UDC: 616-008.854.6-091.82
DOI: 10.52532/2663-4864-2022-4-66-33-37

STAGES OF CYTOLOGICAL EXAMINATION (USING IMMUNOCYTOCHEMICAL EXAMINATION) OF EFFUSION FLUIDS

K.S. PAVLYUK[1], M.G. LEONOV[2,3], A.V. AKOBYAN[1], T.V. SINITSKAYA[1], O.V. GOSPIROVICH[1], E.A. ARTEMOVA[1], Zh.B. YELEUBAYEVA[4]

[1]«State Budgetary Healthcare Institution «Scientific Research Institute – S.V. Ochapovsky Regional Clinical Hospital No. 1» GBUZ, Krasnodar, the Russian Federation;
[2]State Budgetary Healthcare Institution «Oncological dispensary No. 3» GBUZ, Novorossiysk, the Russian Federation;
[3]Kuban State Medical University, Krasnodar, the Russian Federation;
[4]«Kazakh Institute of Oncology and Radiology" JSC, Almaty, the Republic of Kazakhstan» JSC

ABSTRACT

Relevance: *Cytological criteria of tumors in exudate fluids are associated with specific subjective difficulties, one of which is the differential diagnosis of proliferating mesothelial and adenocarcinoma cells.*

The study aimed to *increase the informational value of cytological diagnostics in a multidisciplinary hospital.*

Methods: *From 2018 to 2021, 10,082 serous cavity effusions (pleural – 8,166 (81%), abdominal cavity – 1,512 (15%), pericardial – 404 (4%)) were included in the cytological examination. Microscopic examination of traditional preparations was carried out, and immunocytochemical (ICC) examination was carried out in difficult diagnostic situations.*

Results: *In this study, in women, the traditional cytological examination of effusion fluids revealed metastatic lesions of the serous cavities in 672 cases (58%), mainly due to breast cancer progression (26%). In men, pleurisy was primarily due to metastasis of adenocarcinoma of the lung – 266 cases (23%). ICC research increased the diagnostic accuracy of cytological examination by 62-93% and the specificity – by 95-99%.*

Conclusions: *An algorithm for conducting ICH studies, differing in the number of panels of monoclonal antibodies used to determine the histological form and organ - the source of the tumor, has been developed. In specific cases, conducting ICR studies with 2-3 monoclonal antibodies may be enough to confirm the histological form of cancer and, where necessary, perform additional ICR studies without significant loss of time for obtaining results.*

Keywords: *immunocytochemistry (ICC), monoclonal antibodies, malignant tumors, pleural fluid, ascitic fluid, conventional cytology, liquid-based cytology.*

Relevance: The significance of cytological examination in modern medicine is indisputable. In contrast to histological examination, it is performed not on tissue but on the cellular level. Clinical cytology differs from other clinical laboratory diagnostic methods because it aims to identify atypical cells with cytomorphological diagnoses in non-tumor and tumor processes. Detecting the signs of exudate malignancy at the cellular level is difficult, and one of the challenges is the differential diagnosis of mesothelial cells with signs of proliferation and cells suspicious of adenocarcinoma (ADC). The discernible reactivity of the sulfur cavity cover, the desquamation, and the regenerative ability of the mesothelium cause a great variety of cellular compositions.

Often, malignant cells in exudates of serous cavities cannot be detected even at the late stages of the disease due to their insufficient number in the studied material [1]. Recently, the widespread introduction of liquid cytology and immunocytochemical (ICC) examination methods have significantly reduced the factor of subjectivity [2].

This requires optimal management in morphological and clinical diagnostic laboratories (CDL), establishing cytological criteria for differential tumor diagnosis in examining exudative fluids, and developing an algorithm for ICC examinations. Signs of cell atypia in various lesions intersect with the signs of malignancy, creating difficulties in identifying the nature of the lesion and can cause false-positive or false-negative cytological diagnoses [3, 4].

A cytologist is to inform the clinician how important it is to observe and follow the instructions adopted at a CDL cytology department, which contain an algorithm for referring biological material and preparing cytological material, as well as performing a microscopic examination and interpreting the results.

The study aimed to increase the informational value of cytological diagnostics in a multidisciplinary hospital.

Materials and methods: The study was performed at the clinical diagnostic laboratory of the Center for Thoracic Surgery (CTS) of GBUZ "Scientif-

ic Research Institute – Regional Clinical Hospital No. 1 after S.V. Ochapovsky" (Krasnodar, Russia). In 2018-2021, 10,082 samples of serous cavity effusion fluids (8,166 (81%) pleural, 1,512 (15%) abdominal, and 404 (4%) pericardial) were subjected to cytological examination in outpatient and other hospital departments for suspected malignant neoplasm of thoracic organs (most often, for lung cancer).

Before serous cavity punctures to assess the extent of the tumor process, all patients underwent a comprehensive examination using radiation (ultrasound, X-ray, computed tomography, magnetic resonance imaging) and other diagnostic methods. The surgeons followed standard procedures when performing serous cavity punctures for diagnostic and/or therapeutic purposes.

An anticoagulant (5% sodium citrate solution of 5 ml per 100 ml or heparin of 1 ml (5,000 IU) per 500 ml fluid) was added to the exudative fluid. All the obtained exudate samples were referred to the CDL for examination. At the first preanalytical stage, a medical laboratory technician evaluated the physical and chemical properties and the presence of sediment before and after centrifugation in a standard centrifuge. The glass slides for ICC were prepared by one of the two methods: liquid (using poly-L-lysine-coated slides) on a Cytospin 4 cytocentrifuge or traditional. The obtained micro slides were fixed by the May-Grunwald method and stained by Romanowsky-Giemsa.

The analytical stage included a microscopic examination of traditional preparations. In complicated diagnostic cases, we performed an ICC examination using different manufacturers' mono- or polyclonal antibody panels. The most common antibody panels contained general cytokeratins (AE1/AE3), Ber-EP4 epithelial antigen, cancer-embryonic antigen (CEA), epithelial membrane antigen (EMA), mesothelial antigen HBME-1, Vimentin, Calretinin, Mesothelin, thyroid transcription factor-1 (TTF-1), Cytokeratins (CK) 7, 20, 5/6, Napsin, CA-125, and Wilms tumor marker (WT-1).

Results: The number of cytological examinations performed in the CDL decreased by 34% in 2020 compared to 2019, as shown in Table 1. This was due to epidemiological limitations caused by the new coronavirus infection, COVID-19. In 2021, the number of examinations increased by 12% compared to 2020.

Table 1 – The number of cytological examinations performed in the clinical diagnostic laboratory of GBUZ "Scientific Research Institute – Regional Clinical Hospital No. 1 after S.V. Ochapovsky" (2018-2021)

Cytological material	2018		2019		2020		2021	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Total examinations	48,308	100	47,403	98	31,911	66	38,030	78

According to the retrospective analysis of morphological examination results obtained from 2019 to 2021, metastatic lesions of serous cavities (pleural and abdominal) in the exudative fluids in women were mostly diagnosed by traditional cytology – 672 cases (58%) of the total number of metastatic lesions of serous cavities. This was mainly due to breast cancer progression (26%). The immunocytochemical pattern versus IHC in breast cancer metastases is presented in Figure 1. In men, metastatic pleurisy was mainly caused by lung ADC metastasis – 266 cases (23%). The immunocytochemical pattern of lung cancer metastasis is shown in Figure 2.

By morphological structure, ADCs were more frequent (75, 54.3% of cases) than squamous cell cancer (35, 25.36%). Other histological forms were less frequent and included small cell lung cancer (16, 1.59%) and neuroendocrine tumors (12, 8.7%) (Table 2).

Table 2 – Verification of primary lung cancer diagnosed by cytology and ICC and confirmed by histopathology and IHC

Squamous cell carcinoma	Adenocarcinoma	Small cell cancer	Neuroendocrine tumor	Total
35 (25.36%)	75 (54.35%)	16 (11.59%)	12 (8.70%)	138 (100%)

Table 2 compares the results of the routine cytological examination and ICC examination with histological and IHC examination for cytological material obtained from lung tumors, lymph nodes of the mediastinum, and pleura to estimate the accuracy of cytological and ICC examinations for tumors of various histogenesis.

The most significant difficulties in the differential diagnosis of cells arose in inflammatory processes. In most cases, mesothelium acquires signs of atypia and polymorphism, which may cause an erroneous assumption of tumor presence.

In all cases when cytology supposed a malignant neoplasm, we conducted an ICC examination using Cytokeratin AE1/AE3 and/or CD 45 (LCA) and Vimentin. CK AE1/AE3 (+) and Vimentin (-) indicated a malignant neoplasm of epithelial nature. In those cases, we conducted an ICC exam-

ination with TTF1 antigens (a marker for lung ADC and thyroid cancer). TTF1(+) and thyroglobulin (Tg) (-) indicated lung ADC. Notably, lung tumor cells are always Tg-negative. TTF1 (+) and Tg (+) suggested thyroid cancer.

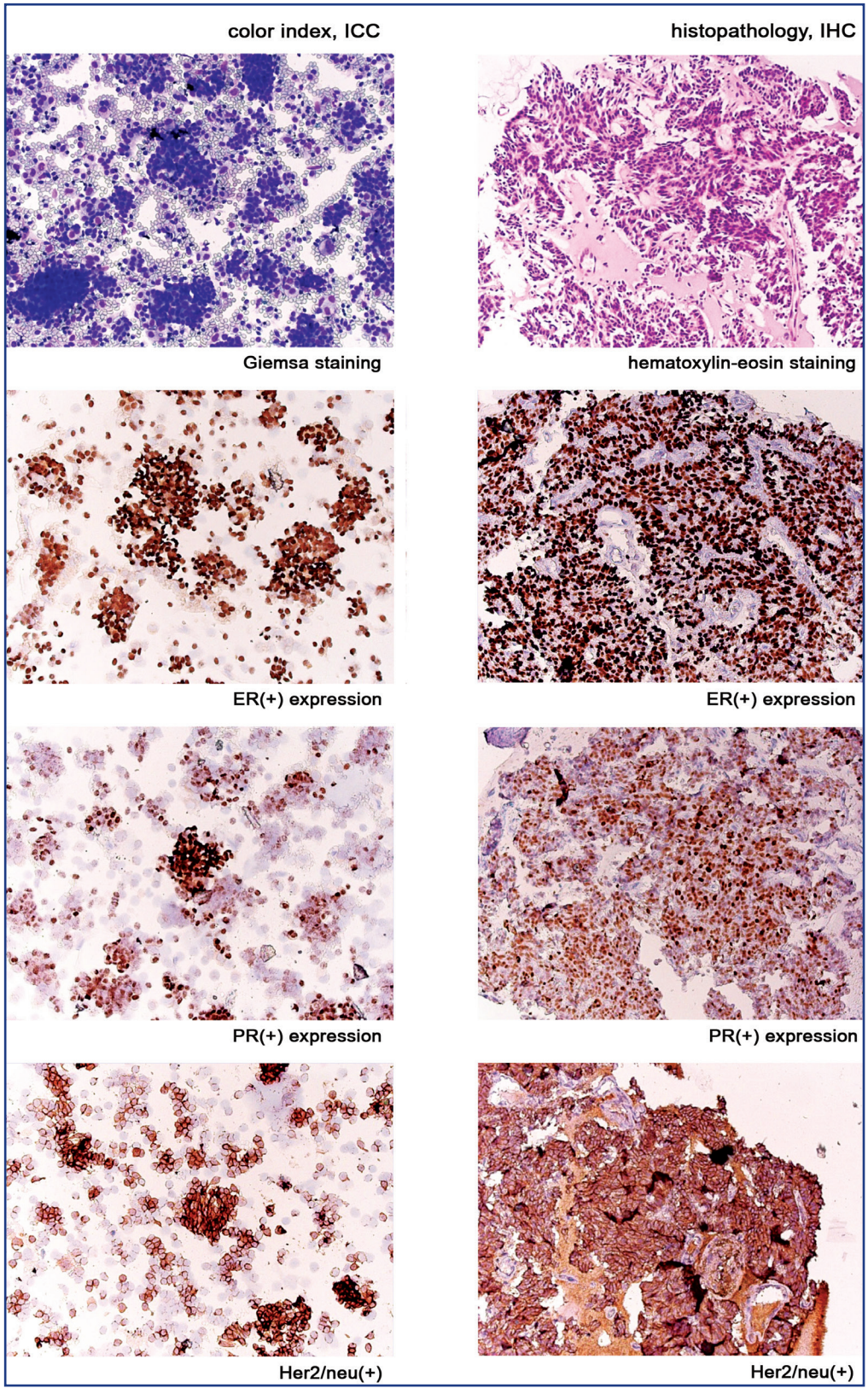

Figure 1 – ICC examination: Breast cancer metastases, ×10

The conducted morphological examination of 5,800 exudates collected over the past three years revealed that the exudative fluid in serous cavities was due to the presence of a malignant process in 20% of cases, had an inflammatory genesis in 30%, and a lymphoid origin – in 35% (Table 3).

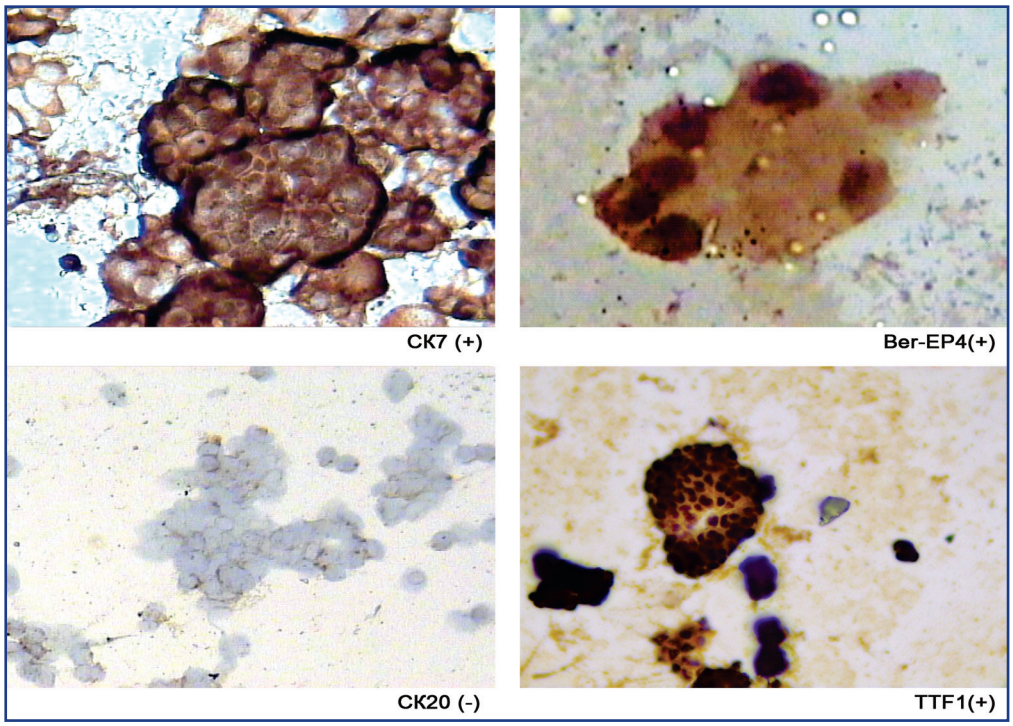

Figure 2 – ICC examination: Lung adenocarcinoma metastases

Table 3 – Morphological characteristic of exudative fluids (n = 5,800)

Nature of the exudate	Quality of examinations	%
Lymphoid exudate	2,030	35
Inflammatory exudate	1,740	30
Exudate with the presence of malignant neoplasm	1,160	20
Other	870	15
Total	5,800	100

Discussion: Thus, in complex cases, ICC increases the diagnostic accuracy of cytology from 62% to 93% and the specificity from 95% to 99%.

In pulmonary tuberculosis, pneumonia-caused pleurisy, and transudate syndrome in patients with cardiovascular insufficiency, a cytological examination is enough for properly classifying pleural exudate by cellular composition. The exudative fluid in patients with non-tumor pleurisy contains mesothelial-lymphocytic, granulocytic-macrophage, and macrophage-histiocytic cells.

Conclusion: Cytological examination of transudates and exudates of serous cavities is a routine daily procedure in cytological laboratories and a method for morphological diagnostics of pathological processes. Examining exudative fluids informs a physician about the exudate pathogenesis to correctly choose treatment tactics and predict the dynamics of disease development.

An established productive clinical-laboratory dialogue significantly increases the informational value of cytological diagnosis. We analyzed the results obtained from different approaches using different sets of mono- or polyclonal antibody panels to determine the tumor histological form and organ affiliation and developed an algorithm for exudative fluids' ICC examination. Namely, a cytologist reviews the material prepared by the traditional method and can make weighted step-by-step decisions on further diagnostic actions. In certain cases, ICC examination with 2-3 antibodies may be enough to determine the tumor's histological form and organ affiliation. If necessary, additional ICC examinations should be performed without significant loss of time in obtaining the results.

References:

1. Leonov M.G., Novik V.I., Belyaeva S.A. Citologicheskaya diagnostika raka yaichnikov: posobie dlya vrachej. - Krasnodar: OOO «Tri-Mil», 2016. - 28 s. [Leonov M.G., Novik V.I., Belyaeva S.A. Cytological diagnosis of ovarian cancer: a guide for physicians. - Krasnodar: Tri-Mil LLC, 2016. - 28 p. (in Russ.)].

2. Egan A.M., McPhillips D., Sarkar S., Breen D.P. Malignant pleural effusion // QJM. – 2014. – Vol. 107 (3). – P.179-184. https://doi.org/10.1093/qjmed/hct245.

3. Borisova O.V. Sovremennye vozmozhnosti citologicheskogo metoda pri issledovanii e'kssudatov iz seroznyx polostej: dis. … kand. med. nauk. - Moskva, 2010. - 194 s. [Borisova O.V. Modern possibilities of the cytological method in the study of exudates from serous cavities: dis. … cand. honey. Sciences. - Moscow, 2010. - 194 p. (in Russ.)]. https://www.dissercat.com/content/sovremennye-vozmozhnosti-tsitologicheskogo-metoda-v-issledovanii-plevralnykh-i-peritonealnyk.

4. Volchenko N.N., Borisova O.V. Diagnostika zlokachestvennyx opuxolej po seroznym polostyam: citologicheskij atlas. - M.: «GE'OTAR-Media», 2017. - 144 s. [Volchenko N.N., Borisova O.V. Diagnosis of malignant tumors by serous cavities: a cytological atlas. - M.: "GEOTAR-Media," 2017. - 144 p. (in Russ.)]. https://www.labirint.ru/books/559863/.

АҢДАТПА

ИЦХ КӨМЕГІМЕН ПЛЕВРАДАН ЭФФУЗИЯЛЫ СҰЙЫҚТЫҚТАРДЫ ЦИТОЛОГИЯЛЫҚ ЗЕРТТЕУ КЕЗЕҢДЕРІ

Қ.С. Павлюк[1], М.Г. Леонов[2,3], А.В. Акобян[1], Т.В. Синицкая[1], О.В. Госпирович[1], Е.А. Артемова[1], Ж.Б.Елеубаева[4]

[1]«Ғылыми-зерттеу институты – С.В. Очаповский атындағы №1 облыстық клиникалық ауруханасы» ГБУЗ, Краснодар, Ресей Федерациясы;
[2]«№3 онкологиялық диспансер» ГБУЗ, Новороссийск, Ресей Федерациясы;
[3]«Кубан мемлекеттік медицина университеті» Федералдық мемлекеттік бюджеттік жоғары оқу орны, Краснодар, Ресей Федерациясы;
[4]«Қазақ онкология және радиология ғылыми-зерттеу институты» АҚ, Алматы, Қазақстан Республикасы

Өзектілігі: *Экссудатты сұйықтықтардағы ісіктердің цитологиялық критерийлері белгілі бір субъективті қиындықтармен байланысты, олардың бірі пролиферацияланатын мезотелий жасушалары мен аденокарцинома жасушаларының дифференциалды диагностикасы болып табылады.*

Зерттеудің мақсаты *– көпсалалы аурухана жағдайында цитологиялық диагностиканың ақпараттылығын арттыру.*

Әдістері: *2018-2021 жылдар аралығы Цитологиялық зерттеуге 10 082 серозды қуыстар эффузиясы (плевра – 8 166 (81%), құрсақ қуысы – 1512 (15%), перикардиальды – 404 (4%)) жатқызылды. Дәстүрлі препараттарды микроскопиялық зерттеу жүргізілді, қиын диагностикалық жағдайларда ІСС зерттеуі жүргізілді.*

Нәтиже: *Зерттеудің талдауы көрсеткендей, әйелдерде эффузия сұйықтығында дәстүрлі цитологиялық әдіспен 672 жағдайда (58%), негізінен сүт безі қатерлі ісігінің (26%) өршуіне байланысты серозды қуыстардағы метастатикалық зақымданулар, ал ерлерде, плеврит негізінен өкпенің аденокарциномасының метастазына байланысты болды – 266 жағдай (23%). ІСС зерттеулерін қолдану цитологиялық әдістің диагностикалық дәлдігін 62%-дан 93%-ға және ерекшелігін 95%-дан 99%-ға дейін арттырады.*

Қорытынды: *Гистологиялық пішінді және органды - ісік көзін анықтау үшін қолданылатын моноклональды антиденелердің панельдерінің санымен ерекшеленетін ІСС зерттеулерін жүргізу үшін алгоритм әзірленді. Арнайы жағдайларда 2-3 моноклоналды антиденелермен ІСС зерттеулері ісіктің гистологиялық түрін растау үшін жеткілікті болуы мүмкін және қажет болған жағдайда нәтижелерді алу үшін уақытты айтарлықтай жоғалтпай қосымша ІСС зерттеулерін жүргізеді.*

Түйінді сөздер: *иммуноцитохимия (ІСС), моноклоналды антиденелер, қатерлі ісіктер, плевра сұйықтығы, асциттік сұйықтық, дәстүрлі цитология, сұйықтық негізіндегі цитология.*

АННОТАЦИЯ

ЭТАПЫ ЦИТОЛОГИЧЕСКОГО ИССЛЕДОВАНИЯ (С ПРИМЕНЕНИЕМ ИЦХ) ВЫПОТНЫХ ЖИДКОСТЕЙ

К.С. Павлюк[1], М.Г. Леонов[2,3], А.В. Акобян[1], Т.В. Синицкая[1], О.В. Госпирович[1], Е.А. Артемова[1], Ж.Б. Елеубаева[4]

[1]ГБУЗ «Научно-исследовательский институт – Краевая клиническая больница №1 им. проф. С.В. Очаповского», Краснодар, Российская Федерация;
[2]ГБУЗ «Онкологический диспансер №3», Новороссийск, Российская Федерация;
[3]ФГБОУ ВО «Кубанский государственный медицинский университет», Краснодар, Российская Федерация;
[4]АО «Казахский научно-исследовательский институт онкологии и радиологии», Алматы, Республика Казахстан

Актуальность: *Цитологические критерии опухолей в выпотных жидкостях связаны с определенными субъективными трудностями, одной из которых является дифференциальная диагностика клеток пролиферирующего мезотелия и клеток аденокарциномы.*

Цель исследования *– повышение информативности цитологической диагностики в условиях многопрофильной больницы.*

Методы: *За период 2018-2021 гг. цитологическому исследованию было подвергнуто 10 082 образца выпотных жидкостей серозных полостей (плевральной – 8 166 (81%), абдоминальной – 1 512 (15%), перикардиальной – 404 (4%)). Проводилось микроскопическое исследование традиционных препаратов, в сложных диагностических случаях выполнялось иммуноцитохимическое (ИЦХ) исследование.*

Результаты: *Традиционный цитологический метод анализа выпотных жидкостей показал наличие метастатических поражений серозных полостей у женщин в 672 случаях (58%), главным образом за счет прогрессирования рака молочной железы (26%). У мужчин в основном регистрировались плевриты за счёт метастазирования аденокарциномы легкого – 266 случаев (23%). Применение ИЦХ исследования повышало диагностическую точность цитологического метода с 62% до 93% и специфичность с 95% до 99%.*

Заключение: *Разработан алгоритм проведения ИЦХ исследований, отличающихся по количеству используемых панелей моноклональных антител, для определения гистологической формы и органа – источника опухоли. В конкретных случаях проведения ИЦХ исследований с 2-3 моноклональными антителами может быть вполне достаточно для подтверждения гистологической формы опухоли. При необходимости, можно выполнить дополнительные ИЦХ исследования без значительных потерь времени на получение результатов.*

Ключевые слова: *иммуноцитохимия (ИЦХ), моноклональные антитела, злокачественные новообразования, плевральная жидкость, асцитическая жидкость, традиционная цитология, жидкостная цитология.*

Transparency of the study: *Authors take full responsibility for the content of this manuscript.*
Conflict of interest: *Authors declare no conflict of interest.*
Financing: *Authors declare no financing of the study.*
Authors' input: *contribution to the study concept – Pavlyuk K.S., Yeleubayeva Zh.B.; study design – Leonov M.G.; execution of the study – Pavlyuk K.S., Gospirovich O.V., Artemova E.A.; interpretation of the study – Akobyan A.V., Sinitskaya T.V.; preparation of the manuscript – Pavlyuk K.S., Leonov M.G.*
Authors' data:
Pavlyuk K.S. – biologist at CDL, GBUZ "Scientific Research Institute – Regional Clinical Hospital No. 1 after S.V. Ochapovsky," Leading Specialist of the CE&ICC Department, Krasnodar, Russian Federation, tel. +79189785349, e-mail: karlygash@nextmail.ru, ORCID ID: https://orcid.org/0000-0001-5059-0289;
Leonov M.G. – M.D., Associate Professor, Department of Oncology with a Course of Thoracic Sur-gery, Federal State Budgetary Educational Institution of Higher Professional Education "Kuban State Medical University," Head Physician of Oncological dispensary No. 3, Novorossiysk, Russian Federation, tel. +79184839444, e-mail: novonko@yandex.ru, ORCID ID: https://orcid.org/0000-0001-9658-4247;
Akobyan A.V. – Thoracic Surgeon of Thoracic Surgery Department-2, GBUZ "Scientific Research Institute – Regional Clinical Hospital No. 1 after S.V. Ochapovsky," Krasnodar, Russian Federation, tel. +79182496652, e-mail: Akobyan_84@mail.ru, ORCID ID: https://orcid.org/0000-0002-2710-2651;
Sinitskaya T.V. – Pulmonologist, GBUZ "Scientific Research Institute – Regional Clinical Hospital No. 1 after S.V. Ochapovsky," Krasnodar, Russian Federation, tel. +79280421650, e-mail: t_sinickaya@mail.ru, ORCID ID: https://orcid.org/0000-0001-6292-7491;
Gospirovich O.V. – Biologist at CDL, GBUZ "Scientific Research Institute – Regional Clinical Hospi-tal No. 1 after S.V. Ochapovsky," Krasnodar, Russian Federation, tel. +79180923729, e-mail: olga-cito@rambler.ru, ORCID ID: https://orcid.org/0000-0002-2777-8205;
Artemova E.A. – Biologist at CDL, GBUZ "Scientific Research Institute – Regional Clinical Hospital No. 1 after S.V. Ochapovsky," Krasnodar, Russian Federation, tel. +79180423442, e-mail: ale-ka.krd@gmail.com, ORCID ID: https://orcid.org/0000-0002-2620-2917;
Yeleubayeva Zh.B. (corresponding author) – Cytopathologist at the Center for morphological studies of "KazIOR" JSC, **Almaty, 050000, Abai Ave., 91, the Republic of Kazakhstan**, tel. +77051339279, e-mail: zhana-ra66@gmail.com, ORCID ID: https://orcid.org/0000-0001-6565-4695.