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Gastric Precancerous Lesions: From Progenitor Cell and Microsatellite Instability to Clinical Interpretation of Gastric Cancer Risk

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Aim: to evaluate the possibility of the MMR-system status, microsatellite instability (MSI) usage in the differential diagnosis of gastric mucosa dysplasia, determination of the gastric adenocarcinoma development risk.
Material and methods. The study included gastric mucosa specimens of 75 patients: 25 with high-grade dysplasia, 25 with low-grade dysplasia, 25 were indefinite for dysplasia. Gastrobiopsy specimens were examined histologically, immunohistochemically using mouse monoclonal antibodies (Diagnostic BioSystems, USA) to the MMR system proteins: MLH-1 (clone G168-15, dilution 1:50), MSH2 (clone DBM15.82, dilution 1:100), MSH6 (clone 44, dilution 1:50), PMS2 (clone A16-4, ready to use). MSI was studied with multiplex PCR evaluation of DNA microsatellites (NR-21, NR-24, NR-27, BAT-25, BAT-26) from paraffin sections, their analysis with capillary electrophoresis. The obtained data were processed with the Statistica 10.0 (StatSoft, USA), presented using descriptive, analytical statistics. VOSviewer (1.6.20) was used to visualize the bibliometric analysis.
Results. MMR-deficient cases were found in low (2.8 %) and high-grade (2.8 %) dysplasia with the immunohistochemical evaluation of MMR-system proteins in gastric mucosa specimens. In all indefinite for dysplasia cases MMR-system proteins remained unaffected. Three MSI-positive cases (6.5 %) were detected by PCR with two low-grade dysplasia, one high-grade dysplasia cases. All identified cases were also immunohistochemically MSI-positive. Conclusion. Determination of MSI can be used as an auxiliary study within a panel of biomarkers aimed to support the decision-making of a pathologist in the alternative of “indefinite for dysplasia” or “definite dysplasia — obligate precancer”.
Keywords: microsatellite instability, chronic gastritis, precancerous lesions, intestinal metaplasia, atrophy, gastric cancer, cancer prediction
Conflict of interest: the study was carried out with the financial support of a grant from the Russian Science Foundation “Microsatellite instability in the gastric mucosa during early and pronounced precancerous changes as a molecular genetic basis for personalized assessment of the risk of developing stomach cancer”, registration No. 23–25–10036; the agreement with the Ministry of Industry and Scientific and Technical Development of the Omsk Region No. 33-s dated 19.06.2023.

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Предраковые изменения слизистой оболочки желудка: от прогениторной клетки и микросателлитной нестабильности к клинической интерпретации риска рака желудка

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Цель: оценка возможности использования статуса белков системы mismatch repair (MMR), микросателлитной нестабильности при предраковых изменениях слизистой оболочки желудка в дифференциальной диагностике дисплазии, определении риска развития рака желудка.
Материал и методы. В исследование включены биоптаты слизистой оболочки желудка 75 пациентов, у 25 из которых диагностирована дисплазия слизистой оболочки желудка высокой степени, у 25 — дисплазия низкой степени, у 25 — неопределенная дисплазия. Гастробиоптаты исследовали гистологическим, иммуногистохимическим методами с использованием мышиных моноклональных антител (Diagnostic BioSystems,

США) к белкам системы MMR: MLH-1 (клон G168-15, разведение 1:50), MSH2 (клон DBM15.82, разведение 1:100), MSH6 (клон 44, разведение 1:50), PMS2 (клон A16-4, готовые к применению). MSI исследовали методом мультиплексной ПЦР с получением ДНК-фрагментов микросателлитов (NR-21, NR-24, NR-27, BAT-25, BAT-26) из парафиновых срезов и их анализа методом капиллярного электрофореза. Полученные данные обработаны с применением пакета Statistica 10.0 (StatSoft, США), представлены с использованием методов описательной, аналитической статистики. Для визуализации библиометрического анализа использовали VOSviewer (1.6.20).

Результаты. При иммуногистохимической оценке экспрессии белков системы mismatch repair в биоптатах слизистой оболочки желудка MMR-дефицитные случаи обнаружены при дисплазии низкой (2,8 %) и высокой (2,8 %) степени. Во всех случаях неопределенной дисплазии сохранялся профицит системы MMR. При оценке методом ПЦР обнаружено три MSI-позитивных случая (6,5 %), из которых два соответствовали дисплазии низкой степени, один — высокой степени. Все выявленные случаи также расценены иммуногистохимически как MSI-позитивные.

Выводы. Определение статуса микросателлитной нестабильности может быть использовано как вспомогательное исследование в рамках панели биомаркеров, направленной на поддержку принятия решения врачом-патологоанатомом в альтернативе «неопределенная дисплазия эпителия» или «определенно дисплазия — облигатный предрак».

Ключевые слова: микросателлитная нестабильность, хронический гастрит, предраковые изменения, кишечная метаплазия, атрофия, рак желудка, канцерпревенция

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It is widely believed that progress in gastric cancer research was such significant in the last 20 years that it has retreated. However, despite great attention to this disease, gastric cancer still occupies the sixth place in the structure of malignant neoplasms in Russia and is among the ten most common malignant tumors in the world [1]. The mortality rate from this cancer remains dramatically high: about 43 % of patients die within a year of diagnosis and only 30 % overcome the five-year survival barrier [1–3]. The reason is the high level of untimely diagnosis: in 2021 the average rate of tumor detection at stages III–IV in Russia was 60.7 % according to the P. Hertsen Moscow Oncology Research Institute [1, 2].

Microsatellite instability (MSI) is a consequence of an absolute deficiency or defect in the DNA mismatch repair system (MMR) gene function: MSH2, MSH3, MSH5, MSH6, MLH1, PMS1 (MLH2), MLH3 and/or PMS2 (MLH4) [4]. The role of MSI was first identified in the molecular pathogenesis of hereditary nonpolyposis colon cancer study [5]. The function of the MMR system is to eliminate errors in the DNA structure appearing in tandem stereotypical repeats of nucleotide groups (microsatellites) during replication, leading to the formation of extra chains of unpaired nucleotides [4, 6]. The MMR system proteins encoded by the eponymous genes form dimeric complexes recognizing DNA defects and remove them, thereby ensuring the restoration of DNA by its daughter strand.

The MSI presence in some gastric cancer cases is not surprising. Some studies have shown an association between *Helicobacter pylori (H. pylori)* infection and aberrant DNA methylation of the genes encoding MMR system proteins, which is probably due to the ability of bacteria to aberrantly methylate host cell DNA [7]. The MMR system defects inevitably lead to the accumulation of genetic aberrations, including those in the coding regions of the genome and consequently lead to the emergence of mutations in oncogenes, tumor suppressor genes, the cell cycle genes and pro-apoptotic genes. Moreover, sometimes MSI is a secondary event in carcinogenesis. For example, the mutant *p53* protein, a product of the eponymous gene (commonly referred to as the «guardian of the genome») is capable of affecting the MMR system and causing disruption of its functioning. This theory is supported by the discovery of correlations between some polymorphisms of the p53 gene and the MSI status [8–10].

There is a suggestion that MSI can be detected not only in cancer, but also in the gastric mucosa (GM) precancerous lesions. This observation naturally raises the question: could it be the cause of tumor transformation of incomplete intestinal metaplasia foci, which is often associated with adenocarcinoma according to epidemiological studies?

The aim of this study is to evaluate the possibility of the MMR-system status, microsatellite instability (MSI) usage in the differential diagnosis of gastric mucosa dysplasia and determination of the gastric adenocarcinoma development risk.

Material and methods

The study included gastric mucosa specimens of 75 patients (41 women, 34 men): 25 — with high-grade dysplasia, 25 — with low-grade dysplasia, 25 were indefinite for dysplasia. Exclusion criteria were insufficient volume of diagnostically significant material in the paraffin block, pronounced artificial changes and fragmentation of GM specimens. The patients age at the time of biopsy ranged from 28 to 87 years (median — 66 years). The biopsy sites localization was antral in 56 cases, in the gastric corpus in 19 cases.

The study protocol was approved by the Local Ethics Committee of the Omsk State Medical University (protocol No. 04 dated March 24, 2023).

Low-grade, high-grade, and indefinite for dysplasia diagnosis was carried out based on the histological differential diagnosis criteria (Table).

In high-grade dysplasia, 23 cases were diagnosed as intestinal-type adenoma, 1 case as foveolar-type adenoma, and 1 — as pyloric gland adenoma.

In low-grade dysplasia, 16 cases were diagnosed as intestinal-type adenoma, 4 cases as foveolar-type adenoma, 4 — as pyloric gland adenoma, and 1 case had serrated phenotype.

Cases were classified as indefinite for dysplasia in the absence of histological features required to reliably differentiate dysplasia from reactive or regenerative GM changes, in small biopsy specimens and/or in cases of high inflammation. Intestinal metaplasia was detected in 13 samples indefinite for dysplasia: complete (type I) in 9 cases, incomplete (type II, III) in 4 cases. In 5 samples hyperproliferative intestinal metaplasia was noted. *H. pylori* colonization was detected in 7 of 25 cases. In 5 cases inflammation was weak, in 14 — moderate, and in 6 cases — severe. Inflammation was absent in 8 cases, weak — in 12 cases, moderate — in 4 cases, and severe — in 1 case.

Histological processing, embedding in paraffin, preparation of paraffin sections and staining with hematoxylin and eosin were carried out according to the generally accepted method. The severity of inflammation and atrophy in GM biopsies was assessed using visual analogue scales of the Russian revision of the OLGA staging system [12]. Histochemical subtyping of intestinal metaplasia was carried out using a combined histochemical technique of brush border staining, typing of mucins (Alcian blue, Periodic acid—Schiff stain (PAS reaction), iron diamine) [13].

Immunohistochemical reactions were performed on paraffin sections using mouse monoclonal antibodies (Diagnostic BioSystems, USA) to the MMR system proteins: MLH-1 (clone G168-15, dilution 1:50), MSH2 (clone DBM15.82, dilution 1:100), MSH6 (clone 44, dilution 1:50), PMS2 (clone A16-4, ready to use). The PolyVue Plus HRP/DAB detection system (Diagnostic BioSystems, USA) was used. Deparaffinization and rehydration, high-temperature antigen retrieval using EDTA buffer (pH 8.0) and incubation with antibodies were performed according to the manufacturer's protocol. Lymphocytes of the inflammatory infiltrate, as well as stromal cells, were used as an internal positive control.

Nuclear immunohistochemical staining of all four MMR proteins (MLH1, PMS2, MSH2 and MSH6) in epithelial cells, lymphocytes of the GM inflammatory infiltrate were considered as an intact MMR system (MMR-proficient, pMMR). Absent immunohistochemical staining of one or more MMR proteins in the nuclei of epithelial cells and the presence of staining in the inflammatory infiltrate lymphocytes, GM stromal cells were considered as a deficient MMR system (MMR-deficient, dMMR). The intensity of nuclear staining was not considered.

MSI diagnostics were performed by multiplex PCR in the PCR laboratory of Vector-Best JSC, Novosibirsk. DNA extraction was performed using the RbMag automated complex (BRAF-600 protocol). To obtain DNA fragments of microsatellites (NR-21, NR-24, NR-27, BAT-25, BAT-26) after multiplex amplification, the amplicons mixture was analyzed by capillary electrophoresis on an Applied Biosystems 3500 genetic analyzer with POP-7 polymer and 50-cm capillaries.

The obtained data visualization was performed using the GeneMapper program, microsatellite markers were visualized in blue (NR-24, BAT-26) and yellow (NR-27, NR-21, BAT-25) detection channels. Instability in two or more markers was considered as the MSI-positive case, instability of one marker or less was assessed as an MSI-stable case.

Microphotographs were taken with an Axiocam 503 color camera, an Axioscope 40 microscope, and image processing was performed using the ZEISS ZEN software package (Carl Zeiss, Germany).

Table. Histopathology of differential diagnosis of neoplasia/dysplasia of the columnar epithelium of the digestive tract mucosa [11]

Таблица. Гистопатология дифференциальной диагностики неоплазии/дисплазии цилиндрического эпителия слизистой оболочки пищеварительного тракта [11]

Feature ***Признак***	**Neoplasia category / *Категория неоплазии***			
	Indefinite neoplasia ***Неопределенная неоплазия***		**Low grade neoplasia** ***Неоплазия низкой степени***	**High grade neoplasia** ***Неоплазия высокой степени***
	Atypical foveal hyperplasia ***Атипическая фовеолярная гиперплазия***	**Hyperpro liferative intestinal metaplasia/ atrophy** ***Гиперпролиферативная кишечная метаплазия/ атрофия***		
1. Histoarchitecture *Гистоархитектоника*	Pits enlarged, widened, impression of an increase in their number; glands are not changed *Ямки увеличены, расширены, складывается впечатление об увеличении их числа; железы не изменены*	Focal or total replacement by intestinal epithelium *Очаговое или тотальное замещение кишечным эпителием*	The glands are round in shape, sometimes oval, irregular in shape, grouped into distinct foci that differ from the surrounding mucosa *Железы округлой формы, встречаются овальные, неправильных очертаний, группируются в отчетливые фокусы, отличающиеся от окружающей слизистой оболочки*	Dense arrangement of glands “back to back”, false and true papillae, only a few unchanged glands are found *Плотное расположение желез «спина к спине», ложные и истинные сосочки, встречаются лишь единичные неизмененные железы*
2. Localization of atypical cells *Локализация атипичных клеток*	Foveal zone *Фовеолярная зона*	Only the deep parts of the glands *Только глубокие отделы желез*	Foveal zone, superficial and deep glands *Фовеолярная зона, поверхностные и глубокие отделы желез*	Foveal zone, superficial and deep glands *Фовеолярная зона, поверхностные и глубокие отделы желез*
3. Zone of location of differentiated cells *Зона расположения*	Below the glands and at the top of the ridges *Внизу желез и на верхушке валиков*	Only at the top of the rollers *Только на верхушке валиков*	May be present in the superficial parts of the mucous membrane *Может быть представлена в поверхностных отделах слизистой оболочки*	Usually absent *Обычно отсутствует*
4. Shape and size of cells *Форма и размеры клеток*	Any type *Любая*	Elongated *Удлиненная*	Elongated *Удлиненная*	Polymorphic *Полиморфная*
5. Mucus secretion *Секреция слизи*	Retained *Сохранена*	Retained *Сохранена*	Reduced but present *Снижена, но присутствует*	Usually absent *Обычно отсутствует*
6. Size of the nucleus *Размеры ядра*	Moderately enlarged, large ones occur *Умеренно увеличены, встречаются крупные*	Moderately enlarged, large ones occur *Умеренно увеличены, встречаются крупные*	Moderately enlarged, large ones occur *Умеренно увеличены, встречаются крупные*	Marked increase *Выраженное увеличение*

End of table. Histopathology of differential diagnosis of neoplasia/dysplasia of the columnar epithelium of the digestive tract mucosa [11]

Окончания таблицы. Гистопатология дифференциальной диагностики неоплазии/дисплазии цилиндрического эпителия слизистой оболочки пищеварительного тракта [11]

7. Shape of the nucleus *Форма ядра*	Round *Округлая*	Round *Округлая*	Round or elongated *Округлая или вытянутая*	Polymorphic *Полиморфная*
8. Mitoses *Митозы*	Absent *Нет*	Occur *Встречаются*	Occur *Встречаются*	Occur frequently *Встречаются часто*
9. Nucleoli *Ядрышки*	Rarely encountered, indistinct *Встречаются редко, неотчетливые*	Rarely encountered, indistinct *Встречаются редко, неотчетливые*	Rarely encountered, distinct, no more than two *Встречаются редко, отчетливые, не более двух*	Occurs frequently, more than two can be encountered *Встречаются часто, бывает более двух*
10. Nucleus polarity *Полярность ядра*	Present: basal *Присутствует: базальная*	Present: basal *Присутствует: базальная*	Typically present: basal *Как правило, присутствует: базальная*	Loss of polarity: middle and/or apical part of the cell (pseudostratification) *Утрата полярности: средняя и/или апикальная часть клетки (псевдостратификация)*
11. Nuclear membrane *Ядерная мембрана*	Regular structure *Регулярного строения*	Regular structure *Регулярного строения*	Usually of regular structure *Обычно регулярного строения*	Often irregular in structure, there are areas of thickening *Часто нерегулярного строения, есть зоны утолщения*
12. Nuclear hyperchromia *Гиперхромия ядра*	Expressed *Выраженная*	Moderate *Умеренная*	Moderate *Умеренная*	Severe or hypochromia *Выраженная или гипохромия*

The obtained data were processed with the Statistica 10.0 (StatSoft, USA). Descriptive statistics were presented as median, interquartile range, maximum and minimum values, percentage ratio (nominal data). Analytical statistics were performed using Fisher's exact test, Mann — Whitney test and contingency tables. VOSviewer (1.6.20) was used to visualize the bibliometric analysis.

Results

Assessment results of immunohistochemical expression of MMR system proteins in gastric mucosa specimens with low-grade, high-grade and indefinite for dysplasia

Immunohistochemical evaluation of MMR system proteins expression in GM biopsies revealed MMR-deficient cases in both high- and low-grade dysplasia. MMR system deficiency was detected in only 4 of 50 samples (8 %) with low- and high-grade dysplasia (Fig. 1).

Comparing the incidence of MMR system deficiency in high-grade (2 cases) and low-grade (2 cases) dysplasia, the incidence was the same, no significant differences were found. All MMR-deficient cases had the intestinal dysplasia phenotype according to the WHO-2019 criteria.

Immunohistochemical expression of the MLH1, MSH2, MSH6 and PMS2 proteins was preserved in all GM specimens, indefinite for dysplasia regardless of the inflammation severity, activity, presence or absence of intestinal metaplasia. This allowed us to conclude that all cases indefinite for dysplasia was MMR-proficient (Fig. 2).

PCR assessment of microsatellite instability in gastric mucosa specimens with low-grade, high-grade and indefinite for dysplasia

There were 23 indefinite for dysplasia (intraepithelial neoplasia) cases with valid results in the PCR MSI status assessment. All cases were microsatellite-stable (MSS) regardless of the presence of atrophy, the presence and type of intestinal metaplasia, and the severity of the GM lamina propria inflammation.

In GM specimens with pronounced precancerous lesions (low- and high-grade dysplasia) valid PCR results were obtained for 46 cases. There

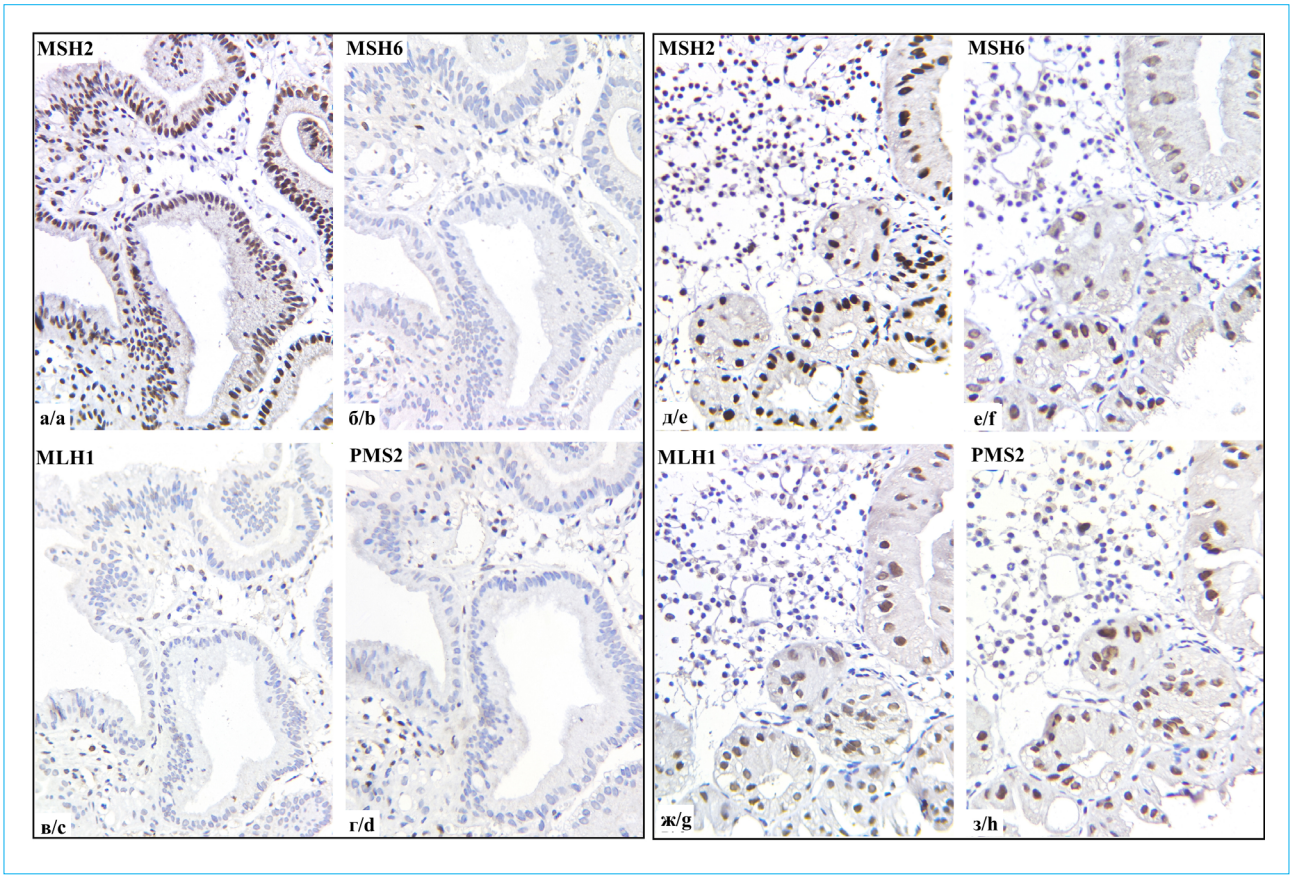

Figure 1. Immunohistochemical expression of MMR system proteins in gastric mucosa specimen with low-grade dysplasia (a–d) and gastric mucosa specimen with high-grade dysplasia (e–h): a — preserved nuclear expression of the MSH2 protein, ×400; b — loss of MSH6 protein expression, ×400; c — loss of MLH1 protein expression, ×400; d — loss of PMS2 protein expression, ×400; e — preserved nuclear expression of the MSH2 protein, ×400; f — preserved nuclear expression of the MSH6 protein, ×400; g — preserved nuclear expression of the MLH1 protein, ×400; h — preserved nuclear expression of the PMS2 protein, ×400

Рисунок 1. Иммуногистохимическая экспрессия белков системы MMR в образце СОЖ с дисплазией низкой степени (а–г) и образце СОЖ с дисплазией высокой степени (д–з): а — сохранная ядерная экспрессия белка MSH2, ×400; б — утрата экспрессии белка MSH6, ×400; в — утрата экспрессии белка MLH1, ×400; г — утрата экспрессии белка PMS2, ×400; д — сохранная ядерная экспрессия белка MSH2 , ×400; е — сохранная ядерная экспрессия белка MSH6, ×400; ж — сохранная ядерная экспрессия белка MLH1, ×400; з — сохранная ядерная экспрессия белка PMS2, ×400

were three MSI cases (6.5 % of the group specimens). Two MSI cases were detected in GM specimens with low-grade dysplasia, and one in GM specimens with high-grade dysplasia. All MSI cases were classified as intestinal-type adenomas according to the phenotype. The distribution of MSI and MSS cases in the subgroups of low- and high-grade dysplasia had no significant differences ($p = 0.56$).

When compared, three of the four cases of dysplasia with MMR deficiency according to the immunohistochemical examination were also microsatellite instable by PCR. One case of high-grade dysplasia with loss of MMR expression detected by immunohistochemistry was microsatellite stable (MSS) by PCR.

When assessing the distribution depending on the patients' gender, all cases with MSI and dMMR were found in women. Microsatellite unstable and dMMR cases were statistically significantly found in older patients (median — 78 years) compared to MSS and pMMR cases (median — 66 years), $p = 0.027$ (Fig. 3).

The absence of MMR-deficiency detected among the indefinite for dysplasia (intraepithelial neoplasia) cases both by immunohistochemistry and by PCR supports the initial hypothesis of the MMR system defect occurrence specificity only in "definite" dysplasia, so it may be useful in the complex differential diagnostics approach of GM regenerative, reactive changes (specimens indefinite for dysplasia) and low-grade/high-grade

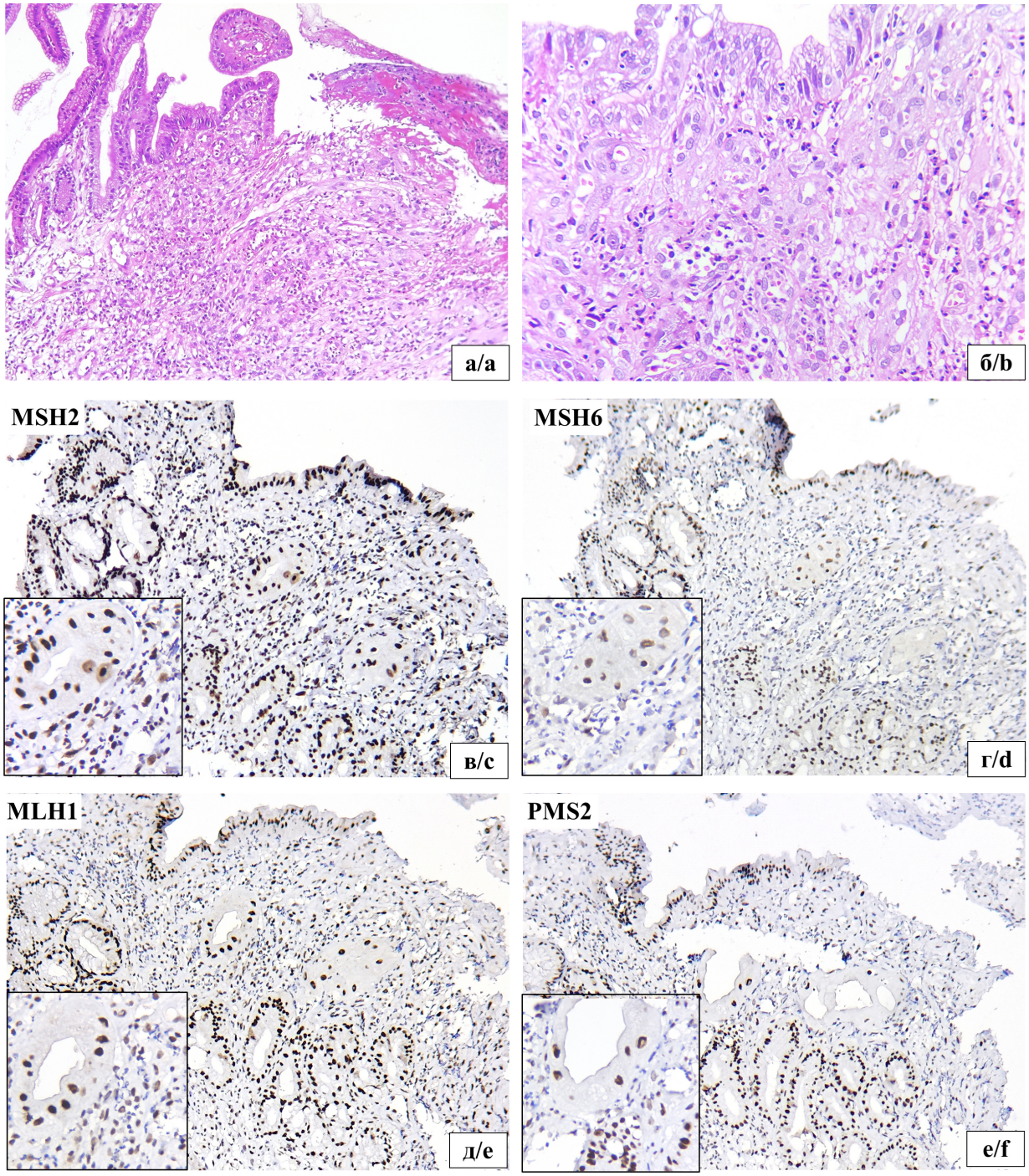

Figure 2. Immunohistochemical expression of MMR system proteins in gastric mucosa specimen with indeterminate dysplasia: a — hematoxylin and eosin staining, ×200; b — hematoxylin and eosin staining, ×400; c — preserved nuclear expression of the MSH2 protein; d — preserved nuclear expression of the MSH6 protein; e — preserved nuclear expression of the MLH1 protein; f — preserved nuclear expression of the PMS2 protein, ×200 (×400 insertion)

Рисунок 2. Иммуногистохимическая экспрессия белков системы MMR в образце СОЖ с неопределенной дисплазией: а — окраска гематоксилином и эозином, ×200; б — окраска гематоксилином и эозином, ×400; в — сохранная ядерная экспрессия белка MSH2; г — сохранная ядерная экспрессия белка MSH6; д — сохранная ядерная экспрессия белка MLH1; е — сохранная ядерная экспрессия белка PMS2, ×200 (врезка ×400)

dysplasia. However, the detection of MSI in both high-grade and low-grade GM dysplasia indicates the fair guess of its occurrence at the early stages of gastric carcinogenesis and may also be important for the MSI-associated gastric cancer development prognosis.

Discussion

The professional community of gastroenterologists, oncologists and pathologists pays close attention to GM early precancerous lesions. The ideas about them are based on the research of the American pathologist of Portuguese origin Pelayo Correa, who in 1988 suggested that the development of intestinal-type gastric cancer is preceded by a cascade of changes including chronic gastritis, GM intestinal metaplasia, atrophy and, finally, dysplasia (intraepithelial neoplasia) [14, 15]. Ideas about the GM cascade precancerous lesions have changed little over the past 35 years, having undergone only editorial revision. However, in recent decades, the emphasis has shifted from severe to early precancerous lesions (GM atrophy and intestinal metaplasia) as a "launch pad" for carcinogenesis, the most significant from the standpoint of cancer prediction [16–19].

While the role of absolute atrophy (absolute deficiency of the GM glands) in carcinogenesis has been repeatedly proven and recognized, the role of intestinal metaplasia (metaplastic atrophy) is more complex and is still being discussed [12, 20, 21]. Even the very essence of the process has repeatedly changed. Currently, metaplasia is understood as a process when a stem or progenitor cell of one tissue becomes a precursor of another progenitor cell.

What influenced the viewpoint of researchers on the problem of gastric cancer and why did the idea of intestinal metaplasia as an event significant for carcinogenesis arise again?

Gastric cancer is an extremely heterogeneous group of tumors (Fig. 4). There was a number of attempts to classify it in terms of its molecular profile. The most notable are the classifications of the Asian Cancer Research Group (ACRG) and the Cancer Genome Atlas (TCGA) [22, 23]. Interestingly, despite the differences between these classifications, both distinguish a separate category — gastric cancer with MSI [22–24]. Even the first ever Russian molecular classification of gastric cancer identifies cancer with MSI as a separate group [25].

Initially, attempts to attribute intestinal metaplasia as a possible direct precursor of adenocarcinoma were made, based on phenotypic similarity among other things. However, ideas about the role of metaplasia in the gastric cancer development quickly underwent significant changes. Leading researchers agreed that intestinal metaplasia does

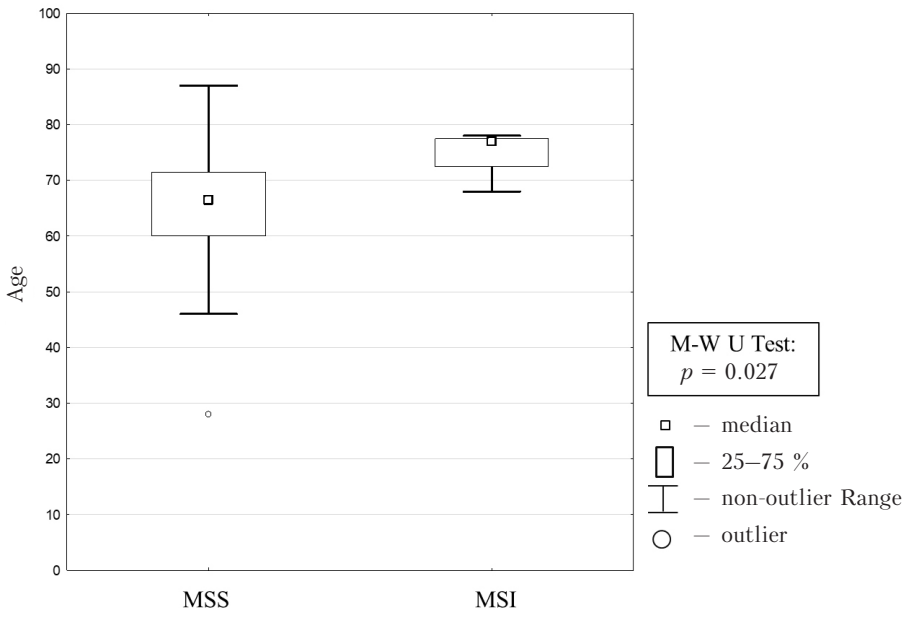

Figure 3. Age distribution of patients with MSS and MSI in the gastric mucosa with low- and high-grade dysplasia

Рисунок 3. Распределение пациентов по возрасту при MSS и MSI в слизистой оболочке желудка при дисплазии низкой и высокой степени

not act as a direct precursor of cancer, but only serves as a marker of persistent inflammation regardless of its etiology [27]. Long-term existence of inflammatory infiltrate is accompanied by damage to the genetic apparatus of gastric epithelial cells by cytokines and the development of genomic changes [28–30]. Accordingly, detection of intestinal metaplasia in gastric biopsy specimens is advisable only from the standpoint of determining the gradation of atrophy, and typing of metaplasia is meaningless [31, 32].

According to WHO experts the further investigations in the field of the gastric cancer risk assessment is in the study of molecular markers: "The clinical usefulness of ancillary markers is still under investigation … predictive biomarkers include immunohistochemistry for p53; target sequencing of TP53, ARID1A, APC, ARID2, and RNF43; MSI; and promoter methylation of p16…" [24].

A search in the PubMed yielded 1214 publications devoted to MSI gastric cancer. However, after specifying the search parameters to study the MSI status in GM precancerous lesions we obtained only 39 publications (Fig. 5).

According to published data, the MSI level in intestinal metaplasia varied from 3.2 to 63.3 % [33–35]. It is especially interesting that in a number of studies an identical MSI profile in foci of intestinal metaplasia located near an already established microsatellite-unstable tumor was found [36]. It should be noted that from the standpoint of the MSI status assessment the stages of the carcinogenesis cascade have been studied extremely unevenly: not a single group of authors has focused their attention on assessing such an early precancerous lesion as absolute (non-metaplastic) GM atrophy.

Does this mean that immunohistochemical study of MMR system proteins supplemented by PCR can be used as an additional predictive marker of gastric cancer? This is hardly true. Despite of the fact that intestinal metaplasia is well recognized by pathologists even with routine histological sections staining with hematoxylin and eosin,

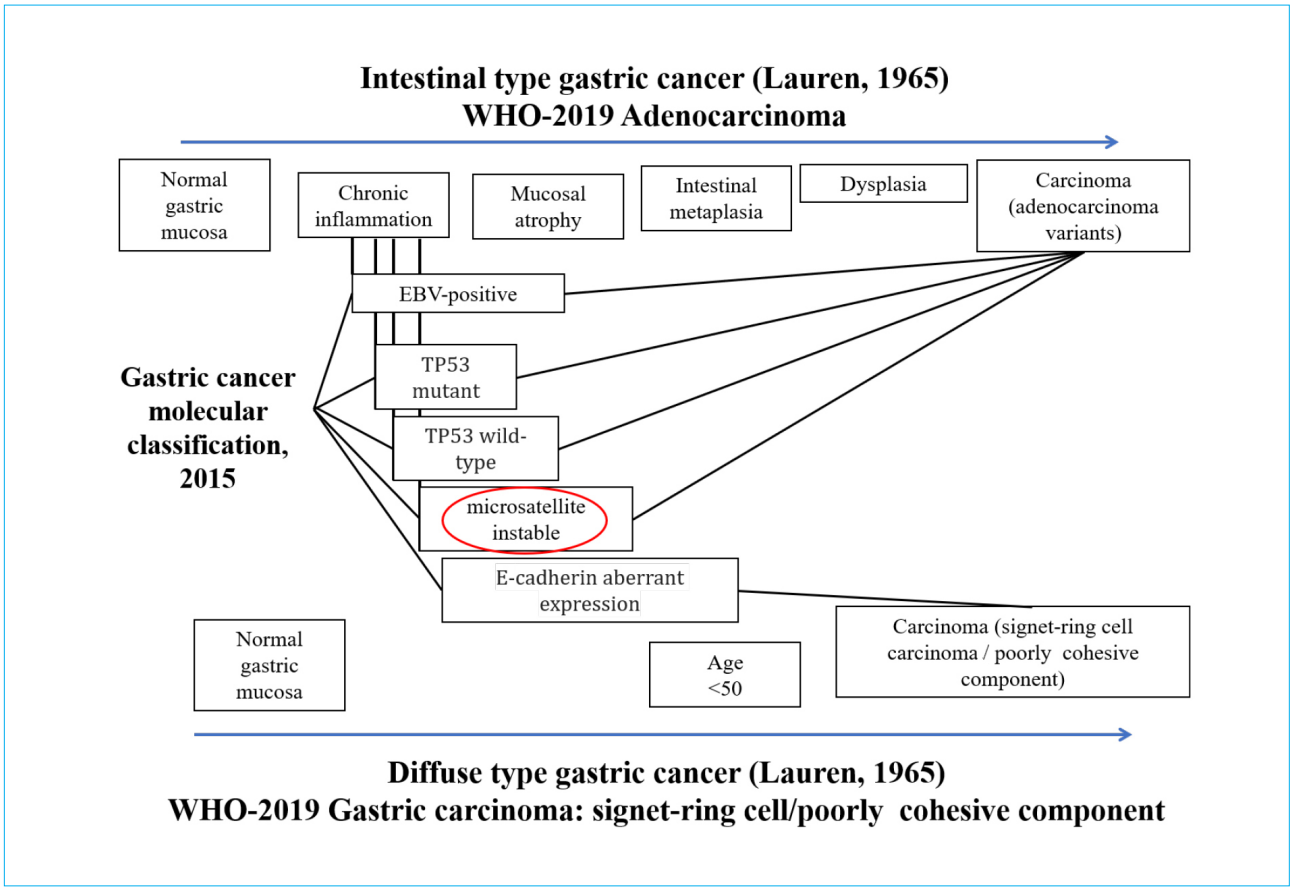

Figure 4. Classification of gastric cancer. Molecular genetic definitions are correlated with classical histological forms: intestinal type gastric cancer and diffuse type gastric cancer (according to P.A. Lauren, 1965) [26]

Рисунок 4. Классификация рака желудка. Молекулярно-генетические дефиниции соотнесены с классическими гистологическими формами: рак желудка кишечного типа и рак желудка диффузного типа (по P.A. Lauren, 1965 г.) [26]

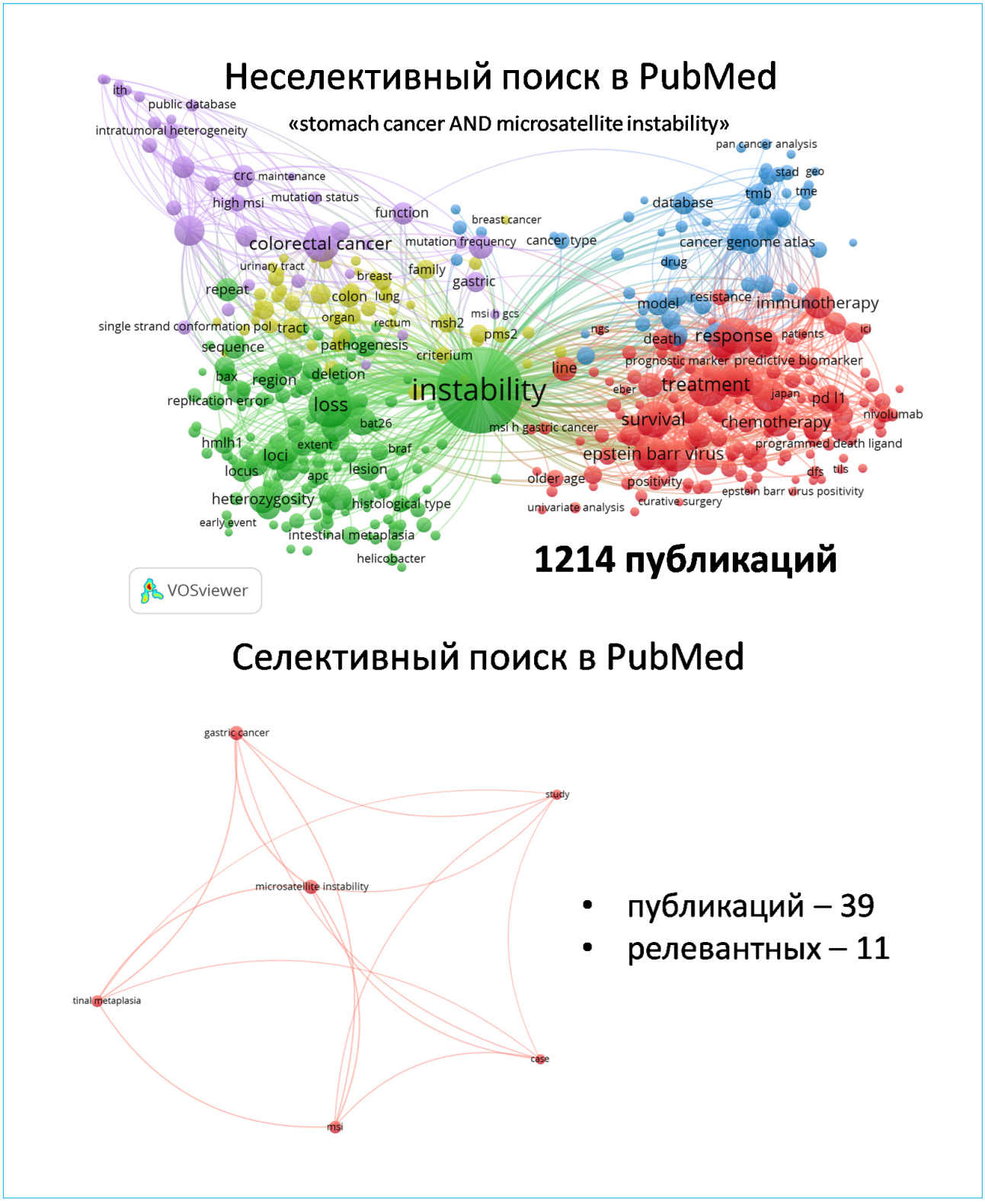

Figure 5. PubMed search query reflecting the pool of publications for the queries “gastric cancer and microsatellite instability” (left) and “precancerous changes in the gastric mucosa/intestinal metaplasia/atrophy and microsatellite instability” (right)

Рисунок 5. Поисковый запрос в системе PubMed с использованием программы VOSviewer, отражающий пул публикаций по запросам «рак желудка и микросателлитная нестабильность» (слева) и «предраковые изменения слизистой оболочки желудка / кишечная метаплазия/атрофия и микросателлитная нестабильность» (справа)

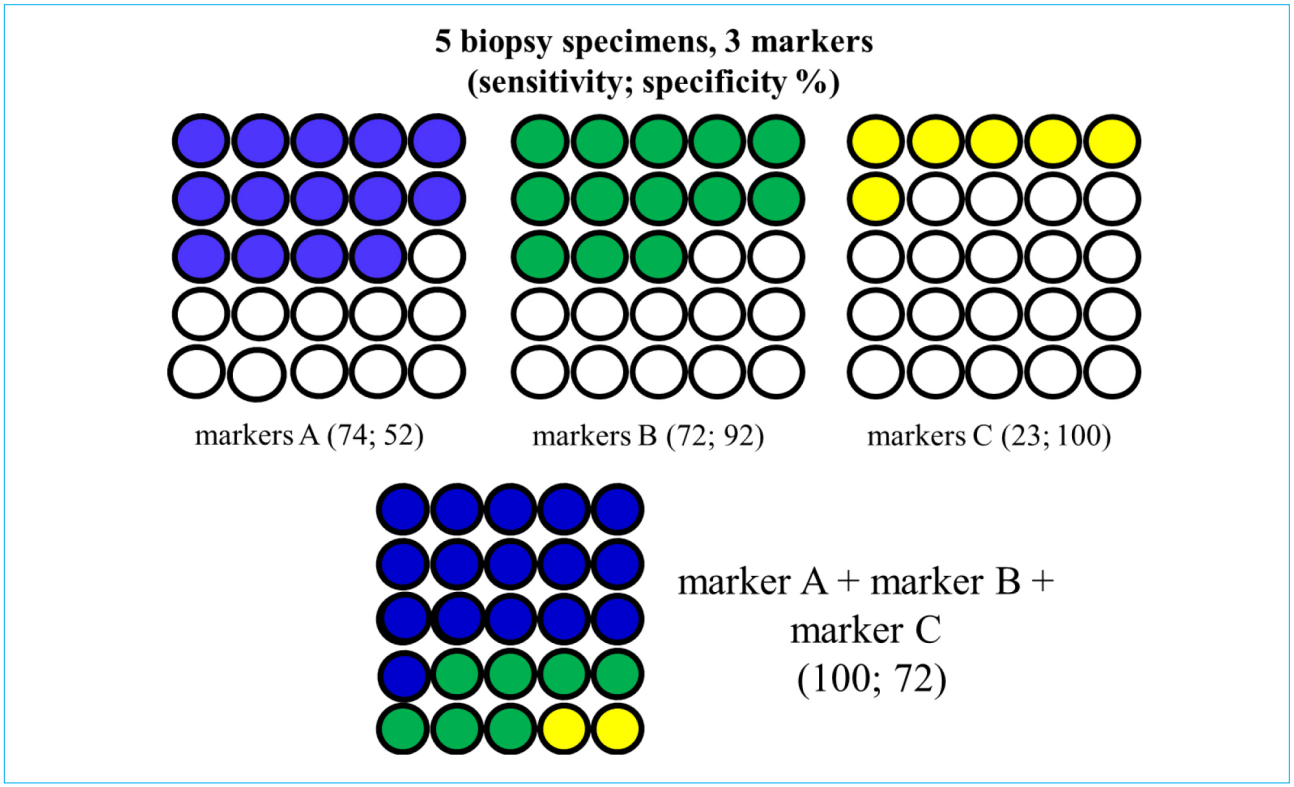

Figure 6. Model of a decision support system in the diagnosis of dysplasia (intraepithelial neoplasia) of the gastric mucosa

Рисунок 6. Модель системы поддержки принятия решений в диагностике дисплазии (интраэпителиальной неоплазии) слизистой оболочки желудка

as well as nuclear immunohistochemical label in the GM epithelium, there is insufficient information on the predictive value of such a finding.

But what about the role of pronounced GM precancerous lesions in the gastric mucosa, namely dysplasia (intraepithelial neoplasia)? After all, it would be natural to assume that the further we move along the cascade of precancerous changes, the higher the level of genomic instability becomes and the more likely MSI can be detected — and therefore, the more likely it can be used as a predictor of gastric cancer development risk.

The situation is complicated by the difficulty of detecting the GM dysplasia (intraepithelial neoplasia) morphological phenomenon. Histological criteria for its diagnosis are cumbersome and difficult to apply for everyday diagnostics (Table). Additional confusion is caused by the presence of a diagnostic category «indefinite for dysplasia» which is actually a reflection of the epithelium regenerative transformation due to the persistent inflammation.

In these terms the use of an additional marker may be appropriate not even from the point of view of gastric cancer prediction, but from a diagnostic standpoint. After all, the probability of the MSI presence in specimens indefinite for dysplasia is minimal because of its biological meaning whereas in truly neoplastic processes (low and high-grade dysplasia) a high level of MSI is expected.

However, in our pilot study the percentage of MMR-deficient cases among diagnostic observations was low, which does not allow us to consider MSI as a predictive marker that could be used by itself. However, the study of MMR system proteins along with other markers included in the general panel may potentially have high sensitivity and specificity and be applicable (Fig. 6).

Conclusion

Microsatellite instability is a common phenomenon registered in gastric cancer. At the same time, the detection of MMR deficiency and microsatellite instability in the gastric mucosa severe precancerous lesions indicates that its detection has possible diagnostic and predictive role. The detection of microsatellite instability in dysplasia (intraepithelial neoplasia) only in some cases does not allow us to consider MSI status as the only marker of carcinogenesis but makes it possible to recommend its use within a panel of biomarkers aimed to support the decision-making of a pathologist in the alternative of "indefinite for dysplasia" or "definite dysplasia — obligate precancer" which requires verification using a large array of data at sequentially traced stages of the Correa cascade.

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