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

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

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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 = 395759) 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 = 588192). 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.
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 (Krebserfassungs- 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 1990s. 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 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 this analysis.

**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 fixed 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 in use 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 reassessed 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.

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.

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**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%**    |

**Table 2** Distribution of HPV types.

| HPV-positive test | 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%     |
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-p       | 15653| 3.96%| 896            | 5.72%          | 14757          | 94.28%         |
| II-g       | 3890| 0.98%| 535            | 13.75%         | 3355           | 86.25%         |
| II-e       | 974 | 0.25%| 261            | 26.80%         | 713            | 73.20%         |
| III-D1     | 299 | 0.08%| 266            | 88.96%         | 33             | 11.04%         |
| III-D2     | 4293| 1.08%| 496            | 11.55%         | 3797           | 88.45%         |
| III-p      | 3355| 0.03%| 1187           | 95.73%         | 577            | 4.27%          |
| III-g      | 610 | 0.15%| 577            | 94.59%         | 125            | 5.41%          |
| III-e      | 169 | 0.04%| 125            | 73.96%         | 0              | 26.04%         |
| III-x      | 39  | 0.01%| 39             | 100.00%        | 0              | 0.00%          |
| IVa-p      | 14  | 0.00%| 11             | 78.57%         | 3              | 21.43%         |
| IVa-g      | 575 | 0.15%| 563            | 97.91%         | 3              | 2.09%          |
| IVb-p      | 35  | 0.01%| 34             | 97.14%         | 1              | 2.86%          |
| IVb-g      | 62  | 0.02%| 56             | 90.32%         | 6              | 9.68%          |
| V-p        | 9   | 0.00%| 7              | 77.78%         | 4              | 22.22%         |
| V-g        | 38  | 0.01%| 34             | 89.47%         | 5              | 10.53%         |
| V-e        | 9   | 0.00%| 5              | 55.56%         | 6              | 44.44%         |
| V-x        | 6   | 0.00%| 6              | 0.00%          | 0              | 100.00%        |
| 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          | 569130| 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        | 588192| 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 = 82107) were Pap II-p/g (~ASC-US/AGC), compared to 1.07% of women aged 51–60 years (n = 109219) 1.07% and just 0.67% of women aged 71–80 years (n = 26915). 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 10489 (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            | 383144| 96.81%| 368289         | 96.12%         | 14855          | 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             | 395759| 100.00%| 370394        | 93.59%         | 25365          | 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 was found to be significantly lower compared to the category of 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 = 62,408 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 = 84,671 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.

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 = 62,408 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 = 84,671 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 18,340,028 samples investigated in 2015 from all of Germany categorised using MN III, with the samples...
(screening and further investigation) obtained from 15124043 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/AIDS): 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). 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].

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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 examination results 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.

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**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.
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