Koilocytosis and squamous (pre)neoplasia as detected in population-based cervical screening: practice and theory

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Summary

Introduction: Koilocytosis (cavitation of the cytoplasm due to active HPV infection) can be detected in the screening process for cervical carcinoma.

Objective: To report the practice of detection of koilocytosis and (pre)neoplasia in population screening and to exploit the collected data to propose an explanation for the relationship between HPV infection and nuclear precancerous changes.

Study design: Centrally collected and stored (SBBW, Leiden, the Netherlands) data from all smears of six regional pathology laboratories (1995-2002), coded according to KOPAC (the national cervical smear coding system; S1: normal thru S9: invasive carcinoma) were accessed. Prevalences per 100,000 smears were calculated for koilocytosis and for squamous abnormalities after stratification for country of origin of screenees. The relative risk (RR) for the *ethnic (age)* groups was computed by dividing the prevalence of the relevant *ethnic (age)* group by the prevalence of *all* women.

Results: Surinamese women featured the highest prevalence of koilocytosis and of all squamous abnormalities. Moroccan women the lowest. The RR for koilocytosis was highest at 30 years (1.84) and lowest at 60 (0.26). RR dependence on age of S5-S9 lesions was similar.

Compared to nonkoilocytotic smears, koilocytosis was 104 times more frequent in the 1,500 S4 smears, 36× more frequent in the 6,700 S2-S3 smears, and 24× more frequent in the 1,740 S5-S9 smears. In all three categories this difference is statistically significant.

Conclusion: High prevalences for both koilocytosis and for preneoplasia were detected in Surinamese immigrants, however, it still does not exclude HPV infection as a confounder linked to sexual lifestyle. The presence of koilocytosis in cervical smears may serve to identify patients with an increased risk for cervical cancer and perhaps warrant more intensive surveillance than what is provided through five-yearly screening.

Key words: Koilocytosis; Cervical screening; Immigrants.

Introduction

In the decades when DNA techniques such as PCR for the detection of DNA of human papillomavirus (HPV) were not yet available for the cytologist, it was known that when a cavity (koilos) was found around the nucleus of a squamous epithelial cell of the cervix, this nucleus often was "atypical", that is, enlarged and sometimes hyperchromatic. The term "warty atypia" was used for such changes [1], "warty" because such koilocytosis was common in virus-induced warty lesions of the external genital epithelia, the macroscopically visible condylomata acuminata. As early as 1956, koilocytotic atypia was reported by Koss and Durfee [2] and this phenomenon is illustrated in the book of Papanicolaou published in 1960 [3]. However, in Canada [4] and Finland [5], it took a further decade before the event of koilocytosis, as observed in cells exfoliated from colposcopically detected flat cervical lesions, was related to sexual transmission of a virus. Transmission electron microscopy (TEM) pictures of the (papilloma) viral particles in a koilocytotic cell derived from a colposcopically detected flat cervical lesion were published by Laverty *et al.* from

Australia [6]. Sato *et al.* [7] detected HPV-like particles in the TEM images of nine out of ten koilocytotic lesions. In the electron microscopy images it is clearly visible that the koilos observed is not a fixation artifact but is virtually an empty space, almost completely lacking cytoplasmic components, a cavity encircling the nucleus loaded with viral particles. Koilocytosis is exclusively found in mature squamous cells [8] and it can be observed both in histological sections [7, 9-11] and in smears [12-14].

There are many studies concerning koilocytosis, HPV and cervical carcinogenesis [15-18]. Koilocytotic cervical epithelium may contain numerous abnormal mitotic figures, having a reverse relationship with the expression of HPV-capsid antigen [19]. HPV-capsid antigen is frequently found in cells within koilocytotic cervical lesions [7]. According to some studies there is possibly a relationship between koilocytosis and specifically high-risk HPV [14]. In this context it is of interest to mention the results of the longitudinal study of Mittal *et al.* [20], describing that koilocytosis preceded carcinoma in situ of the cervix and disappeared from the smears by the time the carcinoma was diagnosed. In human cervical tissues implanted in mice, koilocytosis has been observed during carcinogenesis: dysplastic tissues exhibited both increased levels of proliferation and koilocytosis [21].

The Dutch national coding system for pathology findings in cervical cytology, KOPAC, was introduced in the 80s and especially designed to store the cytopathologic findings of large numbers of smears in an unequivocal manner needed for computer databases [22-25]. Thus the presence or absence of koilocytosis is reported for all smears in the Dutch national screening program. The Dutch screeners are taught how to recognize koilocytosis and how to differentiate it from mimicking cytoplasmic changes such as glycogen storage and fixation artifacts. In the KOPAC system, the (pre)neoplastic squamous epithelial changes are stratified from S2-S3 (ASCUS or borderline) to S9 (clearly invasive squamous cell carcinoma) and recorded separately from koilocytosis, allowing the calculation of the occurrence of koilocytosis in each category.

In the Western region of the Netherlands population-based cervical screening is carried out by six laboratories and all KOPAC cytology reports are centrally stored in the computers of the SBBW. The central organization, the SBBW, pays the general practitioners and the laboratories for their screening efforts on completion of the assessment. In this way, Dutch women are offered complimentary screening seven times in their life.

This database, containing information on almost 450,000 smears in the period of 1995-2002, is of an appropriate size to carry out an informative study on the relationship between koilocytosis and epithelial changes. In view of the size of the study population, stratification to assess a possible relationship with age and other risk factors related to sexual-social behavior, differ considerably in immigrant Surinamese women and monogamic Moroccan immigrants.

Material and Methods

The data of 445,080 smears screened between 1995 and 2002 in the Western region of the Netherlands were included in the study. As they reach the age of 30, 35, 40, 45, 50, 55, and 60, women living in the Western region (population of around 2 million) receive a letter of invitation to have a smear taken by their own general practitioner resulting in seven age cohorts, screened at 5-year intervals. Over the study period, compliance was almost 70%. Of the 445,080 smears, the age and the ethnicity of the women were entered into the database. Ethnicity was based on the country of birth: The Netherlands, Morocco, Turkey, Surinam and others.

All smears, screened by six pathology laboratories, were coded with the KOPAC, the Dutch national coding system for cervical cytology. The O stands for "ontsteking" (inflammatory changes) and within this category, koilocytosis is coded as O1. The remaining smears are classified nonkoilocytotic or non-O1. The P stands for "plaveiselepitheel" (squamous epithelium). Within this category S1 stands for normal or benign, S2-S3 for ASCUS (atypical cells of undetermined significance), S4 for mild dysplasia, S5 for moderate dysplasia, S6 for severe dysplasia, S7 for carcinoma in situ, S8 for microinvasive carcinoma, and S9 for frank invasive squamous cell carcinoma. All diagnoses of S5 or higher generate a referral to the hospital for a colposcopy and if required a biopsy. For the purpose of the study we grouped smears with S5 to S9 into a single category S5-S9. Thus for each smear there is both an O code (O1 or not-

Table 1. — *Data stratified by ethnicity: prevalence per 100,000 for koilocytosis and for squamous abnormalities (S2-S3, S4, S5-S9), relative risk (RR#).*

Ethnicity	Koilos		S2-S3		S4		S5-S9	
	Prev.	RR	Prev.	RR	Prev.	RR	Prev.	RR
Dutch (n = 396,300)	167	0.92	1,524	1.01	333	1.02	389	1.01
Moroccan (n = 3,600)	140	0.77	1,453	0.96	335	0.97	251	0.64*
Turkish (n = 4,300)	233	1.28	1,302	0.86	279	0.81	442	1.13
Surinamese (n = 12,700)	315	1.74*	1,520	1.01	488	1.42*	464	1.19
Other (n = 28,800)	250	1.38	1,367	0.90	451	1.31*	392	1.00
All women (n = 445,080)	181	1.00	1,511	1.00	344	1.00	391	1.00

* Statistically significant difference.
\# The RR is computed by dividing the prevalence of the relevant *ethnic* group (A) by the prevalence of *all* women (B); so, RR = A/B.

Table 2. — *Data stratified by age: prevalence per 100,000 for koilocytosis and for squamous abnormalities (S2-S3, S4, S5-S9), relative risk (RR#).*

Ethnicity	Koilos		S2-S3		S4		S5-S9	
	Prev.	RR	Prev.	RR	Prev.	RR	Prev.	RR
30 (n = 58,586)	333	1.84*	1,516	1.00	614	1.79*	707	1.81*
35 (n = 81,049)	259	1.43*	1,525	1.01	442	1.28*	601	1.54*
40 (n = 79,497)	204	1.12	1,477	0.98	384	1.11	443	1.13
45 (n = 75,566)	140	0.77*	1,736	1.15	302	0.88	303	0.77*
50 (n = 71,353)	108	0.60*	1,863	1.23*	241	0.70	209	0.53*
55 & 60 (n = 79,605)	47	0.26*	999	0.66*	139	0.41*	141	0.36*
All women (n = 445,656)	181		1,511		344		391	

* Statistically significant difference (P is on a 95% confidence level, binomial test).
\# The RR is computed by dividing the prevalence of the relevant *age* group (C) by the prevalence of *all* women (B); so, RR = C/B.

Table 3. — *Cross tabulation: squamous abnormality and koilocytosis.*

Squamous abnormality	Koilocytosis		No koilocytosis		Frequency§
	n	%	n	%	%
S1 (normal)	71	9.0	435,012	97.9	0.02
S2-S3 (ASCUS)	408	51.8	6,316	1.4	6.07
S4 (LSIL)	238	30.2	1,294	0.3	15.54
S5-S9 (HSIL & carcinoma)	71	9.0	1,670	0.4	4.08
S1-S9	788	100.0	444,292	100.0	0.18

§ Frequency = the number n of smears with koilocytosis divided by the total number (koilos + nonkoilos).

Table 4. — *Relationship of the S-codes of the Dutch KOPAC and other classification systems.*

Codes of the KOPAC system	Description - other systems	Bethesda system
S1	Normal	WNL
S2-S3	Borderline changes	ASCUS
S4	Mild dysplasia	LGS
S5	Moderate dysplasia	HGS
S6	Severe dysplasia	HGS
S7	Carcinoma in situ	HGS
S8	Microinvasive carcinoma	Carcinoma
S9	Squamous cell carcinoma	Carcinoma

O1) and an S code (S1, S2-S3, S4, and S5-S9) allowing for study of relationships.

The prevalences per 100,000 smears were calculated for koilocytosis and for squamous abnormalities (S2-S3, S4, and S5-S9). Relative risk (RR) was calculated for the data stratified by ethnicity by dividing the prevalence of the relevant ethnic group by the prevalence of the total group of 445,080 smears. The RR for the *ethnic (age)* groups was computed by dividing the prevalence of the relevant *ethnic (age)* group by the preva-
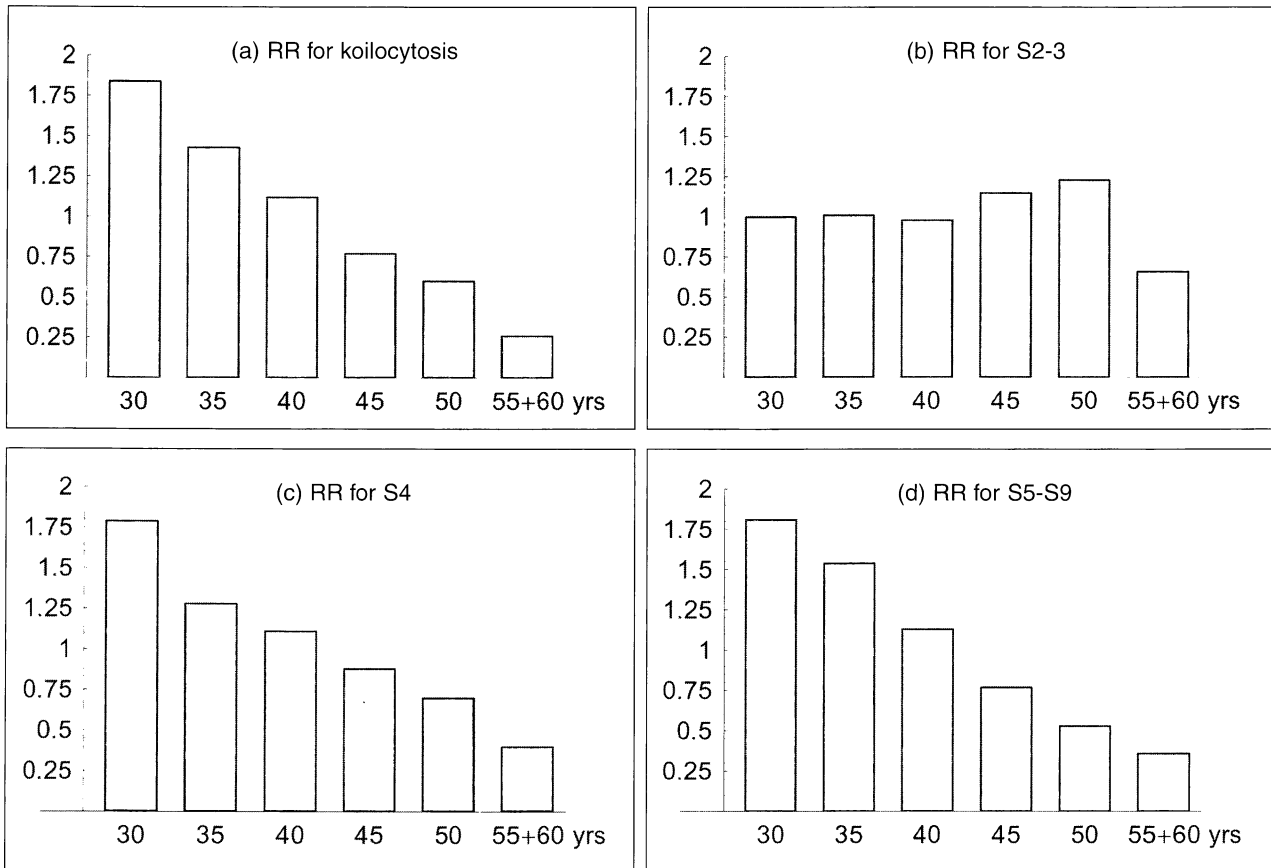
Figure 1(a) – (d). — RR for, respectively, koilocytosis (Figure 1(a)), S2-S3 (Figure 1(b)), S4 (Figure 1(c)), and S5-S9 (Figure 1(d)) of the six cohorts (30, 35, 40, 45, 50, 55, and 60 yrs old). For the calculations of the RR see Tables 1 and 2.

lence of *all* women. The probability of (non)difference of calculated RR between these groups was calculated using the Poisson test in case of low absolute numbers, and a binomial test in other cases. Cases of statistically significant differences are indicated in Tables 1 and 2 by asterisks.

Koilocytosis and squamous abnormalities are presented in a cross table (Table 3). The frequency of koilocytosis in each S-group was calculated as the number of smears with koilos divided by the total number (koilos + nonkoilos). The koilocytotic ratio in each S-group was computed by dividing the percentage of koilocytotic smears by the percentage of nonkoilocytotic smears. The relationship of the S-codes of the KOPAC with the Bethesda system is shown in Table 4.

Results

In Table 1, the prevalence per 100,000 smears for koilocytosis and for squamous abnormalities (S2-S3, S4, S5-S9) and the corresponding RRs are presented for the five ethnic groups. Surinamese women had both the highest prevalence of koilos and squamous abnormalities, with significantly high RRs for all lesions except those with S2-S3, while Moroccan women had strikingly low prevalences. The statistical significance of these differences (Tables 1 and 2) is mainly dependent on the number of cases within each ethnic group with the relevant symptom *and* the relative difference in prevalence (e.g., only 5 out of 3,600 Moroccan women had koilos.

In spite of the fact that the prevalence was 18% lower, there was no significance; the Poisson test estimated with 95% a number of one up to eight Moroccan women, so a prevalence of 180 may be realistic; with numbers higher than 20, e.g., Surinamese with severe dysplasia, S5-S9, the binomial test was used).

In Table 2, the data of respectively koilocytosis and squamous abnormalities, as stratified by age, are presented. The RR for a koilocytotic smear is highest in the 30-year-olds (1.84) and lowest in the oldest cohort of 60 years old (0.26), with significant differences in five of the six age cohorts. The findings in the smears with an S5-S9 code are quite similar.

The gradual decrease of koilocytosis with advancing age is not found with pathology of grade S2-S3, see Figures 1(a)-1(d).

The results of cross tabulation are presented in Table 3.

Discussion

The (monogamic) Moroccan women had the lowest RR for serious squamous lesions (S5-S9) and Surinamese women (often having had more than one sex partner) the highest. The difference in RR for squamous cell pathology between these two ethnic groups was even more pronounced for koilos, with values of 0.77 for Moroccan women and 1.74 for Surinamese women, while these

values were 0.64 and 1.19, respectively, for S5-S9. In theory this might indicate that koilocytosis is more closely linked to multiple sexual partners than neoplasia.

Both koilocytosis and more severe preneoplastic squamous cellular changes (S5-S9) were most frequently encountered in the youngest cohort, with a slightly larger difference in the oldest cohort for koilocytosis (RR decreasing from 1.84 to 0.26) than for (pre)neoplasia (RR decreasing from 1.81 to 0.36). Theoretically, it is possible that the RR for mild dysplasia (S4) was highest (with a value of 104) because of the presence of very large nuclei in these koilocytotic smears. These polyploid nuclei are very striking and easily detected by the screener. It is attractive to turn from practice to theory. It might be that HPV-infection in its active phase causes both koilocytosis and polyploidy. In histologic sections it was shown that such cells have high DNA values [19]. The excess DNA compromises the cell DNA replication mechanism resulting in subsequent abnormal and occasionally even multipolar mitotic figures. The zones of cell proliferation and viral DNA synthesis are often located in the same cell layer [26]. These polyploid mitotic spindles occur in various cell layers [18]. In the cells of S5-S9 smears, koilocytosis is less pronounced compared to the S4 smears (Table 3).

Our practical observations of high prevalences for koilocytosis and preneoplasia in the subgroup of Surinamese women does not exclude HPV infection as a confounder linked to the sexual lifestyle of these immigrants.

The presence of koilocytosis in cervical smears may serve to identify patients with an increased risk for cervical cancer and perhaps warrant more intensive surveillance of Surinamese immigrants than what is provided through five-yearly screening [27, 28].

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