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THE DIAGNOSTIC VALUE OF THE P16INK MARKER FOR VERIFICATION OF TUMORS OF UNKNOWN PRIMARY SITE IN WOMEN WITH ISOLATED LESION OF INGUINAL LYMPH NODES

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Abstract. The diagnostic value of the p16^{ink} marker for verification of tumors of unknown primary site in women with isolated lesion of inguinal lymph nodes. Poslavska O.V., Shponka I.S., Babiy H.S. Carcinomas with an unknown primary site are a heterogeneous group of metastatic tumors, numbering from 3% to 5% of all malignant carcinoma phenotypes. An isolated lesion of the inguinal lymph nodes is relevantly associated with primary localization of tumors in the pelvis, anal canal, lower abdomen, lower limbs, and more towards in the reproductive system (vulva, vagina and cervix for women, penis for men). But in the absence of clinical signs of cancer after careful research, the histological biopsy of the lymph node with additional immunohistochemical staining with organ-specific markers becomes the diagnostic method of choice. A retrospective analysis of the histological, morphometric and immunohistological characteristics of the biopsy material of 59 patients with isolated lesions of the inguinal lymph nodes (35 women and 24 men) aged from 20 to 87 years (mean 59.26±15.86; median 62 years) was conducted. The distribution of variants of an isolated lesion of the inguinal lymph nodes in women showed 14 of 35 (40.00%) metastatic tumors (the other 21 are lymphoproliferative conditions that require phenotyping, but not finding the primary localization). Of 14 metastatic lesions in women, 6 cases demonstrated a p16^{ink} (+) phenotype. For comparison, of 24 cases in men, only 6 (25.00%) were of metastatic origin, of which p16^{ink} (+/-) was partially 1 metastasis of melanoma (16.67%). After a thorough immunohistochemical study with a panel of organo-specific markers among isolated metastatic lesions of the inguinal lymph nodes of tumors in women, half of the revealed localized sites (7 out of 14) were in reproductive organs, namely: 4 p16^{ink} (+) to squamous cervical cancers, 2 p16^{ink} (+) to serous ovarian adenocarcinoma, 1 p16^{ink} (-) to leiomyosarcoma of the uterus. Other localizations did not have a clear location, as they related to metastases of carcinoma from Merkel cells and melanomas, as a result of frequent reduction of the primary tumor lesion in the skin.

Реферат. Диагностическое значение маркера p16^{ink} для верификации опухолей неизвестной первичной локализации у женщин с изолированным поражением паховых лимфатических узлов. Пославская А.В., Шпонька И.С., Бабий А.С. Карциномы с неизвестной первичной локализацией являются гетерогенной группой метастатических опухолей, насчитывающих от 3% до 5% всех злокачественных фенотипов карцином. Изолированное поражение паховых лимфатических узлов релевантно связывают с первичной локализацией опухолей в малом тазу, анальном канале, нижней части брюшной полости, нижних конечностях и, в большей степени, в репродуктивной системе (vulva, vagina и cervix для женщин, penis для мужчин). Но в случае отсутствия клинических признаков происхождения рака после тщательного исследования, диагностическим методом выбора становится гистологическое исследование биопсии лимфатического узла с дополнительным иммуногистохимическим окрашиванием органоспецифическими маркерами. В работе проведен ретроспективный анализ гистологических, морфометрических и иммуногистохимических характеристик биопсийного материала 59 пациентов с изолированным поражением паховых лимфатических узлов (35 женщин и 24 мужчины) в возрасте от 20 до 87 лет (среднее 59,26±15,86; медиана 62 года). Распределение вариантов изолированного поражения паховых лимфатических узлов у женщин продемонстрировало 14 из 35 (40,00%) метастатических опухолей (другие 21 - лимфопролиферативные состояния, требующие

фенотипирования, но не нахождения первичной локализации). Из 14 метастатических поражений у женщин 6 карцином имели p16^{ink} (+) фенотип. Для сравнения, у мужчин из 24 случаев только 6 (25,00%) имели метастатическое происхождение, из них p16^{ink} (+) частично оказался 1 метастаз меланомы (16,67%). После тщательного иммуногистохимического исследования с панелью органоспецифических маркеров среди изолированных метастатических поражений паховых лимфатических узлов опухолей у женщин, половина выявленных локализаций (7 из 14) относились к репродуктивным органам, а именно: 4 p16^{ink} (+) к плоскоклеточным ракам шейки матки, 2 p16^{ink} (+) к серозным аденокарциномам яичников, 1 p16^{ink} (-) к лейомиосаркоме тела матки. Другие локализации не имели четкого местонахождения, так как относились к метастазам карциномы из клеток Меркеля и меланомы, в результате частой редукции первичного опухолевого очага в коже.

Carcinomas with unknown primary site are a heterogeneous group of metastatic tumors that account for 3% to 5% of all malignant phenotypes of carcinomas [7]. The isolated damage of the inguinal lymph nodes is associated with the primary localization of the tumors in the small pelvis, the anal canal, the lower abdominal region, the lower limbs and to a greater extent in the reproductive system (vulva, vagina and cervix for women, penis for men) [5]. However, in the absence of clinical signs of cancer after a thorough study, the diagnostic method of choice becomes a histological examination of the lymph node biopsy with additional immunohistochemical staining with organ-specific markers [3, 6].

p16^{ink} is a specific marker for identifying HPV-dependent cervical neoplasia. Inhibitor of p16^{ink} cyclokinase is a protein that blocks the cell cycle by terminating the retinoblastic oncogene (Rb) phosphorylation. In the cells infected with oncogenic HPV, overexpression of p16^{ink} is determined. However, despite this, cells infected with HPV continue to proliferate because Rb is inactivated by the oncoprotein E7 of HPV. Since the immunohistochemical response to Ki-67 and p16^{ink} highly correlates with HPV-infection, the detection of these markers is used to confirm the diagnosis in cases of suspicion of intraepithelial damage of high oncogenic risk [3]. But in the case of metastatic carcinoma of women, p16^{ink} acquires a differential diagnostic value. According to the literature, the overexpression of p16^{ink} is an absolute indicator of cervical carcinoma is found partially in adenoarcinomas of the ovaries, up to 95% of pancreatic carcinoma, as well as in squamous cell carcinoma of the oropharynx with HPV-dependent carcinogenesis [4, 6].

The aim is to investigate the complex of morphological, morphometric and immunohistochemical characteristics of cases of isolated metastases in the inguinal lymph nodes of female and male patients separately for the improvement of algorithms for diagnostics of the primary source; to find out the frequency of expression of p16^{ink} in metastases without primary site in females.

MATERIALS AND METHODS OF RESEARCH

In this work, a retrospective analysis of histological, morphometric and immunohistological characteristics of the biopsy material of 59 patients with isolated lesion of the inguinal lymph nodes (35 women and 24 men) aged 20 to 87 years (mean 59.26±15.86, median 62 years) was performed. For the morphometric method, the Zeiss Primo Star-Axiocam ERC 5s microscope camera with the licensed ZEN 2 blue edition software was used, informative fields of view were recorded in .jpg format and processed in the ImageJ program with the definition of the perimeter, area and roundness of the nuclei, according to the technique described in previous publications [1, 2]. Immunohistochemical research was carried out in accordance with the protocols of the company ThermoScientific (TS), (USA) on the basis of the morphological department of the treatment and diagnostic center of Pharmacies of the Medical Academy Ltd. (Dnipro) for the period from 2015 to 2018. In sections with a thickness of 4 microns, a lab visualization system Vision Quanto (TS, USA) with the detection of the protein chain using DAB Quanto Chromogen (TS, USA) was used. Characteristics of monoclonal antibodies that were used are listed in Table 1.

Statistical analysis of the parameters of areas, perimeters and coefficient of roundness of cells was carried out in the programs ImageJ and Microsoft Excel with the calculation of minimum, maximum, median, arithmetic mean and standard deviation. The analysis of the distribution of observation groups by average values of the cell nucleus size was carried out using the t-criterion. The statistically significant difference was considered at p<0.05.

RESULTS AND DISCUSSION

According to the algorithm for the diagnosis of tumors without primary site after the primary immunohistochemical panel all observations were divided into 4 groups, which showed differences in age and gender (Table 2).

Table 1

Primary monoclonal antibodies

Primary antibodies	Clone (dilution)	Localization of reaction
Cytokeratin, Pan	AE1/AE3(1:50)	Cytoplasm
Vimentin	Ab-2/sp20 (1:200)	Cytoplasm
CD45	Ab-3 (1:200)	Membrane
S100	Ab-1 (1:100)	Cytoplasm
CA125	Ab-1 (1:10)	Membrane
Chromogranin A	sp12 (1:400)	Cytoplasm
Synaptophysin	sp11 (1:200)	Cytoplasm
Cytokeratin 7 (CK7)	RCK105 (1:100)	Cytoplasm
Cytokeratin 20 (CK20)	Ks 20.8 (1:100)	Cytoplasm
WT-1	Ab-1 (1:500)	Nucleus
ER	sp1 (1:400)	Nucleus

According to Table 2, except lymphoproliferative lesions, immunophenotypes of other tumors were considered as metastases, the origin of which became a diagnostic task. Analysis of metastatic carcinomas of women revealed the positivity of the marker p16^{ink} in 6 out of 7 (85.71%) observations. Among those, 3 (42.86%) had moderate squamous cell differentiation (CKHMW +), 1 – low squamous

cell (Fig. A-B), which, together with positive p16^{ink}, made the primary localization of these observations in the cervix probable, and 2 more cases had signs of serous moderately differentiated adenocarcinoma of the ovary, which also apart from p16^{ink}+ demonstrated positivity of CA125, WT-1 and CK7 (Fig. Г-Д).

Table 2

Distribution of immune phenotypes of inguinal lymph nodes lesions after primary immune-histochemical panel by gender and age, $\chi \pm SD$

Immune phenotypes of lesions of inguinal lymphatic nodes, n (%)	Gender n (%)	Age $\chi \pm SD$ (mean), years
Cyt. AE 1/3(-) / Vim.(+) / CD45(+) / S100(-), Lymphoproliferative states, 39 (66.10)	W 21 \pm 39 (53.85)	57,085 \pm 17,318 (58.75)
	M 18 \pm 39 (46.15)	55,722 \pm 12,351 (58.50)
Cyt. AE 1/3(+) / Vim.(-) / CD45(-) / S100(-), Metastases of carcinomas without primary site, 11 (18.64)	W 7 \pm 11 (63.64)	64,166 \pm 11,409 (64.00)
	M 4 \pm 11 (36.36)	59,750 \pm 11,324 (64.00)
Cyt. AE 1/3(-) / Vim.(+) / CD45(-) / S100(+), Metastases of melanoma, 7 (11.86)	W 6 \pm 7 (85.71)	61,500 \pm 22,006 (69.5)
	M 1 \pm 7 (14.29)	70
Cyt. AE 1/3(-) / Vim.(+) / CD45(-) / S100(-), Metastases of mesenchymal tumors, 2 (3.39)	W 1 \pm 2 (50.00)	61
	M 1 \pm 2 (50.00)	42

Note: $\chi \pm SD$ – mean value \pm standard deviation, w- women, m - men.

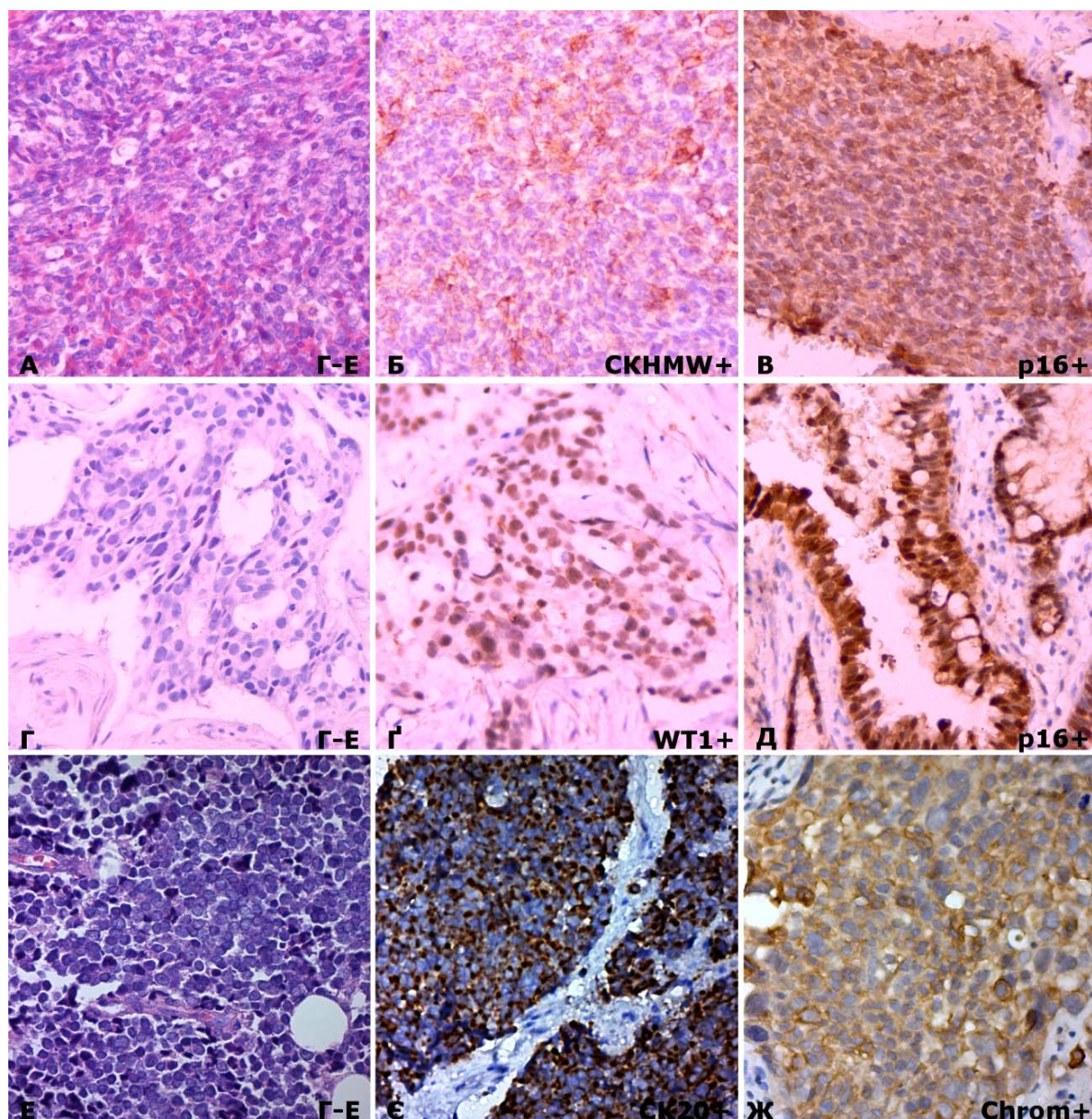


Fig. Histologic types of structure of isolated metastatic carcinomas in inguinal lymph nodes of women.
A. Metastasis of low-differentiated squamous cell carcinoma, Г-Е, ($\times 400$). **Б.** Weak expression of CKHMW, IHC, ($\times 400$). **В.** Positive nuclear-cytoplasmic reaction with p16, IHC, ($\times 400$). **Г.** Metastasis of moderately differentiated AC of ovary, Г-Е, ($\times 400$). **Д.** Positive intranuclear reaction with WT-1, IHC, ($\times 400$).
Д. Nuclear-cytoplasmic reaction with p16 +, IHC, ($\times 400$). **Е.** Metastases of carcinoma from Merkel cells, Г-Е, ($\times 400$). **Е.** Paranuclear expression of CK20, IHC, ($\times 400$). **Ж.** Weak cytoplasmic expression of Chromogranin A, IHC, ($\times 400$)

The only case of p16^{ink} negative metastasis into inguinal lymph nodes in women was the carcinoma from Merkel cells that had specific features of the CK20 + (paranuclear), chromogran +/-, synaptophysin +/-, CD117 +, TTF1- (Fig. E-Ж). Differences in the histological structure of p16^{ink} (+) and p16^{ink} (-) of metastatic carcinomas were reflected in the indicators of their morphometric study with the ImageJ program (Table 3).

Immunohistochemical profile of metastatic carcinomas of men with isolated lesion of the inguinal

lymph nodes determined the similar distribution of tumor nosologies, but the negativity of the marker p16^{ink} in all observations. Namely: 1 out of 4 (25%) observations had low squamous cell differentiation (CKHMW +), without positive organ-specific markers, 1 – low-differentiated neuroendocrine cancer, 1 – metastasis of carcinoma from Merkel cells, 1 – seminal carcinoma (CD117 +, PLAP +, vitmentin) (Table 4).

Table 3

**Indicators of morphometric study of metastatic carcinomas
of the inguinal lymph nodes in women in the ImageJ program**

Types of primary ovarian tumors	Area (mcm ²) $\bar{x} \pm SD$	Perimeter (mcm) $\bar{x} \pm SD$	Coefficient of «roundness» (parameter ImageJ) $\bar{x} \pm SD$
Squamous cell carcinoma (1)	55.480±9.929	29.735±3.421	0.790±0.046
Serous AC of ovary (2)	36.077±8.156	22.450±2.470	0.810±0.126
Carcinoma from Merkel cells (3)	40.576±7.350	24.680±2.012	0.811±0.0542
Lymphocytes of lymphatic node	16.738±2.693	15.993±1.541	0.746±0.098
p	p(1)<0.05, p(2)<0.05, p(3)<0.05,	p(1)<0.05, p(2)<0.05, p(3)<0.05,	p(1)>0.05, p(2)>0.05, p(3)>0.05

Note. $\bar{x} \pm SD$ – mean value \pm standard deviation, statistically significant difference was considered in $p < 0.05$.

In the group of melanoma metastases, women dominated (6:1), but it should be noted that, along with the standard immunohistochemical responses on melanoma S100 +, Vimentin +, such lesions of the inguinal lymph nodes also had partial cyto-

plasmic expression of p16^{ink}, but significantly lower, compared to nevi which proves the pathway for carcinogenesis of loss or translocation of the CDKN2A gene. Morphometric indices of melanoma metastases are presented in Table. 5

Table 4

**Indicators of morphometric study of metastatic carcinomas
of the inguinal lymph nodes in men in the ImageJ program**

Types of tumors	Area (mcm ²) $\bar{x} \pm SD$	Perimeter (mcm) $\bar{x} \pm SD$	Coefficient of «roundness» (parameter ImageJ) $\bar{x} \pm SD$
Squamous cell carcinoma (1)	55.480±9.929	29.735±3.421	0.790±0.046
Neuroendocrine cancer (2)	18.735±2.075	16.500±1.128	0.839±0.052
Carcinoma from Merkel cells (3)	49.533±8.622	27.349±3.042	0.819±0.023
Seminoma (4)	47.651±4.619	32.612±4.714	0.841±0.076
Lymphocytes of lymphatic node	16.738±2.693	15.993±1.541	0.746±0.098
p	p(1)<0.05, p(2)>0.05, p(3)<0.05, p(4)<0.05	p(1)<0.05, p(2)>0.05, p(3)<0.05, p(4)<0.05	p(1)>0.05, p(2)>0.05, p(3)<0.05, p(4)>0.05

Note. $\bar{x} \pm SD$ – mean value \pm standard deviation, statistically significant difference was considered in $p < 0.05$.

The third subgroup of mesenchymal metastatic tumors included 2 cases: 1 metastasis of leiomyosarcoma in woman and 1 – mesenchymal tumor with

undetermined malignant potential in man, both cases had p16^{ink} (-) negative status. Morphometric indicators are listed in Table 6.

Table 5

**Indicators of morphometric study of metastatic melanomas
into the inguinal lymph nodes in the ImageJ program**

Types of primary ovarian tumors	Area (mcm ²) $\bar{x} \pm SD$	Perimeter (mcm) $\bar{x} \pm SD$	Coefficient of «roundness» (parameter ImageJ) $\bar{x} \pm SD$
Melanoma metastasis	31.106±6.694	22.376±4.113	0.600±0.065
Lymphocytes of lymphatic node	16.738±2.693	15.993±1.541	0.746±0.098
p	p<0.05	p<0.05	p>0.05

Note. $\bar{x} \pm SD$ – mean value \pm standard deviation, statistically significant difference was considered in $p < 0.05$.

**Indicators of morphometric study of metastases of mesenchymal tumors
in the inguinal lymph nodes in the ImageJ program**

Types of primary ovarian tumors	Area (mcm ²) $\bar{x} \pm SD$	Perimeter (mcm) $\bar{x} \pm SD$	Coefficient of «roundness» (parameter ImageJ) $\bar{x} \pm SD$
Leiomyosarcoma (1)	35,254±12,831	25,168±5,643	0,467±0,130
Mesenchymal tumor with undetermined malignant potential (2)	74,293±19,595	33,476±4,060	0,816±0,123
Lymphocytes of lymphatic node	16,738±2,693	15,993±1,541	0,746±0,098
p	p(1)<0,05, p(2)<0,05	p(1)<0,05, p(2)<0,05	p(1) <0,05, p(2)>0,05,

Note. $\bar{x} \pm SD$ – mean value \pm standard deviation, statistically significant difference was considered in $p < 0.05$.

CONCLUSIONS

1. Distribution of variants of isolated lesion of the inguinal lymph nodes in women demonstrated 14 out of 35 (40.00%) metastatic tumors (other 21 - lymphoproliferating states requiring phenotyping, but not finding the primary localization). Of 14 metastatic lesions in women, 6 observations (42.86%) demonstrated p16^{ink} (+) phenotype (6 metastases of carcinomas from the reproductive system). For comparison: in men out of 24 cases, only 6 (25.00%) were of metastatic origin, of which 1 metastatic melanoma was found to be partially positive for p16^{ink} (+/-) (16.67%).

2. After a thorough immunohistochemical study with a panel of organ-specific markers in isolated metastatic lesions of the inguinal lymph nodes in women, half of the detected localizations (7 out of 14) related to reproductive organs, namely: 4 p16 (+) – to

squamous cell carcinoma of the cervix, 2 p16^{ink} (+) – to serous adenocarcinomas of ovaries, 1 p16^{ink} (-) – to leiomyosarcoma of the uterus body. Other locations did not have a clear site because they belonged to metastases of carcinoma from Merkel cells and melanoma due to the frequent reduction of the primary tumor cell in the skin.

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REFERENCES

1. Poslavska OV, Shponka IS, Gritsenko PO, Alekseenko OA. [Morphometric analysis of pancytokeratin-negative neoplastic damages of the lymphatic nodes of the neck]. *Medicni perspektivi*. 2018;23(1):30-37. Ukrainian. doi: <https://doi.org/10.26641/2307-0404.2018.1.124915>
2. Poslavska OV. [Determination of linear dimensions and square square surfaces areas of morphological objects on micrographs using ImageJ software]. *Morphologia*. 2016;10(3):377-81. Ukrainian. doi: <https://doi.org/10.26641/1997-9665.2016.3.377-381>
3. Fatemeh Sari Aslani, Akbar Safaei, Masoumeh Pourjabali, Mozhdan Momtahan. Evaluation of Ki-67, h16 and CK17 markers in differentiation cervical intraepithelial neoplasia and benign lesions. *Iran J Med Sci*. 2013;38(1):15-21.
4. Greco FA. Molecular diagnosis of the tissue of origin in cancer of unknown primary site: useful in patient management. *Curr Treat Options Oncol*. 2013;14(4):634-42. doi: <https://doi.org/10.1007/s11864-013-0257-1>
5. Pai V, Kattimani K, Manohar V, Ravindranath S. Inguinal lymph node squamous cell carcinoma of unknown primary site: a case report. *Journal of surgery and operative care*. 2016;1(2):208. doi: <https://doi.org/10.15744/2455-7617.1.207>
6. Lin F, Haiyan Liu. Immunohistochemistry in Undifferentiated Neoplasm / Tumor of Uncertain Origin. *Arch Pathol Lab Med*. 2014;138:1583-610. doi: <https://doi.org/10.5858/arpa.2014-0061-RA>
7. Vajdic CM, Goldstein D. Cancer of unknown primary site. *Aust Fam Physician*. 2015;44(9):640-43.

СПИСОК ЛІТЕРАТУРИ

1. Пославська О. В., Шпонька І. С., Гриценко П. О., Алексеєнко О. А. Морфометричний аналіз панцітокератин-негативних неопластичних ушкоджень лімфатичних вузлів шийї. *Медичні перспективи*. 2018. Т. 23, № 1. С.30-37. DOI: <https://doi.org/10.26641/2307-0404.2018.1.124915>
2. Пославська О. В. Визначення лінійних розмірів та площ окремих морфологічних об'єктів на мікрофотографіях за допомогою програми ImageJ. *Морфологія*. 2016. Т. 10, № 3. С. 377-381. DOI: <https://doi.org/10.26641/1997-9665.2016.3.377-381>
3. Aslani Fatemeh Sari, Safaei Akbar, Pourjabali Masoumeh, Momtahan Mozhdan. Evaluation of Ki-67, h16 and CK17 markers in differentiationg cervical intraepithelial neoplasia and benign lesions. *Iran J Med Sci*. 2013. Vol. 38, N 1. P. 15-21.
4. Greco F. A. Molecular diagnosis of the tissue of origin in cancer of unknown primary site: useful in patient management. *Curr Treat Options Oncol*. 2013. Vol. 14, N 4. P. 634-642. DOI: <https://doi.org/10.1007/s11864-013-0257-1>
5. Pai V., Kattimani K., Manohar V., Ravindranath S. Inguinal lymph node squamous cell carcinoma of unknown primary site: a case report. *J. surgery and operative care*. 2016. Vol. 1, N 2. P. 208. DOI: <https://doi.org/10.15744/2455-7617.1.207>
6. Lin F. Liu H. Immunohistochemistry in Undifferentiated Neoplasm / Tumor of Uncertain Origin. *Arch Pathol Lab Med*. 2014. Vol. 138. P. 1583-1610. DOI: <https://doi.org/10.5858/arpa.2014-0061-RA>
7. Vajdic C. M., Goldstein D. Cancer of unknown primary site. *Aust Fam Physician*. 2015. Vol. 44, N 9. P. 640-643.

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