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IMMUNOLOGICAL FEATURES OF MACROPHAGES ASSOCIATED WITH METASTASIS OF PRIMARY BREAST CARCINOMA INTO REGIONAL LYMPH NODES

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Introduction. Breast cancer (BC) is a heterogeneous disease with 20 histological types, 8 molecular genetic and 6 genomic subtypes, characterized by specific molecular and/or biochemical properties, different clinical course and prognosis (let alone the intratumoral heterogenicity within different sections of one tumor) [1,2]. Recently, much attention has been paid to the study of components and cells of the BC stroma, which influence the development of such processes as providing the tumor vascularization, transepithelial transport, invasion and migration, intravasation and metastasis of tumor cells [3,4]. Under the selective "pressure" of microenvironment, the intratumoral heterogeneity develops. This issue has been discussed in several in-depth reviews of literature [5,6]. It is possible that among the mechanisms of this particular system, we will find a more versatile and reliable target for diagnosis, prognosis or treatment of BC [7].

Therefore, it is promising and important to study tumor-associated macrophages (TAMs) as the cells of innate immunity, which supposedly is able to regulate immune surveillance of the tumor and does not necessarily require the innate link [8].

The aim of the research was to analyze the quantitative characteristics of TAM and M2-like macrophages, which infiltrate the primary focus of BC, in metastasis into the regional lymph nodes and without it, in two groups, balanced by the immunohistochemical type of tumors, to find out the significance of these macrophages in metastases.

Object and methods. Biopsy samples and clinical data were obtained from patients undergoing treatment at Poltava Regional Clinical Dispensary. The study was approved by the Ethics Commission of Ukrainian Medical Stomatological Academy.

Materials of the study were intraoperative tissues of tumors and ipsilateral lymph nodes in radical mastectomy.

Immunohistochemical characteristics of removed tumors (ER, PR, HER2, Ki67) were used for organizing two balanced groups of patients with primary BC, one of which did not reveal metastases into the regional lymph nodes, N0, whereas in the second one there was primary-metastatic BC, N1. Morphological and IHC findings were obtained in certified pathoanatomical laboratories. Each group included three patients with non-luminal HER2+, luminal A, luminal B HER2-negative, luminal B HER2+, and triple-negative BC, with 15 patients in each group, with a total of 30 patients.

The average age of patients was: in the first group – 60 years, from 29 to 79; in the second one – 59, from 30 to 76.

All patients included in the study did not undergo any treatment. Treatment began with radical mastectomy.

According to the data of meta-analysis [9], the use of CD68 as a biomarker of TAM for IHC evaluation has its priorities, as compared with individual definition of CD163 or CD206.

The IHC studies to define TAM and M2-like macrophages were performed using the streptavidin-peroxidase method. Paraffin sections, 5 μm in thickness, obtained by the standard technique of the automated cycle at the pathoanatomical laboratory, were deparaffinized and dehydrated; antigens were restored in citrate buffer (pH 6.0) in a microwave oven (at a power of ≈600 W, 3 cycles for 7 minutes with 1 minute interval), cooled for 20 min, washed in distil water and phosphate buffered saline (PBS, pH 7.2-7.4) for 2 minutes; endogenous peroxidase was blocked with a reagent from PolyVue HRP/DAB Detection System (For Mouse & Rabbit Primary Antibodies, Diagnostic BioSystems, USA) and washed at PBS for 3 min. The sections were then incubated at 4°C overnight with murine anti-CD68 monoclonal antibodies (clone PG-M1, REF PD M065-S, Diagnostic BioSystems, USA) and anti-CD163 (clone 10D6, REF Mob460-01, diluted 1: 100 in antibody diluent buffer for the IHC method (Antibody Diluent, Dako, USA). The following sections were processed in two steps with Mouse/Rabbit PolyVueTM HRP/DAB Detection System (Diagnostic BioSystems, USA), detector system for visualizing the response by DAB chromogen, the nuclei were bleached with Meyer's hematoxylin and enclosed under a cover glass in cedar balsam. Antibody diluent buffer was used instead of primary antibodies as a negative control value, the lymph node tissue – as a positive one.

The evaluation of IHC staining was carried out by counting CD68+TAM and CD163+M2-like TAMs under a light microscope (Biolam, LOMO, Russia: lens × 40, eyepiece × 7) in 7-10 fields of view of intensive IHC reaction of each section, calculating the arithmetic mean within the tumor nests and tumor stroma. Calculation included immune-positive cells with macrophage morphology. Microphotographs were obtained using the Leica DM500, Leica, Germany (lens × 40) microscope.

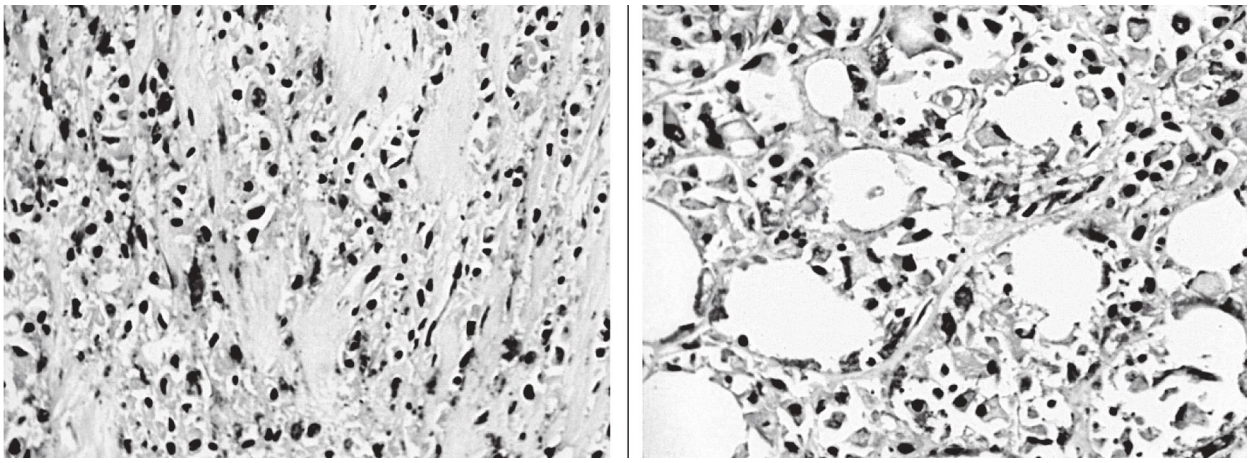

Figure 1 – Immunohistochemical detection of TAM (CD68+) (A) and M2 (CD163+) (B) in the biopsy sample of invasive non-luminal HER2+ breast carcinoma. Immunosuppressive cells appear to lie in the tumor nests (arrows), but adhere to the fibrous structures of the stroma.

Statistical analysis was performed using the GraphPad Prism 5 software by means of nonparametric and parametric methods.

Results. As stated above, the calculation of immune-positive TAM was performed in 5-17 fields of view at high magnification (40×lens) of each section, within the tumor nests, tumoral and peri-tumoral stroma, calculating the arithmetic mean. There have been several reports on different significance of the amounts of TAM, localized in different sections of BC, in particular, separately in the stroma, and their interrelation with the prognosis [4,10]. When calculating separately in different tumor sections, it is necessary to measure the relative area of certain structures on the specimen, which is rather laborious and does not yield rapid results in practice [11]. Such an approach will not ensure the accuracy of representation, at least due to different amounts of intercellular substance in different sections of tumor stroma. In addition, a number of samples do not provide a reliable basis for relating the TAM localization to the connective tissue structure or tumor nest in the IHC study, as represented on **fig. 1.**

Calculation of macrophages in relation to the number of cells / nuclei in a particular section area also has certain inaccuracies, because the measurements of atypical cells differ even within the same specimen. In addition, circular cell infiltration resembles the tissue structure.

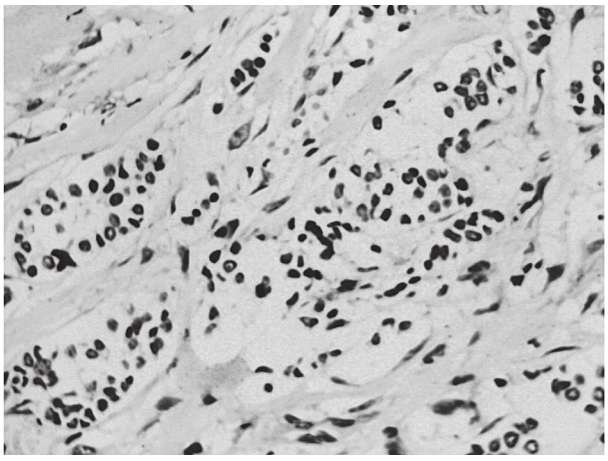

Figure 2 – Immunohistochemical detection of M2-like macrophages (CD163+) in the biopsy sample of invasive duct lumen A breast carcinoma, N0. The section area with tumor complexes free of immune-positive cells.

On the other hand, TAM is a representative of the host macroorganism, and the ways of their penetration into the tumor, understandably, are provided by connective tissue. Therefore, TAMs are necessarily present in this area. Nevertheless, in a number of specimens, we discovered areas of tumor complexes (along with connective tissue), where TAMs were absent, or isolated **(fig. 2).**

Therefore, the method for counting immune-positive cells was selected – over the entire field of view (by the way, this method is used to count the number of tumor mitoses for determining its G-grade), which could serve as a diagnostic express method if it was possible to develop a clinical interpretation of individual parameters of the amount of TAM. However, the reliable quantitative changes in CD68+ TAM between the groups were detected by the nonparametric method, the interquartile interval (IQR) for CD68+TAM of group N1 showed a significant variation spread, and the scope of quantitative results (from 0 to 43, **fig. 1**) reflects a high probability of extreme values, as will be discussed below.

The average quantitative indicators for each group (over 122-146 fields of view at high magnification, 40×lens) are calculated as median and IQR (based on the asymmetric and inclined to the right distribution of variation series **(table 1).** In general, IQR shows an average of 50% of the sample values and allows us to describe the extent of variation in the asymmetric distribution without the effect of extreme values. As can be seen from **table 1,** the largest IQR for CD68+ AM in the second group, N1, reflects the broadest variability of quantitative indicators **(table 1).**

The main idea of the work was to find the differences between the quantities of CD68+ and CD163+ macrophages, localized in tumor biopsy samples between the groups of patients with non-metastatic BC (N0) and with metastases (N1), balanced by the clinical values of ER, PR, HER2 (non-luminal HER2+, luminal A, luminal B HER2+, luminal B HER2-, and triple-negative BC).

The results of statistical processing of average quantitative indicators of CD68+TAM, CD163+M2-like macrophages and CD68/CD163 (macrophage index M1) between groups N0 and N1 did not reveal significant differences **(table 2).** However, when comparing the calculated immune-positive macrophages from all fields of view (40×lens), which were 5-17 for each specimen, a

significant increase in CD68+TAM in the metastasis group was established **(table 3).**

We determined the IHC characteristics of BC for each biopsy sample, including Ki67 and G-grade. Therefore, a statistical check of their correlation relationships with average quantitative indicators of CD68+, CD163+ and CD68/CD163 macrophages was conducted, which revealed a reliable relationship between CD68+ and Ki67, expressed as a percentage, and between ER/PR (in the clinical interpretation: +/-) and CD68+, CD163+, but not the CD68/CD163 indicator **(table 4).**

Discussion. We conducted the calculation of immune-positive macrophages in the stroma and tumor nests, because the localization of macrophages depends on the presence of hypoxic or necrotic regions in the tumor [12], which constitute a prognostic factor, as well as due to the impossibility of precisely attributing the localization of TAM in a number of specimens to the stroma or tumor nest.

We chose a relatively simple way of counting immune-positive cells – over the entire field of view, which could serve as a diagnostic express method if it was possible to develop a clinical interpretation of individual parameters of the amount of TAM. However, the reliable quantitative changes in CD68+TAM between the groups were detected by the nonparametric method, the IQR 15 for CD68+TAM of the second group showed a large variation spread, and the extent of quantitative results (from 0 to 43) shows a high probability of extreme values. In addition, the established increase in CD68+TAM in the primary metastasis is contrary to a number of clinical studies that reflect the fact that CD163+M2 contributes to metastases in BC. However, these studies took into account the amount of CD163+cells binomially: as high and low, and did not determine the amount of CD68+PM [13].

Therefore, the interpretation of personal data regarding the amount of CD68+TAM and CD163+M2 macrophages is not yet adapted to routine clinical use, despite the reliable differences.

We found direct reliable correlation relationships between the number of CD68+TAM (but not CD163+M2-macrophages) and Ki67. The Ki-67 expression level allows us to estimate the proliferative potential of the tumor, since it detects cells in the process of preparation for division and in the phase of mitosis. Thus, it can be assumed that the tumor proliferation and the amount of CD68+TAM synergistically reflect its progression, taking into account the functions of M1-classic macrophages (as subpopulations of CD68+TAM), such as cleansing the necrotic sites from cellular decay [14]. However, other authors, in the study with a larger number of participants, have found a positive correlation between CD163+macrophages located in the tumor stroma with immune-positive Ki67 [15], which is also well explained by the properties of M2 macrophages, such as providing vascularization and immunosuppression [13,16], and also agrees with our own observations of the typical CD163+M2 localization in the tumor stroma.

Together with the results of a significant increase in CD68+TAM (but not CD163+M2) during metastases, their correlation with Ki67 (but, again, not with CD163+M2), one may predict the pro-tumoral potential of M1-like tumor-associated macrophages. The results of a recent in vitro study indicate that production of IL-1β by macrophages plays an important role in the migration of BC cells and their adhesion to blood cells and lymphatic endothelial cells, as well as their transmigration [17,18]. M1 macrophages, in turn, are well known as a significant source of IL-1 [18].

Taken together, this data reflects that the issue concerning the contribution of pro- and anti-tumoral activity of TAM in BC still remains understudied: some authors believe that in the early stages of tumor development, M1 macrophages are favorable, whereas in the later stages – M2 [8], and according to other sources, M1 – are attributed strictly anti-tumoral tumoricidal activity [19].

In BC, signaling through ER and PR plays an important role in the progression of the disease [20]. We determined a reverse correlation between CD68+ TAM, CD163+M2-like macrophages and the clinical value of ER/PR. The above-cited research [15] has revealed a correlation between CD163+ macrophages, which are localized precisely in tumor stroma with a negative status for ER and PR. Similar interrelations have been revealed by other authors [13]. This data virtually coincides with our findings, which is explained by the localization of CD163+M2-like macrophages typically in the stroma (in

Table 1 – Average quantitative indicators of TAM

CD68/163 TAM	The first group, N0		The second group, N1
	Median	IQR	Median
CD68+TAM	7	8	9
CD163+M2	4	5	3

Table 2 – Comparison of average quantitative indicators of CD68+, CD163+ and CD68/CD163 between the groups of BC N0 and N1 showed no significant differences (the Mann-Whitney test)

Indicators	Reliability, p
CD68+ N0 against CD68+ N1	0.36
CD163+ N0 as against CD163+ N1	0.58
CD68/CD163 N0 as against CD68/CD163 N1	0.93

Table 3 – Comparison of quantitative data from each field of view of CD68+, CD163+ between the groups of BC N0 and N1 (the Mann-Whitney test)

Indicators	Reliability, p
CD68+ N0 as against CD68+ N1*	Increase in the second group N1*, p=0.015
CD163+ N0 as against CD163+ N1	Unreliable increase in the second group N1, p=0.93

Note: * – hereinafter indicates a reliable statistical result.

Table 4 – Correlation relationships between the average quantitative indicators of CD68+, CD163+, CD68/CD163 and indicators of the IHC characteristics of BC and G-grade (analysis of groups N0+N1) (the Spearman/Pearson correlation)

Pairs for checking correlation relationships	Correlation coefficients	Reliability
CD68+ and: ER, PR, HER2, G	-0.24; -0.2; -0.28; 0.14	p>0.05
CD68+ and Ki67 (%)*	0.38*	p=0.04
CD68+ and ER/PR(clinical interpretation)*	-0.42*	p=0.02
CD163+ and: ER, PR, HER2, Ki67, G	-0.33;-0.33;-0.15; 0.2; 0.14	p>0.05
CD163+ and ER/PR(clinical interpretation)*	-0.47*	p=0.009
CD68/CD163 and: ER, PR, HER2, Ki67, G	-0.006; 0.06; -0.16; 0.13; -0.08	p>0.05

the nests, we observed that their number was always noticeably smaller, or they were absent **(fig. 2).** The reverse correlation shows that changes in the number of TAMs (in particular, M2) in ER+/ PR+BC, may not be related to the presence of metastases [6].

The revealed patterns refer to the tumoral process and were not influenced by any treatment, since it was initiated by surgical intervention.

Conclusions

1. The number of CD68+TAM was significantly higher in the focus of the primary BC of patients with metastases in the regional lymph nodes.

2. We detected a reverse correlation between CD68+TAM, CD163+M2-like macrophages and the clinical value of ER/PR. Consequently, an increase in the number of TAMs (in particular, M2) in the primary ER+/ PR+BC with metastases may not have a predictive value for metastasis, or ER+/PR+ BC possibly metastasizes without a significant increase in TAM.

3. The revealed differences are confirmed by reliable statistical calculations and relate to the effect of an exceptionally pathological process, since patients did not receive treatment before radical mastectomy.

4. Interpretation of personal data on the number of CD68+TAM and CD163+M2-like macrophages still needs to be developed.

Prospects for further research. To develop the application of quantitative indicators in the clinical practice of TAM as an index of the prognosis of recurrence-free period and overall survival in breast cancer patients.

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ІМУНОЛОГІЧНІ ОСОБЛИВОСТІ МАКРОФАГІВ ПОВ'ЯЗАНІ З МЕТАСТАЗУВАННЯМ ПЕРВИННОЇ КАРЦИНОМИ ГРУДНОЇ ЗАЛОЗИ У РЕГІОНАЛЬНІ ЛІМФОВУЗЛИ

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Резюме. *Мета.* Рак грудної залози (РГЗ) є гетерогенним захворюванням, який розвивається під селективним «тиском» мікрооточення пухлини. Тому перспективно і важливо вивчати пухлинні макрофаги як клітини вродженого імунітету.

Об'єкт і методи. Матеріалом дослідження були інтраопераційні тканини пухлин та іпсілатеральних лімфовузлів при радикальному видаленні молочних залоз. Імуногістохімічні (ІГХ) характеристики видалених пухлин (ER, PR, HER2, Ki67) використовували для організації двох врівноважених груп пацієнток з первинним РГЗ, в одній з яких не було виявлено метастазів в регіональні лімфовузли, N0, а у другій – спостерігався первинно-метастатичний РГЗ, N1.

Результати. Основна ідея роботи полягала у пошуку відмінностей між кількостями CD68+, CD163+ макрофагів, локалізованих у біоптатах пухлин між групами пацієнток з РГЗ без метастазів (N0) і з метастазами (N1), врівноваженими за клінічними значеннями ER, PR, HER2 (нелюмінальним HER2+, люмінальним A, люмінальним B HER2+, люмінальним B HER2-, та тричі негативним РГЗ). Результати статистичної обробки середніх кількісних показників CD68+ ПАМ, CD163+M2-подібних макрофагів та CD68/CD163 (показник M1 макрофагів) між групами N0 та N1 не досягли достовірних відмін. Але, при порівнянні підрахованих імунопозитивних макрофагів з усіх полів зору (об. х 40), яких було 5-17 для кожного препарату, встановлено достовірне збільшення CD68+ПАМ у групі з метастазами.

Висновки. 1. Кількість CD68+ПАМ була достовірно вища у вогнищі первинного РГЗ хворих з метастазами у регіональні лімфовузли. 2. Встановлена зворотна кореляція між CD68+ПАМ, CD163+M2-подібними макрофагами та клінічним значенням ER/PR. Отже, збільшення числа ПАМ (зокрема M2) у вогнищі первинного ER+/

PR+ РГЗ з метастазами може не мати прогностичного значення щодо метастазування, або ER+/PR+ РГЗ, можливо, метастазує без визначного збільшення ПАМ. 3. Виявлені відмінності підтверджені достовірними статистичними розрахунками і відносяться до ефекту виключно патологічного процесу, оскільки пацієнтки не отримували лікування до операції радикальної мастектомії. 4. Інтерпретацію персональних даних про кількість CD68+ПАМ та CD163+ М2-подібних макрофагів ще належить розробити.

Ключові слова: пухлинно-асоційовані макрофаги, імуногістохімічні дослідження, CD68+ПАМ і CD163+М2-подібні макрофаги.

ИММУНОЛОГИЧЕСКИЕ ОСОБЕННОСТИ МАКРОФАГОВ СВЯЗАННЫЕ С МЕТАСТАЗИРОВАНИЕМ ПЕРВИЧНОЙ КАРЦИНОМЫ МОЛОЧНОЙ ЖЕЛЕЗЫ В РЕГИОНАЛЬНЫЕ ЛИМФОУЗЛЫ

Айкян А. З., Шинкевич В. И., Кайдашев И. П.

Резюме. *Цель.* Рак груди (РМЖ) – гетерогенное заболевание, которое развивается под избирательным «давлением» микросреды опухоли. Поэтому перспективно и важно изучать опухолевые макрофаги как клетки врожденного иммунитета.

Объект и методы. Материалом исследования послужили интраоперационные ткани опухолей и ипсилатеральные лимфатические узлы при радикальной мастэктомии. Иммуногистохимические характеристики удаленных опухолей (ER, PR, HER2, Ki67) были использованы для организации двух сбалансированных групп пациентов с первичным РМЖ. В первой группе не было метастазов в регионарные лимфатические узлы, N0, а во второй – первичный метастатический РМЖ, N1.

Результаты. Основная идея работы заключалась в том, чтобы найти различия между количеством макрофагов CD68+ и CD163+, локализованных в образцах биопсии опухоли, между группами пациентов с неметастатическим РМЖ (N0) и с метастазами (N1), уравновешенные клинические значения ER, PR, HER2 (непросветный HER2+, просвет А, просвет В HER2+, просвет В HER2- и тройной отрицательный ВС). Результаты статистической обработки средних количественных показателей CD68+ТАМ, CD163+М2-подобных макрофагов и CD68/CD163 (индекс макрофагов М1) между группами N0 и N1 не выявили достоверной разницы. Однако при сравнении рассчитанных иммуноположительных макрофагов со всех полей зрения (40 × линза), которые составляли 5-17 для каждого образца, было установлено значительное увеличение CD68+ТАМ в группе метастазов.

Выводы. 1. Количество CD68+ТАМ было достоверно выше в очаге первичного РМЖ у пациентов с метастазами в регионарные лимфатические узлы. 2. Мы обнаружили обратную корреляцию между CD68+ТАМ, CD163+М2-подобными макрофагами и клиническим значением ER/PR. Следовательно, увеличение количества ТАМ (в частности, М2) в первичном ER+/PR+ РМЖ с метастазами может не иметь прогностической ценности для метастазирования, или ER+/PR+ РМЖ, возможно, метастазирует без значительного увеличения ТАМ. 3. Выявленные различия подтверждаются достоверными статистическими расчетами и относятся к эффекту исключительно патологического процесса, поскольку до радикальной мастэктомии пациенты не получали лечения. 4. Интерпретация персональных данных о количестве CD68+ТАМ и CD163+ М2-подобных макрофагов все еще требует разработки.

Ключевые слова: опухолевые макрофаги, иммуногистохимические исследования, CD68+ТАМ и CD163+М2-подобные макрофаги.

IMMUNOLOGICAL FEATURES OF MACROPHAGES ASSOCIATED WITH METASTASIS OF PRIMARY BREAST CARCINOMA INTO REGIONAL LYMPH NODES

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Abstract. *Aim.* Breast cancer (BC) is a heterogeneous disease which develops under the selective “pressure” of the tumor microenvironment. Therefore, it is promising and important to study tumor-associated macrophages as the innate immunity cells. The innate immunity system supposedly is able to regulate tumor immune surveillance and does not necessarily require the innate link.

Object and methods. Materials of the study were intraoperative tissue of tumors and ipsilateral lymph nodes in radical mastectomy. Immunohistochemical characteristics of removed tumors (ER, PR, HER2, Ki67) were used for organizing two balanced groups of patients with primary BC. In the first group, there were no metastases into the regional lymph nodes, N0, whereas in the second one there was primary metastatic BC, N1. The groups included three patients with non-luminal HER2+, luminal A, luminal HER2-negative, luminal HER2+, and triple-negative BC, with 15 patients in each group, with a total of 30 patients.

Results. The main idea of the work was to find the differences between the quantities of CD68+ and CD163+ macrophages, localized in tumor biopsy samples between the groups of patients with non-metastatic BC (N0) and with metastases (N1), balanced by the clinical values of ER, PR, HER2 (non-luminal HER2+, luminal A, luminal B HER2+, luminal B HER2-, and triple-negative BC). The results of statistical processing of average quantitative indicators of CD68+TAM, CD163+M2-like macrophages and CD68/CD163 (macrophage index M1) between groups N0 and N1 did not reveal a significant difference. However, when comparing the calculated immune-positive macrophages from all fields of view (40×lens), which were 5-17 for each specimen, a significant increase in CD68+TAM in the metastasis group was established. Comparison of average quantitative indicators of CD68+, CD163+ and CD68/CD163 between groups BC N0 and N1 showed no significant differences (the Mann-Whitney test). We determined the IHC characteristics of BC for each biopsy sample, including Ki67 and G-grade. Therefore, a statistical check of their correlation relationships with average quantitative indicators of CD68+, CD163+ and CD68/CD163 macrophages was conducted, which revealed a reliable relationship between CD68+ and Ki67, expressed as a percentage, and between ER/PR (in the clinical interpretation: +/-) and CD68+, CD163+, but not CD68/CD163.

Conclusions. 1. The number of CD68+TAM was significantly higher in the focus of the primary BC of patients with metastases in the regional lymph nodes. 2. We detected a reverse correlation between CD68+TAM, CD163+M2-like macrophages and the clinical value of ER/PR. Consequently, an increase in the number of TAMs (in particular, M2) in the primary ER+/ PR+BC with metastases may not have a predictive value for metastasis, or ER+/PR+ BC possibly metastasizes without a significant increase in TAM. 3. The revealed differences are confirmed by reliable statistical calculations and relate to the effect of an exceptionally pathological process, since patients did not receive treatment before radical mastectomy. 4. Interpretation of personal data on the number of CD68+TAM and CD163+M2-like macrophages still needs to be developed.

Key words: Tumor-associated macrophages, immunohistochemical studies, CD68+TAM and CD163+M2-like macrophages.

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ОСОБЕННОСТИ ПРОЛИФЕРАТИВНОЙ АКТИВНОСТИ ЦЕРВИКАЛЬНОЙ ТКАНИ ПРИ НАЛИЧИИ АЛКОГОЛЬНОЙ ЗАВИСИМОСТИ

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Связь публикации с плановыми научно-исследовательскими работами. Данная работа является фрагментом НИР Одесского национального медицинского университета «Оптимізація патоморфологічних досліджень з метою удосконалення діагностики, профілактики, лікуванні та реабілітації жінок з екстрагенітальною та геніальною патологією та ускладненнями перинатального періоду», № государственной регистрации 0115U006638.

Вступление. Одним из важных когнитивных факторов влияющих на функцию иммунной системы является употребление алкоголя. Алкоголь был и остается одним из наиболее распространенных веществ употребления и злоупотребления в истории человечества и поражает многие органы [1], при этом употребление алкоголя является основным фактором риска развития болезней [2,3] и получения травм [4,5]. Злоупотребление алкоголем часто упоминается как один из ключевых факторов, лежащих в основе повышенной уязвимости к ВИЧ-инфекции [6,7]. Злоупотребление алкоголем связано с риском в связи с незащищенным сексом, множественными сексуальными партнерами [8], а также физическое и сексуальное насилие [9]. Женщины, употреблявшие алкоголь, чаще имеют инфекции передающиеся половым путем (ИППП).

В тоже время фоновые заболевания ШМ (ФЗШМ) относят к факультативным предраковым процессам, несвоевременная диагностика и неэффективное лечение которых является угрожающим учитывая вероятность трансформации патологически измененных тканей в предраковые и опухолевые процессы. По данным литературы, распространенность патологии ШМ составляет почти 50% среди гинекологических больных, а доброкачественные процессы в структуре патологии ШМ составляют около 80%, наиболее частыми причинами их возникновения являются родовая, или связана с абортами травма (разрыв, изнанку ШМ), инфицирование, реже – гормональные расстройства [10,11].

Одним из эффективных путей снижения заболеваемости и смертности от рака шейки матки является раннее выявление его предраковых поражений или цервикальной интраэпителиальной неоплазии [12] с обнаружением клеточной дисрегуляции, одним из проявлений которых является повышение пролиферативной активности.

В связи с изложенным выше, **целью** нашей **работы** явилось выявление особенностей пролиферативной активности цервикальной ткани при наличии алкогольной зависимости. Решение данной задачи позволит оказывать адекватную гинекологическую помощь, понять механизмы тканевой трансформации, клинически интерпретировать морфологические данные.

Объект и методы исследования. Мы отобрали 50 случаев секционного материала злоупотребляющих алкоголем женщин, умерших от хронического алкоголизма (в основном алкогольный цирроз печени). Возраст женщин колебался от 24 до 46 лет, в среднем 32,7 года. Сформирована группа сравнения из 50 женщин того же возраста. Курение табака, контрацептивы (оральные противозачаточные таблетки), возраст первого полового акта, соматическая патология, связанная (не связанная) с употреблением алкоголя, количество беременностей не учитывались т.к. при наборе обеих групп был использован принцип рандомности.

Материал фиксировали в 10% нейтральном забуференном формалине, после чего заливали парафином. Из подготовленных блоков изготовлены срезы толщиной $5x10^{-6}$ м. Срезы окрашивали гематоксилином и эозином [13]. Иммуногистохимическое исследование (ИГХ) проводилось непрямой иммунопероксидазной реакцией [14] с моноклональными антителами (mAb) к Ki-67 (компания Thermo Scientific, США). Визуализацию реакции проводили с помощью набора UltraVision LP Detection System HRP Polymer & DAB Plus Chromogen (Thermo Scientific, США).

Микроскопическое исследование проводили на микроскопе «Olympus BX41» с последующим морфометрическим исследованием с помощью программы «Olympus DP-soft 3.12». Определяли толщину эпителиального слоя шейки матки. Окрашивание Ki67 оценивалось независимо двумя наблюдателями, и