this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ygyno.2023.04.022>.

References

- [1] E.M. Burd, Human papillomavirus and cervical cancer, *Clin. Microbiol. Rev.* 16 (2003) 1–17.
- [2] F.X. Bosch, S. de Sanjose, Chapter 1: human papillomavirus and cervical cancer—burden and assessment of causality, *J. Natl. Cancer Inst. Monogr.* (2003) 3–13.
- [3] M.H. Stoler, E. Baker, S. Boyle, S. Aslam, R. Ridder, W.K. Huh, et al., Approaches to triage optimization in HPV primary screening: extended genotyping and p16/Ki-67 dual-stained cytology-retrospective insights from ATHENA, *Int. J. Cancer* 146 (2020) 2599–2607.
- [4] T.C. Wright, M.H. Stoler, C.M. Behrens, A. Sharma, G. Zhang, T.L. Wright, Primary cervical cancer screening with human papillomavirus: end of study results from the ATHENA study using HPV as the first-line screening test, *Gynecol. Oncol.* 136 (2020) 189–197.
- [5] G. Ronco, J. Dillner, K.M. Elfstrom, S. Tunesi, P.J. Snijders, M. Arbyn, et al., Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials, *Lancet* 383 (2014) 524–532.
- [6] G.S. Ogilvie, D. van Niekerk, M. Krajden, L.W. Smith, D. Cook, L. Gondara, et al., Effect of screening with primary cervical HPV testing vs cytology testing on high-grade cervical intraepithelial neoplasia at 48 months: the HPV FOCAL randomized clinical trial, *JAMA* 320 (2018) 43–52.
- [7] D.C. Rijkaart, J. Berkhof, L. Rozendaal, F.J. van Kemenade, N.W. Bulkman, D.A. Heideman, et al., Human papillomavirus testing for the detection of high-grade cervical intraepithelial neoplasia and cancer: final results of the POBASCAM randomised controlled trial, *Lancet Oncol.* 13 (2012) 78–88.
- [8] J.C. Gage, M. Schiffman, H.A. Katki, P.E. Castle, B. Fetterman, N. Wentzensen, et al., Reassurance against future risk of precancer and cancer conferred by a negative human papillomavirus test, *J. Natl. Cancer Inst.* 106 (2014).
- [9] D. Saslow, D. Solomon, H.W. Lawson, M. Killackey, S.L. Kulasingam, J. Cain, et al., American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer, *Am. J. Clin. Pathol.* 137 (2012) 516–542.
- [10] H.A. Katki, W.K. Kinney, B. Fetterman, T. Lorey, N.E. Poitras, L. Cheung, et al., Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: a population-based study in routine clinical practice, *Lancet Oncol.* 12 (2011) 663–672.
- [11] G. Koliopoulos, V.N. Nyaga, N. Santesso, A. Bryant, P.P. Martin-Hirsch, R.A. Mustafa, et al., Cytology versus HPV testing for cervical cancer screening in the general population, *Cochrane Database Syst. Rev.* 8 (2017) CD008587.
- [12] WHO WHO, Cervical Cancer Screening Response by Country. Global Health Observatory Data Repository, <https://apps.who.int/gho/data/viewmain/UHC/CERVICALCANCER> 2021.
- [13] X.W. Jin, L. Lipold, J. Foucher, A. Sikin, J. Brainard, J. Belinson, et al., Cost-effectiveness of primary HPV testing, cytology and co-testing as cervical cancer screening for women above age 30 years, *J. Gen. Intern. Med.* 31 (2016) 1338–1344.
- [14] M. Schiffman, W.K. Kinney, L.C. Cheung, J.C. Gage, B. Fetterman, N.E. Poitras, et al., Relative performance of HPV and cytology components of cotesting in cervical screening, *J. Natl. Cancer Inst.* 110 (2018) 501–508.
- [15] M.H. Stoler, T.C. Wright Jr., V. Parvu, L. Vaughan, K. Yanson, K. Eckert, et al., The Onclarity human papillomavirus trial: design, methods, and baseline results, *Gynecol. Oncol.* 149 (2018) 498–505.
- [16] P. Guan, R. Howell-Jones, N. Li, L. Bruni, S. de Sanjose, S. Franceschi, et al., Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer, *Int. J. Cancer* 131 (2012) 2349–2359.
- [17] P.E. Gravitt, The known unknowns of HPV natural history, *J. Clin. Invest.* 121 (2011) 4593–4599.
- [18] S. Shanmugasundaram, J. You, Targeting persistent human papillomavirus infection, *Viruses* 9 (2017) 229.
- [19] W.K. Huh, K.A. Ault, D. Chelmow, D.D. Davey, R.A. Goulart, F.A. Garcia, et al., Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance, *Gynecol. Oncol.* 136 (2015) 178–182.
- [20] L.C. Cheung, D. Egemen, X. Chen, H.A. Katki, M. Demarco, A.L. Wiser, et al., 2019 ASCCP risk-based management consensus guidelines: methods for risk estimation, recommended management, and validation, *J. Low Genit. Tract. Dis.* 24 (2020) 90–101.
- [21] R.B. Perkins, L.N. Fuzzell, P. Lake, M. McIntyre, R. Nayar, M. Saraiya, et al., Incorporating stakeholder feedback in guidelines development for the management of abnormal cervical cancer screening tests, *J. Low Genit. Tract. Dis.* 24 (2020) 167–177.
- [22] P.E. Castle, M.H. Stoler, T.C. Wright Jr., A. Sharma, T.L. Wright, C.M. Behrens, Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study, *Lancet Oncol.* 12 (2011) 880–890.
- [23] T.C. Wright Jr., M.H. Stoler, V. Parvu, K. Yanson, C. Cooper, J. Andrews, Risk detection for high-grade cervical disease using Onclarity HPV extended genotyping in women, ≥ 2 years of age, with ASC-US or LSIL cytology, *Gynecol. Oncol.* 154 (2019) 360–367.
- [24] J.H. Bonde, M.T. Sandri, D.S. Gary, J.C. Andrews, Clinical utility of human papillomavirus genotyping in cervical cancer screening: a systematic review, *J. Low Genit. Tract. Dis.* 24 (2020) 1–13.
- [25] F. Song, P. Yan, X. Huang, C. Wang, H. Du, X. Qu, et al., Roles of extended human papillomavirus genotyping and multiple infections in early detection of cervical precancer and cancer and HPV vaccination, *BMC Cancer* 22 (2022) 42.
- [26] M.H. Stoler, T.C. Wright Jr., V. Parvu, K. Yanson, C.K. Cooper, J. Andrews, Stratified risk of high-grade cervical disease using onclarity HPV extended genotyping in women, ≥ 25 years of age, with NILM cytology, *Gynecol. Oncol.* 153 (2019) 26–33.
- [27] T.C. Wright Jr., M.H. Stoler, V. Parvu, K. Yanson, C. Cooper, J. Andrews, Risk detection for high-grade cervical disease using Onclarity HPV extended genotyping in women, ≥ 2 years of age, with ASC-US or LSIL cytology, *Gynecol. Oncol.* 154 (2019) 360–367.
- [28] T.C. Wright Jr., M.H. Stoler, J. Ranger-Moore, Q. Fang, P. Volkir, M. Safaiean, et al., Clinical validation of p16/Ki-67 dual-stained cytology triage of HPV-positive women: results from the IMPACT trial, *Int. J. Cancer* 150 (2022) 461–471.
- [29] T.C. Wright Jr., C.M. Behrens, J. Ranger-Moore, S. Rehm, A. Sharma, M.H. Stoler, et al., Triage HPV-positive women with p16/Ki-67 dual-stained cytology: results from a sub-study nested into the ATHENA trial, *Gynecol. Oncol.* 144 (2017) 51–56.
- [30] M.A. Clarke, L.C. Cheung, P.E. Castle, M. Schiffman, D. Tokugawa, N. Poitras, et al., Five-year risk of cervical precancer following p16/Ki-67 dual-stain triage of HPV-positive women, *JAMA Oncol.* 5 (2019) 181–186.
- [31] N. Wentzensen, Doeberitz M. von Knebel, Biomarkers in cervical cancer screening, *Dis. Markers* 23 (2007) 315–330.
- [32] C.-C. Chang, R.-L. Huang, Y.-P. Liao, P.-H. Su, Y.-W. Hsu, H.-C. Wang, et al., Concordance analysis of methylation biomarkers detection in self-collected and physician-collected samples in cervical neoplasm, *BMC Cancer* 15 (2015) 418.
- [33] A.D. Olivas, J.E. Barroeta, R.R. Lastra, Overview of ancillary techniques in cervical cytology, *Acta Cytol.* 67 (2023) 119–128.

- [34] M. Safaeian, T.C. Wright Jr., M.H. Stoler, J. Ranger-Moore, S. Rehm, S. Aslam, et al., The IMproving primary screening and colposcopy triage trial: human papillomavirus, cervical cytology, and histopathologic results from the baseline and 1-year follow-up phase, *Am. J. Obstet. Gynecol.* 225 (278) (2021) e1–e16.
- [35] T.M. Darragh, T.J. Colgan, J.T. Cox, D.S. Heller, M.R. Henry, R.D. Luff, et al., 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, *Arch. Pathol. Lab. Med.* 136 (2012) 1266–1297.
- [36] E.C. Pirog, B. Kleter, S. Olgac, P. Bobkiewicz, J. Lindeman, W.G. Quint, et al., Prevalence of human papillomavirus DNA in different histological subtypes of cervical adenocarcinoma, *Am. J. Pathol.* 157 (2000) 1055–1062.
- [37] E.C. Pirog, B. Lloveras, A. Molijn, S. Tous, N. Guimera, M. Alejo, et al., HPV prevalence and genotypes in different histological subtypes of cervical adenocarcinoma, a worldwide analysis of 760 cases, *Mod. Pathol.* 27 (2014) 1559–1567.
- [38] S. de Sanjose, W.G. Quint, L. Alemany, D.T. Geraets, J.E. Klaustermeier, B. Lloveras, et al., Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study, *Lancet Oncol.* 11 (2010) 1048–1056.
- [39] M.A. Smith, M. Sherrah, F. Sultana, P.E. Castle, M. Arbyn, D. Gertig, et al., National experience in the first two years of primary human papillomavirus (HPV) cervical screening in an HPV vaccinated population in Australia: observational study, *BMJ (Clin. Res. Ed.)* 376 (2022), e068582.
- [40] M. Arbyn, L. Xu, F. Verdoodt, J. Cuzick, A. Szarewski, J.L. Belinson, et al., Genotyping for human papillomavirus types 16 and 18 in women with minor cervical lesions: a systematic review and meta-analysis, *Ann. Intern. Med.* 166 (2017) 118–127.
- [41] J. Bonde, F. Bottari, A.D. Iacobone, C.E. Cocuzza, M.T. Sandri, F. Bogliatto, et al., Human papillomavirus same genotype persistence and risk: a systematic review, *J. Low Genit. Tract. Dis.* 25 (2021) 27–37.
- [42] O.G.W. Wong, I.F.Y. Ng, O.K.L. Tsun, H.H. Pang, P.P.C. Ip, A.N.Y. Cheung, Machine learning interpretation of extended human papillomavirus genotyping by Onclarity in an Asian cervical cancer screening population, *J. Clin. Microbiol.* 57 (2019) e00997–19.
- [43] A. Latsuzbaia, D. Vanden Broeck, S. Van Keer, S. Weyers, G. Donders, J. Doyen, et al., Validation of BD Onclarity HPV assay on vaginal self-samples versus cervical samples using the VALHUDES protocol, *Cancer Epidemiol. Biomark. Prev.* 31 (2022) 2177–2184.
- [44] J.U.H. Lam, M. Rebolj, D.M. Ejegod, H. Pedersen, C. Rygaard, E. Lynge, et al., Prevalence of human papillomavirus in self-taken samples from screening nonattenders, *J. Clin. Microbiol.* 55 (2017) 2913–2923.