Thieme

Two Years of Cytology and HPV Co-Testing in Germany: Initial Experience

Zwei Jahre Co-Testung Zytologie und HPV in Deutschland: erste Erfahrungen

OPEN ACCESS

Authors
Arjola Xhaja[1], André Ahr[1,2], Ilona Zeiser[1], Hans Ikenberg[1]

Affiliations
1 CytoMol, MVZ für Zytologie und Molekularbiologie, Frankfurt, Germany
2 Universitätsfrauenklinik Frankfurt, Frankfurt, Germany

Key words
cervical cancer prevention, co-testing, HPV, cytology, LBC

Schlüsselwörter
Prävention Zervixkarzinom, Co-Testung, HPV, Zytologie, LBC

received 15.2.2022
accepted after revision 23.6.2022
published online 16.8.2022

Bibliography
Geburtsh Frauenheilk 2022; 82: 1378–1386
DOI 10.1055/a-1886-3311
ISSN 0016-5751
© 2022. The Author(s).
This is an open access article published by Thieme under the terms of the Creative Commons Attribution-NonDerivative-NonCommercial-License, permitting copying and reproduction so long as the original work is given appropriate credit. Contents may not be used for commercial purposes, or adapted, remixed, transformed or built upon. (https://creativecommons.org/licenses/by-nc-nd/4.0/).
Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence
PD Dr. med. Hans Ikenberg
CytoMol, MVZ für Zytologie und Molekularbiologie
Berner Straße 76
60437 Frankfurt, Germany
hans.ikenberg@cytomol.de

Deutsche Version unter:
https://doi.org/10.1055/a-1886-3311.

ABSTRACT

Introduction On 1 January 2020 the screening programme for the prevention of cervical cancer in women from the age of 35 years of the Statutory Health Insurance (GKV) in Germany changed from an annual cytology examination to cytological and HPV co-testing carried out every three years. A large standard diagnostics laboratory has been using liquid-based cytology (LBC) with computer-assisted screening (CAS) since 1 January 2020 to assess the samples.

Patients and Methods The cytological and HPV results for all cases examined with co-testing from 01.01.2020 to 31.12.2021 (n = 395 759) are reported and the cytology results obtained using co-testing are compared with the results obtained using only conventional primary cytology screening from the two previous years (n = 588 192). Cytology tests were carried out using LBC and computer-assisted screening. A DNA PCR test which can identify 14 types of HPV was used for HPV testing. The cytology results are reported using the Munich Nomenclature III, which is mandatory in Germany, and converted to The Bethesda System (TBS). Problems occurring during the implementation phase are described here.

Results A total of 983 951 cases who had primary screening between 01.01.2018 and 31.12.2021 were analysed. The HR HPV-positive rate with co-testing for all age groups was 6.41 %. Of this group, 16.31 % were positive for HPV-16, 4.43 % for HPV-18, and 71.40 % had one or more of the other 12 HR HPV types. Several different HPV types were identified in 7.86 % of cases. The HPV-positive rate for cases with unremarkable cytological findings was 4.03 %. 0.46 % of tests were technically invalid. The results of primary cytology screening for 2020/21 (LBC) were: Pap 0 (TBS: unsatisfactory) 0.09 %, Pap I and Pap II-a (NILM) 96.82 %, Pap II-p/g (~ASC-US/AGC) 1.23 %, Pap III-p/g (~ASC-H/AGC) 0.19 %, Pap III D1 (LSIL) 1.08 %, Pap III D2 (HSIL) 0.31 %, Pap IVa/b-p/g (HSIL/AIS) 0.18 %, and Pap V-p/g (carcinoma) 0.01 %. The rates for 2018/19 (conventional cytology without routine testing for HPV) were significantly higher for Pap II-p/g (1.64 %) and significantly lower for Pap III-p/g (0.13 %), Pap III D1 (0.45 %), Pap III D2 (0.10 %) and Pap IVa/b-p/g (0.05 %).

Conclusion Evaluation of the data for the two first years of cytology and HPV co-testing from a standard diagnostics laboratory found low HR HPV-positive rates. As regards the cytology tests, the Pap II-p/g rate was significantly lower and the ≥ Pap III rate was significantly higher compared to the two previous years. This points to a probable higher sensitivity and specificity of the new method.

ZUSAMMENFASSUNG

Einleitung Zum 01.01.2020 hat das Screeningprogramm der gesetzlichen Krankenversicherung (GKV) zur Zervixkarzinomprävention in Deutschland bei Frauen ab 35 Jahren den Wechsel von einer jährlichen zytologischen Untersuchung zur Co-Testung Zytologie-HPV alle 3 Jahre vollzogen. Ein großes Routinelabor setzt seit 01.01.2020 in diesen Fällen Dünnschichtzytologie (LBC) mit Computerassistenz (CAS) ein.

Patientinnen und Methoden Die zytologischen und HPV-Ergebnisse aller Fälle der Co-Testung vom 01.01.2020 bis 31.12.2021 (n = 395 759) werden berichtet und die zytologischen Ergebnisse mit dem rein konventionellen zytologischen Primärscreening der vorausgehenden 2 Jahre verglichen (n = 588 192). Die zytologische Untersuchung wurde mit LBC und Computerassistenz durchgeführt. Als HPV-Test wurde eine DNA-PCR eingesetzt, die 14 Typen nachweist. Die zytologischen Befunde werden nach der in Deutschland verbindlichen Münchner Nomenklatur III berichtet und in das Bethesda-System (TBS) übertragen. Probleme der Implementationsphase werden dargestellt.

Ergebnisse Insgesamt wurden 983 951 Fälle des Primärscreenings vom 01.01.2018 bis zum 31.12.2021 analysiert. Der Prozentsatz der HPV-HR-Positivität bei der Co-Testung über alle Altersgruppen betrug 6,41 %. HPV-16 kam in 16,31 %, HPV-18 in 4,43 % und die Gruppe der anderen 12 getesteten HPV-HR-Typen in 71,40 % vor. Mehrere HPV-Typen wurden in 7,86 % nachgewiesen. Bei unauffälligem zytologischem Befund lag die HPV-Positivität bei 4,03 %. 0,46 % der Tests waren technisch ungültig. Die Ergebnisse der Zytologie im Primärscreening 2020/21 (LBC) waren: Pap 0 (TBS: unsatisfactory) 0,09 %, Pap I und Pap II-a (NILM) 96,82 %, Pap II-p/g (~ASC-US/AGC) 1,23 %, Pap III-p/g (~ASC-H/AGC) 0,19 %, Pap III D1 (LSIL) 1,08 %, Pap III D2 (HSIL) 0,31 %, Pap IVa/b-p/g (HSIL/AIS) 0,18 % und Pap V-p/g (carcinoma) 0,01 %. Die Raten 2018/19 (konventionelle Zytologie ohne Routine-HPV-Testung) waren signifikant höher für Pap II-p/g (1,64 %) und signifikant niedriger für Pap III-p/g (0,13 %), Pap III D1 (0,45 %), Pap III D2 (0,10 %) und Pap IVa/b-p/g (0,05 %).

Schlussfolgerung Die Auswertung der Daten eines Routinelabors aus den ersten 2 Jahren der Co-Testung Zytologie und HPV zeigt eine niedrige Rate an HPV-HR-Positivität. Auf zytologischer Ebene wurde im Vergleich zu den 2 Jahren zuvor eine signifikant niedrigere Rate an Pap II-p/g und eine signifikant höhere Rate an Befunden ≥ Pap III gefunden. Daraus resultiert wahrscheinlich eine höhere Sensitivität und eine höhere Spezifität.

Preliminary Note

In the following text, HPV always stands for high-risk HPV.

Introduction

Cervical cancer prevention screening has been part of statutory healthcare in Germany funded by Statutory Health Insurance (*gesetzliche Krankenversicherung* [GKV]) since 1971. About 90 % of the general population in Germany are insured in the Statutory Health Insurance system. The cervical screening examination has remained unchanged since its first introduction and is carried out annually in the form of a conventional cytology smear taken from the cervix. Regular screening has led to a sharp drop in the incidence of invasive cervical cancer to about one third of previous rates [1]. As a consequence of numerous extensive epidemiological and clinical studies, proposed changes to the methodology and algorithm have been discussed – at times very controversially – for more than 10 years [2]. This may be due to the fact that cervical screening in Germany is carried out almost exclusively by private gynaecological practices and the smear is taken by a doctor. Moreover, the cytological findings are still largely evaluated in laboratories run by gynaecologists. These laboratories are often quite small and are neither qualified to carry out molecular tests nor entitled to invoice such tests. Based on its standard criteria, the German preventive programme used to date must be considered opportunistic. Even after the introduction of co-testing, patients are not invited for screening but are only sent a comprehensive information booklet; patients are not recalled if they do not participate and there is no organised follow-up. The data obtained so far have not been systematically collected and evaluated. Nevertheless, according to surveys by the Central Research Institute of Ambulatory Health Care in Germany, the screening participation rate for a three-year period was already 70% in the years 2002–2004, rising to more than 80% in the group aged between 20 and 40 years [3].

In 2008, the National Cancer Plan demanded for the first time that screening for the prevention of cervical cancer should be revised [4]. This led to a decision, incorporated in the Cancer Screening and Register Act (*Krebsfrüherkennungs- und Registergesetz* [KFRG]) of 2013, to introduce an organised screening programme by 2016 [5]. In 2014, the Institute for Quality and Efficiency in Health Care (IQWiG) recommended testing for HPV during primary screening as beneficial [6]. Following this, the Joint National Committee (*Gemeinsamer Bundesausschuss* [G-BA]), which is responsible for introducing new procedures and represents health insurance companies, physicians and hospitals, decided that, in future, women from the age of 30 years would be able to choose between being tested for HPV every 5 years or having an annual conventional cytology examination as before [7]. Numerous objections against both approaches were raised during the subsequent mandatory comments procedure. Finally, the decision of the G-BA was revised in November 2018 and cytological and HPV co-testing for women above the age of 35 years every three years was adopted as the new standard [8]. This approach somewhat contradicts the S3 Guideline “Prevention of Cervical Cancer”, which recommends HPV-based screening and does not answer the question whether screening should start at the age of 30 rather than 35 years, given that comparative data are lacking [9, 10]. After six

years at the earliest (which corresponds to two rounds of screening), the approach will be evaluated. What has also changed is that liquid-based cytology (LBC), generally referred to in Germany as thin-layer cytology, is now explicitly accepted alongside conventional cytology procedures, although the compensation for LBC procedures is not higher than that provided for conventional cytology examinations.

Women between the ages of 20 and 35 years are still entitled to have an annual cytology examination. Clinical screening to detect other cancers of the genitalia and breast cancer screening in women remain the same. After a transition phase, a consultation will be held to consider the upper age limit, using the data obtained from monitoring.

The data presented here were collected in a standard laboratory for cytology and molecular biology specialising in the prevention of cervical cancer (MVZ CytoMol, Frankfurt am Main, Germany). The laboratory has used liquid-based cytology (LBC) (thin-layer cytology) for cytology examinations since 2000, and since 2007 it additionally uses two computer-assisted (CAS) processes. Testing for HPV has been used to complement cytology examinations since the 1990 s. Already in November 2019, when it was clear that co-testing would be introduced on 01.01.2020, the laboratory committed to providing cytology results using LBC with CAS in all cases in where cytology was paired with testing for HPV.

As the implementation of co-testing amounts to a paradigm change which is still controversially discussed, there is a significant interest in studying data obtained before and after this change. Because of the large case numbers which were examined in this laboratory within a relatively short space of time, such data are available. They are reported and discussed below.

Patients and Methods

Patients

The women whose Pap smears form the basis of this study were examined in around 400 gynaecological practices in the context of the cervical cancer screening programme of the GKV. Around 70 % of the gynaecological practices are located within a 100-kilometre radius of Frankfurt, while the other practices are scattered across the rest of Germany. The gynaecological practices are more or less evenly distributed in urban and rural areas. The percentage of single-partner and joint practices and of male and female physicians corresponds to the average for the Federal Republic of Germany. The variation in these variables for gynaecological practices and the examined women between the years 2018/19 and 2020/21 is less than 10 %. Only primary care cases were included in the evaluation. They can be clearly identified by the billing codes used. 60.4 % of women examined in 2020/21 had also attended screening examinations in the years 2018/19. This rate is of the same order of magnitude as that reported in the only study on this issue in Germany for the years 2002–2004 [3].

Tests and their implementation

In contrast to standard practice of only using conventional cytology for mandatory screening, the laboratory which investigated the smears obtained from the cases presented here has offered to use LBC with CAS since 01.01.2020 for all smears from women with statutory health insurance where testing for HPV is also required. In 99 % of cases, this offer was accepted. Only cases in which LBC with CAS was used and testing for HPV was carried out were included in this analysis.

Pap smears investigated in the years 2018/19 using conventional cytology were obtained and sent in by gynaecologists using the decades-old standard procedure, while smears examined with LBC were obtained in accordance with the manufacturer's (ThinPrep, Hologic, Wiesbaden, Germany) instructions, available in all practices as an illustrated instruction manual. As all of the practices already had many years' experience of taking LBC smears in women with private health insurance and in women who paid directly, it can be safely assumed that they were sufficiently familiar with the technique required to obtain the smear. As the LBC procedure employed is FDA-certified to allow the diagnostic workup for HPV to use material from the same cytology container, it is not necessary to take a separate sample. After the LBC container arrives at the laboratory, first an aliquot of 400 µl is taken for the diagnostic HPV workup (cobas test, Roche Diagnostics, Mannheim, Germany). Testing for HPV is carried out in accordance with manufacturer's instructions. Until the end of March 2020, the diagnostic HPV workup was done using the cobas-8800 system. Thereafter, as a consequence of the pandemic, these systems were exclusively supplied with reagents to diagnose coronavirus, meaning that subsequent smear sample analyses had to be carried out using cobas 4800 systems. The two systems provide equivalent results [11]. The cytology evaluation is always only carried out after the result of the HPV test has come in, as the purpose of co-testing is to evaluate the cytology sample while knowing the patient's current HPV status. The cobas system provides results which are grouped into four groups: negative, HPV-16, HPV-18, positive for 12 further HPV types not separately listed. In each case, a fragment of the human β-globin gene is amplified to serve as an internal control. If this cannot be achieved and no HPV amplification is carried out, the result is rated as "invalid" = "unrateable".

The submitted, conventional cytology preparations are fixated with standard methods, dyed, and undergo computer-assisted pre-examination with the BD FocalPoint system (BD, Heidelberg, Germany). The 15 most noticeable visual fields of the smear are identified. After calibration, these fields are examined further under a microscope by cyto-assistants and physicians and a definitive assessment is made.

The LBC samples are prepared in accordance with manufacturer's instructions using the ThinPrep 5000 processor (Hologic, Wiesbaden, Germany). The preparations then undergo computer-assisted analysis using the IMAGING system. The 22 most noticeable visual fields of the smear are identified. These are then analysed further under a microscope by cyto-assistants and physicians and a definitive assessment is made. Both CAS systems have been approved by the US Food and Drug Administration (FDA).

Cytology samples are rated using the Munich Nomenclature III which has been used in Germany since 2014 [12]. The results are then converted into the internationally used Bethesda System (TBS) [13]. Cytology findings classified as ≥ Pap II-p/g (ASC-US/AGC) by cyto-assistants, where the preliminary findings were al-

ready abnormal or where the patient had a prior medical history of abnormality are passed on to physicians for a second assessment. 10% of smears initially assessed as unremarkable were re-assessed a second time as a quality control.

Data collection and statistical evaluation

The data obtained as described above were processed using a specialised computer system and stored (Nexus / Zytologie, nexus, Donaueschingen, Germany). The system has been certified by the National Association of Statutory Healthcare Physicians.

The datasets were analysed descriptively, and Chi² test with continuity correction was used for a retrospective comparison of the frequencies of the cytology results obtained using the two procedures; the results from the years 2018/2019 were compared with those for 2020/2021.

Results

Case numbers

A total of 395 759 cases for the period from 01.01.2020 to 31.12.2021 of women with statutory healthcare insurance above the age of 35 years were examined using cytology and HPV co-testing (LBC with CAS). In the corresponding period from 01.01.2018 to 31.12.2019, a total of 588 192 screening examinations of women with statutory healthcare insurance above the age of 35 years were carried out using only conventional cytology procedures. The data for both periods are reported and analysed here. The figures for 2020/21 are lower, as patients are only entitled to have a co-testing every 3 years, whereas up until 2019, patients were entitled to have an annual conventional cytology smear. Only cases of primary screening were included for both periods. Control examinations and multiple examinations were not included in the study.

HPV

The HPV-positive rate for all age groups was 6.41 %. The rate for women between the ages of 35 and 40 years was 9.60 %, whereas only 3.41 % of women between the ages of 70 and 80 Jahren were HR HPV-positive. See ▶ **Table 1** for all age groups.

▶**Table 1** HPV status according to age group.

Age groups	HPV-negative	HPV-positive
35 to 40	90.35 %	9.65 %
41 to 50	92.84 %	7.16 %
51 to 60	94.53 %	5.47 %
61 to 70	95.92 %	4.08 %
71 to 80	96.59 %	3.41 %
81 to 90	96.70 %	3.30 %
91 to 100	97.53 %	2.47 %
Total	93.59 %	6.41 %

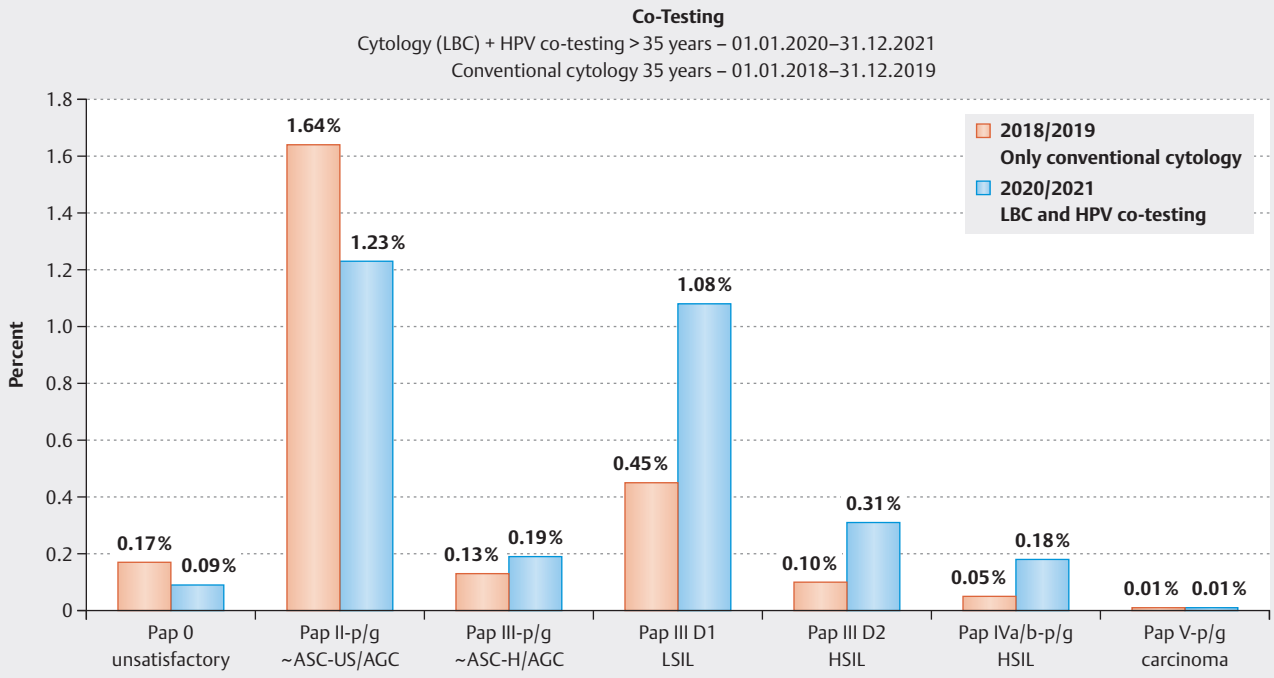
The HPV-positive rate in women with no current cytological abnormality (Pap I and II-a [NILM], n = 14 855) was 4.03 %. HPV-16 alone was detected in 16.31 % of HPV-positive women, HPV-18 alone in 4.43 % and the group of the other 12 types of HR HPV alone in 71.40 %. Several HPV types (16 and 18 or 16 and/or 18 and the group of the 12 other types) were detected in 7.86 % of HPV-positive cases. Overall, HPV-16 was found in 22.32 %, HPV-18 in 6.89 % and the group of 12 other HPV types in 78.96 % of all HPV-positive women. 0.46 % of the HPV tests were technically invalid. For details, see ▶ **Table 2**.

Cytology

Cytological findings were reported using the Munich Nomenclature III, which is mandatory in Germany and, as far as possible, were converted into the standard international Bethesda System (TBS). For the years 2020/21 (co-testing), the following findings were recorded: Pap 0 (TBS: unsatisfactory) 0.09 %; Pap I 92.86 %; Pap II-a 3.96 %; Pap I and Pap II-a together (NILM) 96.82 %; Pap II-p/g (~ASC-US/AGC) 1.23 %; Pap III-p/g (~ASC-H/AGC) 0.19 %; Pap III D1 (LSIL) 1.08 %; Pap III D2 (HSIL) 0.31 %; Pap IVa/b-p/g (HSIL/AIS) 0.18 %; and Pap V-p/g (carcinoma) 0.01 %. The rates for the previous two-year period of 2018/19 (conventional cytology without routine testing for HPV) were significantly higher for Pap II-p/g (1.64 %) and significantly lower for Pap III-p/g (0.13 %), Pap III D1 (0.45 %), Pap III D2 (0.10 %) and Pap IVa/b-p/g (0.05 %) ($p < 0.0001$, respectively). The results for Pap V-p/g were unchanged at 0.01 %. The rate for Pap I und Pap II-a was 97.35 %. For details on all of the Pap groups, see ▶ **Table 3** and ▶ **Table 4**. ▶ **Fig. 1** provides a comparison of the cytology findings for both periods.

▶**Table 2** Distribution of HPV types.

HPV-positive test							
Type	**16**	**16, 18**	**18**	**Other**	**Other, 16**	**Other, 16/18**	**Other, 18**
%	16.31 %	0.30 %	4.43 %	71.40 %	5.40 %	0.31 %	1.85 %



▶ **Fig. 1** Cytology (LBC und CAS) + HPV co-testing results for women > 35 years for the period 2020/21 vs. conventional cytology for the period 2018/19.

▶**Table 3** Pap groups (LBC and CAS) classified using MN III and related HPV findings obtained with co-testing for the period 2020/2021.

Pap groups	n	%	HPV-negative n	HPV-negative %	HPV-positive n	HPV-positive %
0	351	0.09%	330	94.02%	21	5.98%
I	367491	92.86%	367393	99.97%	98	0.03%
II-a	15653	3.96%	896	5.72%	14757	94.28%
II-p	3890	0.98%	535	13.75%	3355	86.25%
II-g	974	0.25%	261	26.80%	713	73.20%
II-e	299	0.08%	266	88.96%	33	11.04%
IIID1	4293	1.08%	496	11.55%	3797	88.45%
IIID2	1240	0.31%	53	4.27%	1187	95.73%
III-p	610	0.15%	33	5.41%	577	94.59%
III-g	169	0.04%	44	26.04%	125	73.96%
III-e	39	0.01%	39	100.00%	0	0.00%
III-x	14	0.00%	11	78.57%	3	21.43%
IVa-p	575	0.15%	12	2.09%	563	97.91%
IVa-g	35	0.01%	1	2.86%	34	97.14%
IVb-p	62	0.02%	6	9.68%	56	90.32%
IVb-g	9	0.00%	2	22.22%	7	77.78%
V-p	38	0.01%	4	10.53%	34	89.47%
V-g	9	0.00%	4	44.44%	5	55.56%
V-e	6	0.00%	6	100.00%	0	0.00%
V-x	2	0.00%	2	100.00%	0	0.00%
All	395759	100.00%	370394	93.59%	25365	6.41%

▶**Table 4** Pap groups (conventional cytology) for the period 2018/2019 classified using MN III.

Pap groups	n	%
0	1009	0.17 %
I	569 130	96.76 %
II-a	3502	0.60 %
II-p	6873	1.17 %
II-g	2786	0.47 %
II-e	432	0.07 %
IIID1	2670	0.45 %
IIID2	605	0.10 %
III-p	538	0.09 %
III-g	226	0.04 %
III-e	31	0.01 %
III-x	26	0.00 %
IVa-p	206	0.04 %
IVa-g	17	0.00 %
IVb-p	59	0.01 %
IVb-g	8	0.00 %
V-p	49	0.01 %
V-g	15	0.00 %
V-e	3	0.00 %
V-x	7	0.00 %
All	588 192	100.00 %

When the findings were classified in accordance with the standard international Bethesda nomenclature (TBS), the percentages for the period 2020/21 and the period 2018/19 (in brackets) were as follows: ASC-US/AGC 1.23 % (1.64 %), LSIL 1.08 % (0.45 %), ASC-H/AGC 0.19 % (0.13 %), HSIL/AIS 0.49 % (0.15 %). These differences were also significant ($p < 0.0001$, respectively). See ▶**Table 5** and ▶**Table 6**.

The rate of cytologically abnormal findings decreased with increasing age. For the period 2020/21, 1.75 % of women aged 35–40 years (n = 82 107) were Pap II-p/g (~ASC-US/AGC), compared to 1.07 % of women aged 51–60 years (n = 109 219) 1.07 % and just 0.67 % of women aged 71–80 years (n = 26 915). The corresponding figures for the respective age groups are 1.89 %, 0.89 % and 0.22 % for Pap III D1 (LSIL); 0.65 %, 0.20 % and 0.06 % for Pap III D2 (HSIL); 0.34 %, 0.09 % and 0.06 % for Pap IVa-p/g (HSIL/AIS).

Correlation between HPV and cytology for the period 2020/21

86.25 % of cases in the Pap II-p group (~ASC-US) (n = 3890) and 73.2 % of cases in the II-g group (~AGC) (n = 974) were HPV-positive.

In the Pap III-p group (~ASC-H) (n = 610), the HPV-positive rate was 94.59 %, and in the Pap III-g group (~AGC) (n = 169) the HPV-positive rate was 73.96 %.

In the Pap III D1 group (LSIL) (n = 4293), 88.45 % were HPV-positive, while 95.73 % of the Pap III D2 group (HSIL) (n = 1240) were HPV-positive.

97.91 % of IVa-p cases (HSIL) (n = 575) were HPV-positive and 97.14 % of IVa-g cases (AIS) (n = 35) were HPV-positive.

While 90.32 % of the Pap IVb-p cases (HSIL) (n = 62) and 89.47 % of the Pap V-p cases (carcinoma) (n = 38) were HPV-positive, 77.78 % of the limited number of cases with Pap IVb-g (AIS) (n = 9) and only 55.56 % of cases with Pap V-g (carcinoma) (n = 9) were HPV-positive.

HPV DNA was found in 11.04 % of cases in the Pap II-e group (NILM) (n = 299) and no HPV DNA was found in the Pap III-e group (AGC) (n = 39).

Overall, 12 264 (3.09 %) of 395 759 co-testing cases had abnormal Pap findings (≥II-p). Of these, 1775 (14.47 %) were HR HPV-negative, and 10 489 (85.52 %) were HR HPV-positive. For more details, see ▶**Table 3**.

▶**Table 5** Pap groups (LBC und CAS) classified according to TBS and related HPV findings obtained using co-testing in the period 2020/2021.

TBS	n	%	HPV-negative n	HPV-negative %	HPV-positive n	HPV-positive %
Unsatisfactory	351	0.09 %	330	94.02 %	21	5.98 %
NILM	383 144	96.81 %	368 289	96.12 %	14 855	3.88 %
Endometrial cells	299	0.08 %	266	88.96 %	33	11.04 %
ASC-US/AGC	4864	1.23 %	796	16.37 %	4068	83.63 %
LSIL	4293	1.08 %	496	11.55 %	3797	88.45 %
ASC-H/AGC	832	0.21 %	127	15.26 %	705	84.74 %
HSIL	1921	0.49 %	74	3.85 %	1847	96.15 %
Carcinoma	55	0.01 %	16	29.09 %	39	70.91 %
All	395 759	100.00 %	370 394	93.59 %	25 365	6.41 %

▶**Table 6** Pap groups (conventional cytology) for the period 2018/2019 classified with TBS.

TBS	n	%
Unsatisfactory	1009	0.17%
NILM	572632	97.35%
Endometrial cells	432	0.07%
ASC-US/AGC	9659	1.64%
LSIL	2670	0.45%
ASC-H/AGC	821	0.14%
HSIL	895	0.15%
Carcinoma	74	0.01%
All	588192	100.00%

Discussion

As cytology and HPV co-testing is being used as standard in primary screening for the prevention of cervical cancer, there has been a highly significant increase in the rates of both lower and higher grade cytologically abnormal findings, categorised using the standard Munich Nomenclature III used in Germany or the international Bethesda System (TBS). However, the frequency of borderline cases categorised using either of the nomenclatures decreased and this drop was highly significant. It can therefore be assumed that both sensitivity and specificity have increased with this most commonly used screening procedure.

This is probably a consequence of the fact that the women's current HPV status is always investigated when the cytology examination is carried out, and the HPV results are always available to the cyto-assistant or physician. It can be assumed that the categorisation of borderline or low-grade cytological abnormality is often subjective and the person evaluating cytology will also look at the woman's HPV status when making a decision. If the woman is HPV-negative, then clearly the findings will be usually classified as Pap I (NILM), whereas if the woman is HPV-positive she will be categorised as Pap II-p/g (~ASC-US/AGC) or higher. If the evaluator has the impression that a higher-grade abnormality is present although the negative HPV-status is negative, the findings will be re-examined, e.g., by reviewing the sample [14]. Accordingly, this has led to very high rates of HPV positivity for all Pap groups starting with II-p (~ASC-US). While 86.25% of Pap II-p cases were HPV-positive, the same figure for the unofficial Pap II-w category used in a study carried out in Hanover and Tübingen in 2006/07 (which approximately corresponds to what is now classified as Pap II-p) was only 10.8% [15]. It can be assumed that colposcopy combined with a histopathological investigation of these cases would result in a significantly higher number of relevant findings which are ≥ CIN 2 compared to investigating patients who only have a cytological abnormality.

It should be noted that in the more clearly defined Pap II-p (~ASC-US) und III-p (~ASC-H) groups, the HPV-positive rate was higher by 13% and 21% respectively compared to the less stringently defined II-g (~AGC) and III-g (~AGC) groups.

The very low HPV-positive rate with associated with groups categorised as II-e (NILM) (11%) and III-e (AGC) (0%) points to a surprisingly high accuracy for these cytological groups, as endometrial carcinomas are always HPV-negative [16].

Overall, we can conclude from these results that co-testing, when carried out as part of routine screening in Germany, appears to have a high sensitivity for histologically confirmed CIN 2+ lesions as evidenced in earlier studies [14] and – just as importantly for screening – should have a higher specificity [17]. This is even more probable, in view of the fact that in the approach described here, conventional cytology carried out in the years 2018/19 also included pre-screening using CAS (FocalPoint). As this technique is associated with a higher sensitivity [18], if there had been no pre-screening, the difference between the results of conventional cytology and the results obtained with LBC, CAS and testing for HPV would have been even greater.

It is difficult to ascertain whether, in addition to testing for HPV, the consistent use of LBC and a CAS imaging system also contributed to the increase in sensitivity and specificity. But that is what was indicted in the Rhine-Saar study which used results obtained in a routine setting [19]. The study was a randomised controlled approach which compared LBC with conventional cytology (without parallel testing for HPV) and found that histologically confirmed cases with HSIL increased significantly by a factor of 2.74 (CIN 2+) and 3.02 (CIN 3+), respectively. When CAS was used, this increased again by 15% (CIN 2+) to 20% (CIN 3+). Because of the limited numbers of cases with histologically confirmed CIN 2+, this increase was not significant. However, the impact of testing for HPV and the use of LBC/CAS in the population analysed here cannot be separately determined as both methods were always used together.

The large increase in findings which were ≥ Pap III (~ASC-H/AGC) on primary screening in the years 2020/21 compared to 2018/19 found in our study is not just due to the impact of LBC, CAS and testing for HPV. It must be assumed that many of the primary screening findings from 2018/19 were not the final results for the respective patients. Previously, low-grade abnormalities were investigated further by carrying out additional cytological examinations, by testing for HPV, and by using biomarkers, usually p16/Ki-67. When all cases examined in one year are taken into consideration, this resulted in a significantly higher rate of cytological diagnoses which were ≥ Pap III (~ASC-H/AGC) compared to the number of cases identified during primary screening. In 2018/19, for example, non-screening cytology examinations carried out in women above the age of 35 years (n = 62408 cases) found 4.71% of cases with Pap II-p/g (~ASC-US/AGC), 3.19% of cases with Pap III D1 (LSIL), 1.76% with Pap III D2 (HSIL), and 1.14% with Pap IVa/b-p/g (HSIL/AIS). Accordingly, in 2020/21 after co-testing had been introduced in non-screening cytology examinations carried out in women above the age of 35 years (n = 84671 cases), there were more cases with Pap II-p/g (4.88%) and Pap III D1 (3.54%) than in 2018/19 but slightly fewer cases with Pap III D2 (1.60%) and significantly fewer cases with Pap IVa/b-p/g (0.58%). This indicates that high-grade abnormalities are identified more easily with the new procedure.

Out of a total of 18340028 samples investigated in 2015 from all of Germany categorised using MN III, with the samples

(screening and further investigation) obtained from 15 124 043 women from all age groups, the frequencies for the different categories were as follows: Pap II-a (NILM): 1.07 %; II-p/g (~ASC-US/AGC): 1.34 %; III (~ASC-H/AGC): 0.21 %; III D1 (LSIL): 0.74 %; III D2 (HSIL): 0.41 %; IVa/b-p/g (HSIL/AIS): 0.17 %, V (carcinoma): 0.01 % [20]. Although these figures also include all additional investigative cytology examinations, the figures are similar to the rates obtained with co-testing. This is a further indication that using cytology and HPV co-testing for primary screening results in higher abnormality rates [17].

When evaluating the low HR HPV-positive rate of 6.41 % compared to prognoses and earlier data [21], it is important to remember this rate also includes cases with prevalent cytological abnormalities, the majority of which are HPV-positive. Without these cases, only 4.03% of women without cytological abnormalities were HPV-positive.

An important aspect of introducing co-testing was the lack of preparedness for the new situation found in many of the facilities and persons involved.

Although the Joint National Committee (G-BA) took the fundamental decision to introduce co-testing in November 2018, the details on how to implement this decision were only made public one year later.

Because of the coronavirus pandemic, at times numerous companies faced extreme difficulties when supplying specimen sample vials. From March 2020, because of the pandemic, analytical instruments were no longer available at extremely short notice and materials were no longer supplied. The preparations by software suppliers supplying laboratories and private practices were also completely inadequate. This led, for example, to the problem that the originally intended statistical recording of the data generated with co-testing could not be carried out from the beginning and could therefore only be concluded by February 28, 2022. Quite apart from the quality of the data collected under such unsatisfactory conditions, the originally intended analysis of the first cycle of the reorganised screening procedure for the prevention of cervical cancer is now unrealistic or will have to be carried out belatedly.

Also the gynaecological practices faced significant problems when implementing co-testing. Often, co-testing samples were sent in from women not entitled to the procedure (age, interval). These samples were not included in this analysis.

The limitations of this study are as follows. It is a retrospective analysis of data obtained during routine procedures carried out by a commercial laboratory. But such comprehensive figures obtained in a “realistic setting” are also an advantage. A slight variance between the populations in the two comparative periods cannot be excluded. But because of the close temporal relationship of the investigated periods and the limited fluctuations in the two large populations, this should not have any material impact on the results. Moreover, the screening participation rate of the investigated cohort was about the same as that reported in the only available study on this issue in Germany [3].

Cytological findings were not correlated with related histological findings. This was partly because no histological results were available for most borderline and low-grade findings, as either no colposcopy examination was carried out or colposcopy did not result in a biopsy. There is also the issue that colposcopy examinations are often carried out some considerable time after the cytological diagnosis. The variance in the re-examination rate and the time periods are also too big. Finally, colposcopy examinations and potential treatment as well as subsequent histopathological examinations are carried out in a large number of cases by external institutions. The cytology laboratory is responsible for obtaining the corresponding findings, but despite all efforts they are not always successful. Going forward the aim will nevertheless be to obtain and correlate as much corresponding data as possible.

Conclusion

The data for the first two years after the introduction of cytology and HPV co-testing in women from the age of 35 years in a large standard population presented here for the first time indicate that this could lead to improvements in the prevention of cervical cancer – at least, if cytology examinations are exclusively carried out using a liquid-based computer-assisted procedure which combines information about patients’ HPV status as described for the cohort presented here. The rate of borderline cytology findings was significantly lower and the rate of higher-grade findings was significantly higher than in previous years when only conventional cytology examinations were carried out. In addition, the HPV-positive rate was low. It is therefore probable that co-testing has a higher sensitivity and a higher specificity for the detection of precancerous cervical lesions requiring treatment.

Acknowledgement

Grateful thanks go to Ms Christiane Schön, BioTeSys GmbH, Esslingen for her assistance with statistical data analysis and critical review of the manuscript and to Mr Arnd Liman, nexus, Donaueschingen for his assistance with data collection. We would also like to sincerely thank Prof. Stefanie Klug, chair of Epidemiology at TUM, Munich for her valuable suggestions regarding the concept and evaluation of the analysis. Special thanks go to Ms Heike Cipura, CytoMol, Frankfurt am Main for her valuable assistance in creating the graph and tables. / Herzlicher Dank gilt Frau Christiane Schön, BioTeSys GmbH, Esslingen, für die Unterstützung bei der statistischen Auswertung der Daten und kritische Durchsicht des Manuskripts sowie Herrn Arnd Liman, nexus, Donaueschingen, für die Unterstützung bei der Datenerhebung. Ebenso herzlich danken wir Frau Prof. Stefanie Klug, Lehrstuhl Epidemiologie TUM, München, für wertvolle Ratschläge zu Konzeption und Auswertung der Analyse. Besonderer Dank an Frau Heike Cipura, CytoMol, Frankfurt am Main für ihre wertvolle Unterstützung bei der Erstellung der Illustration und der Tabellen.

Conflict of Interest

The authors are partners (HI) or employees (AX, IZ, AA) of a cytology and molecular biology laboratory (MVZ CytoMol) which mainly investigates samples obtained during cervical cancer screening. / Die Autoren sind Gesellschafter (HI) bzw. Angestellte (AX, IZ, AA) eines zytologischen und molekularbiologischen Labors (MVZ CytoMol), welches überwiegend Untersuchungen zur Prävention des Zervixkarzinoms durchführt.

References/Literatur

[1] Schneider V. Gynäkologische Krebsvorsorge in Deutschland. Pathologe 2012; 33: 286–292. doi:10.1007/s00292-012-1579-7

[2] Wentzensen N, Arbyn M. HPV-based cervical cancer screening- facts, fiction and misperceptions. Prev Med 2017; 98: 33–35. doi:10.1016/j.ypmed.2016.12.040

[3] Kerek-Bodden H, Altenhofen L, Brenner G. Inanspruchnahme der Früherkennung auf Zervixkarzinom in den Jahren 2002–2004. Zentralinstitut für die kassenärztliche Versorgung in der Bundesrepublik Deutschland Wissenschaftliche Reihe: 62. Köln: Deutscher Ärzte-Verlag; 2010.

[4] Bundesministerium für Gesundheit. Nationaler Krebsplan. . Accessed August 17, 2021 at: https://www.bundesgesundheitsministerium.de/fileadmin/Dateien/5_Publikationen/Praevention/Broschueren/Broschuere_Nationaler_Krebsplan.pdf

[5] Anonymous. Gesetz zur Weiterentwicklung der Krebsfrüherkennung und zur Qualitätssicherung durch klinische Krebsregister (Krebsfrüherkennungs- und -registergesetz – KFRG). . Accessed August 17, 2021 at: https://www.bgbl.de/xaver/bgbl/start.xav?start=//*%5B@attr_id=%27bgbl113s0617.pdf%27%5D#__bgbl__%2F%2F*%5B%40attr_id%3D%27bgbl113s0617.pdf%27%5D__1629198905885

[6] IQWiG. Rapid Report S13–03. HPV-Test im Primärscreening des Zervixkarzinoms. Accessed August 17, 2021 at: https://www.iqwig.de/download/s13–03_rapid-report_kurzfassung_hpv-test-im-primaerscreening-des-zervixkarzinoms.pdf

[7] Gemeinsamer Bundesausschuss. Beschluss des Gemeinsamen Bundesausschusses über eine Beauftragung des Instituts für Qualität und Wirtschaftlichkeit im Gesundheitswesen: Erstellung von Einladungsschreiben und Versicherteninformationen zum Zervixkarzinomscreening. . Accessed August 17, 2021 at: https://www.g-ba.de/downloads/39–261–2224/2015–03–19_IQWiG-Beauftragung_Einladung-Info-Zervixkarzinom-Sc_WZ.pdf

[8] Gemeinsamer Bundesausschuss. Beschluss des Gemeinsamen Bundesausschusses über eine Änderung der Krebsfrüherkennungs-Richtlinie und eine Änderung der Richtlinie für organisierte Krebsfrüherkennungsprogramme: Programm zur Früherkennung von Zervixkarzinomen. Accessed August 17, 2021 at: https://www.g-ba.de/downloads/39–261–3597/2018–11–22_oKFE-RL_Zervixkarzinom.pdf

[9] Hillemanns P, Friese K, Dannecker C et al. Prevention of Cervical Cancer. Guideline of the DGGG and the DKG (S3 Level, AWMF Register Number 015/027OL, December 2017) – Part 1 with Introduction, Screening and the Pathology of Cervical Dysplasia. Geburtshilfe Frauenheilkd 2019; 79: 148–159. doi:10.1055/a-0818-5440

[10] Hillemanns P, Friese K, Dannecker C et al. Prevention of Cervical Cancer. Guideline of the DGGG and the DKG (S3 Level, AWMF Register Number 015/027OL, December 2017) – Part 2 on Triage, Treatment and Follow-up. Geburtshilfe Frauenheilkd 2019; 79: 160–176. doi:10.1055/a-0828-7722

[11] Frayle H, Gori S, Rizzi M et al. HPV testing for cervical cancer screening: technical improvement of laboratory logistics and good clinical performance of the cobas 6800 in comparison to the 4800 system. BMC Womens Health 2019; 19: 47. doi:10.1186/s12905-019-0743-0

[12] Griesser H, Breinl H, Jordan B. Münchner Nomenklatur III: Gynäkologische Dysplasien werden klar zugeordnet. Dtsch Arztebl Ausg A 2014; 111: A640

[13] Nayar R, Wilbur DC. The Bethesda System for Reporting Cervical Cytology: A Historical Perspective. Acta Cytol 2017; 61: 359–372. doi:10.1159/000477556

[14] Martins TR, Longatto-Filho A, Cohen D et al. Influence of Prior Knowledge of Human Papillomavirus Status on the Performance of Cytology Screening. Am J Clin Pathol 2018; 149: 316–323. doi:10.1093/ajcp/aqx163

[15] Klug SJ, Hukelmann M, Hollwitz B et al. Prevalence of Human Papillomavirus Types in Women Screened by Cytology in Germany. J Med Virol 2007; 79: 616–625. doi:10.1002/jmv.20863

[16] Olesen TB, Svahn MF, Faber MT et al. Prevalence of Human Papillomavirus in endometrial cancer: a systematic review and meta-analysis. Gynecol Oncol 2014; 134: 206–215. doi:10.1016/j.ygyno.2014.02.040

[17] Wright TC, jr., Stoler MH, Aslam S et al. Knowledge of Patients' Human Papillomavirus Status at the Time of Cytologic Review Significantly Affects the Performance of Cervical Cytology in the ATHENA Study. Am J Clin Pathol 2016; 146: 391–398. doi:10.1093/ajcp/aqw125

[18] Ikenberg H. Computerassistierte Diagnostik in der Zervixzytologie. Pathologe 2011; 32: 476–483. doi:10.1007/s00292-011-1477-4

[19] Klug SJ, Neis KJ, Harlfinger W et al. A randomized trial comparing conventional cytology to liquid-based cytology and computer assistance. Int J Cancer 2013; 132: 2849–2857. doi:10.1002/ijc.27955

[20] Marquardt K, Kossowski I, Pfandzelter R. Münchner Nomenklatur III. Bundesweite Jahresstatistik nach der Nomenklaturreform. Frauenarzt 2017; 58: 706–712

[21] Dominik S, Klimas D. Wie sicher ist der HPV-Test in der Praxis? Falsch-Negativ-Befunde bei Präkanzerosen der Cervix uteri. Frauenarzt 2014; 10: 986–989