RESEARCH ARTICLE

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High Expression of Vascular Endothelial Growth Factor, *Ki-67*, and Protein 53 are Risk Factors for Poor Response of Paclitaxel-Carboplatin Neoadjuvant Chemotherapy in Stadium IB3, IIA2, and IIB Cervical Cancer

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Abstract

Background: The therapeutic strategy for stage IB3, IIA2, and IIB cervical cancer is still controversial. The modalities are chemoradiation, radical hysterectomy surgery, or administration of neoadjuvant chemotherapy followed by radical hysterectomy. Response to chemotherapy is determined by tumor vascularization or angiogenesis, proliferative activity, and genetic instability of cervical cancer. The marker of tumor cell proliferation is the *Ki-67* protein. In cervical cancer, the *p53* gene is suppressed by human papillomavirus (HPV). The HPV E6 protein promotes the degradation of *p53* thereby inhibiting stabilization and activation of *p53*. This study aimed to prove that high expression of *VEGF*, *Ki-67*, and *p53* are risk factors for a poor response to neoadjuvant chemotherapy. **Methods:** This was a case-control study that was conducted at the Department of Obstetrics and Gynecology in one tertiary hospital in Denpasar from October 2021 to April 2022. There were 56 samples included in this study, which were divided into two equal groups, namely good response and poor response to neoadjuvant chemotherapy. Data were analyzed using the software SPSS-24 including the Kolmogorov-Smirnov normality test, Chi-square, and multiple regression logistics. Data were presented in tables and described narratively. **Results:** It was found that the risk of a poor response to chemotherapy on the expression of *VEGF VEGF*, *Ki-67*, and *p53* were 11.5, 15.0, and 8.33 times, respectively. We obtained a formula for calculating chemotherapy response, y = -7.3+ 1.6 *VEGF* + 1.6 *Ki-67* + 1.8 *p53*. High *VEGF*, *Ki-67*, and *p53* expressions were scored 1, and low expressions were scored 2. The limit value used is 0.05. The result y < 0.05 means the risk of poor response to chemotherapy and the value of y > 0.05 means good response. **Conclusion:** This formulation can be used as a parameter to assess the risk of poor response to neoadjuvant chemotherapy in stage IB3, IIA2, and IIB cervical cancer which can be applied in clinical practice in the treatment of cervical cancer.

Keywords: Cervical cancer- *Ki-67*- paclitaxel-carboplatin- protein 53- vascular endothelial growth factor

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Introduction

Cervical cancer is a malignant tumor of the cervix caused by infection of human papillomavirus (HPV). It is the most common gynecological cancer and is a major cause of morbidity and mortality in women, especially in developing countries. The incidence of cervical cancer has increased over the last two decades. The global incidence of cervical cancer is 13.1 cases per 100,000 women. The incidence of cervical cancer in the world based on the International Agency for Research on Cancer (IARC) in 2015 was 17 cases per 100,000 women [1,2]. The incidence of cervical cancer tends to increase every year, which is an average of 528,000 new cases each year and an average death rate of 266,000 cases each year [3,4].

The management of cervical cancer at an early stage (stage IA to IIA) based on the 2016 National Comprehensive Cancer Network (NCCN) is a radical hysterectomy and bilateral pelvic lymphadenectomy while at an advanced stage which is still localized in the pelvic area (stage IIB to IVA), is chemoradiation [1]. Until now the therapeutic strategy for stage IB3, IIA2, and IIB cervical cancer is still controversial, in which chemoradiation, radical hysterectomy surgery, or administration of neoadjuvant chemotherapy can be carried out which can then be followed by radical hysterectomy [1]. NCCN recommends treatment modalities of direct radical hysterectomy, radical hysterectomy after neoadjuvant chemotherapy, or concurrent chemoradiotherapy [5,6].

Neoadjuvant chemotherapy regimens that are often used include cisplatin, paclitaxel, topotecan, vinorelbine, gemcitabine, and ifosfamide [7]. However, the most widely used combination that provides good results is a combination of platinum-based chemotherapy,

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namely carboplatin and paclitaxel with a duration of administration of two to three cycles. In addition, the combination of paclitaxel and carboplatin has a lower toxicity effect, easy to procure, and more economical in price [8,9].

Response to chemotherapy is determined by tumor vascularization or angiogenesis, cell proliferative activity, and genetic instability of cervical cancer. *VEGF* has an important role in angiogenesis and oxygenation in tissues which is one of the important mechanisms of tumor growth [10]. As a marker of cell proliferation, *Ki-67* can be used as a predictor of potentially malignant tumors [11]. In cervical cancer, the *p53* gene is suppressed by HPV. The HPV E6 protein promotes the degradation of *p53* thereby inhibiting the stabilization and activation of *p53*.

Based on the description, it is not known certainly that there is an effect of a combination of angiogenesis factors, cell proliferation activity, and genetic instability of cervical cancer on the response to neoadjuvant chemotherapy for cervical cancer especially stage IB3, IIA2, and IIB. Therefore, we were interested in conducting research to analyze the effect of a combination of angiogenesis factors, cell proliferative activity, and cancer genetic instability on response to neoadjuvant chemotherapy for cervical cancer stages IB3, IIA2, and IIB.

Materials and Methods

This was a case-control study that was conducted at the Department of Obstetrics and Gynecology of one tertiary hospital in Denpasar. The samples were patients of stage IB3, IIA2, and IIB cervical cancer in the period October 2021 to April 2022 who had been given three cycles of carboplatin-paclitaxel neoadjuvant chemotherapy and consented to participate in the study. Patients with poor responses to chemotherapy were allocated to the case group and good responses was allocated to the control group. For each group, samples were selected by randomized systematic sampling until the sample size of 28 was reached. The exclusion criteria were previously undergone surgery, chemotherapy, or radiotherapy, whether related to gynecological malignancy or not.

The expression of *VEGF*, *Ki-67*, and *p53* were examined by immunohistochemistry of paraffin block from cervical cancer tumor biopsies in the Department of Anatomical Pathology. This measurement of expression was not part of routine examination and was done purposely for this study. Demographics, cancer stage, and histopathological diagnosis data were retrieved from the medical records. Semi-quantitative quantification of *VEGF* stained by immunohistochemistry using the American Monoclonal Antibody Abcam ab1316 was performed and visualized on the cell membrane in a large microscopic field of view. High expression was defined when the value was > 6 and low when the value was < 6 [12]. Semi-quantitative counts of *Ki-67* stained by immunohistochemistry using Monoclonal Mouse Anti-Human *Ki-67* Antigen Biogen Denmark were performed and visualized in the cell nucleus in a large microscopic field of view. High expression was defined when the value was > 2 and low when the value was < 2 [13]. Semi-quantitative quantification of *p53* protein stained by immunohistochemistry using Lab-Vision America's Monoclonal Antibody Clone DO-7 in a large microscopic field of view. High expression was defined when the value was > 7 and low when the value was < 7 [14].

The size of the primary tumor was the longest diameter of the tumor in centimeters (cm), measured based on a computed tomography scan. Tumor size was dichotomized into ≥ 6 cm and < 6 cm. The degree of differentiation was an assessment of the mitotic rate of the tumor and the degree of difference between cancer cells and normal cells. It was classified into good and poor degrees of differentiation. The good degree of difference consisted of well-differentiated and moderately differentiated. While the poor degree of difference consisted of poorly differentiated and undifferentiated. The histopathological type of cervical cancer was cell type based on the histopathological result, which further classified into squamous cell carcinoma (SCC) and non-squamous cell carcinoma (NCSS).

Results

A total of 56 samples were divided into 28 samples into the case group and 28 samples into the control group. The characteristics of the sample can be seen in Table 1.

As can be seen in Table 2, there were 20 samples

Table 1. Characteristics of Sample

Variables	Case (n = 28)		Control (n = 28)		P-value
	Mean	SD	Mean	SD	
Age	45.36	9.87	44.86	10.69	0.85
Parities	3		3		0.93
Body mass index	23.86	1.87	24.87	2.77	0.52
Primary tumor size	5.23	0.87	5.5	1.04	0.79
Degree of differentiation					
Good	12 (42.80)		18 (64.28)		0.18
Poor	16 (57.14)		10 (35.71)		
Histopathology type					
Squamous cell carcinoma	21 (75.0)		22 (78.57)		0.65
Non squamous cell carcinoma	7 (25.0)		6 (21.42)		

Table 2. Expression of *VEGF* in Case and Control

Expression	Case	Control	OR	95% CI	P
High	20	5	11.5	3.23 – 40.86	0.04
Low	8	23			

Table 3. Expression of *Ki-67* in Case and Control

Expression	Case	Control	OR	95% CI	P
High	24	8	15	3.93- 57.22	0.02
Low	4	20			

(71.4%) with high *VEGF* expression in the case group, and there were 5 samples (17.8%) with high *VEGF* expression in the control group. Therefore, high *VEGF* expression had 11.50 times (OR = 11.50, 95% CI 3.23 - 40.86 p = 0.04) higher risk of poor response to neoadjuvant paclitaxel-carboplatin chemotherapy in stage IB3, IIA2, and IIB cervical cancer compared to low *VEGF* expression.

As can be seen in Table 3, there were 24 samples (85.7%) with high *Ki-67* in the case group and there were 8 samples (28.5%) with high *Ki-67* expression in the control group. Thus, high expression of *Ki-67* had 15 times (OR= 15.00; 95% CI = 3.93 - 57.22; p 0.04) higher risk of a poor response to neoadjuvant paclitaxel-carboplatin chemotherapy in stage IB3, IIA2, and IIB cervical cancer by 15 times compared to low *Ki-67* expression.

Table 4, shows that in the case group, there were 25 samples (89.2%) with high *p53* expression and in the control group there were 10 samples (35.7%) with high *p53* expression. Thus high expression of *p53* had 8.33 times (OR= 8.33; 95% CI = 3.60 - 62.39; p = 0.001) higher risk of having a poor response to neoadjuvant paclitaxel-carboplatin chemotherapy in stage IB3, IIA2, and IIB cervical cancer compared to low *p53* expression.

In the multivariate analysis (Table 5), it can be seen that all p-values for each variable were less than 0.05. It can be concluded that *VEGF*, *Ki-67*, and *p53* were independent risk factors for poor response to neoadjuvant chemotherapy in stage IB3, IIA2, and IIB cervical cancer. The sequence of independent variables that were strongest in predicting bad compared to good chemotherapy response was the expression of *p53*, *VEGF*, and *Ki-67* with an OR value of 6.28, 5.17, and 5.01, respectively.

Table 4. Expression of *p53* in Case and Control

Expression	Case	Control	OR	95% CI	P
High	25	10	8.33	3.60 – 62.39	0.01
Low	3	18			

Table 5. Multivariate Analysis of High Expression of *VEGF, Ki-67*, and *p53* to the Poor Response of Neoadjuvant Paclitaxel-Carboplatin in Stage IB3, IIA2, and IIB Cervical Cancer

Variable	Adjusted OR	95% CI	p
High VEGF	5.17	1.14 – 23.27	0.032
High Ki-67	5.01	1.04 – 24.03	0.044
High p53	6.28	1.20 – 32.8	0.029

Discussion

In this study, high *VEGF* expression was the risk factor for poor chemotherapy response. The results of this study were in accordance with research conducted by Edianto et al. (2019) where *VEGF* expression is closely related to tumor size and stage after neoadjuvant chemotherapy, positive *VEGF* expression was found in tumors > 4 cm with poor chemotherapy response ($p < 0.005$). Neoadjuvant chemotherapy in stages IB – IIA cervical cancer, especially large tumors, can shrink the tumor mass and make radical surgery easier. Partial responses were also found in stage IB-IIA cervical cancer and tumor sizes > 4 cm with high *VEGF* expression [15]. Another study stated that *VEGF* expression could predict chemotherapy response, with a correlation that the higher *VEGF* expression predicts a worse response to chemotherapy [16].

Tumor cells secrete abundant pro-angiogenic factors which contribute to the formation of abnormal vascular tissue, indicated by the irregular shape of blood vessels, immature, accompanied by high permeability, thus worsening tumor perfusion. Poor tumor vascular function has major consequences for the tumor environment and leads to hypoxia, reduced infiltration and immune system activity, and increased risk of metastases. Compared with healthy vessels, tumor vessels exhibit atypical morphology. The tumor vascular network is characterized by dilation, tortuousness, and irregularity. Immaturity and inadequate mural cell support lead to high permeability, poor perfusion, and exacerbation of hypoxia [17].

VEGF secretion by tumor cells can suppress PDGFRb signaling in vascular smooth muscle cells through the assembly of a receptor complex consisting of PDGFRb and *VEGFR2*. Barrier integrity is also compromised following the loss of VE-cadherin function. Tumor cells release proteolytic enzymes such as MMP, elastase, or trypsin to support the breakdown of VE-cadherin. Tumor cells then secrete inflammatory factors that affect vascular permeability. *VEGF* can increase vascular permeability through modulation of the activity of several GTPases such as RhoA, which is the main role of VE-cadherin in cancer-related vascular permeability. All of these factors can affect vascular permeability, leakage, and metastasis by facilitating the entry of tumor cells into the bloodstream [17].

VEGF triggers the expression of the anti-apoptotic protein Bcl-2, inhibits down-regulation, and induces Bcl-2. Increased expression of Bcl-2 in response to *VEGF* is associated with increased proliferation and survival of tumor cells even in the presence of anti-hormones. This suggests that *VEGF* stimulates the proliferation of *VEGF*R2-positive tumor cells, enhances survival through Bcl-2 expression and activity, and overrides the growth-suppressive effects of anti-hormones [18].

In this study, high *Ki-67* expression increased the risk of a poor chemotherapy response by 15 times (OR=15.0; 95% CI = 3.93 – 57.22; p=0.002) compared to low *Ki-67*

expression. These findings are consistent with a study conducted by Minckwitz which showed that patients with high *Ki-67* expression had a risk of poor chemotherapy response ($p < 0.0001$) and had a higher mortality rate compared to patients who had low or moderate *Ki-67* expression ($p < 0.0001$) [19].

On the other hand, a prospective cohort study conducted by Yamashita et al. reported no significant relationship between the expression of *Ki-67* protein and the 5-year prognosis of patients receiving chemotherapy [20]. In a study conducted by Vosmik et al. [21] also found that *Ki-67* expression did not significantly correlate with chemotherapy response in patients with SCC (OR = 1.01; 95% CI 0.97–1.05; p=0.55) [21]. Research by Mahayasa et al. (2016) stated that there was no relationship between *Ki-67* and Caspase-3 expression in neoadjuvant chemotherapy response in stage IB2 and IIA2 cervical cancer patients [22]. In post-chemotherapy patients at Dokuz Eylul University Hospital, no correlation was found between *Ki-67* expression and response to therapy [23]. A case-control study by Arens et al. (2005) involving 60 cancer patients also obtained similar results, where there was no significant relationship between *Ki-67* expression and response to therapy in cancer patients undergoing neoadjuvant chemotherapy [24]. Thus, in this study, *Ki-67* expression could not be used as a predictive factor for tumor mass reduction.

Ki-67 is a nuclear antigen that plays a role in cell proliferation in both normal and tumor cells. *Ki-67* has become a clinical marker that can predict tumor size, invasiveness, cancer stage, response to therapy, and patient survival. The higher expression of *Ki-67* indicates that tumor cells have a fast cell cycle so they proliferate quickly [25]. In a recent meta-analysis, *Ki-67* co-joined with the p16 biomarker has been shown to be effective in detecting HPV-induced cervical cancer compared to pap cytology and HPV DNA testing [26]. A study conducted on cervical cancer cases by comparing *Ki-67* expression before and after chemotherapy showed that reduced expression of *Ki-67* after chemotherapy is a good predictor of response to neoadjuvant chemotherapy in cervical cancer [27]. The poor response can be explained by the mechanism of increased survivin expression which is closely correlated with increased expression of *Ki-67*. This was proven in a study by Zhang et al. [28] where there was an increase in survivin which had a positive correlation with increased expression of *Ki-67* in cervical cancer ($p<0.01$) [28].

Survivin is a protein known as an Inhibitor of Apoptosis Protein (IAP) which plays a role in regulating the process of apoptosis and cell division. Survivin is expressed in the G2/M phase of the cell cycle which regulates the mechanism of chromosome division during the cell division process. Survivin also plays a role in inhibiting the process of apoptosis both directly and indirectly by inhibiting caspase 3/7-p21 interactions (apoptotic terminal effector enzymes) [6]. Survivin through CDK4/2 cyclin kinase binding can inhibit the apoptotic activation pathway. Survivin binding to CDK4 will trigger CDK2/cyclin-E activation and ribosomal phosphorylation. Through ribosomal phosphorylation, cells rapidly enter the G1/S phase of the cell cycle, causing the release of p21 from the survivin-CDK4 complex together with mitochondrial pro-caspase 3 to inhibit caspase-3 activity and preventing cytochrome c release from mitochondria. In addition, survivin can inhibit the apoptotic proteins Bax and Fas ligands [29]. Survivin also plays a role in triggering resistance to paclitaxel chemotherapeutic agents through the mechanism of blocking the apoptotic pathway [28].

The results of bivariate analysis stated that high *p53* expression increased the risk for poor chemotherapy response by 8.3 times (OR = 8.33; 95% CI = 2.038 – 34.070; p = 0.003) compared to low *p53* expression. The results of this study were in accordance with research conducted by Amijaya et al. [30], where mutant *p53* expression is associated with resistance to neoadjuvant chemotherapy compared to wild-type *p53* expression [30]. Of the 35 subjects, 77.14% were resistant to chemotherapy, 17.41% with positive mutant *p53* expression. However, the results of multivariate analysis in this study showed that in stage II cervical cancer, expression of mutant *p53* was not associated with a worse response to neoadjuvant chemotherapy (OR = 3.121; 95% CI = 0.193-50.601, p = 0.423). Furthermore, a poor degree of differentiation was associated with a worse response to neoadjuvant chemotherapy (OR = 12.863; 95% CI = 1.723-96.052, p=0.013) [30].

Research conducted by Muhartono [31] also stated that there was a significant correlation between the level of *p53* immunoexpression and the response rate of neoadjuvant chemotherapy (p = 0.000) [31]. The higher the level of *p53* immunoexpression, the worse the response to neoadjuvant chemotherapy. Another study by Aminah et al. (2011) reported that there was a significant correlation between *p53* immunoexpression and clinical response to cisplatin chemotherapy (OR = 2.8, 95% CI = 1.387 – 5.654, $p < 0.05$) [32]. Cervical cancer cells with positive *p53* immunoexpression have a risk of poor cisplatin chemotherapy response by 2.8 times [33].

In the interpretation of the immunohistochemical staining results in this study, stained *p53* was interpreted as normal or wild-type *p53* if it was stained weakly to moderately in the nucleus of tumor cells and was comparable to the intensity of the stain in normal tissue. Meanwhile, mutant or abnormal *p53* is a staining type that shows 2 types of features: 1) strong overexpression in the entire tumor, or 2) absence of staining (complete absent staining) in tumor cells with internal control comparisons showing a wild type or normal pattern. Thus, the stained *p53* which showed overexpression in this study was a mutant *p53* protein. This study used *p53* antibody (DO-1) which is able to detect either mutant or wild-type *p53* protein. In the early phase of cervical carcinogenesis, the *p53* protein is inactivated by the E6 oncoprotein through proteasomal degradation. In the advanced phase of carcinogenesis, a *p53* gene mutation occurs so this event is a late event. More than 75% of mutations in *p53* cause impaired function of *p53* wild type which reduces the effectiveness of the protein and increases the function of wild type *p53* protein. The *p53* mutant usually has the ability to survive protein degradation, so overexpression

of *p53* can assess the prognosis in some malignancies [34].

Deregulation of wild-type *p53* function and mutations in the *p53* gene will have an effect on the apoptotic pathway through deregulation of the expression of apoptotic proteins such as Bax, Bcl-2, Nova and Puma. Mutant *p53* triggers increased expression of anti-apoptotic proteins such as Bcl-2. Bcl-2 further prevents the release of cytochrome c from mitochondria resulting in an obstacle to the process of apoptosis. In addition, there was a decrease in Bax protein expression which further prevented the formation of Mitochondrial Apoptosis Induced Channel (MAC) in the outer mitochondrial membrane. In addition to inhibiting the apoptotic process, *p53* overexpression can activate several signaling pathways involved in cell survival, such as the NF-kB and PI3/AKT pathways. Activation of the NF-kB signaling pathway plays a role in the processes of immunoregulation, inflammation, cell proliferation, apoptosis and carcinogenesis [35]. This pathway regulates the expression of proteins important for cell survival such as Survivin, Bcl-2, Bcl-X, and XIAP which in turn triggers resistance to chemotherapeutic agents. This was proven in vivo and in vitro studies conducted by Haiyan Zhu et al. [12] where activation of the NF-kB pathway inhibited the effectiveness of chemotherapy drugs through an inhibition mechanism in the cell apoptosis pathway [12]. The PI3K/AKt signaling pathway also regulates cell survival, cell cycle progression, growth, and metabolism of cancer cells. Overexpression of Akt is seen in almost all solid tumors and has been shown to trigger paclitaxel chemotherapy resistance in tumors. Akt also triggers phosphorylation of Bcl protein at Ser136/Ser112 residues which in turn triggers inhibition of pro-apoptotic protein expression [35].

Research conducted by Huang et al. [36], stated that when wild-type *p53* changes to mutant *p53*, this can affect intrinsic apoptosis in three ways by regulating BAX expression and activity [36]. First, *p53* will lose its regulatory function of BAX transcription, which directly causes a decrease in BAX protein expression and inhibits apoptosis. Second, *p53* will lose the regulatory function of PUMA, which is an important upstream activator of BAX, thereby reducing BAX activity and apoptosis. Mutant *p53* cannot bind to the PUMA promoter to initiate transcription due to conformational changes, therefore, *p53* will lose its function resulting in resistance to chemotherapy. Third, previous studies have shown that wild-type *p53* can directly activate BAX through its interaction and change the conformation of BAX [36].

Several studies have shown that mutations in *p53* result in function-of-function activity in which *p53* no longer behaves as a tumor suppressor but rather as an oncogene. For example, some mutations in *p53* help cancer cells maintain proliferation and become more aggressive thereby enabling metastasis and resistance to chemotherapy. Mutant *p53* can induce miRNA expression resulting in inhibition of genes involved in the cell cycle. MiR128-2 expression is induced by mutant *p53* resulting in cell cycle inhibition involving the transcription factor E2F5. Inhibition of E2F5 by miR128-2 eventually leads to chemotherapy resistance. Mutant *p53* has also been shown to increase drug resistance through increasing *MDR1* gene transcription although it does not bind directly to the MDR1 promoter, but instead interacts and cooperates with the ETS-1 transcription factor to promote *MDR1* expression and enhances cell survival in various chemotherapeutic agents [34].

In conclusion, the risk of having an adverse response to paclitaxel-carboplatin neoadjuvant chemotherapy in cervical cancer patients with stages IB3, IIA2, and IIB with high expression of *VEGF*, Ki67, and *p53* is 11.50, 15, and 8.33 times higher, respectively. Expression of *VEGF*, *Ki-67*, and *p53* are moderate risk factors for poor response to paclitaxel-carboplatin neoadjuvant chemotherapy in stage IB3, IIA2, and IIB cervical cancer. The total effect of high *VEGF* levels on the risk of poor chemotherapy response was calculated by adding up the direct effects of 28% and the good indirect effects through Ki67 of 14.5% and *p53* of 7.5% and obtained a result of 50%. The total effect of high Ki67 levels on the risk of having a bad chemotherapy response is 44% which is the sum of the direct contribution of 29% and the indirect contribution of 15%. The contribution of high *p53* expression to the risk of having a bad chemotherapy response directly is 30%.

Author Contribution Statement

All authors contributed equally in this study.

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References

1. Hacker NF, vermorken Jb. Cervical cancer. In: Jonathan s. Berek, neville f (eds.) berek & hacker's gynecologic oncology. 6th edition. Wolters kluwer; philadelphia: 2015. Pp. 326-389.
2. World health organization. Comprehensive cervical cancer control: A guide to essential practice [internet]. 2nd ed. Geneva: World health organization; 2014 [cited 2021 jan 14]. Available from: Http://apps.Who.Int/iris/bitstream/10665/144785/1/9789241548953_eng.Pdf?Ua=1.
3. Bray f, ferlay j, soerjomataram i. Global cancer statistics 2018: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca Cancer J Clin. 2018;68:394–424.
4. World health organization. Who guidelines for screening and treatment of precancerous lesions for cervical cancer prevention. Geneva: World health organization; 2013. 40 p.
5. Shen Y, Yang L, Wang Z. Treatment of early bulky cervical cancer with neoadjuvant paclitaxel, carboplatin and cisplatin prior to laparoscopical radical hysterectomy and pelvic lymphadenectomy. Oncol Lett. 2012;3(3):641-5. https://doi.org/10.3892/ol.2011.529.
6. Wang Y, Wang G, Wei LH, Huang LH, Wang JL, Wang SJ, et al. Neoadjuvant chemotherapy for locally advanced cervical cancer reduces surgical risks and lymph-vascular space

involvement. Chin J Cancer. 2011;30(9):645-54. https://doi.org/10.5732/cjc.011.10050.
7. Iwata T, Miyauchi A, Suga Y, Nishio H, Nakamura M, Ohno A, et al. Neoadjuvant chemotherapy for locally advanced cervical cancer. Chin J Cancer Res. 2016;28(2):235-40. https://doi.org/10.21147/j.issn.1000-9604.2016.02.13.
8. Rieu r, eminowicz g. Chemotherapy for cervical cancer bt - uterine cervical cancer: Clinical and therapeutic perspectives. In s.A. Farghaly (ed.) cham: Springer international publishing: 2019. Pp. 215 – 231.
9. Lee SH, Jeong D, Han YS, Baek MJ. Pivotal role of vascular endothelial growth factor pathway in tumor angiogenesis. Ann Surg Treat Res. 2015;89(1):1-8. https://doi.org/10.4174/astr.2015.89.1.1.
10. Carmeliet P. Vegf as a key mediator of angiogenesis in cancer. Oncology. 2005;69 Suppl 3:4-10. https://doi.org/10.1159/000088478.
11. Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep. 2015;11(3):1566-72. https://doi.org/10.3892/mmr.2014.2914.
12. Zhu P, Ou Y, Dong Y, Xu P, Yuan L. Expression of vegf and hif-1α in locally advanced cervical cancer: Potential biomarkers for predicting preoperative radiochemotherapy sensitivity and prognosis. Onco Targets Ther. 2016;9:3031-7. https://doi.org/10.2147/ott.S104142.
13. Popiel A, Piotrowska A, Sputa-Grzegrzolka P, Smolarz B, Romanowicz H, Dziegiel P, et al. Preliminary study on the expression of testin, p16 and ki-67 in the cervical intraepithelial neoplasia. Biomedicines. 2021;9(8). https://doi.org/10.3390/biomedicines9081010.
14. Liu L, Li XD, Chen HY, Cui JS, Xu DY. Significance of ebp1 and *p53* protein expression in cervical cancer. Genet Mol Res. 2015;14(4):11860-6. https://doi.org/10.4238/2015.October.2.19.
15. Eder P, Korybalska K, Linke K, Witowski J. Angiogenesis-related proteins--their role in the pathogenesis and treatment of inflammatory bowel disease. Curr Protein Pept Sci. 2015;16(3):249-58. https://doi.org/10.2174/1389203716666150224150756.
16. Zhang J, Liu J, Zhu C, He J, Chen J, Liang Y, et al. Prognostic role of vascular endothelial growth factor in cervical cancer: A meta-analysis. Oncotarget. 2017;8(15):24797-803. https://doi.org/10.18632/oncotarget.15044.
17. Zhang L, Chen Q, Hu J, Chen Y, Liu C, Xu C. Expression of hif-2α and vegf in cervical squamous cell carcinoma and its clinical significance. Biomed Res Int. 2016;2016:5631935. https://doi.org/10.1155/2016/5631935.
18. Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. J Clin Oncol. 2005;23(5):1011-27. https://doi.org/10.1200/jco.2005.06.081.
19. Matsuzaki S, Klar M, Mikami M, Shimada M, Grubbs BH, Fujiwara K, et al. Management of stage iib cervical cancer: An overview of the current evidence. Curr Oncol Rep. 2020;22(3):28. https://doi.org/10.1007/s11912-020-0888-x.
20. Yamamoto S, Ibusuki M, Yamamoto Y, Fu P, Fujiwara S, Murakami K, et al. Clinical relevance of ki67 gene expression analysis using formalin-fixed paraffin-embedded breast cancer specimens. Breast Cancer. 2013;20(3):262-70. https://doi.org/10.1007/s12282-012-0332-7.
21. Vosmik M, Laco J, Sirak I, Beranek M, Hovorkova E, Vosmikova H, et al. Prognostic significance of human papillomavirus (hpv) status and expression of selected markers (her2/neu, egfr, vegf, cd34, p63, *p53* and ki67/mib-1) on outcome after (chemo-) radiotherapy in patients with squamous cell carcinoma of uterine cervix. Pathol Oncol Res. 2014;20(1):131-7. https://doi.org/10.1007/s12253-013-9674-5.
22. Mahayasa imw, tobing mdl, harsono ab. Hubungan antara ekspresi ki-67 dan kaspase-3 dengan respons kemoterapi neoajuvan pada pasien karsinoma serviks stadium ib2 dan iia2 di rumah sakit dr. Hasan sadikin, bandung. Indonesian journal of cancer. 2016;10(2):49–54.
23. Kamoi S, Ohaki Y, Okada S, Matsushita N, Kawamura T, Araki T. Mitotic index and ki-67 nuclear antigen labeling index as predictors of chemotherapy response in uterine cervical carcinoma. Gynecol Oncol. 2001;83(3):555-9. https://doi.org/10.1006/gyno.2001.6444.
24. Arsyad nr, siswosudarmo r, kusumanto a. Hubungan antara ekspresi *p53* mutan terhadap operabilitas kanker serviks stadium iib pasca kemoterapi neoajuvan. J Kesehat Reproduksi. 2020; 7(1): 49.
25. Pan D, Wei K, Ling Y, Su S, Zhu M, Chen G. The prognostic role of ki-67/mib-1 in cervical cancer: A systematic review with meta-analysis. Med Sci Monit. 2015;21:882-9. https://doi.org/10.12659/msm.892807.
26. Okoturo E, Rabiu K. Meta-analysis on the diagnostic performance of p16/ki-67 dual immunostaining for cervical cancer screening. Asian Pac J Cancer Biol. 2023;8:91-9. https://doi.org/10.31557/apjcb.2023.8.1.91-99.
27. Kamoi S, Ohaki Y, Amano Y, Mori O, Matsushita N, Doi D, et al. Pre-treatment mitotic index versus computer-quantitated ki-67 nuclear antigen labeling index as predictors of response to neoadjuvant chemotherapy in uterine cervical carcinoma. J Nippon Med Sch. 2003;70(3):219-26. https://doi.org/10.1272/jnms.70.219.
28. Zhang Y, Yan H, Li R, Guo Y, Zheng R. High expression of survivin predicts poor prognosis in cervical squamous cell carcinoma treated with paclitaxel and carboplatin. Medicine (Baltimore). 2019;98(20):e15607. https://doi.org/10.1097/md.0000000000015607.
29. Pal A, Kundu R. Human papillomavirus e6 and e7: The cervical cancer hallmarks and targets for therapy. Front Microbiol. 2019;10:3116. https://doi.org/10.3389/fmicb.2019.03116.
30. Ancuţa E, Ancuţa C, Cozma LG, Iordache C, Anghelache-Lupaşcu I, Anton E, et al. Tumor biomarkers in cervical cancer: Focus on ki-67 proliferation factor and e-cadherin expression. Rom J Morphol Embryol. 2009;50(3):413-8.
31. Muhartono, Hanriko R. Korelasi antara imunoekspresi *p53* dan respons kemoterapi neoadjuvan regimen fluororasil, adriamisin, dan siklofosfamid pada karsinoma duktus payudara invasif. Majalah Kedokteran Bandung. 2012;44. https://doi.org/10.15395/mkb.v44n1.209.
32. Baruah R, Sangappa S. Immunohistochemical expression of ki67 and *p53* in primary breast carcinoma and combined ki67-*p53* status phenotypes in hormone receptor positive breast carcinoma. JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH. 2020;14. https://doi.org/10.7860/JCDR/2020/43546.13581.
33. Meinhold-Heerlein I, Ninci E, Ikenberg H, Brandstetter T, Ihling C, Schwenk I, et al. Evaluation of methods to detect *p53* mutations in ovarian cancer. Oncology. 2001;60(2):176-88. https://doi.org/10.1159/000055316.
34. Bosse T, Lax S, Abu-Rustum N, Matias-Guiu X. The role of predictive biomarkers in endocervical adenocarcinoma: Recommendations from the international society of gynecological pathologists. Int J Gynecol Pathol. 2021;40(Suppl 1):S102-s10. https://doi.org/10.1097/pgp.0000000000000755.
35. Chen CA, Chen TM, Wu CC, Chang CF, Hsieh CY. Human papillomavirus DNA and *p53* status in stage ib bulky cervical cancer. J Cancer Res Clin Oncol. 1994;120(11):678-82.

https://doi.org/10.1007/bf01245381.
36. Huang Y, Liu L, Cai J, Yang L, Sun S, Zhao J, et al. The efficacy and response predictors of platinum-based neoadjuvant chemotherapy in locally advanced cervical cancer. Cancer Manag Res. 2020;12:10469-77. https://doi.org/10.2147/cmar.S270258.