The level of antibody against E6 HPV 16 oncoprotein in blood sera of women with chronic HPV 16 infection and cervical cancer

W. Kędzia[1], A. Olejnik[2], M. Schmidt[2], R. Nawrot[2], A. Goździcka-Józefiak[2], H. Kędzia[1], M. Spaczyński[1]

[1]Department of Gynecology and Obstetrics, Division of Gynecological Oncology, Karol Marcinkowski University of Medical Sciences; [2]Department of Molecular Virology Adam Mickiewicz University, Poznań (Poland)

Summary

The aim of this study was to estimate of the role of chronic HPV 16 infection and the presence of anti E6 HPV 16 in the initiation of the cancerogenesis process of cervical cancer.

Material and Methods: The study included two groups of patients. The first group comprised 323 women observed for three consecutive years (1998-2000), in whom the presence of HPV 16 viruses was estimated by PCR, and the level of anti E6 HPV 16 antibodies was estimated in the plasma with ELISA. A similar test was performed in a group of 46 patients with cervical intraepithelial neoplasia (CIN), 91 patients with invasive cervical cancer and 22 women after hysterectomy and RTG-therapy.

Results: In 32 patients, chronic HPV 16 infection showed a steady rise in the mean absorbance level of anti E6 HPV 16 antibodies from 0.04 in 1998 to 0.06 in 2000, while in HPV-negative women the mean absorbance value was 0.03-0.04.

Mean absorbance value in patients with CIN III and invasive cancer rose with advancing stage of the cancer process and lowered after completion of oncological treatment. The values were 0.14, 0.33 and 0.13, respectively.

Conclusion: The persistence of chronic HPV 16 infection and accompanying steady rise in absorbance index caused by an increase in the level of antiviral antibodies are a clear warning signal preceding in time the histological process of cancerogenesis.

Key words: Cervical cancer; HPV 16; Chronic infection; Antibody.

Introduction

Papillomaviruses are non-enveloped, double-stranded DNA viruses containing a circular genome of approximately 8,000 base pairs and are associated with the majority of cervical carcinomas. The genome can be divided into three functional regions: early, late and control. The early region of HPV 16 is composed of open reading frames, E1, E2, E4, E5, E6, E7, whose gene products are responsible for viral DNA replication, transcriptional control and cellular transformation [1, 3, 23, 25]. The E6 and E7 encode oncoproteins that bind with cellular tumor suppressor gene products, p53 and pRB, respectively, and through their interaction promote cell-cycle progression [6, 7, 19, 24].

Expression of HPV 16 E6 and E7 open reading frames is regulated by the HPV E2 protein. Malignant transformation is usually accompanied by disruption of the E2 gene and consequent deregulated expression of E6 and E7. HPV infection has been diagnosed on the basis of cytological, colposcopic, histological and molecular examination using PCR and nucleic acid hybridization. An alternative method for detecting HPV infection could be serological assays, which may monitor current and past infection. In the present study serum HPV 16 E6 antibodies were measured in healthy women without HPV infection (control group), asymptomatic carriers and women with cervical cancer before hysterectomy and after RTG-therapy.

Revised manuscript accepted for publication May 24, 2005

Material and Methods

Samples

Samples of plasma were collected from two groups of women. The first group of was comprised of women 35-50 years old (mean age 45), who were examined gynecologically for three consecutive years from 1998-2000. All of them had their blood drawn for evaluation of anti HPV 16 E6 antibodies. A Pap smear was done for cytological evaluation and for HPV 16 DNA isolation for further PCR. In 1998 323 women were diagnosed in this manner; in 1999 from the previous group only 220 reported back and in 2000 - only 168. A total of 711 plasma samples were collected. These women comprised a control group for women with cervical intraepithelial neoplasia (CIN) and cervical cancer.

The second group of women was established upon discovering in the first gynecological examination changes within the cervix. In this group of women blood was collected form 46 women with intraepithelial neoplasia; ten from women with CIN I and 36 plasma samples were obtained form women with CIN III. Additionally 91 samples of blood were collected from women with invasive cancer (FIGO Stage I, II) and 22 plasma samples form patients after the end of the therapeutical process. Women with cervical cancer had their blood drawn twice: before the operation and after finishing oncological treatment (brachy- and teletherapy). In the second group of women there were 159 plasma samples collected; altogether the antibodies were measured in 870 samples.

Expression of E6 HPV 16 in Escherichia coli

The E6 coding sequence was cloned after PCR amplification from the reference clone of HPV 16 (Gene Bank Acc. no.

K02817), with primers designed to introduce Bam HI and Hind III restriction enzyme sites. The forward and reverse primer sequences were:
5' AGAGGATCCGATGCACAAAAAGAGAACTGCA3' and 5' CATAAGCTTACAGCTGGGTTTCTCTACG3', respectively. Amplification was performed with 0.2 mM concentration of each primer and 0.4 U of Taq DNA polymerase (DyNAzyme, Finnzymes). Following restriction of enzyme digestion the PCR product was cloned into pET28b + vector (Novagen). DNA sequencing was performed using T7 forward primer. Recombinant vector pET28 - E6 HPV 16 was introduced in E. coli BL21 DE3pLys. Overexpression was induced at (OD = 4.0) by using 1 mM IPTG (isopropylthiogalactozyd). E. coli cells were harvested three hours after induction and pellets were incubated on ice for 15 min in lysis buffer. Cell lysates were centrifuged (10000 g, 30 min) and the soluble fraction of protein was used to purify E6 oncoprotein by affinity chromatography. Purification of recombinant protein was performed in native conditions on HisTrap™ column (Amersham Pharmacia Biotech) on a AKTA Explorer chromatograph (Amersham Pharmacia Biotech).

Antigen E6 HPV 16 binding enzyme-linked immunosorbent assay (ELISA)

The standard assay employed to study antibodies binding to E6 protein was a sandwich enzyme-linked immunosorbent assay. Antigen E6 was diluted to 5 μg/ml in PBS, pH 7.2 and 96-well plates were coated 1hr at 37°C. Plates were incubated in PBST (PBS + 0.05% Tween 20) with 1% gelatine 1hr at 37°C. Bound antigen was incubated 1hr at 37°C with 100 μl diluted to 1:100 serum with PBST. Antibodies were detected with anti-human polyvalent goat immunoglobulin conjugated to horseradish peroxidase. Absorbance was read in ELISA Labsystems Multiscan PLUS rader at 450 nm.

Identification of HPV DNA 16 sequence

Cellular DNA was extracted as described by Fife and examined by polymerase chain reaction with specific primers to target the L1 gene region of HPV 16 (primers HPV 16/L1A, HPV 16/L1B, size product 264 bp) [14].

Results

Of 323 women diagnosed in 1998, HPV 16 infection was diagnosed in 56 women (17.3%). Pap smears in all women were normal. Of 220 women studied in 1999, 159 were HPV 16 negative and among HPV 16 positive patients in 42 women the infection was still present. In all women cytological smears were normal. In 19 women who were HPV negative a year before, HPV 16 infection was found. Of women diagnosed in 2000, in a group of women who were diagnosed HPV 16 positive for two consecutive years 32 still had HPV 16 infection and in five there was a diagnosis of HSIL. Signs of the presence of the virus were not found in 123 women, while in 13 HPV 16 negative patients HPV 16 infection was found in the cervix before occurrence of cervical cancer. Results are demonstrated in Table 1.

Compared to the whole population of 323 studied women HPV 16 infection was persistent for three years in 32 patients, which constitutes 9.9%, while an infection lasting only one year was also present in 32 patients. In a group of women who were HPV 16 positive for three years there was a constant and noticeable rise in absorbance measurement from 0.04 in 1998 to 0.06 in 2000 (Table 2). The mean absorbance in seropositive women from the control group, who were HPV negative was from 0.03-0.04 (Table 3). Relatively constant results of absorbance in this group of women coexisted with lack of signs of viral infection for three successive years. Comparison of mean absorbance in the plasma of seropositive women with CIN I, CIN III (CIS), invasive carcinoma (blood collected before radical hysterectomy) and final treatment clearly shows a rise in the absorbance values with advancement of the cancerogenesis process and lowering after the end of RTG-therapy (Table 4).

It is worth noting that over three years of examination of absorbance with wavelength in plasma from women with chronic viral infection, the mean values were similar to values achieved in women with CIN I (Tables 2 and 4).

Table 1. — *Number of women examined gynecologically for the first time in 1998 and then in 1999 and 2000. Presence of HPV 16 infection and changes in epithelial cells in the cervical smears.*

Year	Number of patients	HPV 16+		HPV16-		HPV16- → HPV16+	
		n/%	HSIL	n/%	HSIL	n/%	HSIL
1988	323	56/17.3	–	267/82.6	–	–	–
1999	220	42/19.0	–	159/72.0	–	19/19.0	–
2000	168	32/19.0	5/16	123/73.0	–	13/8.0	–

Table 2. — *The results of mean absorbance antibody against E6 HPV 16 measurements in the plasma of seropositive women from the control group; HPV positive cases performed with ELISA.*

Year	Number of HPV 16+/seropositive	Mean absorbance
1988	56/47	0.04
1999	42/40	0.05
2000	32/32	0.06

Table 3. — *The results of mean absorbance antibody against E6 HPV 16 measurement in the plasma of seropositive women from the control group; HPV 16 negative cases performed with ELISA.*

Year	Number of HPV16/18-/seropositive [% of all HPV-women]	Mean absorbance
1988	35/267 [13%]	0.04
1999	21/159 [13.2%]	0.03
2000	28/123 [22.7%]	0.03

Table 4. — *The results of mean absorbance values of anti E6 HPV 16 antibodies in the ELISA test in the plasma of women with CIN I, CIN III (CIS), invasive carcinoma and after the end of oncologic treatment.*

Diagnosis	Number of patients	Mean absorbance
CIN I	10	0.06
CIN III (CIS)	36	0.14
Invasive carcinoma	91	0.33
Invasive carcinoma after RTG-therapy	22	0.13

Discussion

Detecting antibodies directed against viral epitopes is one of the methods gaining popularity in the studies of HPV infection spread [4, 10, 21, 28, 36]. There is also a search for correlations between the level of antibodies and cancerogenic processes and cervical cancer. The studies focus mainly on the presence in plasma antibodies directed against viral oncoproteins E6 and E7, highly oncogenic HPV types 16 and 18, in patients with CIN and invasive cervical cancer.

The opening serological results form 323 women conducted in 1998 are inconclusive. Using the PCR method we found in 17.3% (56/323) of women the presence of HPV 16 DNA in normal cytological smears from the cervix. Among 47 women from 56 infected with HPV there was a detectable presence of E6 in the plasma. Of 267 HPV-negative women in 13% (35/267) the presence of anti E6 HPV 16 antibodies was found. The mean absorbance value was 0.04. The results of seropositive women with normal Pap smears are not surprising. Other authors also found antibodies directed against E7 HPV 16 in a group of women HPV 16 negative [8, 16, 19, 20, 22, 27]. There are considerable differences among results interpreting extent of HPV 16 antibodies for studied populations with normal Pap smears ranging from 14% to 59% of seropositive results. This phenomenon is probably caused by the possibility of spontaneous regression of a past infection, which has been observed in 24.8-68% of HPV infected women, especially in young women with a better immunologic system function [16, 18, 30-32]. The mean value of absorbance in women both HPV 16 positive and seropositive evaluated in 1999 and 2000 changed from 0.04 in 1998 to 0.06 in 2000. The mean values of absorbance for the remaining seropositive women from the control group in subsequent years of observation was at or below 0.04. The analysis of the mean absorbance in the three-year period in a group of HPV 16+ and seropositive for protein E6 women selected in 1998 suggests the development of a chronic infection in this group of patients and in consequence progression of the cervical disease. Proof of this was finding HSIL in 2000 in the cervix of five women (Table 1). The results achieved suggest that chronic infection with HPV16 and seropositivity for viral antigens are factors favoring the cancerogenic process.

In our research we found clear differences in the absorbance with 450 nm wavelength in the plasma of women with CIN I and CIN III, 0.06 and 0.14, respectively. Similar results were achieved by other authors [12, 16, 18, 21, 34]. De Sanjose *et al.* [8] also confirmed a clear and noticeable rise in seropositivity values associated with progression of the dysplasia. The highest number of patients with anti E6 HPV 16 antibodies was found in the CIN III group, and was connected with the development of invasive cervical cancer.

There are many reports considering the usefulness of evaluating different antibodies directed against epitopes of highly oncogenic types of HPV [11, 16, 19, 20, 35]. Most of the research focuses on protein E7. The interest in E7 HPV 16 antibodies that has been raised in the last few years gave way to the first attempts to try and estimate the correlation of the increase in cervical cancer with the presence of certain fractions of these antibodies against selected E7 HPV 16 epitopes. De Sanjose and coworkers studied E7/1, E7/2, E7/3 HPV 16 antibodies and suggested that the worse prognosis is associated with the presence in plasma of E7/2 HPV16 antibodies, raising the risk of developing cancer 4.3 times, and the presence of a high titer of the E7/1 HPV 16 antibody raises that risk 22.6 times [8]. Describing such a precise correlation between antibody type and risk of developing cancer seems a little premature at the present state of knowledge. During evaluation of antibodies in women with HPV infection long-term observation seems most important. Our studies with the anti E6 HPV 16 antibodies showed that in patients with invasive cervical cancer the mean absorbance in the ELISA was 0.33, and in patients after RTG-therapy this value decreased to 0.13. Our research suggests a correlation between the mass of neoplastic tissue and rising anti E6 antibodies.

The highest absorbance values were detected in advanced invasive cancers. Highly suggestive is the marked lowering of the absorbance after the end of rtg-therapy from 0.33 to 0.13. In 59% of women (57/91) treated for cervical cancer the presence of anti E6 HPV 16 antibodies was detected in the plasma. This result differs from the mean frequency of seroconversison of antiHPV antibodies in cervical cancer patients in other studies, which never exceeded 50% [8, 16, 19, 20]. The higher number of seropositive women in this study can be attributed to two factors. First, we evaluated the antibodies directed against E6 HPV16, which according to many authors clearly correlates with cervical cancer. Secondly in the studied population a large number of women had cervical cancer in Stage Ib. The phenomenon of the rise of seroconversion with the advance of stage of cervical cancer has been confirmed by various authors [2, 5, 11, 15, 33]. Sun *et al.* utilizing the E6 and E7 protein synthesized in vivo as an antigen source, also confirmed the above-mentioned correlations and stated that using such substrates allows the development of a very specific and sensitive method [29]. There are single papers whose authors point to the presence of anti HPV 16 antibodies as an independent prognostic factor [2, 33]. Our results together with other data prove that there is a clear chance for practical use of serological methods based on detection of viral antibodies in the prophylaxis of cervical cancer. At present the belief, backed up by numerous and trustworthy data, is that malignant cancer starts with changes in the genes. Acknowledging that fact, modern oncologic prophylactic methods should enable the detection of a neoplastic process at the level of activation of protooncogenes and inactivation of suppressor genes together with detection of oncogenic HPV viruses. From a practical point of view, it would involve confirmation of chronic HPV infection (repeated positive PCR, confirming the presence of HPV DNA), which is accompanied by a rise in the absorbance associated with the rise of antiviral antibodies (ELISA). Our data labels such a state a clear warning state, preceding the initiation of a cancerogenesis process. The calcula-

tion of costs of serological and viral tests set against the cost of detecting, diagnosing and treating primary invasive cervical cancer leaves no question about the value of modern cervical cancer prophylaxis.

References

[1] Auvinen E., Crusius K., Steuer B., Alonso A.: "Human papillomavirus type 16 E5-protein (review)". *Int. J. Oncol.,* 1997, *11,* 1297.

[2] Baay M.F., Duk J.M., Groenier K.H., Burger M.P., de Bruijn H.W., Hollema H. *et al.*: "Relation between HPV-16 serology and clinico-pathological data in cervical carcinoma patients: prognostic value of anti-E6 and/or anti-E7 antibodies". *Cancer Immunol. Immunother.,* 1997, *44,* 211.

[3] Burd E.M.: "Human papillomavirus and cervical cancer". *Clin. Microbiol Rev.,* 2003, *16,* 1.

[4] Carter J.J., Galloway D.A.: "Humoral immune response to human papillomavirus infection". *Clin. Dermatol.,* 1997, *15,* 249.

[5] Chee Y.H., Namkoong S.E., Kim D.H., Kim S.J., Park J.S.: "Immunologic diagnosis and monitoring of cervical cancers using in vitro translated HPV proteins". *Gynecol. Oncol.,* 1995, *57,* 226.

[6] Chellappan S., Kraus V.B., Kroger B., Munger K., Howley P.M., Phelps W.C., Nevins J.R.: "Adenovirus E1A, simian virus 40 tumor antigen, and human papillomavirus E7 protein share the capacity to disrupt the interaction between transcription factor E2F and the retinoblastoma gene product". *Proc. Natl. Acad. Sci. USA,* 1992, *89,* 4549.

[7] Daniels P.R., Sanders C.M., Maitland N.J.: "Characterization of the interactions of human papillomavirus type 16 E6 with p53 and E6-associated protein in insect and human cells". *J. Gen. Virol.,* 1998, *79,* 489.

[8] De Sanjose S., Hamsikova E., Munoz N., Bosch F.X., Hofmannova V., Gili M. *et al.*: "Serological response to HPV 16 in CIN III and cervical cancer patients. Case-control studies in Spain and Colombia". *Int. J. Cancer,* 1996, *66,* 70.

[9] Dillner J., Kallings I., Brihmer C., Sikstrom B., Koskela P., Lehtinen M. *et al.*: "Seropositivities to human papillomavirus types 16, 18, or er 33 capsid and and to Chlamydia trachomatis are markers of sexual behavior". *J. Infect. Dis.,* 1996, *173,* 1394.

[10] Dillner J., Lehtinen M., Bjorge T., Luostarinen T., Youngman L., Jellum E. *et al.*: "Prospective seroepidemiologic study of human papillomavirus infection as a risk factor for invasive cervical cancer". *J. Natl. Cancer Inst.,* 1997, *89,* 1293.

[11] Dillner J., Lenner P., Lehtinen M., Eklund C., Heino P., Wiklund F., Hallmans G., Stendahl U.: "A population-based seroepidemiological study of cervical cancer". *Cancer Res.,* 1994, *54*, 134.

[12] Dillner J., Wiklund F., Lenner P., Eklund C., Frederiksson-Shanazarian V., Schiller J. *et al.*: "Antibodies against linear and conformational epitopes of human papillomavirus type 16 that independently associate with incident cervical cancer". *Int. J. Cancer,* 1995, *60*, 377.

[13] Dyson N., Howley P.M., Munger K., Harlow E.: "The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product". *Science*, 1989, *243,* 934.

[14] Fife K.H., Rogers R.E., Zwickl B.W.: "Symptomatic and asymptomatic cervical infections with human papillomavirus during pregnancy". *J. Infect. Dis.,* 1987, *156,* 904.

[15] Fisher S.G., Benitez-Bribiesca L., Nindl I., Stockfleth E., Muller M., Perez-Garcia F *et al.*: "The association of human papillomavirus type 16 E6 and E7 antibodies with stage of cervical cancer". *Gynecol. Oncol.,* 1996, *61,* 73.

[16] Hamsikova E., Novak J., Hofmannova V., Munoz N., Bosch F.X., de Sanjose S., Shah K., Roth Z., Vonka V.: "Presence of antibodies to seven human papillomavirus type 16-derived peptides in cervical cancer patients and healthy controls". *J. Infect. Dis.,* 1994, *170,* 1424.

[17] Hamsikova E., Smahel M., Sapp M., Munoz N., Bosch F.X., Shah K.V., Vonka V.: "Correlation between the presence of anti HPV33 VLP antibodies and HPV DNA in cervical neoplasia patients". *Arch. Virol.,* 1997, *142*, 413.

[18] Lehtinen M., Dillner J., Knekt P., Luostarinen T., Aromaa A., Kirnbauer R., Koskela P., Paavonen J., Peto R., Schiller J., Hakama M.: "Serologically diagnosed infection with human papillomavirus type 16 and risk for subsequent development of cervical carcinoma: nested case-control study". *Br. Med. J.,* 1996, *312,* 537.

[19] Mann V.M., de Lao S.L., Brenes M., Brinton L.A., Rawls J.A., Green M. *et al.*: "Occurrence of IgA and IgG antibodies to select peptides representing human papillomavirus type 16 among cervical cancer cases and controls". *Cancer Res.,* 1990, *50,* 7815.

[20] Muller M., Viscidi R.P., Sun Y., Guerrero E., Hill P.M., Shah F. *et al.*: "Antibodies to HPV-16 E6 and E7 proteins as markers for HPV-16-associated invasive cervical cancer". *Virology,* 1992, *187,* 508.

[21] Nonnenmacher B., Hubbert N.L., Kirnbauer R., Shah K.V., Munoz N., Bosch F.X. *et al.*: "Serologic response to human papillomavirus type 16 (HPV-16) virus-like particles in HPV-16 DNA-positive invasive cervical cancer and cervical intraepithelial neoplasia grade III patients and controls from Colombia and Spain". *J. Infect. Dis.,* 1995, *172,* 19.

[22] Olsen A.O., Dillner J., Gjoen K., Sauer T., Orstavik I., Magnus P.: "A population-based case-control study of human papillomavirus type 16 seropositivity and incident high-grade dysplasia of the uterine cervix". *Int. J. Cancer,* 1996, *15,* 415.

[23] Pim D., Collins M., Banks L.: "Human papillomavirus type 16 E5 gene stimulates the transforming activity of the epidermal growth factor receptor". *Oncogene*, 1992, *7,* 27.

[24] Scheffner M., Werness B.A., Huibregtse J.M., Levine A.J., Howley P.M.: "The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53". *Cell,* 1990, *63,* 1129.

[25] Schmidt M., Kędzia W., Goździcka-Józefiak A.: "Intratype HPV 16 sequence variation within LCR of isolates from asymptomatic carriers and cervical cancers". *J. Clin. Virol.,* 2001, *23*, 65.

[26] Sethi S., Muller M., Schneider A., Blettner M., Smith E., Turek L. *et al.*: "Serologic response to the E4, E6, and E7 proteins of human papillomavirus type 16 in pregnant women". *Am. J. Obstet. Gynecol.,* 1998, *178,* 360.

[27] Strickler H.O., Schiffman M.H., Eklund C., Glass A.G., Scott D.R., Sherman M.E. *et al.*: "Evidence for at least two distinct groups of humoral immune reactions to papillomavirus antigens in women with squamous intraepithelial lesions". *Cancer Epidemiol. Biomarkers Prev.,* 1997, *6,* 183.

[28] Suchankova A., Ritterova L., Krcmar M., Krchnak V., Vagner J., Jochmus I. *et al.*: "Comparison of ELISA and western blotting for human papillomavirus type 16 E7 antibody determination". *J. Gen. Virol.,* 1991, *72,* 2577.

[29] Sun Y., Shah K., Muller M., Munoz N., Bosch F.X., Viscidi R.P.: "Comparison of peptide enzyme-linked immunosorbent assay and radioimmunoprecipitation assay with in vitro-translated proteins for detection of serum antibodies to human papillomavirus type 16 E6 and E7 proteins". *J. Clin. Microbiol.*, 1994, *32,* 2216.

[30] Syrjanen K., Nurmi T., Mantyjarvi R., Ilonen J., Syrjanen S., Surcel H.M., Yliskoski M., Vayrynen M., Chang F., Saarikoski S.: "HLA types in women with cervical human papillomavirus (HPV) lesions prospectively followed up for 10 years". *Cytopathology,* 1996, *7,* 99.

[31] Syrjanen K., Syrjanen S.M.: "Papillomavirus Infections in Human Pathology". Chichester: Wiley, 2000.

[32] Syrjanen K., Vayrynen M., Castren O., Mantyjarvi R., Yliskoski M.: "The relation between the type of immunoreactive cells found in human papillomavirus (HPV) lesions of the uterine cervix and the subsequent behaviour of these lesions". *Arch. Gynecol.,* 1984, *234,* 189.

[33] Viladiu P., Bosch F.X., Castellsague X., Munoz N., Escriba J.M., Hamsikova E *et al.*: "Human papillomavirus DNA and antibodies to human papillomaviruses 16 E2, L2, and E7 peptides as predictors of survival in patients with squamous cell cervical cancer". *J. Clin. Oncol.,* 1997, *15,* 610.

[34] Wideroff L., Schiffman M.H., Nonnenmacher B., Hubbert N., Kirnbauer R., Greer C.E. *et al.*: "Evaluation of seroreactivity to human papillomavirus type 16 virus-like particles in an incident case-control study of cervical neoplasia". *J. Infect. Dis.,* 1995, *172,* 1425.

[35] Zehbe I., Wilander E., Delius H., Tommasino M.: "Human papilloma 16 E6 variants are more prevalent in invasive cervical carcinoma than the prototype". *Cancer Res.,* 1998, *58*, 829.

Address reprint requests to:
W. KĘDZIA, M.D., Ph.D.
ul. Sobieslawa 7
61-062 Poznań (Poland)