Diagnostic value of the photodynamic method in the evaluation of lesions of the uterine cervix

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Summary

Purpose: The purpose of this study was to assess the efficacy of the photodynamic diagnosis (PDD) method in the diagnosis of lesions of the uterine cervix, as well as to establish its place and efficacy among currently available diagnostic techniques.

In the case of lesions located on the cervix, the projected aim of the study was intended to be realised by performing a detailed comparative analysis of the examination results of the following: PDD, cytology, colposcopy and testing for the presence of viruses, as compared with histological evaluation of performed biopsies.

Key words: Photodynamic diagnosis method; Vulvoscopy; Lesions of the uterine cervix.

Introduction

An efficacious comparison was performed evaluating photodynamic diagnosis (PDD), colposcopy, cytology and, HPV-DNA presence in the diagnosis of cervix/vaginal lesions. The evaluation included the sensitivity, specificity, efficacy, and positive and negative prognostic value of the various methods. A high level of consistency was observed between the photodynamic method and histological diagnosis, particularly high regarding precancerous and neoplastic lesions of the cervix, reaching 94.3%. The precise localisation of lesions using PDD may be used in the choice of appropriate treatment methods, thus leading to extension or limitation of the treatment procedure.

Cervix cancer is the most frequent female genital cancer and currently occupies second place in terms of malignant diseases in women in Poland, right behind breast cancer [1-4]. An important portion of diagnoses is made in clinically advanced stages. Therefore only half of the patients are likely to achieve a 5-year disease-fee period [4]. A diagnosis of cervical cancer in its pre-invasive stage (CIN 3) allows for a 100% cure, even when using conservative surgery, such as cervical conization. Thus early diagnosis is an important issue for gynaecological oncology and is based on cytological examinations of vaginal smears, colposcopy examinations and histological examinations of targeted biopsies taken from the cervix and of material sampled from the cervical canal.

The histological examination is the basis for diagnosing a neoplastic process going on in the cervix. A targeted biopsy sampling – taking samples under colposcopic view – helps to improve the accuracy of the diagnosis which is the basis for adequate treatment.

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Recently, procedures helping in the diagnosis of a malignant process have been completed with the photodynamic method. This method uses the property of selective, specific phototoxic activity on neoplastic cells in an area exposed to laser light irradiation [5-10]. Photodynamic diagnosis (PDD) and photodynamic therapy (PDT) are relatively new procedures used in the diagnosis and treatment of neoplastic diseases. In the last few years, a dynamic development in studies on possibilities to introduce the method into routine clinical practice has been observed. The basis for scientists' interest in its use in the diagnostic process of malignant diseases lies both in the biophysical phenomenon occurring in tissues under the influence of light, causing proper luminescence of cells stimulated by laser light – called autofluorescence, and in the luminescence of tissues that have incorporated an exogenous pigment.

Photodynamic therapy is based on a phenomenon of neoplastic cell destruction with conservation of healthy tissue due to physical reactions occurring in an exogenously administered photosensitizer that specifically accumulates in neoplastic tissue. The effect of those reactions is the acquisition of active chemical compounds that will ultimately destroy pathological cells.

Materials and Methods

The material was a group of 124 patients, aged 25 to 79 years, treated at the Department of Obstetrics and Gynaecology, 2nd Medical Department of the Medical University of Warsaw from 1998 to 2003 for observed pathology of the cervix. Indications for diagnostics included cervical lesions – persistent erosions (cytological CIN 2/3 and invasive cancer), exophytic lesions and ulcerations.

Patients underwent colposcopy, cytological and viral examinations.

The sampled and stained material for cytological examination was subject to microscopic evaluation and classification into five groups according to Papanicolaou.

Cytological groups 1 and 2 (CIN 1 and 2, respectively) were considered as normal (60 cases). Groups 3 and 4 (CIN 3 and invasive carcinoma, respectively) were considered as abnormal (64 cases).

Each patient was subjected to an esocervix and endocervix smear designed to detect the presence of HPV-DNA. The smear was placed on transport medium. Identification of human papilloma viruses HPV-DNA was performed using the hybridisation method - Hybrid Capture System (HC2 HPV DNA Test) at the Laboratory of Venereology of the Medical University of Warsaw. This method allows for the detection of viruses classified as so-called carcinogenic "high risk" (sample B) (HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and "low risk" (sample A) (HPV types: 6, 11, 42, 43, 44). The hybridisation method is a combination of a DNA hybridisation test with use of RNA probes and of an immunoenzymatic test, allowing for the detection and marking of created hybrids. Created hybrids reflect the presence of HPV-DNA in the tested sample.

All patients underwent a PDD examination using a 5-ALA photosensitizer with 15% cream (5-aminolevulinic acid hydrochloride) manufactured by Jelfa. The photo-sensitizer was administered onto the cervix or the vulva six hours prior to the PDD examination. For better photosensitizer absorption by tissues, ALA-impregnated swabs were used in the vagina. In order to obtain fluorescence, the tissue was exposed to energy in the form of light from a 300W xenon lamp with a wavelength of 400-420 nm. Tissue fluorescence was considered as a positive result suggesting the possibility of a neoplastic process. All procedures during PDD examinations were performed in a room with diminished lighting in order to minimize degradation of porphyrins.

After the PDD examination, patients underwent tissue sampling from lesions in order to obtain a histological diagnosis. In the case of biopsies taken from the cervix, a microscopic examination result was considered as benign in the following: uterine neck erosion, cervicitis and endocervicitis, CIN 1 and CIN 2. Malignancy was suspected in the following histological diagnoses: CIN 3, uterine neck cancer and eso and endo cervix cancer.

The efficacious assessment of PDD, colposcopy examination and cytological examination in the evaluation of cervical lesions was performed and compared with histological examination results. The following parameters were assessed: sensitivity, efficacy, specificity, and positive and negative prognostic values.

The statistical analysis of obtained results was performed based on the following techniques: Student's-test for independent and dependent assays, chi-square Pearson test and Yates-modified chi-square test as well as Fisher's test.

Results with $p < 0.05$ were considered statistically significant. Analyses were performed using the STATISTICA software package (version 5.5 by StatSoft).

Results

In patients with cervical lesions, according to the study methodology, the assessment included efficiency of the cytological examination, HPV-DNA presence, colposcopy and PDD examination.

Efficiency of cytological examination in the diagnosis of malignant lesions of the vaginal part of the cervix.

Table 1 shows the results of cytological and histological diagnoses obtained in 124 patients who underwent targeted biopsy taken from lesions located on the vaginal part of the cervix.

Table 1. — *Comparison of cytological examination results of smears taken from the uterine cervix with histological examination results.*

No. of patients 124	Cytological group	Histological examination CIN 1	CIN 2	CIN 3	Invasive cancer	Inflammatory lesions
60	2	0	0	0	0	60
53	3	14	8	14	0	17
11	4	0	0	0	11	0

$p < 0.01$.

As presented in the above table, the diagnosis consistency percentage across cytological and histological examinations was similar in cytological groups 2 and 4 reaching 100%. In 60 patients in cytological group 2, the following benign lesions were diagnosed in the material sampled for histological examination: uterine neck erosion, chronic cervicitis. All cases in cytological group 4 (11 patients), had confirmed malignant process evidence. The histological examination established a diagnosis of invasive cervical carcinoma.

In 53 patients in cytological group 3, a histological diagnosis of dysplasia was obtained in 36 cases, which represents 67.9%, with 14 patients being diagnosed with CIN 1, eight with CIN 2 and 14 with CIN 3. In 17 cases (32.1%) benign lesions were diagnosed, such as uterine neck cancer and chronic cervicitis.

HPV-DNA prevalence compared with cytological examination.

Tables 2, 3 and 4 show the results of cytological diagnoses of smears taken from the uterine cervix and targeted histological biopsies taken from lesions of the uterine cervix as well as the results of HPV-DNA prevalence.

Table 2. — *HPV-DNA infection prevalence in patients in cytological group 2 confirmed by histological diagnosis.*

Cytological group 2 No. of patients	Histological diagnosis	HPV-DNA type A	type B	type AB
60	Inflammatory lesions	2	3	0

HPV-DNA low risk – sample A. HPV-DNA high risk – sample B.

Table 3 presents results of smears taken from the uterine cervix in patients in cytological group 3, of HPV-DNA prevalence, as compared with the results of the histological examinations of targeted biopsies taken from lesions of the uterine cervix.

Table 3. — *HPV-DNA prevalence in patients in cytological group 3 confirmed by histological diagnosis.*

Histological diagnosis	No.	HPV-DNA type A	HPV-DNA type B	HPV-DNA type A and B
Inflammatory lesions	17	0	0	0
CIN 1	14	1	2	0
CIN 2	8	2	4	1
CIN 3	14	4	10	3
Invasive carcinoma	0	0	0	0
Overall	53	7	16	4

HPV-DNA low risk – sample A. HPV- DNA high risk – sample B.

Table 4 shows the results of smears taken from the uterine cervix in patients in cytological group 4, of HPV-DNA prevalence, as compared with the results of the histological examinations of targeted biopsies taken from lesions of the uterine cervix.

Table 4. — *HPV-DNA prevalence in patients in cytological group 4 as compared with the histological diagnosis.*

Cytological group 4 No. of patients	Histological diagnosis	HPV-DNA type A	HPV-DNA type B	HPV-DNA type AB
11	Squamous cell invasive cancer	2	8	1

HPV-DNA low risk – sample A. HPV-DNA high risk – sample B.

Tables 2, 3 and 4 show that in the group of patients with HPV infections estimated as low risk – sample A, and high risk – sample B, an increased infection ratio was observed which is consistent with the increase in CIN stage.

Efficiency of the colposcopy examination in the diagnosis of malignant lesions of the uterine cervix.

Table 5 presents the results of colposcopy and histological examinations obtained in 124 patients by targeted biopsy taken from lesions of the uterine cervix.

Table 5. — *Comparison of colposcopy examination results with histological examination results of targeted biopsy of the uterine cervix.*

Colposcopy n - 124	Histological examination CIN 1	CIN 2	CIN 3	Invasive cancer	Inflammatory lesions
Suspicious 47	9	6	14	11	7
Non-suspicious 77	5	2	0	0	70

$p < 0.01$.

Of 47 patients with suspicious colposcopy results, 29 were diagnosed with histological dysplasia representing 61.7% of patients including CIN 1 patients - nine cases, CIN 2 - six cases and CIN 3 - 14 cases. In 11 (23.4%) patients, invasive cancer was diagnosed, while in seven cases (14.9%) the result was false-positive.

In 77 patients with colposcopic images estimated non-suspicious, consistency with histological diagnosis was observed in 70 cases (90.9%).

In the analysed material, there were seven cases of colposcopic false-negative results. In histological examination, CIN 1 type lesions were observed in five cases, while CIN 2 type lesions were seen in two cases.

HPV-DNA prevalence and as compared with colposcopy examination.

Table 6 gives the results of colposcopy examinations, HPV-DNA prevalence tests and histological examination results of targeted biopsies taken from the uterine cervix.

Among nine patients with a CIN 1 diagnosis and suspicious colposcopic images, there was one case showing low carcinogenic potential HPV virus and two cases with high carcinogenic potential HPV virus.

Table 6. — *HPV-DNA presence in patients with suspicious colposcopy image as compared with histological diagnosis.*

Histological diagnosis	No.	HPV-DNA type A	HPV-DNA type B	HPV-DNA type A and B
Inflammatory lesions	7	2	3	0
CIN 1	9	1	2	0
CIN 2	6	2	4	1
CIN 3	14	4	10	3
Invasive carcinoma	11	2	8	1
Overall	47	11	27	5

HPV-DNA low risk – sample A. HPV- DNA high risk – sample B.

Among six patients with a CIN 2 diagnosis and suspicious colposcopic images, two cases were found to have low carcinogenic potential HPV virus and four cases high carcinogenic potential HPV virus. One case was diagnosed with a "mixed" low- and high-risk infection.

In 14 patients with a CIN 3 diagnosis, four cases with low carcinogenic potential virus were found and ten cases with high carcinogenic potential virus. Three patients were diagnosed with a "mixed" low- and high-risk infection.

In 11 patients with suspicious colposcopic images, a histological examination revealed malignancy – an invasive form of cervical cancer. Two of these patients were diagnosed with low-risk virus presence, while eight patients had high-risk carcinogenic infections. One case was diagnosed with a "mixed" low- and high-risk infection.

In seven cases of false suspicious colposcopic images, a low-risk virus infection was found in two cases, while a high-risk infection was observed in three cases.

Among 77 patients with non-suspicious colposcopic images, there were no low- or high-risk virus infections found.

PDD efficacy in the diagnosis of malignant lesions of the uterine cervix.

Table 7 presents the results of photodynamic examinations – PDD and histological examination results obtained in 124 patients by targeted biopsies taken from lesions of the uterine cervix.

Table 7. — *Comparison of PDD examination results with histological examination results.*

PDD n - 124	Histological examination CIN 1	CIN 2	CIN 3	Invasive cancer	Inflammatory lesions
Fluorescence 50	13	7	14	11	5
No fluorescence 74	1	1	0	0	72

$p < 0.01$.

As presented in Table 6, in 50 patients with evidenced fluorescence after laser light activation, a histological confirmation of dysplasia was obtained in 34 cases (68.7%) - of which CIN 1 was diagnosed in 13 cases, CIN 2 in seven and CIN 3 in 14 cases. Eleven patients (22%) were diagnosed with invasive cancer, while five patients (10%) had false-positive results.

Of 74 patients without evidence of tissue fluorescence, the consistency with histological diagnosis was observed in 72 cases (97.3%). In two cases the result was false-negative.

HPV-DNA prevalence as compared with the photodynamic examination.

Table 8 shows the results of photodynamic examinations – PDD, HPV-DNA prevalence and histological results of targeted biopsies taken from the uterine cervix.

Table 8. — *Comparison of PDD examination results with histological examinations and HPV-DNA virus presence tests in patients with tissue fluorescence.*

Histological diagnosis	No.	HPV-DNA type A	HPV-DNA type B	HPV-DNA type A and B
Inflammatory lesions	5	2	3	0
CIN 1	13	0	2	0
CIN 2	7	1	4	1
CIN 3	14	4	10	3
Invasive carcinoma	11	2	8	1
Overall	50	9	27	5

HPV-DNA low risk – sample A. HPV- DNA high risk – sample B.

In 13 patients with a CIN 1 diagnosis and evidence of tissue fluorescence, no evidence was found of low-risk carcinogenic HPV virus presence, while in two cases a high-risk carcinogenic virus presence was observed.

In seven patients with a CIN 2 diagnosis and evidence of tissue fluorescence, there was one case of low-risk virus presence and four cases of high-risk carcinogenic virus presence. One case was diagnosed with a "mixed" low- and high-risk infection.

In 14 patients with a CIN 3 diagnosis and evidence of tissue fluorescence, there were four cases of low-risk carcinogenic virus presence and ten cases of high-risk carcinogenic virus presence. Three cases were diagnosed with a "mixed" low- and high-risk infection.

In 11 patients with evidence of tissue fluorescence, a malignant process was diagnosed at histological examination – an invasive form of cervical cancer. Two patients among them were diagnosed with a low-risk virus, while eight other patients had a high-risk carcinogenic virus. One case was diagnosed with a "mixed" low- and high-risk infection.

In five cases with cervix inflammation diagnosed in the histological examination and evidence of tissue fluorescence, two cases were diagnosed with low-risk virus, with three cases with high-risk virus.

Table 9 presents the results of histological examinations and presence of HPV-DNA virus in patients without evidence of tissue fluorescence.

Table 9. — *Comparison of PDD examination results with histological examinations and presence of HPV virus in patients without tissue fluorescence.*

Histological diagnosis	No.	HPV-DNA type A	HPV-DNA type B	HPV-DNA type A and B
Inflammatory lesions	72	0	0	0
CIN 1	1	1	0	0
CIN 2	1	1	0	0
CIN 3	0	0	0	0
Invasive carcinoma	0	0	0	0
Overall	74	2	0	0

HPV-DNA low risk – sample A. HPV- DNA high risk – sample B.

In two cases that did not demonstrate tissue fluorescence, one with CIN 1 and the other with CIN 2, low-risk virus was found.

Based on statistical analyses assessing the efficiency of the photodynamic examination, performed according to the study method, the following statements may be formulated:

Photodynamic diagnostics has the following characteristics as compared with cytological examination: lower sensitivity – 95.7% compared to 100% for cytological examination ($p = 0.159$), higher specificity – 93.5% compared to 77.9% for cytological examination ($p = 0.0003$), higher efficacy – 94.3% compared to 86.2% for cytological examination ($p < 0.01$), higher positive prognostic value – 90% compared to 73.4% for cytological examination ($p = 0.0125$), lower negative prognostic value – 97% compared to 100% for cytological examination ($p = 0.089$).

The above results are illustrated in Figure 1.

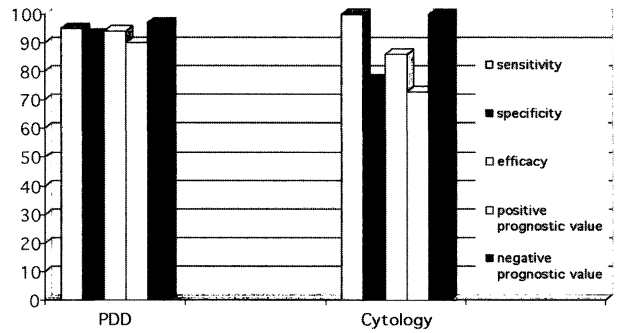

Figure 1. — Comparison of PDD and cytology examinations in terms of sensitivity, specificity, efficacy, and, positive and negative prognostic value in patients in the first group.

Photodynamic diagnosis, as compared with colposcopy examination, has the following characteristics:

– higher sensitivity – 95.7% compared to 85.1% for colposcopy ($p = 0.024$);

– higher specificity – 93.5% compared to 90.9% for colposcopy ($p = 0.159$);

– higher efficacy – 94.3% compared to 88.7% for colposcopy ($p < 0.01$);

– higher positive prognostic value – 90% compared to 85.1% for colposcopy ($p = 0.23$);

– higher negative prognostic value – 97.2% as compared with 90.9% for colposcopy ($p = 0.06$).

The above results are illustrated in Figure 2.

Discussion

Formerly performed studies on cervical cancer clearly indicate that only early diagnosis of this disease offers an opportunity for a complete cure [11, 12]. The diagnostic proceeding has been, until now, based on cytological, colposcopic and viral examinations, and on histological examinations of targeted biopsies taken from the cervix.

Cytodiagnostics is a widely used method in the diagnosis of pathological lesions of the squamous epithelium and early stages of cervical cancer [13-16].

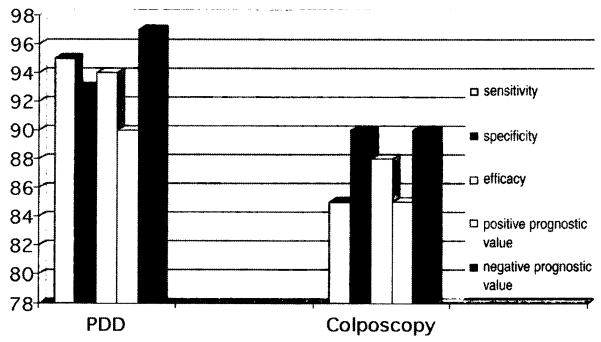

Figure 2. — Comparison of PDD and colposcopy examinations in terms of sensitivity, specificity, and, efficacy, positive and negative prognostic value in patients in the first group.

In our material, we performed an efficiency analysis of cytological examinations in the diagnosis of malignancies of the uterine cervix, based on the histological examination results of targeted biopsies.

In 124 patients with cervical lesions in the form of a non-healing erosion (in cytological groups 2-4), exophytic lesions and ulcerations (Table 1), the percentage of diagnostic consistency of cytological examinations with histological examination results was similar for groups 2 and 4 and reached 100%. In 60 patients in cytological group 2, benign lesions were diagnosed in the material sampled for histological examination. Cases in cytological group 4 (11 patients) had confirmed evidence of a malignant process. The histological examination established a diagnosis of invasive cervical carcinoma. In 53 patients in cytological group 3, a histological diagnosis of dysplasia was obtained in 36 cases (67.9%) with 14 patients being diagnosed with CIN 1, eight with CIN 2 and 14 with CIN 3. In 17 cases (32.1%) benign lesions were diagnosed.

Results of studies analysing the consistency of cytological and histological examinations in patients in cytological group 3, published by Banaczek *et al.* [17], show 76.4% of benign lesions such as erosions, 21.7% of dysplastic lesions and 2.04% of pre-invasive cancer in histological examinations. The consistency was somewhat inferior to the one obtained in our material. A cytological and histological examination consistency study in 130 patients in cytological group 3, presented by Ouaas *et al.* [3], showed a 44.6% initial diagnosis confirmation.

The cytological examination shows a certain percentage of error as compared with histological evaluation, reaching 20% depending on the authors [17-21]. In Gerber's studies [20], false positive results were obtained in 66.4%, dysplastic lesions in 12.3%, while pre-invasive cancer in 1.2%. The consistency of the cytological examination with the histological examination was somewhat inferior to the one obtained in our material. After histological verification, Kasiak [22] observed benign lesions in 21.4% of women in cytological group 3, with dysplastic lesions representing 76.2% and malignancies 2.4%. The consistency of cytological and histological examinations was somewhat inferior to the one obtained in our material.

Obtaining false-positive results of PAP II smears is caused by the existence of many factors such as: experience of the person evaluating the smear, sampling and staining techniques and quite often presence of a symptom-free inflammatory state. Changes occurring in the cytological image of stratified squamous epithelium cells in bacterial, viral and Chlamydia infections may qualify smears to cytological group 3. A certain tendency was observed for over-estimating cytological groups, which in turn increases the number of false-positive diagnoses.

The relationship between infection of the cervix with human papilloma virus (HPV), particularly certain strains of that virus, and cervical cancer morphogenesis is widely known and recognised [23-33]. It was therefore relevant to include infection diagnostics in our study – HPV, based on the hybridization technique.

An analysis of HPV- DNA infection prevalence was performed in a group of 124 women with cervical lesions, in cytological groups 2, 3 and 4, further compared with targeted biopsy histological results (Tables 2-4). An increase infection rate of high-risk HPV-DNA was observed depending on CIN stage: CIN 1 – 22.2%, CIN 2 – 66.6%, CIN 3 – 71.4% and invasive carcinoma – 72.7%.

Studies by other authors confirm our observations. In Kwaśniewska *et al.'s* studies [30, 31] a gradual increase was observed in HPV infection: CIN 1 and CIN 2 – 55.5%, CIN 3 – 70.5% and invasive carcinoma – 83.3%. Basta [34] also obtained results consistent with our findings.

Colposcopy examination is one of the most effective tests in the diagnosis of symptom-free precancerous lesions and malignancies within the cervix. [3, 24, 35].

In our material, apart from the cytological and viral examinations, an analysis was performed on the efficacy of colposcopy examination in the diagnosis of malignant lesions of the uterine cervix, based on histological examination results of targeted biopsies (Table 5).

One hundred and twenty-four patients with cervical lesions in the form of erosion in cytological groups 2, 3 and 4 with exophytic lesions or ulcerations underwent detailed colposcopy examinations. Forty-seven patients had suspected colposcopy images. In that particular group, a histological diagnosis of dysplasia was obtained in 29 cases (61.7%) of which there were: CIN 1 – nine cases, CIN 2 – six cases and CIN 3 – 14 cases. Eleven patients (23.4%) were diagnosed with invasive cervical cancer.

Histological examination results in that group confirmed colposcopy diagnosis in 85.1%, while in 14.9% the result was false-positive, as inflammatory changes were ultimately diagnosed.

In 77 patients with colposcopic images estimated as non-suspicious, consistency with histological diagnosis was observed in 70 cases (90.9%). In seven cases (9.1%), a false-negative colposcopy result was obtained, while in histological examination, CIN-I type lesions were observed in five cases, while CIN-II type lesions were found in two cases.

Massad *et al.* [36] obtained results similar to ours when studying suspicious colposcopy images in 2,825 women, obtaining 80% consistency with the histological examination. Similar results were also reported by Szurkus and Harrison [37]. High result consistency (reaching 92.5%) of colposcopy and histological examinations was obtained by Basta *et al.* [35] in their studies. Study results published by Sobola *et al.* [38], analysing consistency of suspected colposcopy images with histological examination in patients in cytological group 3, showed a 60% accuracy of colposcopic diagnosis. Consistency was inferior to that of our material. In the group of patients with unsuspicious colposcopic images, diagnostic consistency reached 96.3% and was comparable to our results.

Despite the widespread use of cytological, colposcopic and viral examinations in the diagnosis of cervical lesions, cervical cancer still occupies one of the leading positions among neoplasms occurring in women. Therefore, considering the dissatisfactory results of early diagnosis and treatment, we decided to include photodynamic diagnosis in the overall evaluation and to assess its efficacy in diagnosing precancerous lesions and malignancies of the cervix.

In our material, we performed an efficacious analysis of the photodynamic examination in the diagnosis of malignant lesions of the uterine cervix based on the histological results of targeted biopsy (Table 7).

Photodynamic examination was performed in 124 patients with cervical lesions in the form of erosions in cytological groups 2, 3, 4, exophytic lesions and ulcerations.

In 50 patients with evidence of tissue fluorescence after laser light activation, histological confirmation of dysplasia was obtained in 34 cases (68.7%), of which 13 cases were CIN 1, seven CIN 2, while 14 were CIN 3. In 11 patients (22%) a diagnosis of invasive cancer was made, while in five patients (10%) the results were false-positive.

Of 74 patients without evidence of tissue fluorescence, consistency with histological diagnosis was observed in 72 cases (97.3%). In two cases the result was false-negative with a microscopic diagnosis of small and medium grade dysplasia.

Application of the photodynamic method in the diagnostic process of genital tract malignancies constitutes the subject of only a few reports in Polish and the worldwide literature. None of these reports include a comprehensive workup of the method depending on the nature of the cancer, the location and clinical stage. There is also univocal information missing regarding the choice of the photosensitizer, its concentration and application method, as well as regarding the use of optimal laser light sources.

Pelczyński *et al.* [39] published results of local administration of 5-aminolevulinic acid in the diagnosis of cervical lesions in 19 women in cytological group 3. A histological confirmation of dysplasia was obtained in 14 patients (73.6%), of which there were six cases of CIN 1 and four cases of CIN 2 and CIN 3. The published results refer to a relatively limited group of women with formerly diagnosed dysplasia, however, these are consistent with the results we have obtained.

Loning *et al.* [40], evaluating fluorescence and lesion localisation on the cervix in 20 women subsequently subjected to conization due to the presence of neoplasia, obtained results similar to ours. Those observations were also confirmed in 28 women by Hillemanns *et al.*, Wierrani *et al.* [41] and Muroya *et al.* [42]

The photodynamic diagnosis method, similarly to other methods for the early detection of cervical malignancies, bears a certain error. This error may be the result of many factors. In our study, we experienced problems with choosing the right medium and application of the photosensitizer. Its satisfactory penetration into tissues, providing us with an objective fluorescence image, was only obtained when using 15% ALA ointment on eucerine medium and vaginal swabs. Obtaining false results (both false-positive and false-negative results) depends on the time between its application and exposure to laser light as well as on the choice of a proper wavelength for the laser light.

Despite those difficulties, our results, confirmed by the literature data, seem to indicate a high efficacy of the photodynamic diagnosis method in the diagnosis of malignancies within the cervix. However, it should not be forgetton that histological examination is the basis for a definitive diagnosis. The value of the photodynamic examination is undoubtedly unquestionable in the detection of cervical cancer in early stages through tissue fluorescence observation and its localisation, which in turn helps the following targeted biopsy, thus greatly improving the accuracy of pretherapeutic diagnoses. It should also be mentioned that the direct evaluation of fluorescence provides information on the multi-focal character of lesions and on their range, which in turn may constitute an indication for further prognostics.

When evaluating the diagnostic value of the photodynamic examination according to the study methodology, the presence of HPV-DNA virus was assessed with a widely accepted relationship with the morphogenesis of cervical cancer [24-27, 33, 43, 44] in patients with or without tissue fluorescence (Tables 8 and 9). The results confirm our previous observations. In 45 patients with tissue fluorescence after laser light activation, in whom a cytological examination revealed CIN-type lesions, the Hybrid Capture System examination showed the presence of HPV-DNA. In five patients in whom the fluorescence result was false-positive (lesions such as cervicitis and endocervicitis in microscopic examination), there were two cases of low-risk virus and three cases of high-risk virus.

It is possible that the fluorescence phenomenon related to HPV infection precedes CIN-type changes in the histological examination. An increase in HPV-DNA infections was observed in the study group depending on the CIN stage.

In a group of 72 patients without fluorescence after ALA and with CIN 1 type lesions (1 case) and CIN 2-type lesions (1 case), the prevalence of low oncogenetic potential viruses was established.

Photodynamic diagnosis is not a method used for iden-

tification of viral infection, but combined with the Hybrid Capture method, and cytological and colposcopic examinations, it may help to precise the diagnosis.

Based on scarce data from the literature, the photodynamic diagnosis method and the relationship with HPV-DNA virus infection are subjects for studies on photodynamic therapy. While using PDT for dysplastic lesion treatment, a decreased prevalence of the HPV virus was observed, reaching up to 90% [41]. The above observations were also confirmed by Schmidt *et al.* [45] as well as by Mathias *et al.* [46].

Conclusions

1. An important consistency of photodynamic examinations with histological diagnoses was observed, particularly high in cases of cervical precancerous and cancerous lesions, reaching 94.3%.

2. A superior accuracy of PDD was observed, as compared with colposcopy and cytological examination results – 88% and 86%, respectively.

3. The possibility of performing a one-time specific biopsy of lesions found during the photodynamic examination helps improve the accuracy of histological diagnoses.

4. Fluorescence observation and the possibility of evaluating its intensity during the photodynamic examination may constitute the basis for determining the range or multifocal character of the lesions.

5. Precise localisation of lesions using PDD may be used in the choice of appropriate treatment methods, often influencing the extension or limitation of the treatment procedure.

6. No adverse events were observed when performing local PDD.

7. Encouraging proper experiences allows us to popularize the method of photodynamic diagnosis in gynaecological practice in order to improve the diagnostic accuracy, for example in the so-called cumulative analysis of results of various methods.

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