Thieme

Histology Findings after Two Years of Cytology/HPV Co-Testing in Germany

Histologische Befunde nach 2 Jahren Co-Testung Zytologie und HPV in Deutschland

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ABSTRACT

Introduction
Since 1 January 2020, diagnostic confirmation of abnormalities detected in the context of cytology/HPV co-testing in cervical cancer screening under the statutory health insurance scheme in women aged 35 and over has been performed according to predefined algorithms. A colposcopy is indicated even in the case of borderline/low-grade cytological changes and/or HPV persistence. In this article we compare the histology findings after primary screening examinations in 2020/21 with those from 2018/19, thus also comparing the results of two different screening approaches.

Patients and Methods
Our analysis included all of the cytology, HPV, and histology results from all primary screening examinations, as well as the resulting diagnostic confirmation and curative cases, that could be obtained by 30 June 2023. In 2018/19 these comprised 650600 cytology and 1804 histology findings, and in 2020/21 there were 491450 cytology and 7156 histology findings. The absolute numbers of histology findings and the percentage ratios of these to all cytological diagnoses are presented with comparison factors.

Results
In 2020/21 there were 5.2 times more histology findings in relation to all previous cytology examinations than in 2018/19, as well as 10.6 times more biopsies, 3.8 times more conizations, and 1.2 times more hysterectomies. There was a particularly high increase in diagnostic confirmation of borderline/low-grade or only HPV-positive findings. With co-testing, 12.7 times more CIN1, 6.4 times more CIN2, and 3.5 times more CIN3 lesions were diagnosed. The proportion of biopsies without dysplasia was 7.6 times higher than in previous years. Cervical carcinomas were diagnosed 1.8 times more frequently, and endometrial carcinomas 0.7 times less frequently.

Conclusion
More CIN lesions were found with co-testing, but the increase in histology findings of low-grade or no dysplasia was far greater than findings of CIN3. Lesions not requiring treatment accounted for 94.4% of biopsy results in 2020/21. The use of computer-assisted LBC with progression markers could reduce this.

ZUSAMMENFASSUNG

Einleitung
Seit dem 01.01.2020 wird bei Auffälligkeiten im Rahmen der Co-Testung Zytologie/HPV bei der Zervixkarzinomvorsorge der GKV ab 35 Jahren nach vorgegebenen Algorithmen abgeklärt. Bereits bei grenzwertigen/geringgradigen zytologischen Veränderungen und/oder HPV-Persistenz ist eine Kolposkopie vorgesehen. In dieser Arbeit vergleichen wir die histologischen Befunde nach Untersuchungen in der primären Prävention 2020/21 mit 2018/19 und damit auch die Ergebnisse zweier unterschiedlicher Screeningansätze.

Patientinnen und Methoden
Alle zytologischen, HPV- und histologischen Ergebnisse aus allen primären und den daraus resultierenden Abklärungs- und kurativen Fällen, die bis 30.06.2023 in Erfahrung gebracht werden konnten, wurden analysiert. Aus den Jahren 2018/19 waren dies 650 600 zytologische und 1804 histologische Befunde, 2020/21 491 450 zytologische und 7156 histologische Befunde. Die absoluten Zahlen der histologischen Befunde und die prozentualen Verhältnisse dieser zu allen zytologischen Diagnosen werden mit Vergleichsfaktoren dargestellt.

Ergebnisse
2020/21 gab es bezogen auf alle vorhergehenden Zytologien 5,2-mal mehr histologische Befunde als 2018/19, 10,6-mal mehr Biopsien, 3,8-mal mehr Konisationen und 1,2-mal mehr Hysterektomien. Besonders stark nahm die Abklärung grenzwertiger/niedriggradiger oder nur HPV-positiver Befunde zu. Mit der Co-Testung wurden 12,7-mal mehr CIN1, 6,4-mal mehr CIN2 und 3,5-mal mehr CIN3 diagnostiziert. Der Anteil der Biopsien ohne Dysplasie war 7,6-mal höher als die Jahre zuvor. Zervixkarzinome wurden 1,8-mal mehr und Endometriumkarzinome 0,7-mal weniger diagnostiziert.

Schlussfolgerung
Mit der Co-Testung wurden mehr CIN gefunden, aber die Zunahme der histologischen Befunde niederen Grades oder ohne Dysplasie war weit stärker als jene der CIN3. Nicht therapiepflichtige Läsionen machten 2020/21 zusammen 94,4% der Biopsieergebnisse aus. Der Einsatz von LBC mit Computerassistenz und Progressionsmarkern könnte diese reduzieren.

Introduction

COMMENTS
The cytology findings were categorized according to the Munich Nomenclature III, which is mandatory in Germany. They have been converted in each case to the Bethesda system (TBS), and the corresponding diagnoses are reported in brackets; in the text, HPV always means high-risk (HR) HPV.

Since 2020, the new standard in the German statutory health insurance (SHI) program for the prevention of cervical carcinoma in women aged 35 and over has been cytology/HPV co-testing every three years instead of annual conventional cytology. Liquid-based cytology (LBC) techniques and computer assistance (CAS) are now also permitted for cytology, but are not additionally funded [1]. The mandatory algorithms for diagnostic confirmation of abnormal findings established by the Federal Joint Committee, a regulatory body, require expert colposcopy and, if possible, biopsy, even if there are only slight deviations from the norm [1]. This applies, for example, in the case of two positive HPV tests (even if HPV 16 or 18 is not detected), as well as borderline cytology findings (Munich nomenclature): Pap II-p/g; TBS: ASC-US/AGC-NOS) with HPV positivity or two Pap III D1 findings (TBS: LSIL) without HPV positivity. This is in contrast to the S3 guideline "Prevention of cervical carcinoma", which initially recommends a further check-up in these cases, also including testing for the biomarker p16/Ki-67 [2].

The data presented here were collected in a routine cytology and molecular biology laboratory specializing in cervical cancer screening examinations (MVZ CytoMol, Frankfurt am Main, Germany). Since the start of co-testing in 2020, LBC (ThinPrep, Hologic, Wiesbaden, Germany) with computer assistance (Imager, Hologic, Wiesbaden, Germany) together with HPV DNA testing (cobas, Roche Diagnostics, Mannheim, Germany) has been offered as the standard for co-testing. This offer was accepted by over 99% of the eligible individuals. The data collected under these conditions on cytological diagnoses in 2020 and 2021 compared to those based on conventional cytology without routine HPV testing in 2018 and 2019 were previously published in this journal in 2022 [3]. The histology findings collected following either co-testing (2020/21) or cytology examinations in primary screening (2018/19) are now also available, insofar as they could be obtained. In this article, we correlate these findings to previous cytology findings, and compare the results from the periods before and after the introduction of co-testing. Due to the high number of cases that were examined in this laboratory using co-testing and the large number of histology findings that were subsequently

documented, this provides a good basis for an initial critical evaluation of the new screening algorithm.

Patients and Methods

Patients and diagnostic procedures

In Germany, colposcopy examinations and biopsies, as well as therapeutic procedures such as conizations, are performed decentrally in numerous practices and institutions. As at 31 December 2021 (latest available figures), 39 dysplasia units and 247 dysplasia consultations had obtained the relevant certification [4]. However, it is estimated that some of the examinations and procedures are performed outside of these certified facilities. Above all, however, it is neither intended nor possible to include cases treated in facilities that do not do billing using SHI codes for the statutory screening examination (such as university hospitals or non-certified practices) for the purpose of evaluating the results of co-testing. Therefore, at CytoMol, we systematically try to obtain the relevant findings from our submitters. This is also required under the agreement on quality assurance measures pursuant to Section 135 (2), Book V of the German Social Code [SGB V] (Cervical Cytology Quality Assurance Agreement). First of all, for every abnormal cytology finding or finding of HPV persistence that we issue, we ask to be sent the results of any diagnostic examinations or therapeutic procedures. If we do not receive these within one year, the practices in question will be asked to send us the relevant findings via a so-called recall list. If the cytology of a new cytological examination ordered from us indicates that an intervention has taken place, we will request by telephone and/or fax to be provided with the relevant histology findings.

When the medical reports arrive at CytoMol, they are scanned for electronic storage and the core diagnoses are entered into our laboratory system once the reports have been checked by medical and administrative staff. Over a year, these activities are expected to occupy at least one full-time employee. In the course of the first year after histological diagnosis, over 70 % of this data was obtained and stored. For Pap IVa-p in 2021 (SHI), we had received 75.42 % of histology results after one year. After another six months, this increased by at most a further 10 % of findings. In order to cover the first two years of co-testing as completely as possible, 30 June 2023 was therefore chosen as the cutoff date for receipt of the last documented histology findings.

Below we compare the histology findings obtained up to this point in time from women who were found to have abnormal cytology findings and persistent HPV positivity (Pap II-a) during co-testing in 2020 and 2021 with the histology findings from women who were found to have cytological abnormalities in the two previous years, 2018 and 2019, during the annual cytology testing without routine HPV testing that constituted the primary screening examination, performed annually at that time. Here, too, all histology findings that were obtained by 30 June 2023 were included in the evaluation. Only cases detected through primary screening, as well as subsequent diagnostic and curative procedures, were included in the evaluation. These are clearly defined through specific billing codes. It is possible that a patient may have had multiple samples taken, for example first a biopsy after primary screening cytology, then a conisate after diagnostic or curative cytology. This bias applied to both reporting periods. If several biopsies were assessed at the same time, only the highest-grade findings were recorded. In total, 650 600 cytology findings and 1804 histology findings were included in the evaluation for 2018/19, and 491 450 cytology findings and 7156 histology findings were included in the evaluation for 2020/21.

Details of the gynecology practices that performed the examinations on the women whose results form the basis of this paper, as well as details on the patient population and the procedures for cytology and HPV testing, are described in the publication of the cytology and HPV results for the first two years of co-testing [3].

Methods

The histological processing of the tissue obtained during biopsy or treatment was carried out according to medical standards adhered to in over 100 pathology institutes throughout Germany.

Data collection and statistics

The data obtained in this way were processed and stored in a specialized computer system (nexus Cytology, nexus, Donaueschingen, Germany). The data for cytology and HPV detection from the years 2020/21 and 2018/19 were already stored in the same system.

The datasets were processed descriptively, and the ratio of the frequency of histology findings in the 2018/19 vs. 2020/21 periods was compared retrospectively and also presented as a factor.

Results

Case numbers and methods for diagnostic confirmation

In 2020 and 2021, 395 759 primary screening cytology/HPV co-tests were performed; the resulting histology findings are reported here. In addition, the histology results from 11 020 diagnostic cytology and 84 671 curative cytology procedures performed following abnormal co-tests were included in the evaluation.

For the 12 264 (3.09 %) abnormal cytology findings from primary screening (n = 395 759), 2851 (0.72 %) histology findings had been obtained by 30 June 2023. From 11 020 diagnostic cytology examinations, 3064 (27.80 %) abnormal PAP findings resulted in 1673 (15.18 %) histology findings, and from 84 671 curative cytology procedures with 9760 (11.52 %) abnormal results, there were 2632 (3.1 %) histology findings. In total, 7156 (1.45 %) histology findings were obtained following 491 450 PAP tests performed in 2020/21 with 25 088 (5.1 %) abnormal findings (details ▶ **Table 1**).

In 2018 and 2019, with approximately the same number of patients, there were 588 192 cytology findings from primary prevention based on the annual cytology-only screening examination that was performed during this period. From this period up to 30 June 2023, 14 551 (2.47 %) abnormal cytological findings were generated following primary screening. This resulted in 713 (0.12 %) histology findings. After 62 408 curative cytology procedures, which resulted in 7335 (11.75 %) abnormal findings, 1091 (1.74 %) histology findings were obtained. A total of 1804 (0.28 %)

▶**Table 1** Distribution of cytology findings, histology results, and interventions in women > 35 in the years 2018/19 and 2020/21. Percentage factors comparing 2020/21 to 2018/19 in relation to all previous cytology examinations.

Cyto/Histo/Intervention/n	Year		Screening cytology		Curative cytology		Diagnostic cytology		Total		Factor 20/21 vs 18/19
		% of all procedure	n	% of all screening procedures	n	% of all curative procedures	n	% of all diagnostic confirmation procedures	n	% of all cytology procedures	
Cytological diagnoses All	2018/19		588 192		62 408				650 600		0.75
	2020/21		395 759		84 671		11 020		491 450		
Abnormal cytology	2018/19		14 551	2.47 %	7 335	11.75 %	–		21 886	3.36 %	1.52
	2020/21		12 264	3.09 %	9 760	11.52 %	3 064	27.8 %	25 088	5.10 %	
Histology	2018/19		713	0.12 %	1 091	1.74 %	–		1 804	0.28 %	5.17
	2020/21		2 851	0.72 %	2 632	3.1 %	1 673	15.18 %	7 156	1.45 %	
Biopsy	2018/19	29 %	275		255				530	0.08 %	10.63
	2020/21	59 %	1 555		1 399		1 236		4 190	0.85 %	
Conization	2018/19	52 %	258		679				937	0.14 %	3.78
	2020/21	36 %	1 107		1 111		408		2 626	0.53 %	
Hysterectomy	2018/19	19 %	180		157				337	0.05 %	1.2
	2020/21	5 %	189		122		29		340	0.06 %	

histology findings were obtained following 650 600 PAP tests with 21 886 (3.36 %) abnormal cytology findings.

While – due to the longer interval between screening smear tests – the number of PAP smears decreased by a factor of 0.75 in 2020/21 compared to 2018/19, the percentage of abnormal smears increased from 3.36 % to 5.1 %, i.e., by a factor of 1.52. The rate of diagnostic histology procedures increased from 0.28 % to 1.45 %. This represents an increase by a factor of 5.17 (details ▶**Table 1**).

Of the diagnostic procedures from 2018/19, 713 (40 %) were performed following primary cytology screening and 1091 out of 1804 cases (60 %) were performed following curative cytology. In 2020/21, out of 7156 cases, this figure was also 40 % (2851) after primary cytology (co-testing), 23 % (1673) after diagnostic cytology, and 37 % (2632) after curative cytology.

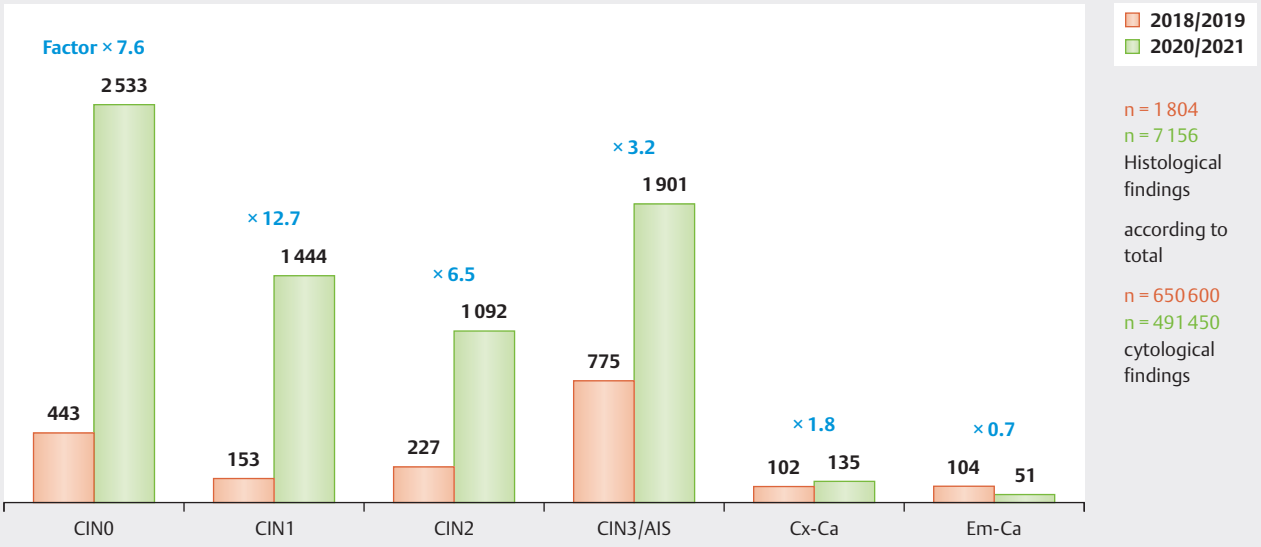
While 29 % of histology findings resulted from a biopsy or curettage in 2018/19, this percentage increased to 59 % in 2020/21. 52 % of the findings were obtained by conization in 2018/19, compared to 36 % in 2020/21. The percentage of histology findings after hysterectomy was 19 % in 2018/19; this decreased to 5 % in 2020/21 (▶**Table 1**).

The absolute number of biopsy procedures increased from 530 to 4190. Their percentage in relation to all previous cytology examinations during and following primary screening increased by a factor of 10.63 (0.08 % >>> 0.85 %). The number of conizations increased from 937 to 2626. This represented an increase by a factor of 3.78 in relation to all cytology examinations (0.14 % >> 0.53 %). The number of hysterectomies as well as their percentage in relation to cytology examinations remained almost the same (337 = 0.05 % and 340 = 0.06 %; factor of 1.2) (▶**Table 1**).

Histology results

With the exception of endometrial carcinomas and other very rare neoplasia, the absolute number of all histological diagnoses increased between 2018/19 and 2020/21, in some cases steeply. For CIN1, for example, the figure increased from 153 to 1444, corresponding to an increase (relative to all cytology examinations during those two years) by a factor of 12.7. CIN2 increased from 227 to 1092 (by a factor of 6.5). For CIN3 and adenocarcinoma in situ (AIS), the number increased by a factor of 3.2, from 775 to 1901. There was a smaller increase in invasive carcinoma of the cervix (from 102 to 135, by a factor of 1.8). Only the absolute number of endometrial carcinomas and other very rare types of neoplasia decreased from 104 to 51 (by a factor of 0.7). The increase in cases without any evidence of histological abnormalities (CIN0) was also very high. In 2018/19 the number was 443, compared to 2533 in 2020/21, a factor of 7.6 (▶**Fig. 1**).

The proportion of CIN1 increased from 8.5 % in 2018/19 to 20.2 % in 2020/21 and that of CIN2 from 12.6 % to 15.3 %, while the rate of CIN3 and AIS fell from 43 % in 2018/19 to 27 % in 2020/21, and the rate of invasive cervical carcinomas from 6 % to 2 %. Endometrial carcinomas and other very rare neoplasia were found in 0.7 % of cases in 2020/21 compared to 6 % in 2018/19. Histological examination showed an absence of any abnormalities (CIN0) in 35.4 % of cases in 2020/21, compared to 24.6 % in 2018/19. Thus, the rate of histological abnormalities in the diagnostic or



Histological findings	2018/2019		2020/2021		Factor related to all cytologies
	n	% of 650600	n	% of 491450	
Without CIN	443	0.068	2533	0.515	7.6
CIN1	153	0.023	1444	0.293	12.7
CIN2	227	0.034	1092	0.222	6.5
CIN3/AIS	775	0.119	1901	0.386	3.2
CC	102	0.015	135	0.027	1.8
EC and others	104	0.015	51	0.010	0.7

▶ **Fig. 1** Histology results from cytology + HPV co-testing > 35 years in 2020/21 vs. cytology screening only in 2018/19.

therapeutic procedures in 2020/21 decreased from 75.4% to 65.6% compared to 2018/19 (▶ **Fig. 2a,b**).

The percentage of histology findings obtained through the various diagnostic and therapeutic interventions was similar in 2020/21 compared to 2018/19 for biopsies in the case of CIN0 and CIN2 (CIN0: 53.5% vs 55.3%; CIN2: 12.6% vs 10.6%). However, it differed significantly for CIN1 (28.3% vs 13.2%) and CIN3 (4.1% vs 9.6%). With regard to conization, the differences were smaller for all CIN groups (for details see ▶ **Fig. 3a,b**). However, the absolute number of cases (see above) rose – in some cases very steeply.

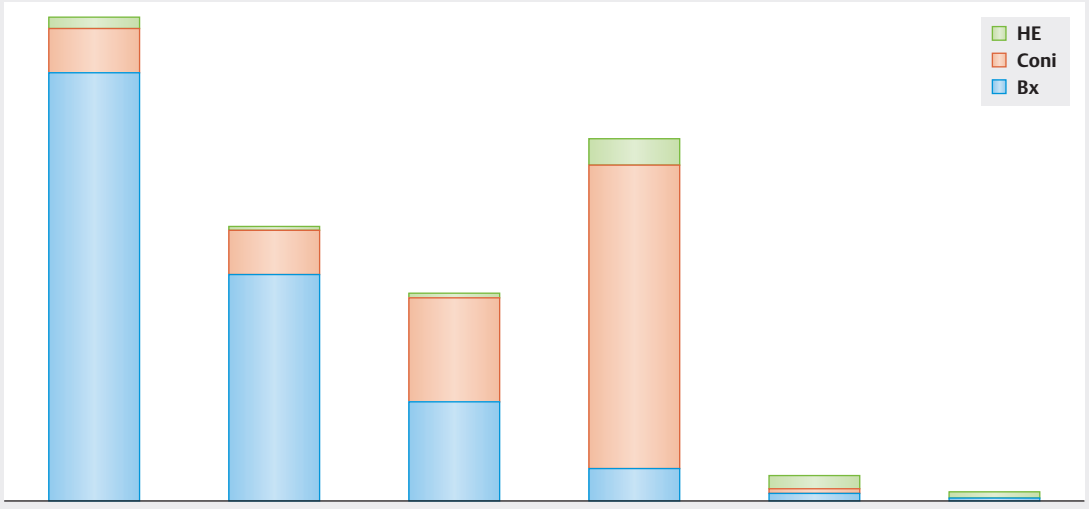
In 2020/21, 88.5% of the 2533 cases without abnormal histology findings (CIN0) were biopsied, as were 82.2% of the 1444 CIN1 cases. In contrast, 48.2% of CIN2 cases were biopsied, as were 9.0% CIN3, and 31% of cervical carcinomas. In the case of conizations, the figures were contradictory: this procedure was performed for 9.2% of CIN0, 16.4% of CIN1, 49.3% of CIN2, 83.7% of CIN3 lesions, and 18% of cervical carcinomas. The corresponding figures for 2018/19 can be found in ▶ **Fig. 2** and ▶ **Fig. 3**. Together, these lesions that did not require therapy accounted for 94.4% of the biopsy results (n = 3956). In 2018/19, this percentage, and especially the absolute number, was significantly lower, at 79.0% (n = 419).

Correlating histology after cytology (co-test) with HPV status

Due to the limitations of the routine laboratory system, the correlation of histology findings to HPV status in the previous examination (for details see [3]) could only be established for cases in which the histology was obtained directly after the primary cytology test (i.e., directly after the co-testing and thus almost always from the first diagnostic colposcopy biopsy). Out of 2851 cases, 93.8% (n = 2673) were HPV-positive, and 6.2% (n = 178) were HPV-negative. HPV positivity was found in 98.1% (n = 537) of CIN1 cases, 98.4% (n = 386) of CIN2, and 97.0% (896) of CIN3. 94.8% of the 77 invasive squamous cell carcinomas, 93.3% of the 15 AIS, 83.3% of the 24 cervical adenocarcinomas, and 0% of the 39 endometrial carcinomas were HPV-positive. The eight vulvar carcinomas and one vaginal carcinoma included in this group were HPV-positive (▶ **Table 2**).

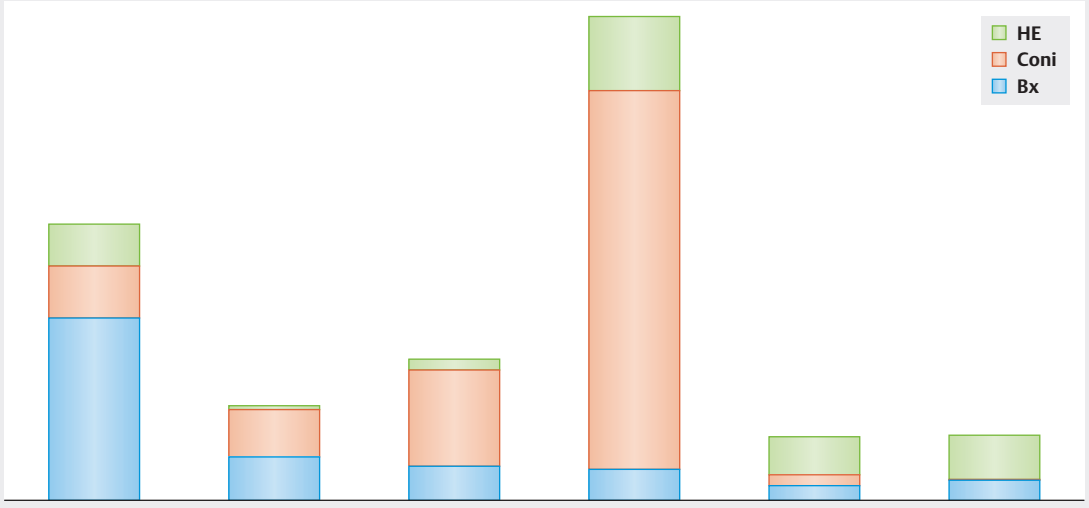
Thus, approximately 245 of the 247 Pap II-p cases (99.2%) and 816 of the 822 Pap III D1 diagnoses (99.3%) were HPV-positive. The rate of HPV positivity was slightly lower in glandular atypia (75 of 84 Pap II-g [89.3%]; 83 of 97 Pap III-g [85.6%]) and in Pap V-p (3 of 34 [91.2%] and V-g (3 of 6 [50%]). All Pap III-e, V-e and V-x cases were HPV-negative (details ▶ **Table 2**).

a



	Without CIN		CIN1		CIN2		CIN3/AIS		Cx-Ca		Em-Ca and others		All
HE	57	2.25%	20	1.39%	28	2.56%	138	7.26%	68	50.37%	29	56.86%	340
Coni	233	9.19%	237	16.41%	538	49.27%	1591	83.69%	25	18.52%	2	3.92%	2626
Bx	2243	88.55%	1187	82.20%	526	48.17%	172	9.04%	42	31.11%	20	39.22%	4190
All	2533	100%	1444	100%	1092	100%	1901	100%	135	100%	51	100%	7156
	35.4%		20.2%		15.3%		26.5%		1.9%		0.7%		100%

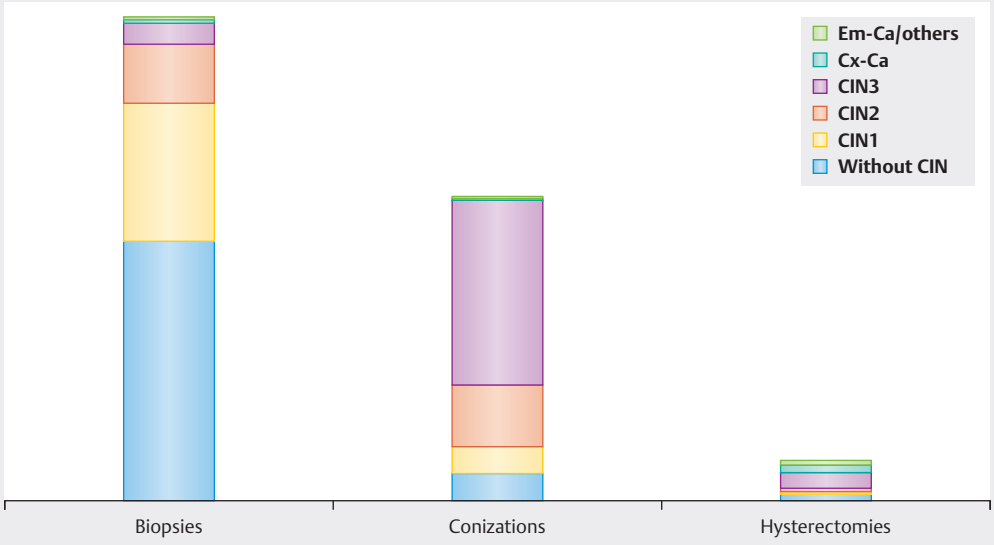
b



	Without CIN		CIN1		CIN2		CIN3/AIS		Cx-Ca		Em-Ca and others		All
HE	67	15.12%	7	4.57%	18	7.93%	117	15.09%	60	58.82%	68	65.38%	337
Coni	83	18.74%	76	49.67%	153	67.40%	607	78.32%	17	16.67%	1	0.96%	937
Bx	293	66.14%	70	45.75%	56	24.67%	51	6.58%	25	24.51%	35	33.65%	530
All	443	100%	153	100%	227	100%	775	100%	102	100%	104	100%	1804
	24.6%		8.5%		12.6%		42.9%		5.6%		5.8%		100%

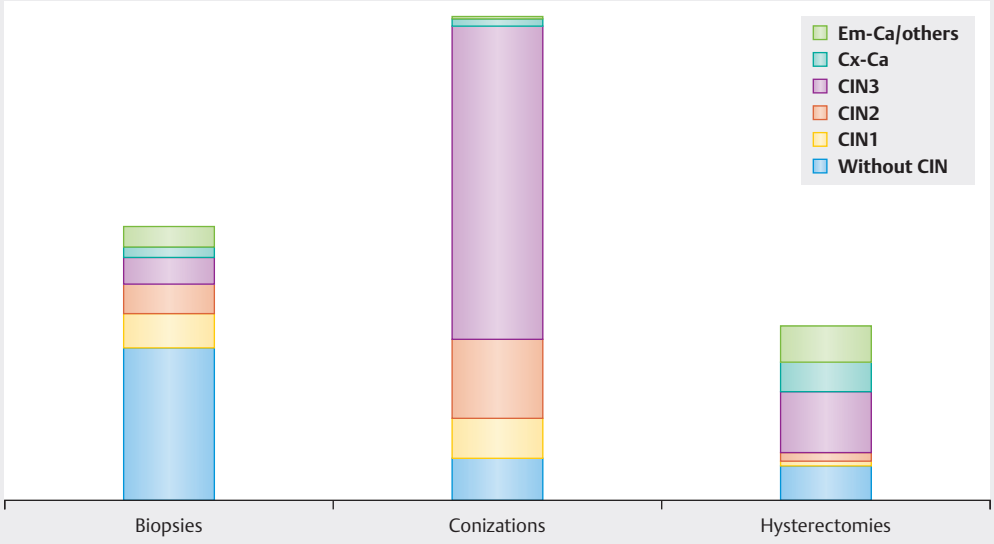
▶ **Fig. 2** **a** Proportion of types of intervention for various histology findings in 2020/21, n = 7156. **b** Proportion of types of intervention for various histology findings in 2018/19, n = 1804.

a



Distribution n = 7 156 2020/2021	Without CIN		CIN1		CIN2		CIN3/AIS		Cx-Ca		Em-Ca and others		All
Biopsies	2 243	53.53 %	1 187	28.33 %	526	12.55 %	172	4.10 %	42	1 %	20	0.47 %	4 190
Conizations	233	8.87 %	237	9.02 %	538	20.49 %	1 591	60.59 %	25	0.95 %	2	0.07 %	2 626
Hysterectomies	57	16.76 %	20	5.88 %	28	8.24 %	138	40.59 %	68	20 %	29	8.53 %	340

b



Distribution n = 1 804 2018/2019	Without CIN		CIN1		CIN2		CIN3/AIS		Cx-Ca		Em-Ca and others		All
Biopsies	293	55.28 %	70	13.20 %	56	10.57 %	51	9.62 %	25	4.72 %	35	6.60 %	530
Conizations	83	8.86 %	76	8.11 %	153	16.33 %	607	64.78 %	17	1.81 %	1	0.10 %	937
Hysterectomies	67	19.88 %	7	2.08 %	18	5.34 %	117	34.72 %	60	17.80 %	68	20.18 %	337

▶ **Fig. 3** **a** Histology findings from different types of intervention in 2020/21, n = 7156. **b** Histology findings from different types of intervention in 2018/19, n = 1804.

▶**Table 2** Histology findings (n = 2851) and HPV status with regard to the different cytology groups (n = 395 759) in primary screening (co-testing) in 2020/21.

Primary screening cytology 2020/21	CIN0		CIN1		CIN2		CIN3		AIS		PE-CC		Ad-CC		Em-Ca		All	HPV results	
Pap/HPV	neg	pos	neg	pos	neg	pos	neg	pos	neg	pos	neg	pos	neg	pos	neg	pos	n	neg%	pos%
I	34		5			1	2				1				6		49	98%	2%
II-a	5	43		5		4		8							4	4	73	12.3%	87.7%
II-p	2	148		56		20		19		1		1					247	0.8%	99.2%
II-g	6	47	1	12	1	9		7							1		84	10.7%	89.3%
II-e	11		1										1		3		16	100%	0
III D1	5	291		283		118	1	119		2		1				2	822	0.7%	99.3%
III D2	9	109	3	98	3	132	4	169		1		2		3			533	3.6%	96.4%
III-p	8	85		41		46	3	139		1		5		2	1	1	332	3.6%	96.4%
III-g	6	28		12		10	3	21	1	6	1	3	1	3	2		97	14.4%	85.6%
III-e	8														6		14	100%	0
III-x	2											1			1		4	75%	25%
IVa-p		27		18	1	34	9	338				23		1			451	2.2%	97.8%
IVa-g		2				2	1	13		3				5			26	3.8%	96.2%
IVb-p		1		1	1	3	3	27			1	13		1			51	9.8%	90.2%
IVb-g												2		5		1	8	0	100%
V-p				1		1	1	8			1	20			1	1	34	8.8%	91.2%
V-g								1				2	1		2		6	50%	50%
V-e													1		1		2	100%	0
V-x															2		2	100%	0
All	96 10.9%	781 89.1%	10 1.9%	527 98.1%	6 1.6%	380 98.4%	27 3.0%	869 97.0%	1 6.7%	14 93.3%	4 5.2%	73 94.8%	4 16.7%	20 83.3%	30 76.9%	9 23.1%	2851 100%	178 6.2%	2673 93.8%

Histology findings for the various PAP groups

The cytology was evaluated according to the Munich Nomenclature III applicable in Germany since 2014 [5]. The findings were then converted to the internationally standardized Bethesda system (TBS) [6].

Details of the evaluation are described in the previous article on cytology during the first two years of co-testing [3]. With regard to the diagnostic or therapeutic procedures to confirm abnormal cytology findings, there was a higher rate of such procedures in all PAP groups (except V-g) in 2020/21 compared to 2018/19. The increase was highest in the case of borderline and low-grade cytology findings, and became smaller as the severity of cytology results increased. The comparison factor for the diagnostic confirmation rates – in relation to the total number of all cytology examinations – was 13 for Pap II-p (ASC-US) and Pap III D1 (LSIL), 7.3 for Pap III D2 (HSIL), 4.3 for Pap III-p (ASC-H), 2.2 for Pap IVa-p (HSIL), 1.5 for IVb-p and 1.3 for V-p (carcinoma) (for details see ▶ **Table 3**).

It should be noted that Pap group II-a (NILM) (factor of 18.7) is not comparable between the two periods, because in 2018/19 there was a predominance of abnormalities relating to the patient's medical history that were not due to a positive HPV result, while in 2020/21 this diagnosis was predominantly assigned after a positive HPV finding (in our laboratory, in order to draw attention to such cases, a Pap II-a was assigned for any type of HPV positivity without cytological abnormality). Accordingly, from among the cases of primary screening cytology (co-testing) in 2020/21, 94.3 % of the Pap II-a patients were HPV-positive [3].

The absolute numbers of histology findings following abnormal cytology also increased – in most cases steeply, with the exception of Pap V (carcinoma). Thus, for Pap II-p (ASC-US) the number increased from 67 to 650, for Pap III D1 (LSIL) from 216 to 2120, for Pap III D2 (HSIL) from 274 to 1523, for Pap III-p (ASC-H) from 211 to 691, and for Pap IVa-p (HSIL) from 534 to 867. For groups IV-b, p, g 73 vs 79 and V-p 38 vs 38, the numbers remained almost unchanged. In the Pap group V-g, the absolute number in 2020/21 vs. 2018/19 actually decreased from 15 to 7 (see ▶ **Fig. 1** and ▶ **Table 3**).

The number of diagnostic and therapeutic interventions for which histological examination did not yield any conspicuous finding (CIN0) increased – in most cases very steeply – (see ▶ **Table 3**).

This was particularly pronounced in the case of borderline and low-grade findings. For example, for Pap II-a (NILM) the number increased from 19 to 410, for II-p (ASC-US) from 45 to 399, for Pap III D1 (LSIL) from 76 to 800 and Pap III-p (ASC-H) from 48 to 197. For Pap II-g (AGC-NOS) from 44 to 116, Pap III-g (AGC-FN) from 46 to 79, Pap IVa-p (HSIL) from 34 to 65, and Pap IVa-g (AIS) from 5 to 7, the increase was smaller or non-existent. For PAP IVb-p (HSIL) and PAP V (carcinoma), there were no CIN0 findings in 2020/21.

The absolute numbers of CIN1 and CIN2 lesions also increased significantly in 2020/21 – again, particularly after borderline and low-grade cytological diagnoses. Following a Pap II-a finding (NILM), CIN1 increased from 5 to 103, and CIN2 from 4 to 39. Following a Pap II-p finding (ASC-US), CIN1 increased from 5 to 143, and CIN2 from 6 to 57. Following a Pap III D1 finding (LSIL), CIN1 increased from 56 to 706 and CIN2 increased from 41 to 318, and following a Pap III-p finding (ASC-H), CIN1 increased from 18 to 103, and CIN2 increased from 35 to 113. The increase was smaller for Pap II-g (AGC-NOS), from 2 to 21 and 2 to 27 respectively for CIN1 and CIN2, for Pap III-g (AGC-FN) CIN1 increased from 3 to 27 and CIN2 increased from 8 to 26, and for Pap IVa-p (HSIL), CIN1 increased from 19 to 41 and CIN2 from 39 to 86. For Pap IVa-g (AIS), the absolute numbers remained unchanged, and for Pap V (carcinoma), there was only one CIN1 and two CIN2 findings in 2020/21.

Absolute numbers of CIN3/AIS have also increased. For Pap II-a (NILM), the number rose from 6 to 44. For Pap II-p (ASC-US) it rose from 2 to 49, for Pap III D1 (LSIL) from 39 to 289, and for Pap III-p (ASC-H) from 97 to 267. The increase was smaller for Pap II-g (AGC-NOS), from 2 to 15, for Pap III-g (AGC-FN), from 26 to 56, for Pap IVa-p (HSIL), from 421 to 639, for Pap IVa-g (AIS), from 25 to 34, and for Pap V (carcinoma), from 5 to 13. For Pap IVb-p (HSIL) and Pap IVb-g, the numbers dropped slightly from 42 to 37 and from 4 to 2 respectively.

Accordingly, the rate of CIN3, now regarded as a key target lesion in cervical cancer screening, increased in groups II-p (ASC-US) and II-g (AGC-NOS), from 3.0 % to 7.5 % and from 3.5 % to 8.3 % respectively. For Pap III D1 (LSIL) and Pap III D2 (HSIL), it dropped from 18.1 % to 13.6 % and from 37.6 % to 29.6 %, respectively. For Pap IVa-p (HSIL), there was only a slight decrease (78.8 % vs. 73.7 %). For Pap II-a (NILM), it was 7.3 %. A comparison with 2018/19 is not possible here (see above).

For details on all PAP groups and all histology findings, including the percentages and their comparison factors, see ▶ **Table 3**.

Within the scope of this study, it was not possible to record the number of colposcopy procedures in which no tissue was removed. Under the Cervical Cytology Quality Assurance Agreement, the agreement on quality assurance measures pursuant to Section 135(2) SGB V, it is in fact required to obtain the results of histological examinations for the purposes of evaluation and follow-up on cytology results; and with considerable organizational effort (see material and methods), this is largely possible to achieve. However, this does not apply to diagnostic colposcopy procedures; often the cytology laboratory is often not even aware of these procedures, and, in particular, the reports from the procedures performed in the dysplasia centers and consultation hours of hospitals and university clinics are usually not provided.

Discussion

In the first two years of cytology/HPV co-testing in 2020/21, the rate of abnormal cytology findings in the cases handled by a large routine laboratory increased by around 50 % [3]. This is probably primarily a consequence of routine parallel HPV testing. In the case of HPV positivity, more attention was evidently paid to cytological abnormalities, and hence a cytological abnormality was more likely to be determined as a final result. The new routine use of LBC with computer assistance may also have played a role. This is indicated by data from a German study [7]. The effects of the two new methods cannot be separated, as they have always been used together. What is particularly remarkable, however, is

▶**Table 3** Histology findings for various PAP groups relating to the total number of cytology examinations in women aged >35 in 2018/19 vs 2020/21 and the corresponding factor.

Cytology	Year	n histologies	CIN0		CIN1		CIN2		CIN3/AIS		Cx-Ca		Em-Ca		Factor
I	2018/19	80	50	62.50%	2	2.50%	0	0%	2	2.50%	2	2.50%	24	30.00%	1.4
	2020/21	84	62	73.8%	11	13.10%	1	1.19%	3	3.57%	1	1.19%	6	7.14%	
II-a	2018/19	42	19	45.24%	5	11.90%	4	9.52%	6	14.29%	1	2.38%	7	16.66%	18.7
	2020/21	605	410	67.77%	103	17.02%	39	6.45%	44	7.27%	0	0%	9	1.49%	
II-p	2018/19	67	45	67.16%	5	7.46%	6	8.96%	2	2.99%	2	2.98%	7	10.44%	13
	2020/21	650	399	61.38%	143	22.00%	57	8.77%	49	7.54%	1	0.15%	1	0.15%	
II-g	2018/19	57	44	77.19%	2	3.51%	2	3.51%	2	3.51%	0	0%	7	12.28%	4.5
	2020/21	181	116	64.09%	21	11.60%	27	14.92%	15	8.29%	1	0.55%	1	0.55%	
IIID 1	2018/19	216	76	35.19%	56	25.93%	41	18.98%	39	18.06%	1	0.46%	3	1.39%	13
	2020/21	2120	800	37.74%	706	33.30%	318	15.00%	289	13.63%	4	0.19%	3	0.14%	
IIID 2	2018/19	274	47	17.15%	36	13.14%	88	32.12%	103	37.59%	0	0%	0	0%	7.3
	2020/21	1523	367	24.10%	282	18.52%	414	27.18%	451	29.61%	9	0.59%	0	0%	
III-p	2018/19	211	48	22.74%	18	8.53%	35	16.59%	97	45.97%	10	4.73%	3	1.42%	4.3
	2020/21	691	197	28.50%	103	14.91%	113	16.35%	267	38.64%	9	1.30%	2	0.29%	
III-g	2018/19	100	46	46.00%	3	3.00%	8	8.00%	26	26.00%	6	6.00%	11	11.00%	2.6
	2020/21	199	79	39.70%	27	13.57%	26	13.07%	56	28.14%	7	3.51%	4	2.01%	
IVa-p	2018/19	534	34	6.36%	19	3.56%	39	7.30%	421	78.84%	20	3.74%	1	0.19%	2.2
	2020/21	867	65	7.50%	41	4.73%	86	9.92%	639	73.70%	34	3.92%	2	0.23%	
IVa-g	2018/19	40	5	12.50%	4	10.00%	3	7.50%	25	62.50%	3	7.50%	0	0%	1.9
	2020/21	57	7	12.28%	4	7.01%	3	5.26%	34	59.65%	9	15.79%	0	0%	
IVb-p	2018/19	62	2	3.22%	1	1.61%	1	1.61%	42	67.74%	16	25.81%	0	0%	1.5
	2020/21	69	1	1.45%	1	1.45%	6	8.70%	37	53.62%	24	34.78%	0	0%	
IVb-g	2018/19	11	0	0%	1	9.09%	0	0%	4	36.36%	5	45.45%	1	9.09%	1.2
	2020/21	10	0	0%	0	0%	0	0%	2	20.00%	7	70.00%	1	10.00%	
V-p	2018/19	38	1	2.63%	0	0%	0	0%	4	10.53%	27	71.05%	6	15.79%	1.3
	2020/21	38	0	0%	1	2.63%	2	5.26%	12	31.58%	21	55.26%	2	5.26%	
V-g	2018/19	15	0	0%	0	0%	0	0%	1	6.67%	8	53.33%	6	40.00%	0.6
	2020/21	7	0	0%	0	0%	0	0%	1	14.29%	4	57.14%	2	28.57%	

the five-fold increase in the number of histology findings compared to the two previous years, 2018/19, in which the screening for cervical carcinoma was based solely on an annual conventional cytology examination. In addition to the higher number of abnormal cytology and now also HPV findings, this can also be attributed to the new diagnostic algorithm which is generally mandatory, and which provides for very low threshold values for the use of diagnostic colposcopy. The absolute number of all histological diagnoses therefore increased – in some cases steeply. However, the increase in CIN3 cases was smaller – in some cases significantly smaller – than the increase in CIN1 and CIN2 cases. In percentage terms, the rates of CIN2 and CIN3 following Pap III D1 (LSIL), Pap III D2 (HSIL), and Pap III-p (ASC-H) actually decreased in 2020/21 compared to 2018/19. This was also the case for CIN3 following Pap IVa-p/g (HSIL/AIS).

The number of CIN1 cases in relation to the total number of all previous cytology examinations was 12.7 times higher in 2020/21 than in 2018/19, and the number of CIN2 cases was 6.5 times higher. With a two-year screening interval, as in our analysis, we should expect to see at least a twofold increase in dysplastic lesions in order to achieve the same efficiency as with annual screening. This is the case in the CIN3/AIS group, with a factor of 3.2. In the carcinoma group, we only find an increase of 1.8 times. Thus, if the interval is prolonged, there are clearly fewer cervical carcinomas detected per unit of time. The detection rate for endometrial carcinomas and other very rare neoplasia was even lower (factor of 0.7). One possible cause for this could be a reduced awareness of HPV-negative cytological changes, particularly of a glandular nature. In addition, the probability of detecting non-HPV-associated lesions is higher with annual cytology.

There was a significant increase, especially in absolute numbers, in the number of cases with no abnormal histology findings (CIN0). It was particularly noticeable that these figures rose sharply after diagnostic confirmation of Pap II-a (NILM) and II-p/g (ASC-US/AGC-NOS), and even more so after Pap III D1 (LSIL).

It would be reasonable to assume that in the next rounds of screening by co-testing, the number of abnormal findings and thus the number of diagnostic colposcopies will decrease. However, the experience to date in our cytology laboratory (as of February 2024) does not indicate this. On the contrary, there is an increasing number of colposcopies, especially for 2 nd, 3 rd, and 4 th procedures.

In 2020/21, there was a significant shift in the frequency of the various interventions for diagnostic confirmation of abnormal findings compared to 2018/19. In accordance with the algorithm of the Federal Joint Committee, biopsies were carried out far more frequently, resulting in a decrease in the proportion of conizations and hysterectomies. The absolute number of biopsies increased eight-fold from 2018/19 to 2020/21; measured against the number of previous cytology examinations, the increase was even more than ten-fold. At the same time, there was a sharp increase in the number of biopsies in which no histological abnormalities or only CIN1 or 2 were found. Together, this amounted to 94.4% of the biopsies. This raises the question of whether it is justified to carry out an invasive diagnostic procedure, following which almost none of the detected lesions are treated.

The rate of HPV positivity in previous co-testing was very high in all cases in which histological diagnoses, especially low-grade, were obtained (with the exception of endometrial carcinoma). Except for invasive carcinomas, it was even slightly higher than the rate for all cytology co-tests combined [3]. This was a consequence of the HPV-based algorithms for diagnostic colposcopy in cases of borderline and low-grade dysplasia and persistent HPV positivity. In routine screening, most borderline/low-grade HPV-negative cases were assessed as cytologically unremarkable. Only morphologically more conspicuous smears were investigated further despite being HPV-negative.

The rate of diagnostic procedures to confirm cytological abnormalities increased sharply in 2020/21 compared to 2018/19. This increase was most pronounced for the borderline and low-grade findings. The corresponding factor ranged from 13 for Pap II-p (ASC-US) to 2.2 for Pap IVa-p (HSIL). For Pap II-a (NILM), it even reached 18.7. As a result, the absolute numbers also increased ten-fold (Pap III D1/LSIL).

The absolute numbers and the rate of CIN3 increased. Again, the increase was slightly larger for borderline and low-grade cytology findings than for glandular and higher-grade abnormalities. However, this increase was associated with a far greater increase in biopsy procedures finding no evidence of dysplasia or lesions not requiring treatment (up to 30 times more CIN1 cases following Pap II-p/ASC-US). For Pap II-p (ASC-US) and Pap II-g (AGC-NOS), the rate of CIN3 was still slightly below the international target value of 10% (also the target value in the S3 guideline), at 7.5% and 8.3% respectively, and for Pap III D1 (LSIL), at 13.6%, it was only slightly above the target figure.

In the only other previously published evaluation of histology results following abnormalities detected through co-testing in a routine laboratory – including data from the first year only, the rate of low-grade or absent histology findings in the diagnostic confirmation of Pap II p/g (ASC-US/AGC-NOS) and Pap III D1 (LSIL) was even more pronounced [8]. In 979 women, CIN3+ was found in only 1.4% of cases of Pap II p (ASC-US) and in 7.3% of cases of Pap III D1 (LSIL). In a recently published registry study (n = 4763) from university or other highly specialized dysplasia clinics or centers investigating the results of colposcopic diagnostic confirmation of abnormal findings after co-testing, significantly higher values were found at 10.8% (II-p/ASC-US), 23.4% (II-g/AGC-NOS), and 11.7% (III D1) respectively [9]. An evaluation of 3118 cases of cytological abnormalities with HPV positivity from a university dysplasia unit in the years 2015 to 2020, i.e., predominantly before co-testing, revealed 22.4% and 14.1% CIN3+ lesions in Pap II-p/ASC-US and Pap III D1/LSIL respectively, all with HPV positivity [10]. Also prior to co-testing, Schenck reports a CIN3 rate of 7.7% for Pap II-p/ASC-US, 8.5% for Pap II-g/AGC-NOS, and 14.2% for Pap III D1/LSIL from the 2019 annual health insurance statistics [11].

A possible explanation for the higher detection rate of CIN3 following borderline and low-grade cytological abnormalities in the cohort reported in this study compared to the only evaluation following routine co-testing that has been published to date, in addition to stringent quality control and the use of LBC and CAS in cytology, is the optional use of the biomarker p16/Ki-67 in cases of HPV positivity, without or with only borderline or low-grade

cytological abnormalities. This has been recommended by CytoMol in many cases since 2012 as a supplement to the procedure for diagnostic confirmation in such constellations. In both periods, this intermediate diagnostic method was used optionally for HPV positivity without or with borderline or low-grade cytology findings. Exact quantification is not possible due to the limitations of the routine laboratory system.

RCTs have shown that for HPV positivity without cytological abnormalities or with ASC-US or LSIL findings, up to 90 % of prevalent CIN2+ can be identified with a p16/Ki-67 positivity rate of 20–30 % and 50–60 %, respectively [12, 13]. However, even with the optional use of p16/Ki-67, in the data reported here for Pap II-p/g (ASC-US/AGC-NOS), the rate of colposcopic diagnostic confirmation, at 8 %, did not quite meet the international minimum rate of 10 %. It would therefore seem sensible to include biomarkers as an obligatory intermediate step in an upcoming revision of the diagnostic algorithm. To date, only p16/Ki-67 appears to be sufficiently validated for this purpose. Methylation-based markers detect all invasive carcinomas, but significantly less CIN3 than p16/Ki-67 [14]. The 2018 German S3 guideline for the prevention of cervical carcinoma has already given a grade C recommendation (evidence level IV) for the use of this marker in cases of borderline cytological abnormalities detected through co-testing [2]. Marker-based diagnostics can be performed directly as a reflex test when using LBC, without the patient having to be called in again. Diagnostic colposcopy, on the other hand, takes a considerable amount of time for the patient and is also more stressful.

The even higher rate of CIN3+ following borderline and low-grade cytological abnormalities in the specialized centers could in turn be explained through patient selection.

The CIN3 rate after high-grade cytology findings (Pap IVa-p) was the same in the cohort reported here, at 82.6 %, as in Stübs [10] at 83.8 %, and in the annual health insurance statistics [11] at 80.5 %, and was significantly higher than in Henes [9], at 67.3 % in the specialized centers.

Screening has become much more complex due to the new algorithm. While previously the diagnosis could be made by the laboratory technician (Chemical Technical Assistant) in up to 94 % of cases, a final assessment by a doctor is now necessary in up to 15 % of cases. The complexity of possible recommendations, even falling outside the algorithm in justified cases, is a serious problem. In the second and third year of co-testing, the number of queries from practices to the laboratory and vice versa increased. In some phases, up to 30 % of cases involved queries. In addition, there were uncertainties regarding the invoicing, in particular for follow-up examinations. Many of these issues have not yet been adequately resolved.

The limitations of this study are as follows: this is a retrospective analysis of routine data from a commercial laboratory. However, this also represents an advantage, as it means a large amount of data is available from a “real life setting”. A small degree of variance in the screening cohorts for both comparison periods cannot be ruled out. However, this should not have a significant effect on the results due to the closeness in time, and the fact that minor fluctuations do not have a significant impact on very large populations. Histological processing of the tissues was carried out in numerous different pathology institutes. It was not possible to perform a central revision of the findings. For many borderline and low-grade cytology results, there are no histology findings available, as colposcopy was either not performed or did not lead to a biopsy.

Conclusion

Due to the requirements of the algorithm specified by the Federal Joint Committee, significantly more diagnostic procedures were performed in 2020/21 to clarify abnormal findings in cervical screening examinations, especially biopsies, for which the increase in relation to all previous cytology examinations was 10.6-fold. There was a particularly high increase in diagnostic confirmation of borderline/low-grade or only HPV-positive findings. However, the rate of CIN3-detection generally remained below 10 %. In total, we received five times the number of histology findings compared to the previous two years. Many more pre-neoplastic lesions were diagnosed. However, the increase in CIN1 and 2 lesions detected was significantly greater than for CIN3 lesions. There was also a dramatic increase in the rate of tissue samples that were found to be dysplasia-free, especially after biopsy. Of the cytological abnormalities for which diagnostic histology was performed, 93.8 % were HPV-positive. The HPV-based algorithm clearly leads to overdiagnosis of borderline and low-grade lesions that are HPV-positive. Out of 4190 biopsies, only 5.6 % of the lesions were found to require treatment.

As an intermediate step towards diagnostic confirmation, it would be useful to use biomarkers so as to reduce the number of unnecessary colposcopy procedures. The materials necessary for their detection are already conveniently available as a reflex test from the LBC.

Our data show that screening with co-testing is more sensitive than screening with a single (cytological) test. However, this is at the expense of specificity. In addition, with annual screening, more carcinomas of both the cervix and endometrium were diagnosed per unit of time.

Conflict of Interest

The authors are partner (HI) and employees (AX, AA, IZ) of a lab for cytology and molecular biology (CytoMol) mainly active on cervical cancer screening.

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