Thieme

HPV High-risk Multiple Infection Is a Key Predictor of Cervical Dysplasia in Diagnostic LEEPs: a Retrospective Cohort Analysis

Mehrfachinfektion mit multiplen Hochrisiko-HPV-Typen ist ein Schlüsselprädiktor für zervikale Dysplasien in diagnostischen LEEPs: eine retrospektive Kohortenanalyse

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ABSTRACT

Objective This study aimed to identify predictors for the presence of cervical dysplasia in diagnostic LEEPs (Loop Electrical Excision Procedure) of the cervix.

Materials/Methods The study was designed as a retrospective single-institution cohort analysis of all patients who underwent LEEP without prior proof of high-grade intraepithelial lesion (diagnostic LEEP) between 2015 and 2020 in the Department of Obstetrics and Gynecology of University Hospital Aachen. In order to identify the most meaningful predictive variables for CIN status (CIN2+ or non-CIN2+), multivariate logistic regression was performed and a machine-learning method was used.

Results A total of 849 patients with an indication for loop excision of the cervix were assessed for eligibility. Finally, 125 patients without prior proof of CIN2+ were included into the study. Based on the final multivariate logistic regression model, multiple high-risk HPV infections ($p = 0.001$), the presence of a T2 transformation zone ($p = 0.003$) and major lesion changes ($p = 0.015$) as a result of the colposcopy examination were found to be statistically significant for CIN status based on the diagnostic LEEP. Subsequent ROC analysis showed a high predictive value for the model of 88.35% (AUC). The machine-learning technique (recursive partitioning) identified similar variables as important for CIN status with an accuracy of 75%.

Conclusion For clinical decision-making, the result of the colposcopy examination (T2, major change) as well as the results of HPV testing (multiple high-risk HPV infections) are stronger indicators for clinicians to perform diagnostic excisional procedures of the cervix than the presence of high-grade cytological abnormalities.

ZUSAMMENFASSUNG

Zielsetzung Ziel dieser Studie war die Identifizierung von Prädiktoren für das Auffinden von zervikalen Dysplasien in diagnostischen zervikalen LEEPs (Loop Electrical Excision Procedure).

Material/Methoden Es handelt sich um eine retrospektive monozentrische Kohortenanalyse aller Patientinnen, die sich einer LEEP ohne vorherigen Nachweis einer hochgradigen intraepithelialen Läsion (diagnostische LEEP) zwischen 2015 und 2020 in der Abteilung für Geburtsheilkunde und Gynäkologie des Universitätskrankenhauses in Aachen unterzogen. Um die aussagekräftigsten prognostischen Variablen für den CIN-Status (CIN2+ oder nicht-CIN2+) zu identifizieren, wurde eine multivariate logistische Regressionsanalyse durchgeführt und eine maschinelle Lernmethode verwendet.

Ergebnisse Insgesamt wurden 849 Patientinnen mit Indikation für eine elektrochirurgische Schlingenexzision des Zervix auf ihre Eignung überprüft. Es wurden schließlich 125 Patientinnen ohne vorherigen Nachweis einer CIN2+ Läsion in die Studie aufgenommen. Das endgültige multivariate logistische Regressionsmodell zeigte, dass Mehrfachinfektion mit multiplen Hochrisiko-HPV-Typen (p = 0,001), das Vorhandensein einer T2-Transformationszone (p = 0,003) sowie Major Lesion Changes (p = 0,015) in Folge der Kolposkopie-Untersuchung statistisch signifikant waren für den auf den diagnostischen LEEP basierenden CIN-Status. Die ROC-Analyse zeigte, dass das Modell einen hohen prognostischen Wert von 88,35 % (AUC) aufwies. Die maschinelle Lernmethode (rekursive Partitionierung) hat ähnliche Variablen als wichtig für den CIN-Status mit einer Genauigkeit von 75 % ermittelt.

Schlussfolgerung Für die klinische Entscheidungsfindung sind die Befunde der Kolposkopie-Untersuchung (T2, große Veränderung der Läsion) sowie die Ergebnisse von HPV-Tests (Infektionen mit mehreren Hochrisiko-HPV-Typen) wichtigere Indikatoren für das Ausführen von diagnostischen zervikalen Exzisionsverfahren als das Vorhandensein hochgradiger zytologischer Auffälligkeiten.

Abbreviations

AGC	atypical glandular cells
AIC	Akaike information criterion
ASC-H	atypical squamous cells, cannot exclude HSIL
AUC	area under the curve
LSIL	low-grade squamous intraepithelial lesion
HPV	human papillomavirus
HSIL	high-grade squamous intraepithelial lesion
LEEP	Loop Electrical Excision Procedure
PCR	polymerase chain reaction
ROC	receiver operating characteristics
SD	standard deviation

Introduction

Infection with human papillomavirus (HPV), and persistent HPV infection in particular, can cause cervical dysplasia (also known as cervical intraepithelial neoplasia [CIN]), which may subsequently lead to cancer [1]. Although the majority of women with HPV infection will never develop CIN or cancer, a relatively large number of women is at risk of developing a CIN.

Nearly all developed countries have implemented cervical cancer screening programs to reduce the incidence of cervical cancer [2, 3, 4, 5, 6, 7, 8, 9, 10]. Due to the introduction of a program in the 1970 s, today cervical cancer is a rare disease in Germany with around 4300 patients diagnosed with cervical cancer every year [11]. The incidence of cervical intraepithelial neoplasia (CIN 1–3) is 50–100 times higher and often requires colposcopic evaluation [12, 13].

Especially since the beginning of 2020 with the introduction of the new cervical cancer screening algorithm in Germany, colposcopy plays a major role. Even patients with one-time low-grade cytologic anomalies are referred for colposcopy if they have had a high-risk HPV infection. Patients with persistent HPV infection over one year are also referred [14]. In cases of inadequate colposcopy (e.g., scars, bleeding or inflammation), suspected intracervical CIN2+ or a history of treatment for cervical dysplasia, the German national guidelines recommend excisional treatment of the cervix (diagnostic LEEP) [15]. The guidelines also state that normal endocervical curettage (in patients with T3 transformation zone) does not reliably rule out the presence of a CIN3+, especially in older patients, and that therefore diagnostic LEEP should be considered in these cases. We were of the opinion that the recommendations still leave a lot of questions unanswered, such as: when should cervical CIN2+ be suspected? When exactly should diagnostic LEEP be performed in elderly patients with T3 transformation zone and normal endocervical curettage? In practice, discrepancies between high-grade cytological abnormalities and colposcopy-directed biopsies or endocervical curettage are usually taken into account. The indication for a diagnostic LEEP should be highly restricted as it entails an invasive surgical procedure for the patient under general anesthesia which carries specific surgical risks. Therefore, in order to keep morbidity rates low, there is a high need for additional markers which can help clinicians make the right indication for diagnostic LEEPs. Our retrospectively designed study of patients who were seen in our standardized, highly frequented and quality-controlled dysplasia (DKG-certified) unit aimed to find predictors for the presence of cervical dysplasia in diagnostic loop excisions of the cervix.

Materials/Methods

Study population

The study was designed as a retrospective analysis of all patients who underwent diagnostic loop excision between 2015 and 2020 at the university hospital in Aachen. Diagnostic loop excisions were defined as loop excisions in patients who underwent colposcopy with biopsy or endocervical curettage (in cases with T3 transformation zone) prior to the LEEP but without proof of high-grade intraepithelial lesions. In order to include all patients who under-

went LEEP without prior proof of HSIL, we retrospectively investigated 849 consecutive patients who underwent LEEP at University Hospital Aachen between 2015 and 2020. During the study period, only LEEP was performed, and cold-knife conization was not carried out.

Data collection

In our department, colposcopies are performed in standardized conditions using a Leisegang 3 MCV colposcope. The general assessment is carried out using the 2011 International Federation for Cervical Pathology and Colposcopy (IFCPC) Terminology for the cervix, with transformation zone types classified accordingly as 1, 2, or 3. A conventional Pap smear of the cervix, a test for human papillomavirus (PCR for HPV DNA) and the application of 5 % acetic acid to the cervix represent the standard of care in our unit, and this procedure is carried out for every woman referred with abnormal cytology. During the whole period of investigation, the Seegene Anyplex II HPV 28 detection kit was used. It simultaneously detects 19 high-risk and intermediate-risk HPV genotypes and 9 low-risk types. The classification of HPV viruses into different categories was in accordance with the IACR (international Association of Cancer Registries) guidelines. The detection of multiple high-risk HPV viruses was defined as multiple high-risk HPV infection. The colposcopic findings were classified in accordance with the IFCPC terminology as "normal," "minor," "major," or "suspicious for invasion/cancer." For cases with T3 transformation zone where no parts of the transformation zone could be visualized even with splaying of the cervix, we used the term "non-satisfactory" colposcopy. Normal findings included, for example, metaplasia, viral warts, and polyps. Minor findings were defined as delicate punctation, thin acetowhite epithelium, and irregular geographic borders. Typical major lesions are represented by sharp borders, an inner border, ridge sign, dense acetowhite epithelium, a coarse mosaic pattern, and coarse punctation. Atypical vessels, fragile vessels, irregular surface, exophytic lesions, necrosis, and ulceration are suspicious for invasion [16]. A colposcopy-directed biopsy was taken from the most suspicious part of the lesion. In some patients with multifocal lesions, more than one biopsy was taken. In cases with T3 transformation zone, endocervical curettage was performed. In addition, known risk factors for the presence of cervical intraepithelial dysplasia were collected for every patient (see ▶ **Table 1**), e.g., smoking, immunosuppression, history of LEEP, and patients' age. All operations were performed under colposcopic view of the cervix and were carried out by experienced, highly qualified, AG-CPC certified staff at the DKG-certified colposcopy unit of University Hospital Aachen. Decisions regarding surgical treatment were based on the German S3 guideline for the prevention of cervical cancer. All included patients had a suspicious cytology on referral (LSIL; HSIL; ASC-H, AGC or carcinoma) and normal findings in the colposcopy-directed biopsies or endocervical curettage. This study aimed to find reliable predictors for the presence of cervical dysplasia in diagnostic loop excisions of the cervix.

Ethical approval

The study was approved by the Ethics Committee of the RWTH Aachen University Faculty of Medicine in May 2021 (EK 182/21). All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Helsinki Declaration and its later amendments.

Statistical analysis

Continuous variables are expressed as mean values ± standard deviation (SD). Categorical data are presented as absolute frequencies and percentages. Differences for each variable between CIN status (CIN2+ or non-CIN2+) groups were summarized by descriptive statistics and investigated by Mann-Whitney U-test or Fisher's exact test.

For the primary analysis, the CIN status was regarded as the primary endpoint. To identify the most meaningful predictor variables for the CIN status and classify patients according to these predictor variables, multivariate logistic regression was performed and a machine-learning method was used.

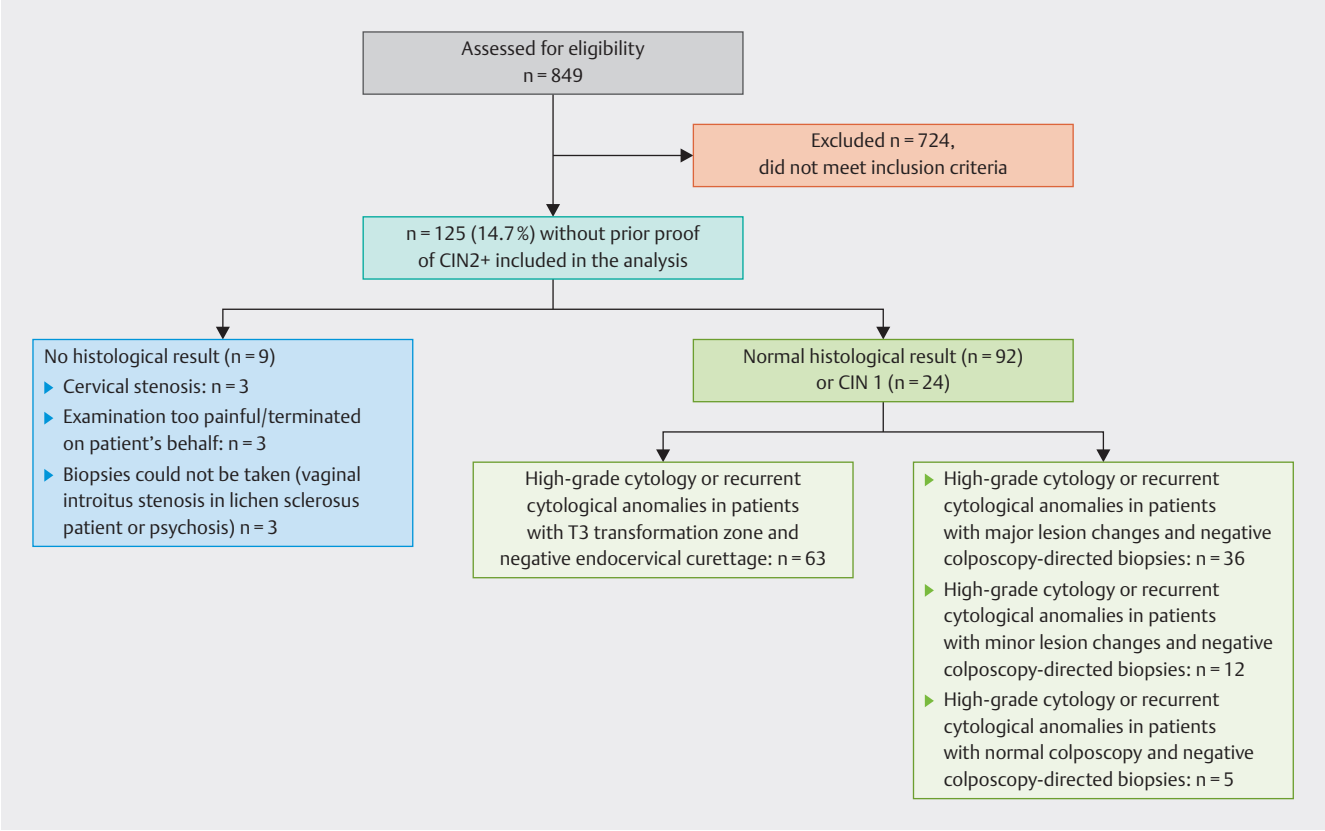
Logistic regression was conducted to investigate the influence of known risk factors (e.g., age, smoking, immunosuppression, history of LEEP) and typical results of the intensified gynecological work-up (e.g., colposcopy, transformation zone, HPV testing and cytological results) on the primary endpoint (CIN2+ or non-CIN2+). Model selection was performed using the Akaike information criterion (AIC) [17]. The AIC is defined as $2k - 2\ln(L)$. where k is the number of predictor variables and L is the maximum value of the likelihood function of the logistic regression model. The better the model fits the data, the higher the value of the likelihood function and thus the lower the AIC. Models with a lower AIC are better. The number of predictor variables is positive, and therefore the 2 k-term is often referred to as a "penalty term", as the addition of extra variables is "penalized", discouraging overfitting. The best-fitting model with the lowest AIC is described in the results section. The area under the curve (AUC) of the receiver operating characteristics curve (ROC) was derived to assess the predictive performance of the final logistic regression. The final logistic regression included 113 patients; 12 subjects were not included due to incomplete information (data).

In addition, recursive partitioning was used to generate a decision tree. Recursive partitioning first finds the best split (CIN2+/non-CIN2+) for all possible covariates and the splitting criteria for that covariate and then recursively applies the same procedure for both new subgroups [18]. The goodness of each split is defined by the "purity" of the new subgroups, i.e., the relative frequency of correct classifications. The available algorithms differ by how the covariate/split point is estimated and when the algorithm terminates. In our analysis, we chose the CART algorithm with a control parameter maximum depth of any node in the final tree of four, after investigating different complexity parameters (cp) and cross-validation results.

▶**Table 1** Patient characteristics.

	CIN2+	Non-CIN2+	P value
Patients	57 (45.6%) ▪ CIN 2: 18 (14.4%) ▪ CIN 3: 38 (30.4%) ▪ CA: 1 (0.08%)	68 (54.4%)	
Age (years)	42.88 ± 10.74	51.40 ± 12.49	< 0.001
History of loop excision	5 (8.77%)	7 (10.29%)	1
Smoker 1. Yes 2. No 3. History of smoking 4. No information	 24 (42.1%) 21 (36.84%) 9 (15.79%) 3 (5.26%)	 19 (27.94%) 30 (44.14%) 14 (20.58%) 5 (2.16%)	0.296
Immunosuppression	2 (3.5%)	2 (2.9%)	1
Cytology on referral ▪ IIID (LSIL) ▪ IIIg (AGC) ▪ IIIp (ASC-H) ▪ IVap (HSIL) ▪ V (carcinoma)	 17 (29.82%) 1 (1.75%) 3 (5.26%) 35 (61.40%) 0	 35 (51.47%) 2 (2.94%) 5 (7.35%) 24 (35.29%) 1 (1.47%)	0.016
Control cytology ▪ I/II ▪ IID1 (LSIL) ▪ IIID2 (HSIL) ▪ IIIg (AGC) ▪ IIIp (ASC-H) ▪ IIIx (AGC favor neoplasia) ▪ IVap (HSIL) ▪ V (carcinoma) ▪ missing	 9 (15.79%) 8 (14.04%) 16 (28.07%) 2 (3.51%) 2 (3.51%) 3 (5.26%) 14 (24.56%) 1 (1.75%) 2 (3.51%)	 26 (38.24%) 19 (27.94%) 16 (23.53%) 0 3 (4.41%) 0 4 (5.88%) 0 0	< 0.001
Result of the colposcopy examination ▪ major change ▪ minor change ▪ unsatisfactory ▪ missing	 40 (70.18%) 5 (8.77%) 11 (19.30%) 1 (1.75%)	 18 (26.47%) 17 (25%) 33 (48.53%) 0	< 0.001
Transformation zone ▪ T1 ▪ T2 ▪ T3 ▪ missing	 11 (19.30%) 20 (35.09%) 25 (43.86%) 1 (1.75%)	 13 (19.12%) 7 (10.29%) 47 (69.12%) 1 (1.47%)	0.002
HPV infection ▪ high-risk (non-specified) ▪ high-risk non-16/18 ▪ high-risk 16/18 ▪ intermediate-risk ▪ low-risk ▪ negative ▪ missing	 4 (7.02%) 25 (43.86%) 22 (38.60%) 2 (3.51%) 0 2 (3.51%) 2 (3.51%)	 2 (2.94%) 31 (45.58%) 19 (27.94%) 4 (5.88%) 2 (2.94%) 10 (14.71%) 0	0.141
Multiple high-risk HPV infection	20 (35.09%)	9 (13.24%)	0.003

We used recursive partitioning as the white-box algorithm in the field of explainable artificial intelligence (XAI) for several reasons. An additional analysis with a completely different approach is useful to investigate the robustness of results, in particular whether the same variables are identified as meaningful predictors. Furthermore, recursive partitioning is able to model non-linear relationships between predictors and outcome (CIN status) well, while logistic regression, by its very nature, is a linear model. Moreover, in contrast to black-box algorithms in the field of machine learning/artificial intelligence such as neural networks, recursive partitioning yields high explainability, an important feature in AI (artificial intelligence). The final decision tree was based on the same dataset that was utilized for the final logistic regression model. To prevent over-parametrization and allow better applica-



▶ **Fig. 1** Patient flow chart showing the indications for diagnostic LEEP.

tion of the results to new patients ("unseen data"), the decision trees underwent a procedure called "pruning". The optimal pruning parameter was identified by thorough cross validation. To assess performance metrics for unseen data, the "leave-one-out" method was used: for each subject, the algorithm was trained on the remaining 112 subjects and the resulting algorithm utilized to classify the subject who was left out during the training. This procedure was repeated for all 113 subjects. The relative frequency of correct classifications is referred to as the accuracy.

A subgroup analysis investigated the distribution of multiple high-risk HPV infections and the result of the colposcopy examination in different HPV-infection subgroups, utilizing the relative row frequencies of the respective contingency tables.

All tests were two-sided and assessed at the 5 % significance level. Because of the exploratory nature of the study, the significance level was not adjusted to account for multiplicity. All statistical analyses were conducted using the statistical software R [19].

Results

A total of 849 patients with an indication for loop excision of the cervix were assessed for eligibility. A total of 125 patients without prior proof of CIN2+ were included in the study. Inclusion criterium was loop excision without prior proof of high-grade intraepithelial lesion. ▶ **Table 1** shows the characteristics of all included patients and ▶ **Fig. 1** shows the indications for diagnostic LEEP. Patients with CIN2+ based on diagnostic LEEP were significantly younger than patients classified as non-CIN2+. Significant differences between the two groups were also identified for control cytology, the result of the colposcopy examination, the transformation zone, and the presence of multiple high-risk HPV infection, respectively.

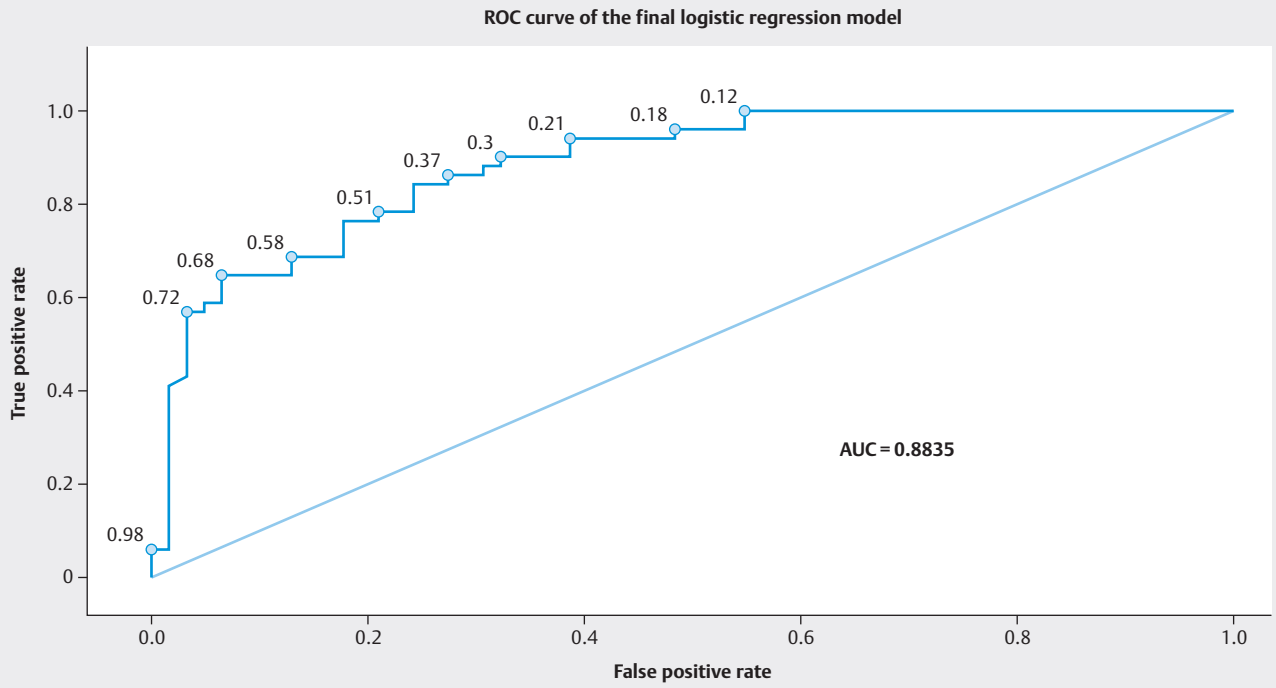
Results of the multivariate analysis

For the multivariate analysis, patients who were PAP IIIg and IIIp on referral were combined into one category as were PAP IIIg, IIIp and IIIx in control cytology.

Model selection based on the AIC revealed that smoking, a history of LEEP, immunosuppression and high-risk HPV 16/18 infection did not contribute sufficiently to the model fit, thus the applied algorithm determined the final model.

Based on the final multivariate logistic regression model, multiple high-risk HPV infection ($p = 0.001$), T2 transformation zone ($p = 0.003$), major lesion change ($p = 0.015$) as the result of the colposcopy examination and control cytology, in particular the change from category I/II to IIIg/p/x ($p = 0.03$), were all found to be statistically significant for CIN status based on the result of the diagnostic LEEP.

Furthermore, the final multivariate logistic regression model could be utilized to predict CIN status. Logistic regression models the probability of CIN2+ in a linear fashion, expressing the relationship between predictors and outcome in a closed (i.e., direct and explicit) form. For our final model, the following formula indicates the probability (p) of CIN2+:



▶ **Fig. 2** Receiver operating characteristics curve of the final multivariate logistic regression.

p=exp(Z)/exp(1+Z), with

Z = − 2.309 − 0.049 × age − 0.716 × cytology on referral Pap III gp + 1.062 × cytology on referral Pap IV ap + 2.206 × major lesion change + 0.646 × non-satisfactory colposcopy − 0.175 × control cytology PAP III D1 + 0.636 × control cytology PAP III D2 + 2.412 control cytology PAP III g/p/x + 1.662 × control cytology PAP IV ap + 2.755 T2 transformation zone + 1.421 T3 transformation zone + 2.195 multiple high-risk HPV infection (see ▶ **Table 2**)

Cytology on referral, control cytology, colposcopy and transformation zone are dummy-coded; thus, if neither of the mentioned categories is true, e.g., control cytology is I/II, a 0 has to be inserted for all mentioned categories. For example, a 60-year-old patient with cytology on referral Pap IIIg/p, a major lesion change, control cytology PAP III g/p/x, a T2 transformation zone and no high-risk multiple HPV infection has an estimated probability of CIN2+ of 80 %. The same patient with high-risk multiple HPV infection has an estimated probability of CIN2+ of 97.29 %.

The overall predictive performance of this multivariate logistic regression model was analyzed using a ROC curve. The AUC value was 88.35 % (see ▶ **Fig. 2**).

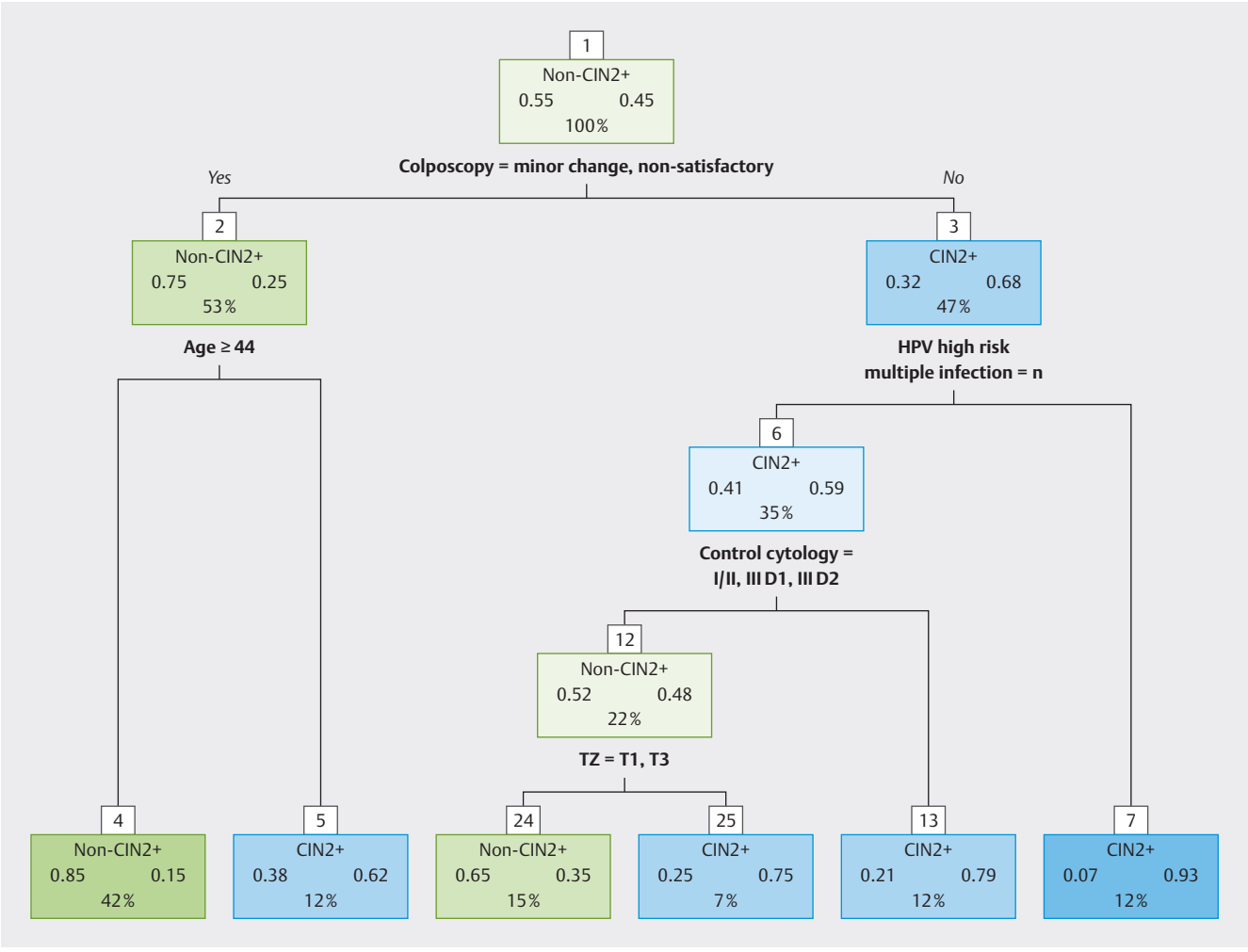
Recursive Partitioning

The "leave-one-out" algorithm was found to have an accuracy of 75 %, meaning that three out of four future patients will be classified correctly with regard to the development of CIN2+. The final decision tree based on the same dataset that was utilized in the final logistic regression model is shown in ▶ **Fig. 3**. The branches of the tree should be followed to classify whether a patient is CIN2 + or not. Thus, at the start of the tree, if the colposcopy examination shows a minor lesion change, the left branch is followed as the colposcopy findings are classified as either "minor change" or "non-satisfactory" (i.e., the "yes" path is chosen). In the underlying dataset, 53 % of subjects had a minor lesion change or non-satisfactory colposcopy, and 75 % of this subgroup were non-CIN2+ while 25 % were CIN2+. This information is provided in the first knot on the left. The final leaves of the tree are determined by following the tree's path all the way to the bottom, using "yes" answers on the left or "no" on the right. The purity of the final leaves is determined analogously, e.g., 93 % of subjects with a major lesion change and multiple HPV infection were CIN2+.

Subgroup analysis for HPV

The subgroup analysis for HPV status showed that multiple high-risk HPV infection was considerably more common in the subgroup of patients with high-risk HPV type 16 or 18 than in the other HPV risk categories (▶ **Table 3**).

Colposcopy findings of a major change lesion was more common in patients with high-risk HPV (▶ **Table 4**).



▶ **Fig. 3** Final decision tree of recursive partitioning classifies patients into two CIN-status classes: CIN2+ and non-CIN2+.

▶**Table 2** Final multivariate logistic regression model.

	Coefficient	p-value
Intercept	−2.309	0.189
Age	−0.049	0.066
Cytology on referral Pap IIIg/p	−0.716	0.515
Cytology on referral Pap IVap V	1.062	0.065
Major lesion change	2.206	**0.012**
Non-satisfactory colposcopy	0.646	0.477
Control cytology PAP IIID1	−0.175	0.820
Control cytology PAP IIID2	0.636	0.375
Control cytology PAP III g/p/x	2.412	**0.030**
control cytology PAP IVap	1.662	0.070
T2 transformation zone	2.755	**0.003**
T3 transformation zone	1.421	0.071
Multiple high-risk HPV infection	2.195	**0.001**

▶**Table 3** Relative frequencies for multiple HPV infection according to HPV status. The subgroup Lower Risk combines the HPV subgroups negative, LR and IR.

	Multiple HPV infection	No multiple HPV infection
Lower Risk	0.000	1.000
HR non-16/18	0.196	0.804
HR 16/18	0.439	0.561

▶**Table 4** Relative frequencies of colposcopy results by HPV status. The subgroup Lower Risk combines the HPV subgroups negative, LR and IR.

	Minor lesion change	Major lesion change	Non-satisfactory colposcopy
Lower Risk	0.158	0.211	0.632
HR non-16/18	0.196	0.482	0.321
HR 16/18	0.171	0.537	0.293

Discussion

In the literature, the reported accuracy of colposcopy-directed biopsy ranges from 60–95 % for HSIL [20, 21]. The histological differences between colposcopy-directed biopsy and loop excision of the transformation zone (LETZ) have been a cause for concern for a long time [22, 23, 24]. The German S3 guidelines list specific indications for carrying out diagnostic loop excisions. In our opinion, the patient cohort with indications for carrying out diagnostic LEEP in accordance with the S3 guidelines is quite large, especially as it includes elderly patients with T3 transformation zone and negative endocervical curettage, and in most colposcopy clinics, the cohort has increased considerably since the start of the new cervical cancer screening in Germany. But it also includes patients with suspected endocervical CIN2+ and discrepant cytology, colposcopy and histology findings. This study aimed to narrow down these groups and find evidence-based influencing factors for the presence of CIN2+ in diagnostic LEEPs [25, 26].

The majority of studies evaluate the concordance rates for preoperative colposcopy-directed biopsies and cone histology. Duesing et al. included 36 patients in their analysis of 166 patients without preoperative detection of CIN2+. In the accuracy analysis of colposcopy-directed biopsies by Stuebs et al., 106 of 642 patient had normal/LSIL findings in the colposcopy-directed biopsies. Thus, “diagnostic LEEP” rates were 21 % and 16 %, respectively [25, 27]. This is in line with our diagnostic LEEP rate of 14.7 %. Unfortunately, no further attention has been given so far to the characteristics of the patient group with negative biopsies prior to LEEP surgery, although this patient collective seems to exist in all colposcopy centres. In the systematic meta-analysis of Underwood et al., the sensitivity of colposcopy-directed biopsies for detecting CIN2+ was 80.1 %. Thus, CIN2+ was found in excisional biopsies (LEEP) in 20 % of cases without prior detection via colposcopy-directed biopsies. A multivariate analysis of potential factors affecting the quality of colposcopy-directed biopsies was not possible because of insufficient data in most of the included studies [20].

We present here a study based on our retrospective data showing that HPV high-risk multiple infection is a key indicator for the presence of HSIL in diagnostic loop excisions. In the final multivariate logistic regression model, the presence of a T2 transformation zone rather than T3 or T1, colposcopy findings of a major lesion change and the presence of multiple high-risk HPV infections had a major influence on the presence of HSIL in diagnostic loop excisions. Interestingly, the machine-learning technique (recursive partitioning) identified similar variables as important for CIN-status classification; thus, the results can be regarded as robust. In cases with negative colposcopy findings (only minor changes or unsatisfactory colposcopy) in patients aged ≥ 44, the estimated probability that diagnostic loop excision will be negative (without a histological finding of CIN2+) was 84 %. On the other hand, in cases with a major colposcopic lesion change and the presence of a multiple high-risk HPV infection, the risk of HSIL is 93 % (See ▶ **Fig. 3**). According to the ASCCP guidelines, the NHSCS, and the German guidelines, excisional procedures are not recommended in patients with CIN 1 or normal biopsies [15, 28, 29]. Exceptions can be made in cases with suspected endocervical dysplasia or unsatisfactory colposcopy in combination with abnormal cytology findings. Accordingly, 57.6 % of our patient collective had a non-visible transformation zone (T3) and 35.2 % an unsatisfactory colposcopy, with a non-visible transformation zone, even upon splaying of the cervix. In practice, colposcopists often face a diagnostic challenge when dealing with patients with a T3 transformation zone. Nevertheless, diagnostic discrepancies also exist in patients with T2 or T1 transformation zone. Of course, in these cases it is rarer, as colposcopy-directed biopsies can be taken with greater accuracy [27]. Interestingly, the presence of a T2 transformation zone rather than T3, and the presence of a major colposcopic lesion change were the main predictors of the presence of CIN2+ in our study. Thus, looking at our data, the presence of diagnostic discrepancies in patients with T2 or T1 transformation zones is a stronger predictor of CIN2+ than in patients with a T3 transformation zone. This can be explained by the higher diagnostic value of colposcopy in patients with T1 or T2 transformation zone. In cases of major colposcopic lesion changes, our data suggests that when this is combined with the presence of other risk factors (e.g., multiple high-risk HPV infection), diagnostic loop excision should be considered even when biopsies are negative. Treating major colposcopy-detected lesion changes without additional risk factors would lead to an overtreatment of patients as shown in the TOMBOLA study [30].

The overwhelming majority of sexually active women and men have been infected with HPV at least once in their lifetime [31]. A woman might have been infected with two HPV types by one partner and become infected with a third HPV type later on. One infection can persist even after several others have cleared [32]. In our study, we found that the presence of multiple high-risk HPV infection is a key predictor of the presence of CIN2+ in diagnostic loop excisions. We performed a sub-analysis for HPV status which showed that infection with high-risk HPV type 16 or 18 (high-risk HPV 16/18 group) is associated with multiple high-risk HPV infection and major colposcopic lesions (▶ **Table 3** and ▶ **Table 4**). Multiple high-risk HPV infection and major colposcopic lesion changes are far more common in the high-risk HPV 16/18 subgroup. Multivariate analysis revealed that, given information on multiple high-

risk HPV infection and major change lesion, the HPV risk status does not have an incremental value and thus does not contribute notably to the model fit. Due to this confounding, the HPV risk status correctly does not appear in the final model, although it is known to have a high carcinogenic potential [32]. Of course, the colposcopist needs a modern HPV DNA detection kit which can identify the different HPV genotypes and classify them in different categories in accordance with the IACR (International Association of Cancer Registries) guidelines. When using tools that only give binary information (HPV 16/18 or other), information which would be of value to the gynecologist (multiple high-risk HPV infection) is unavailable.

Our study has several strengths and limitations that need to be addressed. It is a retrospective study with a limited patient cohort. It will be very interesting to see whether the identified influencing variables will be confirmed in a prospective multicenter study.

The overall predictive performance of this multivariate logistic regression model is very good, as demonstrated by the high AUC value of 88.35 % of the ROC curve. By using machine-learning algorithms on our data, we were able to show that the accuracy for unseen data is 75 %, which is relatively high despite the limited patient cohort.

Conclusion

Our data showed that high-grade cytological abnormalities (PAP IV – HSIL), neither upon referral nor in the control cytology have a major influence. Clinicians should rather focus on the results of the colposcopy examination (T2, major change) and of HPV testing (multiple high-risk HPV infection) when considering diagnostic excisional procedures of the cervix.

Conflict of Interest

The authors declare that they have no conflict of interest.

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