

# Bayesian analysis of baseline risk of CIN2 and $\geq$ CIN3 by HPV genotype in a European referral cohort

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Whereas HPV16 and HPV18 have been the focus in current risk-based cervical cancer screening algorithms using HPV genotype information, mounting evidence suggests that oncogenic HPV types such as HPV31, 33, 52 and 58 pose a  $\geq$ CIN3 risk equivalent to or greater than that of HPV18, and the combined risk of HPV31 and HPV33 rivals even HPV16 in women above 30 years of age. Here, we evaluate the baseline risk of CIN2 and CIN3 by genotype in a colposcopy referral population from Denmark and Italy. In total, 655 women were enrolled upon a referral to colposcopy after a positive screening sample. All samples were HPV analyzed using Onclarity HPV assay with extended genotyping and combined with the histology outcomes, a Bayesian probability modeling was used to determine the risk per genotype assessed. The combined data for this referral population showed that the  $\geq$ CIN2 risk of HPV16 was 69.1%, HPV31 at 63.3%, HPV33/58 at 52.7%, HPV18 at 46.6% and HPV52 at 40.8%. For  $\geq$ CIN3, the risks were 44.3%, 38.5%, 36.8%, 30.9% and 16.8% for HPV16, HPV31, HPV18, HPV33/58 and HPV52, respectively, indicating that the baseline risk of disease arising from HPV16 is, not surprisingly, the highest among the oncogenic HPV genotypes. We find that the HPV genotype-specific  $\geq$ CIN2 and  $\geq$ CIN3 risk-patterns are so distinct that, for example, 35/39/68 and 56/59/66 should be considered only for low intensive follow-up, thereby proposing active use of this information in triage strategies for screening HPV-positive women.

## Introduction

Cervical infection with a high-risk (hr) human papillomavirus (HPV) may result in virus persistence and progression to a precancerous lesion like cervical intraepithelial neoplasia (CIN), which can result in development of invasive cancer.<sup>1,2</sup>

Approximately 30% of women with CIN3 develop invasive cancer if left untreated, and the aim of cervical cancer screening is therefore to detect and surgically treat precancers, such as CIN3, with excision or ablation treatment to avoid its progression to invasive cancer.<sup>3,4</sup>

**Abbreviations:** AGC: atypical glandular cells; ASCCP: American Society for Colposcopy and Cervical Pathology; ASC-H: atypical squamous cells—cannot exclude HSIL; ASCUS: atypical squamous cells of undetermined significance; CIN: cervical intraepithelial neoplasia; ECC: endocervical curettage; FDA: Food and Drug Administration (United States); HPV: human papillomavirus; hr: high-risk; HSIL: high-grade squamous intraepithelial lesions; IEO: European Institute of Oncology; LBC: liquid based cytology; LEEP: loop electrosurgical excision procedure; LSIL: low-grade squamous intraepithelial lesions; MCMC methods: Markov-Chain Monte Carlo methods; SCJ: squamous columnar junction

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**What's new?**

Cervical infection with a high-risk Human Papilloma Virus (HPV) may result in virus persistence and progression to a precancerous lesion and eventually cancer. Primary HPV-based cervical cancer screening holds promise but requires triage of HPV-positive samples to reduce overdiagnosis. HPV genotyping has been proposed to be included in triage screening algorithms on the basis of equal risk, equal treatment. Here, the authors present data from a European study showing that risk of CIN2 and  $\geq$ CIN3 lesions is genotype-specific in a colposcopy referral population of women over 30, demonstrating the utility of HPV genotype information in screening.

Current standard-of-care cervical cytology-based screening has decreased cervical cancer incidence<sup>5–7</sup> by up to two-thirds. However, cytology is being replaced with HPV testing in several countries on the premise that HPV screening confers a higher sensitivity toward detection of lesions in women 30 years or older ( $\geq 30$  years of age).<sup>8–10</sup> Moreover, the negative predictive value of HPV DNA testing is superior to that of cytology,<sup>9</sup> thereby allowing fewer screening rounds per women lifetime at equal or better safety than cytology. The evidence for HPV screening<sup>9</sup> was mainly established using non-discriminatory HPV assays reporting only hrHPV-positive or hrHPV-negative outcomes without ability to differentiate between genotype(s) of an infection.

The caveat of HPV-based screening is a lower specificity necessitating triage of hrHPV-positive screening findings and multiple triage strategies have been proposed. Here the role of triage tests is to delineate clinically significant infection. Given its long use, cytology is the triage with the most follow-up data, however, hrHPV-positive and cytology triage normal outcomes presents a challenge as well does the definition of appropriate retesting intervals and choice of retest type for this group. Underlying all positive HPV test outcomes is one or more hrHPV genotypes, which have a distinct age dependent risk of disease, and the detection of HPV genotype(s) is objective since it is derived from an automated instrument/algorithm vs. human-read cytology slides. Yet, genotyping information is, as of today, not utilized to its fullest in establishing new molecular HPV-based screening algorithms based upon the concept of equal management of equal risk.

Risk of progression and risk of disease are key features in developing new screening HPV algorithms, with the aim of more accurately distinguishing hrHPV-positive women for retesting at a defined interval or direct referral to colposcopy<sup>11–13</sup> with the aim to reduce overtreatment and cost of screening at the same time. In this context, we propose that HPV genotype information derived directly from the screening sample can be utilized as triage information in advanced integrated risk-based referral algorithms.

Our study evaluated the role of extended genotyping using the BD Onclarity™ HPV Assay, not in a screening population, but in a referral population of women with a high prevalence of cervical high-grade lesions which simulates a primary HPV screening situation where the cytology triage outcome combined with a genotype-specific risk-estimate would define the follow-up. We evaluated the prevalence of different HPV

genotypes in  $\geq$ CIN2 and  $\geq$ CIN3 lesions and estimated the risk of  $\geq$ CIN2 and  $\geq$ CIN3 lesions associated with different genotypes. The BD Onclarity™ HPV Assay provides results on an automated platform (BD Viper LT™ System) and offers genotyping with individual reporting of types 16, 18, 31, 45, 51 and 52 and identification of three groups of types: 33/58, 56/59/66 and 35/39/68. The Onclarity HPV test is internationally validated on both SurePath and Thinprep LBC media for use in primary HPV screening<sup>14,15</sup> and has recently received FDA approval for primary screening in women over 25 years in the United States.<sup>16,17</sup> To investigate the risk of disease arising from the individual HPV genotypes, we used Bayesian data analysis which provides a natural and principled way of modeling risk in subjects with multiple genotype infections.

**Materials and Methods****Study population**

The current study is a subanalysis of data previously published and a full description of the study populations can be found in the original publications.<sup>18,19</sup> The subanalysis includes prospective samples from Italian women<sup>18</sup> as well as prospective and retrospective samples from Danish women<sup>19</sup> undergoing cervical cancer screening. A flow chart detailing the study population is found in Figure 1.

**Prospective samples (Denmark/Italy)**

Briefly, the prospective study element was designed as a European (Denmark/Italy) study in which women referred for colposcopy based on positive HPV and/or abnormal cervical cytology were enrolled. All participants underwent cervical sampling, and the specimens were collected in either ThinPrep™ vials (Italy) or BD SurePath™ vials (Denmark) and subsequently tested using the BD Onclarity HPV Assay on the fully automated BD Viper LT system. In addition, liquid-based cytology (LBC) was performed on all samples. All women underwent colposcopy-directed biopsy subsequently. Exclusion criteria were previous treatment for CIN, conization, LEEP, laser surgery or cryosurgery treatment, known pregnancy, partial or complete hysterectomy and application of chemical compounds to the cervix 24 hr prior to study inclusion.

**Retrospective samples (Denmark)**

Residual material from 411 consecutive, unselected residual SurePath samples with abnormal cytology (atypical squamous cells of undetermined significance or worse,  $\geq$ ASCUS) were

collected at the pathology department, Copenhagen University Hospital, Hvidovre, between September and October 2012. After all samples had been collected, 10 samples were excluded due to too little material.

In this analysis, prospective samples from both arms and retrospective samples from the Danish arm were included only if a valid and complete histological follow-up procedure was completed.

**Colposcopy procedures**

In the Danish arm, biopsies of colposcopy-identified lesions or a four-punch random biopsy in each quarter were taken if no lesions were visible. Endocervical curettage (ECC) was always taken in combination with the biopsies (described in detail in Ref. 19). In Denmark, HPV-positive women with ASCUS are referred for follow-up with colposcopy and biopsy-taking as are women with high grade squamous intraepithelial lesion (HSIL), atypical glandular cells (AGC), atypical squamous cells, cannot rule out high-grade squamous

intraepithelial lesion (ASCH) or cytological indications of carcinoma and women with continued ASCUS and low-grade squamous intraepithelial lesion (LSIL) cytology diagnosis. Danish Gynecology Guidelines recommend biopsy taking on all acetowhite lesions observed, or a random four quadrants biopsy set where no lesions are visible upon colposcopy. In the Italian arm, a random biopsy at the squamous columnar junction (SCJ) was performed in all women even if no colposcopic lesion was found, while ECC was taken if the SCJ was not visible (described in detail in Ref. 18). Histology was performed for all women with positive cytology ( $\geq$ ASCUS, where ASCUS stands for atypical cells of undetermined significance) and/or a positive HPV test. Histology was performed either from a specimen obtained by colposcopy with biopsy or from the evaluation of a conization specimen, and in the case of high-grade lesions, a conservative surgical procedure was performed with histology evaluation of the tissue.

For both arms, when excisional treatment was performed, no other biopsy was taken.

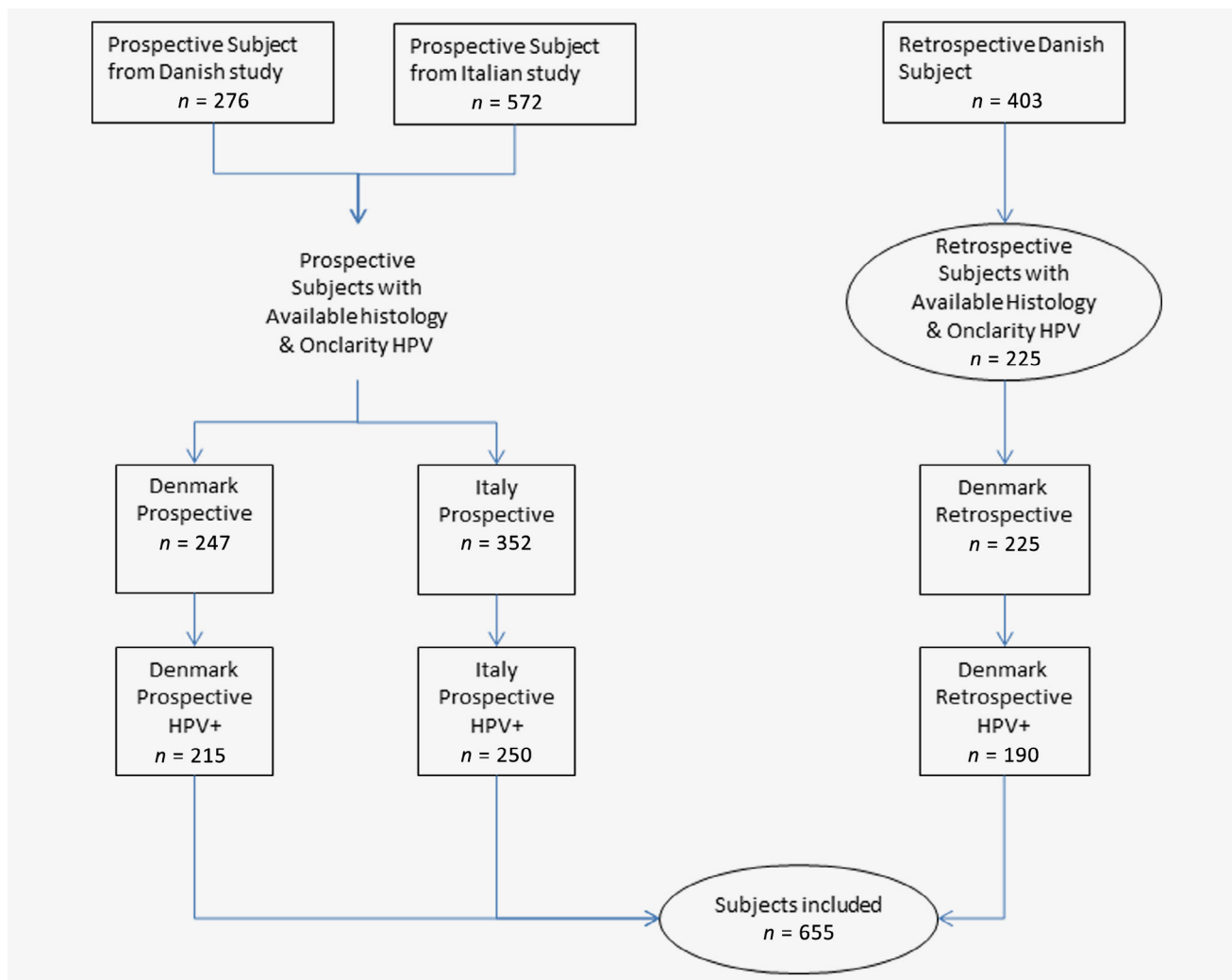


Figure 1. Flow chart detailing the selection of referral samples included in study from both study centers. [Color figure can be viewed at wileyonlinelibrary.com]

### HPV testing

The BD Onclarity HPV Assay is a qualitative target-amplification test that utilizes real-time PCR and fluorescent probe technology to detect E6 and E7 DNA regions of the HPV genome. In terms of reporting, a consensus result is provided (presence or absence of hrHPV, i.e., positive for at least one of the following types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 or 68), as well as delineation of six individual types (types 16, 18, 31, 45, 51 or 52) and three grouped types (types 33/58, 56/59/66 or 35/39/68). The assay also contains a cellular beta-globin control for detection of sample inadequacy or inhibition. All samples were tested on the BD Viper LT system. The tests were performed according to the manufacturer's instructions.

### SurePath and ThinPrep cytology

Concurrent cytology was performed on all included screening samples. In the Danish arm, cytology was prepared from the SurePath collected samples, and the entire processing was done in concordance with manufacturer's specifications. For the Italian arm, ThinPrep based cytology was performed with Thin Prep 5000 slide processor (Hologic, Inc., Bedford, MA) in accordance with manufacturer's specifications. In each arm, cytology outcomes were determined by trained specialist biotechnicians. For the SurePath arm, reading was done following automated focal point evaluation of all slides. All positive diagnosis was confirmed by a specialist pathologist.

### Histology

All histology derived from the two arms was processed in concordance with the parent laboratories standard operating procedures. Resulting histology slides were reviewed and evaluated by specialist pathologist. In the Danish arm, all the original histology results were adjudicated by a senior specialist pathologist. In the Italy arm, histology was adjudicated by two independent pathologists and discrepant results were further adjudicated by a third independent pathologist (Italian arm only).

### Data analysis and statistics

A Bayesian model was used to estimate the baseline risk of disease for each genotype; the model assumes that the risk of disease in subjects with multiple genotype coinfection is determined by the highest risk genotype. Specifically, the risk for each genotype is treated as a Bayesian parameter with a distribution between 0 and 1; subjects with one single genotype "j" detected were modeled to have risk  $R_j$ , subjects with two genotypes j and k detected were modeled to have risk  $\max(R_j, R_k)$ , and in general, subjects with multiple genotypes were modeled to have risk equal to the maximum risk parameter of the detected genotypes. A joint posterior probability distribution for the nine genotype risk parameters was derived by Markov-Chain Monte Carlo (MCMC) methods. The risks of  $\geq$ CIN2 and  $\geq$ CIN3 are calculated with 95% confidence intervals. In addition, the probability that the baseline risk of disease from one genotype is greater than the baseline risk of disease from another genotype was

derived by MCMC methods. Uninformative prior uniform distributions were used for all risk estimates. The data was analyzed using SAS/STAT<sup>®</sup> and R software.

### Medical ethics and data protection approvals

The study was approved by Ethical committees in concordance with National Law in Italy and Denmark, respectively. Informed consent was obtained for all the prospective included women according to local ethical approval (European Institute of Oncology [IEO], Milan, S689/212 study approved and Copenhagen University Hospital, Hvidovre, H-4-2012-070 Danish Capital Regional ethical committee approved).

### Results

Six hundred and fifty-five women referred to colposcopy, based on abnormal cytology and/or positive HPV test, were enrolled and included in this analysis. Samples with inadequate or missing cytology or histology were excluded. Italian, Danish and combined demographical data, cytology and histology results are summarized in Table 1, where the overall data shows an average age of enrolled subjects of 33.7 years, with 90.9% having cytology of  $\geq$ ASCUS on the enrollment sample. In total, of the 655 included women, 347 had  $\geq$ CIN2 at follow up (137 CIN2, 210 CIN3; Table 1). The baseline samples showed that for women with CIN2, 56.2% were HPV16

**Table 1.** Baseline age, cytology and histology of Onclarity HPV-positive subjects with available histological diagnoses

Study population	Denmark n = 405	Italy n = 250	Combined n = 655
Age			
Mean	32.6	35.6	33.7
SD	10	9.2	9.8
Min	18	19	18
Max	74	69	74
Cytology			
Normal	44 (10.9%)	16 (6.4%)	60 (9.2%)
ASCUS	68 (16.8%)	14 (5.6%)	82 (12.5%)
LSIL	73 (18.0%)	72 (28.8%)	145 (22.1%)
ASC-H	27 (6.7%)	8 (3.2%)	35 (5.3%)
HSIL	190 (46.9%)	131 (52.4%)	321 (49.0%)
AGC	0 (0.0%)	3 (1.2%)	3 (0.5%)
AGC, favor neoplastic	0 (0.0%)	1 (0.4%)	1 (0.2%)
Squamous cell carcinoma	1 (0.2%)	2 (0.8%)	3 (0.5%)
Adenocarcinoma	2 (0.5%)	3 (1.2%)	5 (0.8%)
Histology			
NEG	102 (25.2%)	27 (10.8%)	129 (19.7%)
CIN1	113 (27.9%)	66 (26.4%)	179 (27.3%)
CIN2	64 (15.8%)	73 (29.2%)	137 (20.9%)
$\geq$ CIN3	126 (31.1%)	84 (33.6%)	210 (32.1%)

**Table 2.** Prevalence of HPV genotypes in Onclarity HPV-positive women  $\geq 30$  years of age, with available colposcopy and histological diagnosis

Onclarity genotype <i>n</i> (%)	Denmark, Onclarity HPV+			Italy, Onclarity HPV+			Combined		
	<CIN2 <i>n</i> = 116	CIN2 <i>n</i> = 31	$\geq$ CIN3 <i>n</i> = 65	<CIN2 <i>n</i> = 63	CIN2 <i>n</i> = 49	$\geq$ CIN3 <i>n</i> = 65	<CIN2 <i>n</i> = 179	CIN2 <i>n</i> = 80	$\geq$ CIN3 <i>n</i> = 130
16	26 (22.4)	17 (54.8)	29 (44.6)	14 (23.0)	28 (57.1)	34 (52.3)	40 (22.6)	45 (56.2)	63 (48.5)
31	12 (10.3)	0 (0.0)	10 (15.4)	8 (13.1)	2 (4.1)	5 (7.7)	20 (11.3)	2 (2.5)	15 (11.5)
18	11 (9.5)	8 (25.8)	16 (24.6)	13 (21.0)	14 (28.6)	14 (21.5)	24 (13.5)	22 (27.5)	30 (23.1)
33/58	21 (18.1)	7 (22.6)	8 (12.3)	5 (8.1)	10 (20.4)	12 (18.5)	26 (14.6)	17 (21.2)	20 (15.4)
52	19 (16.4)	3 (9.7)	4 (6.2)	11 (17.7)	2 (4.1)	2 (3.1)	30 (16.9)	5 (6.2)	6 (4.6)
45	16 (13.8)	5 (16.1)	9 (13.8)	5 (8.2)	3 (6.1)	2 (3.1)	21 (11.9)	8 (10.0)	11 (8.5)
51	5 (4.3)	3 (9.7)	2 (3.1)	5 (8.1)	2 (4.1)	2 (3.1)	10 (5.6)	5 (6.2)	4 (3.1)
35/39/68	16 (13.8)	1 (3.2)	4 (6.2)	5 (8.1)	6 (12.2)	5 (7.7)	21 (11.8)	7 (8.8)	9 (6.9)
59/56/66	36 (31.0)	6 (19.4)	1 (1.5)	25 (40.3)	3 (6.1)	3 (4.6)	61 (34.3)	9 (11.2)	4 (3.1)

positive, 27.5% were HPV18, 10% were HPV45 and 21.2% were HPV33 and/or 58 (combined detection of these two genotypes). All remaining hrHPV genotypes detected had prevalence below 12% (Table 2). For  $\geq$ CIN3, the combined prevalence was 48.5, 23.1, 15.4 and 11.5% for HPV16, HPV18, HPV33/58 and HPV31, respectively. Most notable prevalence differences observed for  $\geq$ CIN3 between the Italian and Danish study arms were HPV16 with 52.3% vs. 44.6%, HPV31 with 7.7% vs. 15.4% and HPV45 with 3.1% vs. 13.8%, respectively.

Of all genotypes, only HPV16, HPV52 and 33/58 showed risk estimation heterogeneity between Italy and Denmark, and only at  $\geq$ CIN2. When looking at  $\geq$ CIN3, the heterogeneity between the countries was nonsignificant (Table 3).

Applying Bayesian probability modeling to the combined data for this referral population showed that the  $\geq$ CIN2 risk of HPV16 was 69.1%, with HPV31 at 63.3%, HPV33/58 at 52.7%, HPV18 at 46.6% and HPV52 at 40.8% (Table 4). Correspondingly, for  $\geq$ CIN3, the risks were 44.3, 38.5, 36.8, 30.9 and 16.8% for HPV16, HPV31, HPV18, HPV33/58 and HPV52, respectively (Table 4, Fig. 2), indicating that the baseline risk of disease arising from HPV16 is, not surprisingly, the highest among the hrHPV genotypes.

## Discussion

To improve specificity of HPV-based screening algorithms without loss of sensitivity, one may want to assign a certain action to only a subset of hrHPV genotypes to reduce the number of referrals to colposcopy in favor of retesting within a defined interval. The concept of risk-based screening is a paradigm reflected in all available guidelines and the use of HPV genotyping information is merely an extension of this concept.

Here, we present data from a European study demonstrating that risk of CIN2 and  $\geq$ CIN3 is genotype specific in a colposcopy referral population of women  $\geq 30$  years of age. Our study comprises 655 cases with HPV testing with extended genotyping and concurrent cytology from both SurePath collected and ThinPrep collected samples and histologically follow up.<sup>18,19</sup> Our data demonstrate large variations in the risk associated with the detection of different hrHPV genotypes where HPV16, HPV18, HPV31 and HPV33/HPV58 confers the highest risk of underlying disease. At the other end of the spectrum, the combined detection of HPV56/HPV59/HPV66 is associated with low risk of disease (Fig. 2). Further subdividing HPV genotypes as per prevalence (Table 2) and prevalent risk (Table 3) of CIN2 and  $\geq$ CIN3, we applied Bayesian

**Table 3.** Prevalent risk of  $\geq$ CIN2 and  $\geq$ CIN3 by Onclarity genotype and by country, in women  $\geq 30$  years of age

Onclarity genotype	$\geq$ CIN2 risk			$\geq$ CIN3 risk		
	Denmark	Italy	Heterogeneity <i>p</i> value	Denmark	Italy	Heterogeneity <i>p</i> value
16	63.9 (51.7, 74.9)	81.6 (71.0, 89.5)	0.017	40.3 (28.9, 52.5)	44.7 (33.3, 56.6)	0.620
31	68.6 (50.7, 83.1)	68.3 (51.9, 81.9)	1.000	45.7 (28.8, 63.4)	34.1 (20.1, 50.6)	0.352
18	45.5 (24.4, 67.8)	46.7 (21.3, 73.4)	1.000	45.5 (24.4, 67.8)	33.3 (11.8, 61.6)	0.514
33/58	41.7 (25.5, 59.2)	81.5 (61.9, 93.7)	0.002	22.2 (10.1, 39.2)	44.4 (25.5, 64.7)	0.100
52	23.8 (8.2, 47.2)	68.8 (41.3, 89.0)	0.009	19.0 (5.4, 41.9)	31.2 (11.0, 58.7)	0.458
45	46.7 (28.3, 65.7)	50.0 (18.7, 81.3)	1.000	30.0 (14.7, 49.4)	20.0 (2.5, 55.6)	0.696
51	50.0 (18.7, 81.3)	44.4 (13.7, 78.8)	1.000	20.0 (2.5, 55.6)	22.2 (2.8, 60.0)	1.000
39/68/35	26.9 (11.6, 47.8)	26.7 (7.8, 55.1)	1.000	15.4 (4.4, 34.9)	13.3 (1.7, 40.5)	1.000
59/56/66	16.3 (6.8, 30.7)	19.4 (7.5, 37.5)	0.765	2.3 (0.1, 12.3)	9.7 (2.0, 25.8)	0.303
HR+	45.3 (38.5, 52.2)	64.4 (56.9, 71.4)	<0.001	30.7 (24.5, 37.3)	36.7 (29.6, 44.3)	0.235

Table 4. Bayesian analysis for prevalent risk of  $\geq$ CIN2 and  $\geq$ CIN3 in the combined population

Genotype	$\geq$ CIN2 risk % (95% CI)	Probability that row genotype has higher $\geq$ CIN2 risk than column genotype									
		16	31	33/58	18	52	39/68/35	51	45	59/56/66	
16	69.1% (63.4%, 74.8%)		0.85	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
31	63.3% (54%, 71.9%)	0.15		0.93	0.96	1.00	1.00	1.00	1.00	1.00	1.00
33/58	52.7% (42.2%, 62.8%)	0.00	0.07		0.74	0.90	1.00	1.00	1.00	1.00	1.00
18	46.6% (30.5%, 61.6%)	0.00	0.04	0.26		0.69	0.96	0.96	0.96	1.00	1.00
52	40.8% (25.4%, 55.7%)	0.00	0.01	0.10	0.31		0.87	0.92	0.92	1.00	1.00
39/68/35	28.9% (17%, 41.7%)	0.00	0.00	0.00	0.05	0.13		0.65	0.67	1.00	1.00
51	25.3% (10.9%, 42.4%)	0.00	0.00	0.00	0.04	0.09	0.35		0.53	0.99	0.99
45	24.6% (11.1%, 43.4%)	0.00	0.00	0.00	0.04	0.08	0.34	0.47		0.99	0.99
59/56/66	6.1% (1.3%, 14.3%)	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01		

Genotype	$\geq$ CIN3 risk % (95% CI)	Probability that row genotype has higher $\geq$ CIN3 risk than column genotype									
		16	31	18	33/58	52	45	51	39/68/35	59/56/66	
16	44.3% (38.7%, 49.9%)		0.88	0.84	0.99	1.00	1.00	1.00	1.00	1.00	1.00
31	38.5% (29.9%, 47.1%)	0.12		0.58	0.86	1.00	1.00	1.00	1.00	1.00	1.00
18	36.8% (22.6%, 50.6%)	0.16	0.42		0.74	0.98	0.99	0.99	0.99	1.00	1.00
33/58	30.9% (20.9%, 41.3%)	0.01	0.14	0.26		0.97	0.97	0.98	0.99	1.00	1.00
52	16.8% (7.5%, 29.1%)	0.00	0.00	0.02	0.03		0.62	0.64	0.71	1.00	1.00
45	14.7% (4.3%, 28.4%)	0.00	0.00	0.01	0.03	0.39		0.51	0.57	0.99	0.99
51	14.2% (4.4%, 26.8%)	0.00	0.00	0.01	0.02	0.36	0.50		0.55	0.99	0.99
39/68/35	13.1% (5.5%, 22.9%)	0.00	0.00	0.01	0.01	0.29	0.43	0.45		0.99	0.99
59/56/66	1.9% (0.1%, 7%)	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01		

multivariate method (MCMC) modeling to estimate risk of CIN2 and  $\geq$ CIN3 of the individual genotypes and thereby rank them (Table 4). The Bayesian MCMC is similar in concept to the hierarchical method,<sup>20,21</sup> with the added benefit of allowing uncertainty in the hierarchy estimation. For subjects with multiple infections the Bayesian MCMC assigns a risk value equal to the highest risk conferred by each genotype, and the risk ordering of genotypes can vary during the Bayesian MCMC estimation process. The strength of this approach is that probabilistic statements about the genotype risk ordering can be calculated as the Bayesian MCMC can estimate the probability that a given genotype's risk is in the top risk group of genotypes or the probability that a genotype's risk is at least as high as a given threshold. The limitation of Bayesian MCMC on the other hand is that the use of a given prior may influence results if study data are limited.

Based upon the Bayesian MCMC analysis, four groups of hrHPV genotypes can be clustered from our data. Not surprisingly, HPV16 is in its own category with a prevalence at 48.5% and a 44.3% prevalent risk of  $\geq$ CIN3 (Tables 2 and 4). HPV31, 18 and 33/58 constitute the second category at a prevalence of 11.5, 23.1 and 15.4% for  $\geq$ CIN3, respectively, but with 38.5, 36.8 and 30.9% prevalent risk of  $\geq$ CIN3. The third category includes HPV45, 51, 52 and 35/39/68, though the latter is a composite value for the nondiscriminatory detection of any of the three genotypes individually or in combination. Finally, a fourth category containing the combined detection of HPV56/59/66 is prevalent at 3.1% but only has a prevalent risk of  $\geq$ CIN3 of 1.9%. In fact, no

CIN3 was found based upon a single detection of HPV56/59/66 (data not shown). Instead, these genotypes were found as part of multiple infections with genotypes at higher risks questioning which of the infections were the true drivers of transformation.<sup>20</sup> From a risk estimate perspective, a limitation of our study is that data are derived from a referral cohort of mostly HPV-positive women with abnormal cytology and not a random cohort from primary HPV screening. This leads to higher risk estimates, however the risk ordering we observe mirrors what is previously seen in several studies especially when looking to risk-estimates in HPV-positive women with abnormal cytology.<sup>12,22,23</sup>

In theory, different genotypes could have different risk profiles in different populations. Our data originates at two geographically distinct areas, the Milan area of Northern Italy and Capital Region of Denmark but evaluating differences in prevalent risk of CIN by HPV genotypes showed few differences between the sites. Most notably, HPV18 was associated with a higher risk in Denmark (45.5%) compared to what was found in Italy (33.3%; Table 3). On the other hand, HPV52 was associated with a higher risk in Italian women compared to Danish women, though both observations could be due to low numbers, more than a true representation of geographical defined differences in the oncogenicity of the genotypes. Nonetheless, the hypothesis that different genotypes could have different impact in geographical distinct populations should not be discounted. Mirabello *et al.*<sup>24</sup> convincingly showed a large variability in HPV16 variant lineages, suggesting that each

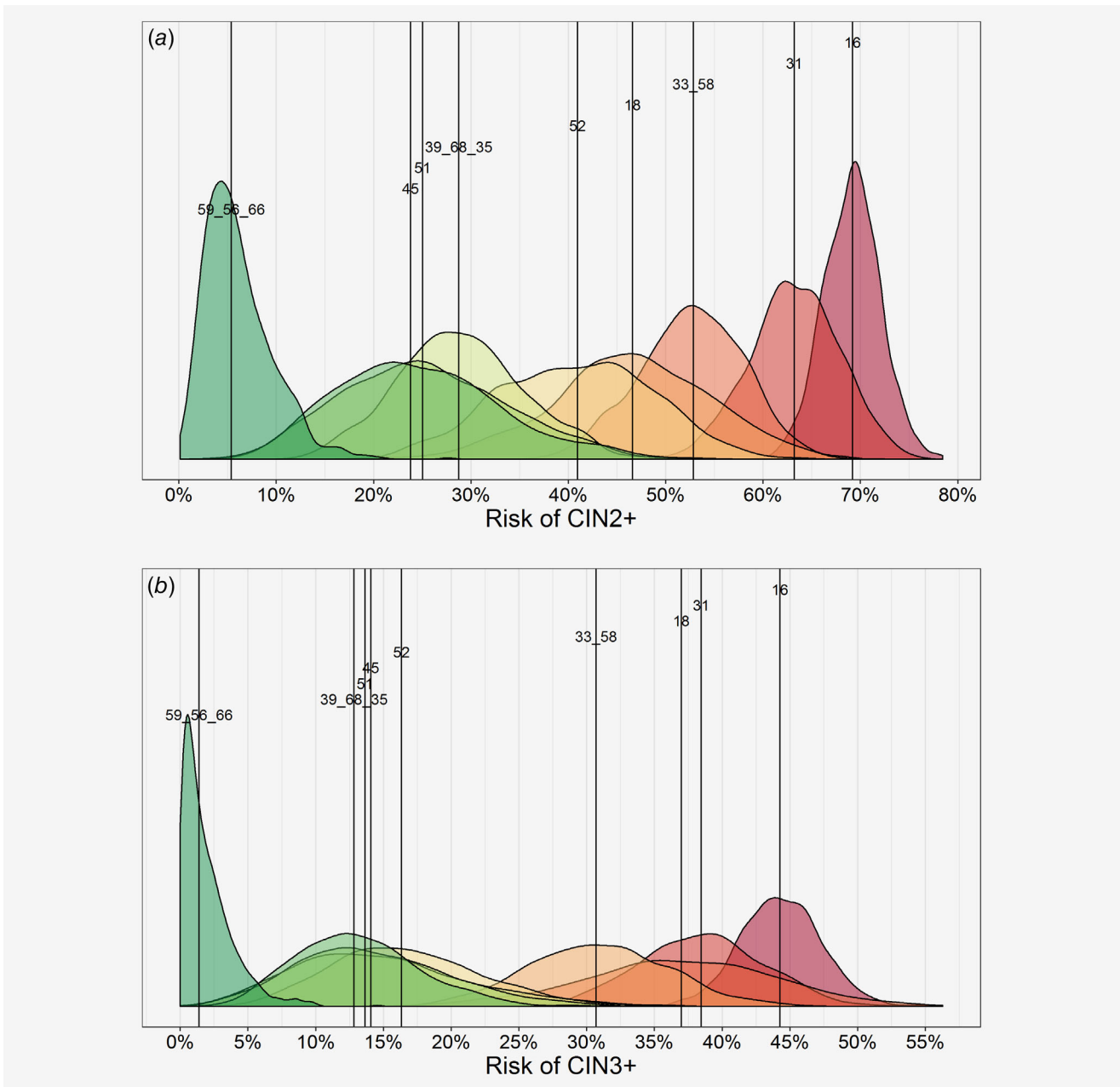


Figure 2. Risk of (a) CIN2+ and (b) CIN3+ by genotype. 95% probability with confidence intervals. [Color figure can be viewed at wileyonlinelibrary.com]

variant could indeed be considered an individual virus, with individual carcinogenic potential and which could give rise to different geographic risk profiles.

Knowing the specific risk of a given genotype for the development of CIN2 or CIN3 will offer screening laboratories, new options to more precisely refer women for follow up without having to conduct a series of different triage tests which are both time-consuming and increase the cost of diagnosis. Together, this allows an individualized risk-of-disease assessment opening new scenarios in HPV management as primary HPV screening will be challenged by a high number of hrHPV-positive samples, not all

of which will result in a diagnosis of disease. Triage strategies, which can delineate the clinically relevant infections, are required but consensus is, at best, only slowly building.<sup>13,25–27</sup> Whereas the discussion of risk stratification by HPV genotypes began almost a decade ago, previous use of HPV genotyping in screening has been restricted to limited genotyping for HPV16/18 as in the ASCCP guidelines,<sup>28</sup> alternatively requiring adjunct testing on a separate HPV genotyping assay. The question thus remains whether we can utilize HPV genotype risk estimates effectively in an HPV-positive screening setting to stratify women for follow-up while reducing the harm of overtreatment due to a

false-positive result. Here, screening guidelines should weigh the longitudinal risk of individual genotypes based on the concept of equal management of equal risk. Women with high enough risk of  $\geq$ CIN3 should be referred to colposcopy, whereas those at lower risk may be referred to less invasive and resource requiring retesting at a defined interval to allow an opportunity for viral clearance and lesion regression to occur. Mounting evidence over the last decade suggests that other hrHPV types such as HPV31, 33, 52 and 58 pose a  $\geq$ CIN3 risk equivalent or greater than that of, that is, HPV18. Cuzick *et al.*<sup>29</sup> evaluated the role of full genotyping in the accuracy of  $\geq$ CIN2 detection and found that in women with abnormal cytology HPV33 was associated with the same positive predictive value (PPV) for  $\geq$ CIN2 and  $\geq$ CIN3 of HPV16. Moreover, they found that HPV18 was associated with a lower PPV, similar to HPV31, 52, 35, 58 and 51. A large Danish study by Thomsen *et al.* confirmed that the long-term risk of  $\geq$ CIN3 by HPV31 and 33 was at par with HPV16 in Danish women  $\geq$ 30 years of age.<sup>30</sup> Moreover, the combined risk of HPV31 and HPV33 rivals even HPV16 in women  $\geq$ 30 years of age.<sup>22,23,30–33</sup>

Thus, extended genotyping can allow for improved risk stratification of patients and offer stronger screening algorithms. To this end, we suggest that genotyping used as part of advanced integrated screening algorithms adds value especially in cases of hrHPV-positive with low-grade cytology triage outcome (ASCUS or LSIL).<sup>34–36</sup> As HPV genotype information is directly available from the screening test it is rather simple to combine with the cytology triage outcome. Yet, to achieve this a firm, risk-based referral algorithm based upon estimations of risk of  $\geq$ CIN2 or  $\geq$ CIN3 by the individual HPV genotypes in referral populations such as our is required<sup>37</sup> combined with risk-estimates from studies detailing the genotype risk in primary screening populations.<sup>11,12,38</sup> Also needed is a discussion of what is an acceptable risk for underlying disease *vs.* the value of a reduced number of colposcopy referrals. In this respect, a cytology triage outcome of  $\geq$ HSIL continues to warrant direct referral to colposcopy in line with current clinical practice.

Moreover, an added benefit of the risk-based approach to clinical management of screening positive women include genotyping as a monitoring tool to reveal persistent *vs.* multiple transient infections by multiple samples over time. Here, persistence determination and screening history combined can be a parameter in the overall risk assessment.<sup>39–41</sup> Finally, from a screening laboratory operational perspective, HPV assays with extended genotyping for cervical cancer screening are already available,<sup>42,43</sup> and these assays have the capacity for returning detailed genotype

results on an HPV-positive sample without further analysis required. On the other hand, assays with limited genotyping reporting HPV16 and 18 individually combined with bulk detection of the remaining hrHPV genotypes will have limited value in such strategy as well as in screening populations with increasing proportions of HPV16/18 vaccinated women reducing the assay to a simple hrHPV “yes/no” assay.<sup>44–48</sup>

## Conclusion and Perspectives

Here, we present data from a European study demonstrating that risk of CIN2 and  $\geq$ CIN3 is genotype specific in a colposcopy referral population of women  $\geq$ 30 years of age. The extended genotyping information was retrieved directly from the clinical sample without the need for adjunct testing. We report that the HPV genotype-specific  $\geq$ CIN2 and  $\geq$ CIN3 risk-patterns are so distinct that, for example, 35/39/68 and 56/59/66 should be considered only for low intensive follow-up, for example, retesting after a defined period thereby proposing to actively use this information in triage strategies for women with HPV screening positive baseline test results.

Similarly, screening guidelines should weigh the longitudinal risk posed by HPV31, HPV33/58 and HPV52 compared to HPV16 and HPV18 based on the concept of equal management of equal risk.

Finally, use of a screening assay with genotyping in a screening algorithm could potentially reduce the need for other technologies to triage HPV-positive screening samples, thereby lowering the turnaround time and cost per screening sample.

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## Author contribution

JBO and MTS designed the study. DME, HP, MF, FB and MTS performed the clinical and laboratory work. DME, MTS, MS, JB, VP, LV and KY performed data analysis. DME, MTS, FB and JB assisted the interpretation of the results. All authors wrote, read and approved the final version of the article. MTS and JB are guarantors for the study.

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